EXPERIMENTAL HOOKWORM INFECTION IN HUMANS WITH METABOLIC DISEASE

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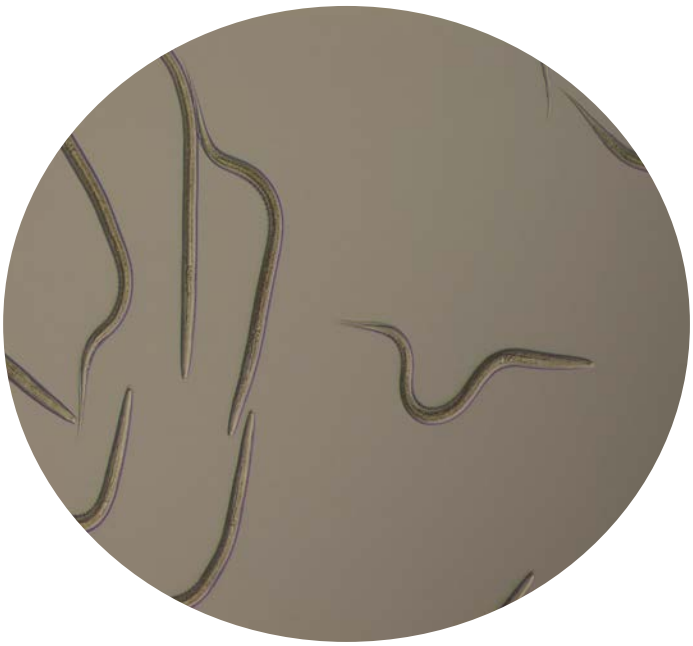

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And just like this, my PhD journey that seemed endless at the start is over. Despite hitting some major roadblocks in my personal life and a few "Are we there yet?" moments, I feel like I won the PhD jackpot. With the most amazing and supportive advisory team, an exciting project that not even a pandemic could derail, and countless opportunities for professional development, I could not have asked for a more positive experience. Here, I would like to acknowledge people who encouraged and supported me along the way, and I apologise in advance if I cannot mention every single individual due to space constraints.

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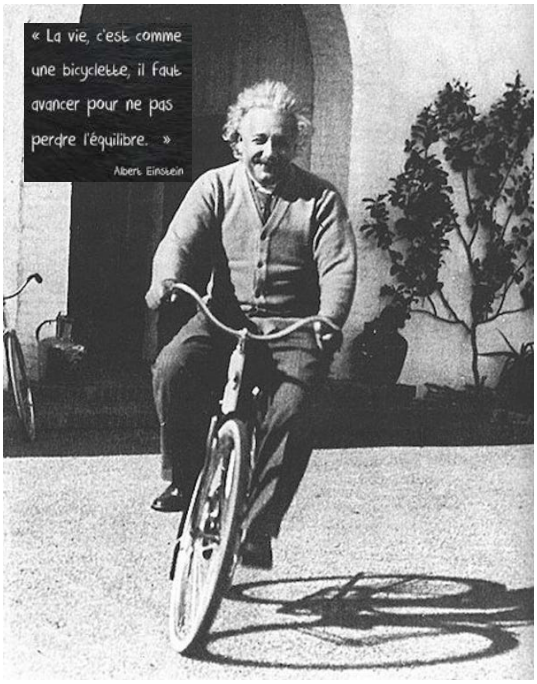

Statement of the Contribution of Other

Nature of Assistance	Contribution	Names, Titles
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	Data management and analysis	Dr Paul Giacomin Fintan Thompson
	Bioinformatics	Dr Matt Field
	Statistical support	Professor Adrian Esterman
	Editorial support	Dr Paul Giacomin
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	Adipokine ELISAs	Dr Paul Giacomin
	DNA extraction and investigational product	Luke Becker
	Human hookworm multiplex qPCR	Rebecca Traub, Patsy Zendajas, Szu Fui Hii (University of Melbourne)
	Trial coordination	Sally McDonald Grace Stanton Jasmine Bell Lynne Saunders
	Trial doctors	Professor Malcolm McDonald Dr Lea Merone Dr Lynne Reid
	Participant recruitment	Sally McDonald Melissa Piontek Tyler Gilstrom

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Published work by the author incorporated into the thesis

Pierce, D., Merone, L., Lewis, C., Rahman, T., Croese, J., Loukas, A., McDonald, M., Giacomin, P., & McDermott, R. (2019). Safety and tolerability of experimental hookworm infection in humans with metabolic disease: study protocol for a phase 1b randomised controlled clinical trial. *BMC Endocrine Disorders*, *19*(1), 136. https://doi.org/10.1186/s12902-019-0461-5

AL, PG, and RM conceived and designed the trial. TR, JC, and MM provided advice on trial design. CL, LM, and DP contributed in writing the study protocol and manuscript. All authors read and approved the final manuscript.

Planned publications

Due to the blinded nature of the trial and the very recent two-year trial completion in June 2020, Chapters 3-5 contain unpublished data that will be submitted for publication in 2022.

Chapters 3&4 - Safety and tolerability & Metabolic responses - will be submitted to Lancet Diabetes & Endocrinology with the proposed authors Pierce D., McDonald M., Thompson F., Esterman A., Croese J., Loukas A., McDermott R. & Giacomin P. The manuscript is in preparation.

Chapter 5 - Immune and adipokine responses – will be submitted to Parasite Immunology with the proposed authors Pierce D., Ryan R., Hebbard L., Loukas A., McDermott R. & Giacomin P. Manuscript preparation will begin later this year.

Abstract

Obesity is a pandemic and was the fourth leading cause of death in 2017. Abdominal obesity, in particular, has been highlighted as the critical risk factor in the development of metabolic syndrome (MetS), which precedes type 2 diabetes (T2DM). Diabetes is a complex, incurable illness requiring lifelong medical care to defer complications and is characterised by insulin resistance in target organs, sustained low-grade systemic inflammation originating from the expanding white adipose tissue, and gut microbiota dysbiosis. Currently, the prevention and management of T2DM rely heavily on pharmaceutical drugs that primarily target hyperglycaemia without considering the underlying inflammation. Lifestyle adaptations, including a healthy diet and greater physical activity, can successfully prevent the development of T2DM but are poorly adopted, and new preventative interventions are needed. Evidence from experimental mouse and human observational studies suggests a protective role of helminth (parasitic worm) infection against metabolic disorders via induction of an anti-inflammatory type 2 immune response. However, causal evidence from experimental human infection was needed to support these findings. Interventional clinical studies, particularly randomised, controlled trials, can provide some of the most robust levels of evidence. I hypothesised that experimental hookworm infection would be safe, tolerable, and acceptable, stabilise or improve determinants of metabolic disease, and induce a biased Type 2 and immune regulatory response.

Our study team designed and implemented a word-first Phase Ib clinical trial of experimental hookworm infection in humans with metabolic disease. In this randomised, double-blind, placebo-controlled trial, adult individuals with MetS were infected with either 20 or 40 infective larvae of the human hookworm *Necator americanus* or the placebo (chilli) sauce and evaluated every six months for two years. I aimed to establish the acceptability, tolerability, and safety of experimental infection, determine the influence of infection on key metabolic and physical parameters, and investigate potential correlates and mechanisms of protection against metabolic disease. The study protocol based on Chapter 2 of this thesis was published in the journal BMC Endocrine disorders in June 2019.

My first results chapter (Chapter 3 of this thesis) details the examination of the primary outcome for this Phase 1b clinical study, that is if hookworm infection is safe, well-tolerated and acceptable in humans with metabolic disease. My results showed that adverse events following hookworm treatment were mainly mild to moderate in nature and largely unrelated to early withdrawal from the trial, with abdominal discomfort in the earl trial stage stemming from worm migration to the intestine well-tolerated. Sixteen out of forty participants dropped out early during the two-year trial, with only three of these for hookworm-related gastrointestinal discomfort. Hookworm treatment almost universally induced patent (faecal egg-positive) infections and eosinophilia. Public interest in the trial was strong, and infected participants became very attached to their new cohabitants. I found that participants' mood

and well-being were stable or improved throughout the two-year trial duration, with compelling trends for people in the hookworm-treated groups to exhibit greater improvements in mood and well-being scores compared to Placebo. Together, I established that hookworm infection was safe and well tolerated by the vast majority of participants.

Chapter 4 of my thesis details the effect of hookworm infection vs Placebo on metabolic health parameters. Critically, this study demonstrated that infection with a low dose of 20 hookworm larvae significantly improved insulin resistance as assessed by the homeostatic model assessment for insulin resistance (HOMA-IR), the trial's primary metabolic outcome. Further, body mass was reduced by approximately 10% in the low-dose hookworm treatment group. The higher dose of 40 worms induced trends in improved metabolism, but the magnitude of these improvements was lesser than treatment with 20 hookworms. Despite improvements in measures of glucose homeostasis, hookworm treatment did not associate with improvements in blood lipid profiles. My results provide the first causal evidence for a protective role of hookworm infection against metabolic disease. Additionally, the magnitude of improvement in insulin resistance well exceeded previously reported effects found in cross-sectional and deworming human studies and suggested hookworm infection as a promising preventative intervention for T2DM.

In Chapter 5 of this thesis, I interrogated potential correlates and mechanisms of protection against insulin resistance. Sex, age, and physical activity were not significantly associated with changes in insulin resistance. In contrast, body mass changes correlated significantly with HOMA-IR changes in hookworm-treated individuals, where people who exhibited lower HOMA-IR typically saw greater body mass reductions. Improved HOMA-IR was also positively associated with greater adherence to a Mediterranean diet. Further, hookworm infection induced a biased type 2 immune response in the blood, characterised by increased serum IL-5 levels and expansion of eosinophil and basophil counts. I also observed that hookworm infection was associated with elevated adiponectin levels, which positively correlated with improvements in HOMA-IR, but this association was not significant.

In summary, this study has added some much-needed causal support for a beneficial role of helminth infection in humans with metabolic disease. My findings should be confirmed in future trials that can also address the current trial's limitations regarding participant numbers, generalisability, and consistency of worm quality. The stored Biobank samples will allow further exploration of immune responses and gut microbiota composition and functional changes that may have moderated HOMA-IR improvements. While live worm therapy might not appeal to everyone, worm-derived molecules could provide an acceptable alternative in the future.

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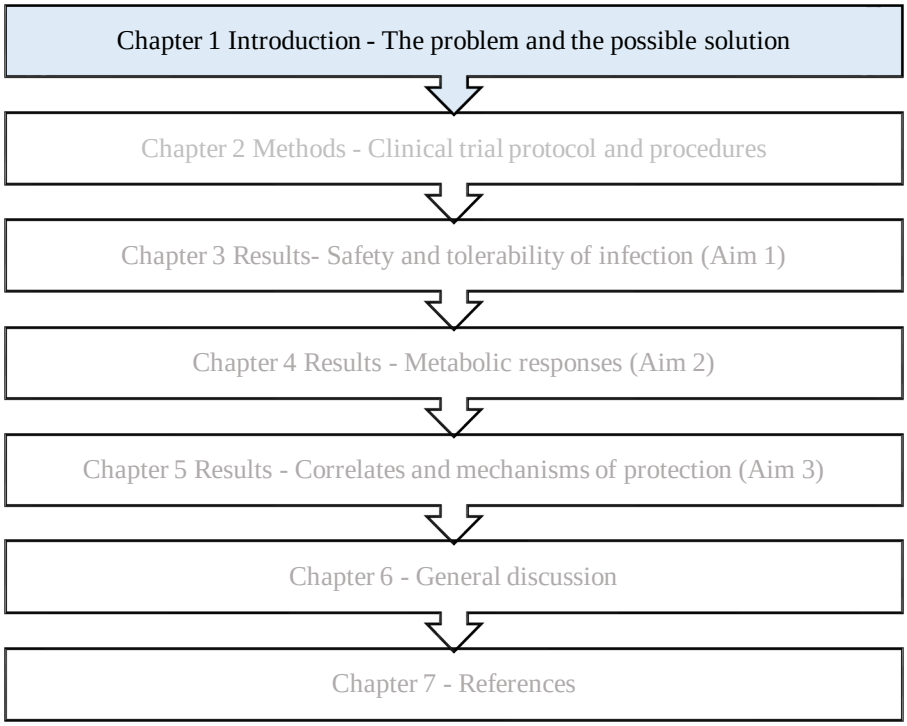
List of abbreviations used throughout the document

AAMs	alternatively activated macrophages
AE	adverse event
AT	adipose tissue
BMI	Body mass index
CVD	cardiovascular disease
DAMPS	damage-associated molecular patterns
ES	excretory-secretory
FFAs	free fatty acids
GLUT	glucose transporter
HbA1c	glycated haemoglobin
HOMA-IR	homeostatic model assessment of insulin resistance
IL	interleukin
IQR	interquartile range
ITT	intention to treat
KW	Kruskal-Wallis
L3	stage-three larvae
MetS	metabolic syndrome
Na	*Necator americanus*
PP	per protocol
PPAR-γ	peroxisome proliferator-activated receptor gamma
PRR	pattern recognition receptor
PUFA	poly-unsaturated fatty acids
SAE	serious adverse event
SCFA	short-chain fatty acids
STH	soil-transmitted helminths
T2DM	type 2 diabetes mellitus

Th2	T helper type 2
Tregs	regulatory T cells
WAT	white adipose tissue
WC	waist circumference

1 INTRODUCTION



This chapter introduces obesity as a critical risk factor in the development of metabolic disorders. I have reviewed the leading processes in the pathophysiology Type 2 diabetes and outlined how helminth infection could attenuate these processes and protect against metabolic diseases. My hypotheses and aims conclude the chapter.

1.1 Obesity, metabolic syndrome, and type 2 diabetes

1.1.1 Obesity – a chronic disease?

Obesity is a pandemic and was the fourth-leading cause of death worldwide in 2017 (G. B. D. Risk Factor Collaborators, 2018). Excess body fat storage (adiposity) generally results from a long-term, positive energy imbalance. Multiple factors, including genetics, dietary intake, physical activity patterns, and environmental elements, are implicated in its aetiology. Obesity was declared a chronic medical disease by the American and Canadian Medical Associations in 2013 and 2015, respectively (Sharma & Campbell-Scherer, 2017). More recently, The Obesity Society (the leading professional society focused on obesity science, treatment and prevention) endorsed the classification of obesity as a worldwide, non-communicable chronic disease in their 2018 position statement (Jastreboff et al., 2019). The society's top leaders argued that "Obesity meets all criteria for being a disease, and, therefore, should be characterised as such". The society argued that recognition as a debilitating disease would likely attract increased funding for the prevention, treatment, and research of obesity.

Furthermore, obese people may experience less discrimination and receive improved care if obesity is taken seriously rather than frowned upon as the self-inflicted consequence of personal shortcomings: gluttony, laziness, and lack of willpower (Jastreboff et al., 2019). In contrast, the American Medical Association's Council on Science and Public Health disagreed and questioned whether recognising obesity as a disease rather than a condition or disorder would improve health outcomes for the obese (MedPage Today, 2013. Accessed 31 May 2019). Many nations, including Australia and most European countries (except for Portugal), have yet to recognise obesity as a stand-alone chronic disease associated with substantially reduced quality of life and increased risk of overall morbidity.

1.1.2 Obesity-associated morbidity and mortality

High body-mass index (BMI) was attributable to 4.72 million deaths and 148 million disability-adjusted life-years (DALYs) in 2017. These figures represent an increase of 36.3% and 36.7% in deaths and DALYs, respectively, from 2007 to 2017 (G. B. D. Risk Factor Collaborators, 2018). Notably, three of the five leading risk factors for death and disability were metabolic, including high BMI, high fasting glucose, and high systolic blood pressure. Since 1975, the number of obese or overweight adults and children has nearly tripled worldwide and grown to just under 2.3 billion people in 2016, equating to 39% of adults that were overweight and 13% that were obese (World Health Organization, 2018). Although current trends point towards stabilising obesity prevalence in developed countries, transitioning and lower-income countries with traditionally lower prevalence of obesity are now seeing levels increasingly similar to those in the United States (Afzal et al., 2016).

Type 2 diabetes is a progressive condition in which the body becomes resistant to the normal effect of insulin and/or gradually loses the capacity to produce enough insulin in the pancreas. Type 2 diabetes is associated with modifiable lifestyle risk factors and has strong genetic and family-related risk factors.
Impaired glucose tolerance: Blood glucose levels are higher than normal but not high enough to be classified as diabetes.
Impaired insulin sensitivity: Insulin sensitivity describes how sensitive the body is to the effects of insulin. Individuals with impaired insulin sensitivity (also called insulin resistance) will require larger amounts of insulin to lower blood glucose levels than someone with high sensitivity. Insulin resistance is associated with the development of Type 2 diabetes.

Greater adiposity impairs the body's structural and functional integrity and heightens the risk of comorbidities (Jastreboff et al., 2019). Significantly, the presence of obesity considerably reduces the number of disease-free years (Nyberg et al., 2018) and favours the development of more than 200 chronic conditions (Jastreboff et al., 2019), including non-alcoholic fatty liver disease (Vernon et al., 2011), cardiovascular disease (CVD) (Lavie et al., 2016; Parto & Lavie, 2017), and type 2 diabetes mellitus (T2DM) (Gray et al., 2015; Guilherme et al., 2008) (Figure 1-1). Even acute diseases such as COVID-19 appear to be more severe in the presence of obesity (Tibirica & De Lorenzo, 2020). These comorbidities lead to poorer health outcomes in obese patients compared to leaner comparisons, as demonstrated in a recent systematic review and meta-analysis (Chu et al., 2020). A 2018 study of 120 000 Europeans reported a significant reduction in disease-free years in both mildly and severely obese individuals (Nyberg et al., 2018). Remarkably, the reduction was not mitigated by gender, smoking status, physical activity level, and socioeconomic status, confirming a ubiquitous association between obesity and healthy life years lost. Increasing obesity in both sexes is consistently associated with consequent T2DM diagnosis and a progressively greater risk for all T2DM complications (Gray et al., 2015). A recent analysis of the global trends and disease burden of obesity, which integrated data from 68.5 million overweight and obese children and adults between 1980 and 2015, identified T2DM as the second leading cause of obesity-related deaths in 2015, surpassed only by CVD (G.B.D. Obesity Collaborators, 2017). Notably, obese diabetics with a BMI above 30 kg/m^2 were more than twice as likely (9.5%) to die from diabetes-related complications compared to individuals with a BMI below 30 kg/m^2 (4.5%) (G.B.D. Obesity Collaborators, 2017), highlighting increasing obesity as a serious adverse factor in diabetes-related mortality.

Elevated BMI considerably increases the risk of overall and premature mortality. In 2015, a large American study including 14 657 individuals established a progressively higher risk of adverse health outcomes and death with greater than normal BMI (≥25 kg/m^2) in both sexes (Gray et al., 2015). The Global BMI Mortality Collaboration confirmed these findings in their 2016 meta-analysis of 239 prospective studies from four continents (The Global B. M. I. Mortality Collaboration, 2016). Demonstrating a close relationship between even slightly higher than normal BMI and excess mortality, these more recent data challenged previous suggestions of an *Obesity-survival Paradox* (Bosello et al., 2016), which postulated a potentially protective effect of overweight and low-grade obesity against all-cause mortality (Doehner et al., 2010; Flegal et al., 2013; Hoddy et al., 2021). The Global BMI Mortality

Collaboration proposed improved control for bias in their study compared to the crude analyses conducted in the previous meta-analysis as the likely reason for the discrepant findings. Alarmingly, the adverse effect of excess body fat on healthy life years (life without disability) and total years of life (longevity) appears to be most significant in the young (20-39 years) age bracket and weakens with increasing age (Grover et al., 2015). In the young age bracket, healthy life years were reduced by 18.8 and 19.1 years for very obese men and women, respectively, and years of life lost were estimated to be as high as 11.9 (men) and 6.3 (women) years in this group. In contrast, obesity seemed to shorten lifespan only minimally in the older age bracket (60-79 years).

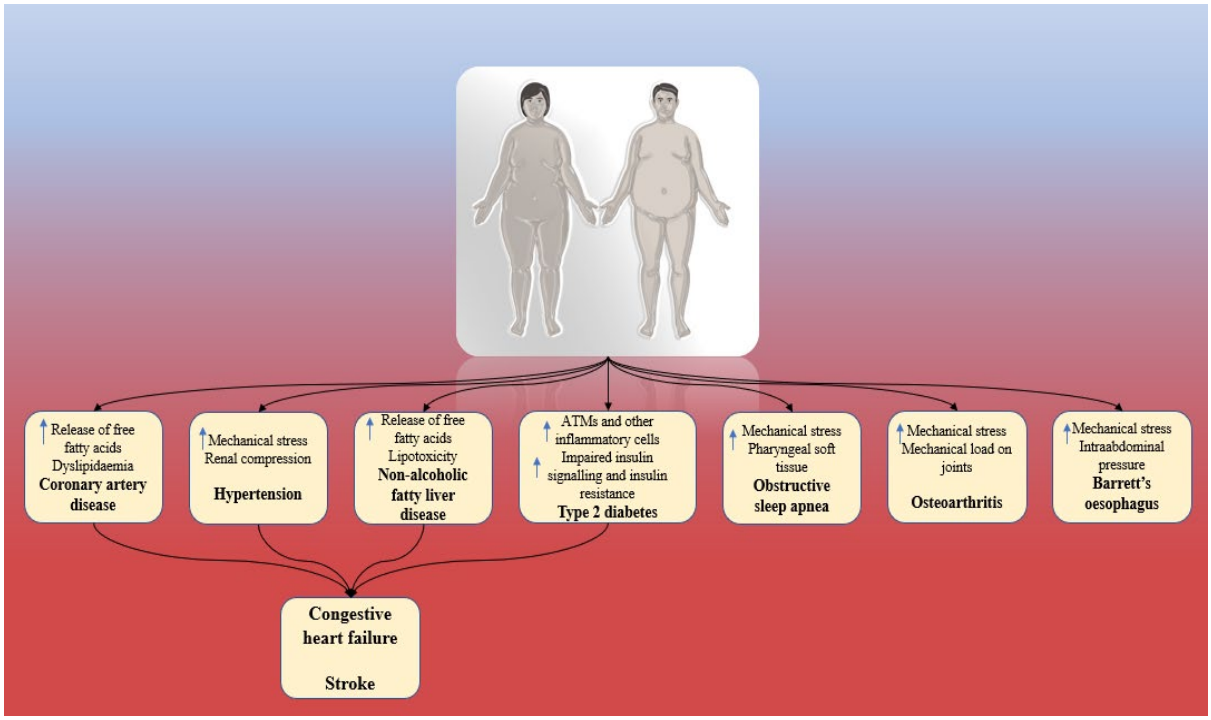


Figure 1-1 Major risk factors and common chronic diseases induced by adiposity
ATMs, adipose tissue macrophages. Adapted from Heymsfield & Wadden, 2017

Particularly concerning is the emerging presence of the negative consequences of obesity in early childhood. For the first time in two centuries, experts predict that life expectancy at birth in the United States may decline during the first half of this century due to escalating obesity in early life and its associated life-shortening complications such as T2DM. (Olshansky et al., 2005). Impaired glucose tolerance and hypertension have been implicated as potential agents in the association between elevated BMI in childhood and premature mortality in adulthood (Engeland et al., 2003; Franks et al., 2010). Interestingly, the greater risk of morbidity and mortality in adults that were obese or overweight in childhood may be largely independent of adult BMI, except for morbidity from diabetes (Must et al., 1992), which further substantiates the importance of overweight and obesity in the development of T2DM and subsequent comorbidities and mortality. Although overweight and obesity are accepted as

significant contributors to greater morbidity and mortality, disagreement still exists about the correct classification of overweight and obesity (Sharma & Campbell-Scherer, 2017).

1.1.3 Classification of obesity – should we keep using BMI?

The sole use of BMI to determine obesity and predict future illness is problematic. Generally, obesity is defined as "abnormal or excessive fat accumulation that may impair health" (World Health Organization, 2018). Previously called the Quetelet index, BMI is a composite measure of body mass in kilograms divided by height in metres squared (kg/m^2). It is routinely used as a surrogate indicator of fatness and obesity (World Health Organization, 2018). Intriguingly, anthropometric measures, such as BMI, were initially not developed to assess body composition or fatness but as a convenient tool to monitor growth (Keys et al., 1972). Nevertheless, the World Health Organisation adopted a BMI classification with three obesity classes as suggested by the 1997 International Obesity Task Force (World Health Organization, 2019) to categorise obesity. With the need to accommodate the ever-growing numbers of people with severe obesity (BMI ≥40 kg/m^2), the American Heart Association proposed two additional obesity classes (Poirier et al., 2011) (Table 1-1). Despite the apparent association between elevated BMI and increased risk of overall morbidity and mortality detailed in 1.1.2, reliance on arbitrary BMI cut-offs as a measure of fatness, predictor of future ill health, and determinant of public health policies is controversial (Nuttall, 2015).

Table 1-1. Classification of obesity by BMI categories

World Health Organization		**American Heart Association**	
Category	**BMI (kg/m^2)**	**Category**	**BMI (kg/m^2)**
Underweight	<18.5	Underweight	<18.5
Normal	18.5-24.9	Normal/acceptable	18.5-24.9
Pre-obesity	25.0-29.9	Overweight	25.0-29.9
Obesity Class 1	30.0-34.9	Obese Grade 1	30.0-34.9
Obesity Class 2	35.0-39.9	Obese Grade 2	35.0-39.9
Obesity Class 3	≥40	Obese Grade 3*	40.0-49.9
		Obese Grade 4*	50.0-59.9
		Obese Grade 5*	≥60

* Severe, extreme, or morbid obesity; BMI, body mass index. Adapted from World Health Organization (2019) and Poirier et al. (2011)

BMI is a poor indicator of body composition and the percentage and distribution of fat, leading to misclassifications (Keys et al., 1972). A crucial shortcoming of BMI is its inability to distinguish between fat and lean (muscle and bone) tissue. This flaw means that individuals with great muscle mass, such as athletes or military personnel, may be falsely classified as obese. Vice versa, metabolically obese people with high body fat percentages and impaired insulin sensitivity can still have a BMI in the normal range (Romero-Corral et al., 2010) and may not be identified as at risk of T2DM. Population-based studies show that the correlation between body fat percentage and BMI is particularly poor in males (Flegal et al., 2010; Keys et al., 1972). Also, females generally have a lower BMI than males despite their higher fat mass (20-45%) relative to their height (Nuttall, 2015), which again illustrates the limitations of BMI as an indicator of obesity and chronic disease.

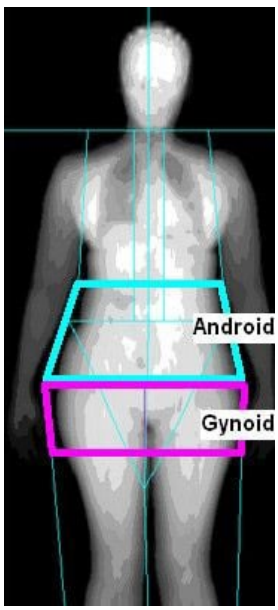


Figure 1-2 Android and gynoid body fat regions

Body fat distribution rather than total body fat percentage more accurately predicts the development of future diseases (Nuttall, 2015; Oliveira-Sequeira et al., 2014). First described by Dr Jean Vague in 1956 (Hanefeld et al., 2007), an android (male) distribution of fat (Figure 1-2) with accumulation of fat in the upper body considerably increases the risk of coronary heart disease, T2DM, gallstones, and gout (Vague, 1956). In contrast, Vague reported no such predisposition with a gynoid (female) fat distribution (Figure 1-2), where fat accumulates in the lower body (gluteal area and thighs). Of note, despite their designation as male and female fat distribution, android and gynoid fat distribution occur and carry the same associations in either sex (Vague, 1956). Subsequent research (Harman-Boehm et al., 2007; Nieves et al., 2003; Pekgor et al., 2019; Pouliot et al., 1992; Ross, Aru, et al., 2002; Ross, Freeman, et al., 2002) has reiterated Vague's observations and highlighted central, that is abdominal and visceral adiposity, as the principal factor in metabolic syndrome (MetS), as it appears to precede all other factors (Cornier et al., 2008). Hence, abdominal waist circumference (WC) or waist-to-hip ratio may be a superior measure to determine the risk for the development of MetS and subsequent life-threatening diseases, including CVD and T2DM (Alberti et al., 2009; O'Neill & O'Driscoll, 2015).

1.1.4 The Metabolic Syndrome

Metabolic syndrome started as a concept of connected metabolic risk factors that increase the risk of CVD, T2DM, and all-cause mortality. Often credited with pioneering MetS (Despres et al., 2008), Professor Gerald 'Jerry' Reaven, in the 1988 Banting lecture, introduced a cluster of risk factors for T2DM and CVD as 'syndrome X'. In his lecture, Reaven linked elevated blood insulin, insulin resistance, glucose intolerance, elevated very low-density lipoprotein triglycerides, reduced high-density lipoprotein cholesterol, and hypertension (Reaven, 1988). His novel concept of insulin

resistance was later confirmed as a critical underlying risk factor in MetS (Alberti & Zimmet, 1998). However, surprisingly, he failed to include obesity or visceral obesity as risk factors, which are now accepted as instrumental in MetS. Nevertheless, the story of MetS did not start with Reaven but much earlier with the Swedish physician Kylin, who first associated high blood pressure, high blood glucose, and gout in 1923 (Kylin, 1923). As mentioned in Section 1.1.3, Vague later connected the presence of metabolic abnormalities that heighten the risk of CVD and T2DM to abdominal obesity (Vague, 1956). Subsequently, in 1965 Avogaro and Crepaldi (Avogaro et al., 1965) again described a syndrome relating to hypertension, hyperglycaemia, and obesity at the European Association for the Study of Diabetes annual meeting. In 1975, Herman Haller, then head of the Department of Medicine at the Medical Academy Dresden, first introduced the term MetS (Haller & Hanefeld, 1975) to define a combination of several related metabolic risk factors, including obesity, impaired glucose metabolism, hypertension, and dyslipidaemia that promote chronic diseases, such as CVD and T2DM, and increased mortality. He also highlighted obesity as the common causative risk factor. The name MetS was officially adopted in 2001 (Expert Panel on Detection, 2001), and MetS has been listed as a disease in the International Classification of Diseases since 2016 (World Health Organisation, 2016. Accessed August 15, 2019).

International organisations, including the International Diabetes Federation, recognise the heightened risk of several chronic diseases in the presence of MetS (International Diabetes Federation, 2006). However, questions remain about the exact definition of MetS and how each component contributes to the development of T2DM and CVD. Diagnostic criteria and thresholds, though overlapping, varied between individual groups (O'Neill & O'Driscoll, 2015) before the agreement on a harmonised definition of MetS in 2009 (Alberti et al., 2009). Even to date, waist circumference thresholds remain national and population-specific (Table 1-2). However, American Heart Association guidelines generally associate a waist circumference of >94cm in males and >80cm in females with a heightened risk for CVD and T2DM. Hence, the guidelines propose these alternative thresholds for individuals with increased insulin resistance regardless of ethnicity and nationality (Alberti et al., 2009).

Interestingly, the harmonised definition of five core criteria (elevated waist circumference, triglycerides, blood pressure, fasting glucose, and reduced high-density lipoprotein cholesterol) and their thresholds do not nominate abdominal obesity and insulin resistance essential for diagnosis despite their widely accepted causative role in the aetiology in MetS. Instead, exceeding the thresholds for three of the five criteria suffices for diagnosing MetS (Alberti et al., 2009). With estimates of almost one-quarter of the world's population affected by MetS in 2006 (International Diabetes Federation, 2006. Accessed Dec 17, 2019), the concurrent prevalence in adults in rural Australia alone was 35.8%. Numbers in the Asia-Pacific region are growing disproportionately (Ranasinghe et al., 2017), likely due to rapid urbanisation and the adoption of the Western diet and sedentary lifestyle (Tahapary et al., 2015). The escalating emergence of non-communicable diseases such as CVD and T2DM associated with MetS constitutes a significant public health crisis for the affected regions (Ranasinghe et al., 2017).

Table 1-2 Criteria and thresholds for the clinical diagnosis of metabolic syndrome

Measure	Thresholds	
Elevated waist circumference (cm)	**Males**	**Females**
• Canada; USA	≥102	≥88
• Europids; Middle Eastern; Sub-Saharan African; Mediterranean	≥94	≥80
• Asians; Japanese; South and Central Americans	≥90	≥80
Elevated triglycerides (drug treatment for elevated triglycerides is an alternative indicator)	≥ 150mg/dL (1.7mmol/L)	
Reduced high-density lipoprotein cholesterol (drug treatment for high-density lipoprotein cholesterol is an alternative indicator)	**Males:** < 40mg/dL (1.0 mmol/L) **Females:** < 50mg/dL (1.3mmol/L)	
Elevated blood pressure (antihypertensive drug treatment in a patient with a history of hypertension is an alternative indicator)	**Systolic:** ≥ 130mmHg and/or **Diastolic:** ≥ 85mmHg	
Elevated fasting glucose (drug treatment for elevated glucose is an alternative indicator)	≥ 100mg/dL	

Adapted from (Alberti et al., 2009)

1.1.5 Rising prevalence and cost of T2DM

Diabetes is a complex, incurable illness requiring lifelong medical care to defer complications. The disease is characterised by elevated blood sugar levels (hyperglycaemia) resulting from insulin resistance in target organs and pancreatic β cell dysfunction. Chronic hyperglycaemia induces endothelial dysfunction, leading to micro-and macrovascular complications such as retinopathy, nephropathy, neuropathy, cardiovascular disease, and stroke (Chatterjee et al., 2017). Although better lifestyle choices, that is, improved diet, greater physical activity, and less sedentary time (Dubé et al., 2012; Hu, 2011) are effective ways to prevent T2DM, rising numbers of people living with T2DM demonstrate that these healthier lifestyle choices are not adopted as recommended.

The cost of T2DM has proliferated during the last 13 years, primarily due to the rising global prevalence of obesity (Seuring et al., 2015). The number of individuals living with T2DM is expected to increase worldwide from 537 million in 2021 to 783 million by 2045, with obesity-associated T2DM accounting for approximately 90% of all cases. Australia's numbers alone are estimated to grow from 1.49 million in 2021 (6.4%) to 1.94 million (8.0%) by 2045. Even applying conservative projections that assume a constant average expenditure per person and constant diabetes prevalence rate, the current estimated global healthcare expenditure on diabetes will climb to one trillion USD by 2045 (International Diabetes Federation, 2022). Of the worldwide four million diabetes-related deaths in 2017 (one every eight seconds), approximately half (46.1%) were in people under 60, which is their most productive age. The loss of family income associated with disability and premature death often has catastrophic consequences for the affected families (International Diabetes Federation, 2022).

The disproportionately more significant economic and personal impact of escalating T2DM prevalence in low- and middle-income countries is particularly concerning. While T2DM prevalence seems to stabilise in high-income countries slowly (Flegal et al., 2010), increases of up to 156% are expected in economies moving from low- to middle-income levels, likely due to rapid urbanisation and adoption of a Western diet and lifestyle (International Diabetes Federation, 2017). Furthermore, individuals in low- and middle-income countries spend higher proportions of their annual income on diabetes care despite the lower direct costs of diabetes (for example, the cost of drugs and physician visits) compared to high-income countries (Seuring et al., 2015). Worryingly, an estimated 50% of all adults living with diabetes worldwide remain undiagnosed and are unaware of their condition. Approximately 85% of these live in low-to middle-income countries, where limited resources and low priority of diagnostic screening hinder early diagnosis. Late diagnosis magnifies the likelihood of future diabetic complications and demand for healthcare services and healthcare expenditure, compounding economic and personal burdens for developing nations (International Diabetes Federation, 2017).

Although direct costs are the primary driver of all diabetes costs, indirect costs, including productivity losses due to absenteeism, presenteeism, mortality, and labour-force dropouts, still amount to approximately 35% of all costs. Equating to 0.6% of the world's gross domestic product, these productivity losses represent a substantial global economic burden (Bommer et al., 2017). Economies willing to invest more in diabetes prevention could potentially see a considerable reduction in direct and indirect costs of T2DM. These preventative investments require fewer financial resources than the consequences they help reduce.

1.1.6 Pathology of metabolic diseases

We can broadly categorise the pathophysiology of obesity-associated metabolic diseases into three main categories. Firstly, metabolic diseases are characterised by sustained low-grade systemic inflammation originating from the expanding white adipose tissue (WAT) (Donath et al., 2019; Hotamisligil et al., 1993; Saltiel & Olefsky, 2017), including neuroinflammation (Robb et al., 2019). Secondly, we observe metabolic disturbances, including adipocyte hypertrophy, systemic insulin resistance, abnormal blood lipids, and elevated blood pressure, as described in Section 1.1.4, predominantly driven by the underlying systemic inflammation (Moyat et al., 2019). Thirdly, the healthy composition of the gut microflora is lost, which alters metabolism (Bäckhed et al., 2004; Ley et al., 2005) and fuels inflammation (Cani et al., 2007). Importantly, these categories are not isolated but form a triad, where each reciprocally affects the other.

In the following sections, I have further detailed the pathophysiologic characteristics of obesity-associated metabolic disorders.

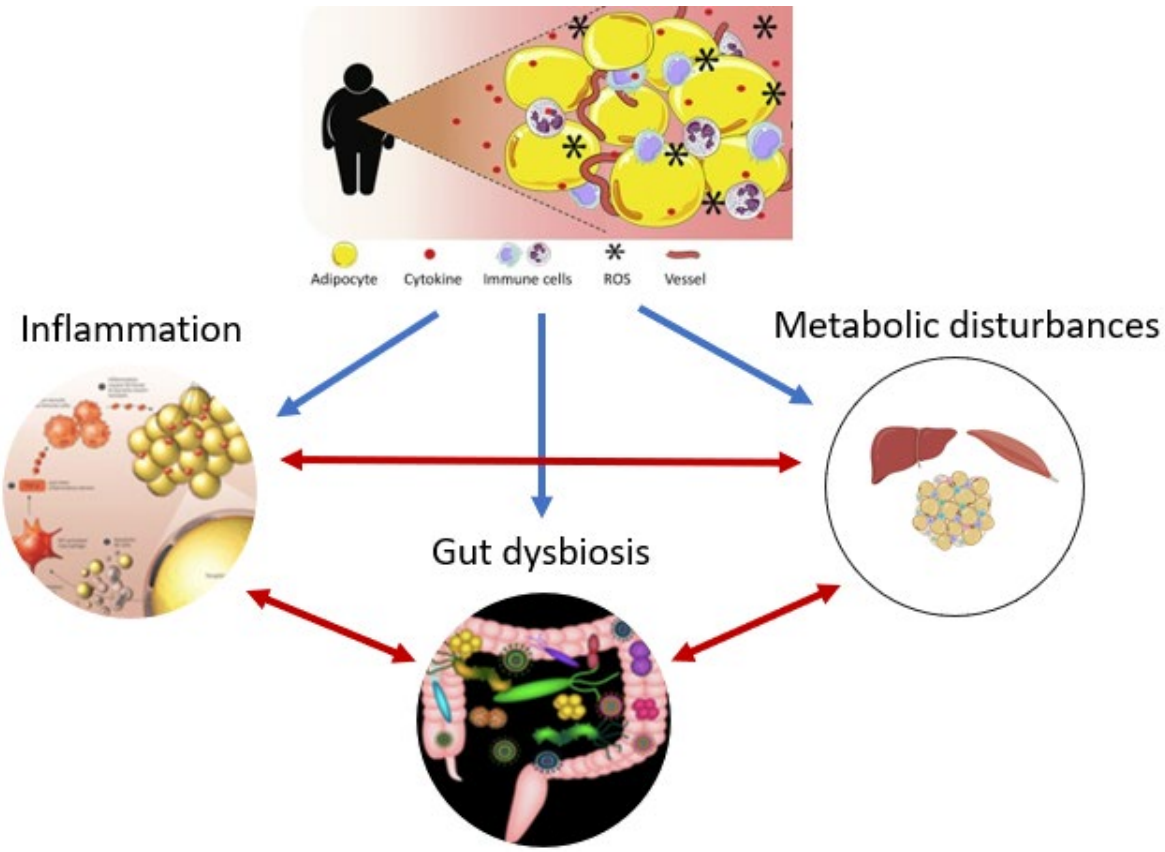


Figure 1-3 The three main pathophysiological categories of metabolic diseases.
The pathophysiology of metabolic diseases can be broadly categorised into three main categories: sustained low-grade systemic inflammation originating from the expanding white adipose tissue; metabolic disturbances, including adipocyte hypertrophy, systemic insulin resistance, abnormal blood lipids, and elevated blood pressure, predominantly driven by the underlying systemic inflammation; and gut dysbiosis, altering metabolism and fuelling inflammation. Importantly, these categories are not isolated but form a triad, in which each reciprocally affects the other.

1.1.6.1 Adipose tissue inflammation

The detrimental role of chronic inflammation is well-established in the pathogenesis of metabolic diseases (Moyat et al., 2019; Tahapary et al., 2015; Wellen & Hotamisligil, 2005). Emanating from the expanding WAT, system-wide inflammation may induce insulin resistance, impair insulin secretion, and dysregulate multiple organs involved in metabolic homeostasis. The sustained activation of the innate immune system seen in obesity-induced inflammation results in similarly sustained disruption of metabolic homeostasis that can last years, decades, or even a lifetime. Unlike self-limiting inflammation during wound healing, obesity-induced inflammation not only alters WAT function but also dysregulates various distal organs such as the liver, skeletal muscle, heart, pancreas, and even the brain (Saltiel & Olefsky, 2017). Areas in the brain that maintain energy homeostasis and regulate appetite, such as the hypothalamus, are particularly affected by inflammatory processes (Jais & Bruning, 2017).

A high-caloric diet triggers rapid remodelling of WAT. Following overfeeding, excess nutrients are stored in adipocytes as lipid droplets. As these droplets expand in size, oxygen diffusion approaches its limit, and mild hypoxia arises. The acute responses to hypoxia are angiogenesis and reshaping of the extracellular matrix to allow further expansion of the WAT and limit hypoxia. In expanding WAT, inflammation may initially support the adaptive responses to overeating by promoting angiogenesis to offset acute hypoxia (Crewe et al., 2017) and increasing adipocyte insulin resistance to prevent excess lipid accumulation (Saltiel & Olefsky, 2017). Acute WAT expansion and remodelling are a healthy response to short periods of overfeeding and are reverted by periods of nutrient shortage. However, chronic overfeeding with continued expansion of WAT and inflammation ultimately results in chronic hypoxia, fibrosis, adipocyte dysfunction and necrosis associated with pathological innate and adaptive immune responses (Crewe et al., 2017).

Pathologic adipocyte hypertrophy poses a major logistical challenge for the resident immune cells. Specifically, macrophages have been highlighted as key players in chronic inflammation and the pathogenesis of metabolic diseases (Ni et al., 2020; Sun et al., 2011). These housekeeping cells represent the largest population of innate immune cells in healthy lean WAT, constituting 5-10% of all leukocytes, and are evenly distributed in lean WAT (Lumeng, Bodzin, et al., 2007). They participate in the clearance (phagocytosis) of apoptotic and necrotic adipocytes, remodelling of the extra-cellular matrix, angiogenesis, and differentiation of adipocyte precursors (Sun et al., 2011; Zaragosi et al., 2010). Furthermore, WAT macrophages may safeguard adipocyte insulin sensitivity via secretion of interleukin (IL)-10, which inhibits the expression of pro-inflammatory chemokines and cytokines, including tumour necrosis factor-alpha (TNF) (Hotamisligil et al., 1993; Smallie et al., 2010). However, the presumed protective role of IL-10 is still unclear. Fuelled by chronic inflammation, WAT macrophage populations appear to proliferate directly proportional to adipocyte size and BMI and may comprise up to 50% of all immune cells in obese WAT (Lumeng et al., 2007; Weisberg et al., 2003).

An anti-inflammatory environment dominates lean**,** healthy abdominal and visceral WAT (Figure 1-4). Lean WAT-resident immune cells and mediators include anti-inflammatory M2-like macrophages, eosinophils, type 2 innate lymphoid cells, regulatory T cells (Tregs), and anti-inflammatory cytokines such as IL-10. Collectively these cells maintain optimal adipocyte mitochondrial function, promote tissue homeostasis, and discourage inflammation and fat accumulation, thereby protecting from insulin resistance (Hotamisligil et al., 1993; Hotamisligil et al., 1997; Lackey & Olefsky, 2016; Pekala et al., 1983). Although macrophages are regularly implicated as the primary regulator of inflammation, eosinophils also play a critical role in this network of adipose tissue-resident immune cells due to their ability to promote and maintain M2-like macrophages via the release of IL-4 (Brigger et al., 2020; Lackey & Olefsky, 2016; D. Wu et al., 2011). As part of the polarised Th2 immune response, alternatively activated M2-like macrophages (AAMs) predominantly participate in parasite clearance (Noel et al., 2004), phagocytosis of debris and apoptotic cells,

smouldering of inflammation, promotion of tissue repair and remodelling, and vasculogenesis (Mantovani et al., 2013; Shapouri-Moghaddam et al., 2018; Wynn, 2004). Recent research suggests that M2-like macrophages can be further sub-divided into four main categories (M2a, M2b, M2c, and M2d), each induced by different stimuli and functioning in a distinct anti-inflammatory manner (Cortes-Selva & Fairfax, 2021; Martinez et al., 2008; Russo & Lumeng, 2018). Of note, recent studies using single-cell RNA-seq approaches have highlighted macrophage heterogeneity and niche-specificity, describing new subsets that may play a pathophysiological role (e. g. lipid-associated macrophages) (Jaitin, 2019). In healthy WAT, crosstalk between the resident immune cells ensures a quick collective response to resolve inflammation resulting from normal tissue remodelling. This delicate balance between pro-and anti-inflammatory processes is perturbed in obese abdominal and visceral WAT (Stivers et al., 2020).

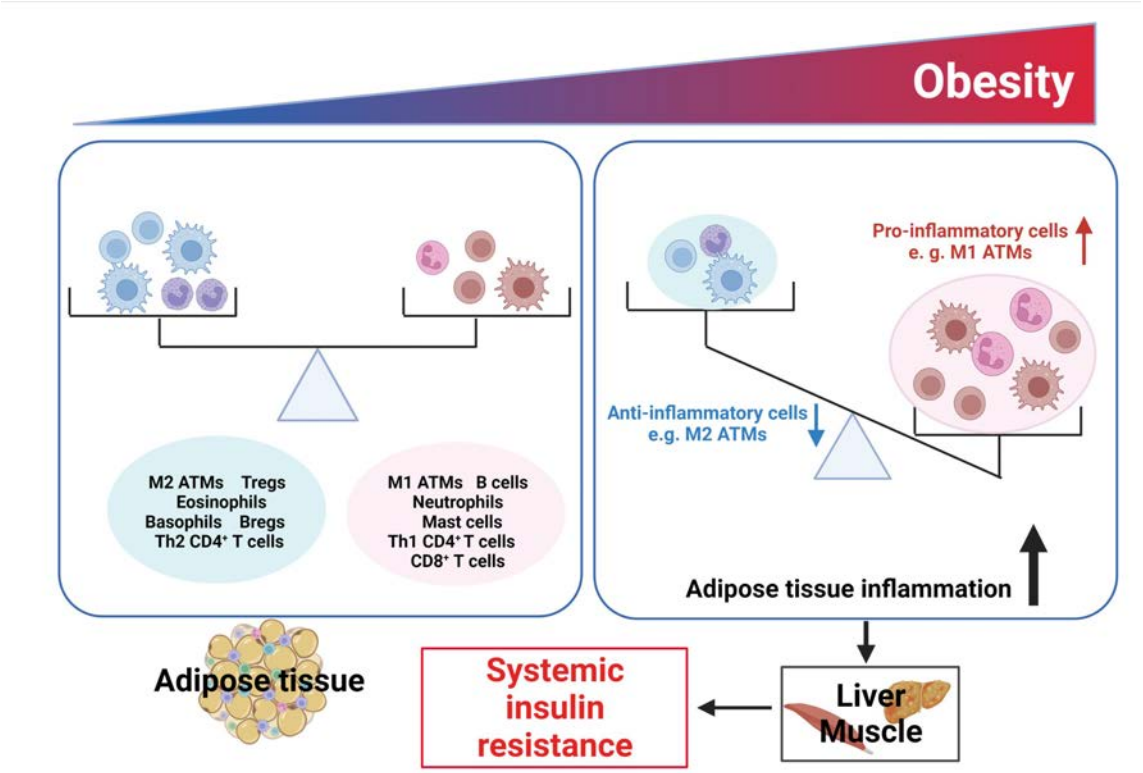


Figure 1-4 Regulation of inflammation and insulin resistance in adipose tissue
M2 ATMs, alternatively activated adipose tissue macrophages; M1 ATMs, classically activated adipose tissue macrophages. Adapted from Kim et al., 2021 and created with BioRender

Quantitative and qualitative differences in macrophage populations occur in obese adipose tissue **(**AT). Macrophages display high plasticity, and their surge in obese WAT is coupled with macrophage polarisation, a switch in their phenotype and functional profile (Lumeng, Bodzin, et al., 2007; Lumeng, Deyoung, et al., 2007; Xu et al., 2003). Besides hypoxia, lipids, and stress, adipocyte death is a leading modulator of immune responses and consequent inflammation in obese WAT (Cinti

et al., 2005; Giordano et al., 2013; Lindhorst et al., 2021; Rausch et al., 2008; Shi et al., 2006; Suganami et al., 2007). In response to the death of hypertrophied adipocytes, lipid-laden pro-inflammatory M1-like macrophages localise to and aggregate in crown-like structures (CLS) that often fuse to form giant multi-nucleated cells around the dead adipocytes to phagocytise their lipid content (Bremer et al., 2011; Cinti et al., 2005; Lindhorst et al., 2021). Cinti et al. (2005) used electron microscopy to determine the ultrastructural features of dead adipocytes inside the CLS. They observed lost plasma membrane integrity, small lipid droplets in the cytoplasm, dilated endoplasmic reticulum, and cell debris in the extracellular space - features generally seen in necrosis rather than apoptosis.

Of note, recent biochemical research has led to a reclassification of necrosis. Whereas previously apoptosis was considered the only pathway of regulated cell death, new details have emerged about pathways of regulated necrosis, which are mechanistically similar to the apoptosis pathway, including necroptosis, pyroptosis, and ferroptosis (Tonnus et al., 2019). However, a critical difference between apoptosis, during which the plasma membrane does not rupture, and necrosis is the loss of plasma membrane integrity and release of intracellular organelles and damage-associated molecular patterns (DAMPs) during necrosis (Sarhan et al., 2018; Scaffidi et al., 2002). High mobility group box1, heat shock proteins, adenosine triphosphate, uric acid, and genetic material such as DNA and RNA are typically released DAMPs following necrosis (Chen & Nunez, 2010). Although generally non-inflammatory in the intracellular space, once exposed, DAMPs initiate an inflammatory response termed necroinflammation, a sterile inflammation independent of pathogen infection (Sarhan et al., 2018). In contrast, during apoptosis, DAMPs are retained in plasma membrane blebs (blister-like protrusions) and deactivated via several processes, resulting in silent cell death that is overall not inflammatory (Garcia-Martinez et al., 2015).

Although the precise pathway of hypertrophic adipocyte death is still unclear, recent evidence implicates pyroptosis as a likely candidate. Mediated by caspase-1 activation, pyroptosis is a swift cell death featuring pore formation in the cell membrane with consequent dissipation of cellular ionic gradients. Meanwhile, the retention of larger constituents in the cytoplasm raises intracellular osmotic pressure. Consequently, water influx causes cell swelling and ultimately ruptures the cell membrane (osmotic lysis), resulting in leakage of the pro-inflammatory cytosolic content (Fink & Cookson, 2006). Furthermore, caspase-1 (also known as IL-1β-converting enzyme) promotes cleavage, activation and secretion of IL-1β and IL-18. These pro-inflammatory mediators induce fever, recruit leukocytes, and stimulate interferon (IFN)-γ production by Th1-type $CD4^+$ T-cells and natural killer cells (Delaleu & Bickel, 2004; Dinarello, 1998; Nakanishi et al., 2001). Interestingly, the release of IL-1β and IL-18 already occurs through the membrane pores prior to lysis (Fink & Cookson, 2006), meaning that the hypertrophied adipocytes advance inflammation even before their death.

Once released from the dying cell, DAMPs boost the pro-inflammatory environment by activating numerous pattern recognition receptors (PRRs) and non-PRRs. Pattern recognition receptors

are present on innate and adaptive immune cells and non-immune cells such as epithelial and endothelial cells and fibroblasts. Besides the classic PRRs, transmembrane non-PRRs, such as receptor for advanced glycation end products, G-protein coupled receptor, and ion channels also sense DAMPs and trigger the migration and activation of immune cells, including macrophages (Gong et al., 2020).

The binding of DAMPs to their receptor triggers an inflammatory cascade and stimulates M1-like polarisation of WAT macrophages. In physiological conditions, AAMs express arginase 1, IL-1, receptor antagonist (IL-1Ra), transforming growth factor-beta (TGF-β), and the transcription factor peroxisome proliferated-activator receptor gamma (PPAR-γ). These factors enable them to perform their housekeeping functions, promote insulin sensitivity, and contribute to lipid buffering in a lipid-rich environment (Russo & Lumeng, 2018). The ligation of DAMPs to PRRs activates transcriptional programs and the polarisation of metabolically activated M1-like macrophages (Stout et al., 2005). M1-like polarisation, in turn, is associated with enhanced antigen-presenting capacity and the production of potent pro-inflammatory mediators, including IL-6, IL-1β, TNF, monocyte chemoattractant protein-1, and reactive oxygen species (Afzal et al., 2016; Lumeng, Bodzin, et al., 2007; Orliaguet, Dalmas, et al., 2020; Weisberg et al., 2003). Concurrently, IL-10, which plays a critical protective role against TNF-induced insulin resistance, declines (Lumeng, Bodzin, et al., 2007). Additionally, the two macrophage populations express different surface markers (CD206, CD209, CD301 on M2-like macrophages; $CD11c^+$ on M1-like macrophages) (Lumeng, Bodzin, et al., 2007). Notably, >90% of metabolically activated macrophages congregate in the CLS, while interstitial WAT macrophages mostly retain their alternatively activated M2-like profile (Giordano et al., 2013; Lindhorst et al., 2021).

Early research suggested that the accumulation of proinflammatory macrophages in obese WAT primarily results from infiltrating monocytes (Weisberg et al., 2003; Xu et al., 2003). However, Lindhorst et al. (2021) recently demonstrated the formation of CLS containing M1-like macrophages around dead adipocytes *ex vivo*, without recruitment from blood monocytes. Their findings indicate that the accumulation of pro-inflammatory macrophages in obese WAT is likely the result of both polarisation and infiltration of circulating monocytes. Once established, this pro-inflammatory environment promotes the migration of additional macrophages and other leukocytes to the WAT, potentiating the inflammatory response.

In summary, while the exact regulatory mechanisms driving macrophage polarisation remain to be characterised, vascular dysfunction resulting from chronic inflammation enhances the risk of diabetic complications such as myocardial infarction, peripheral arterial disease, diabetic nephropathy, and retinopathy (Endemann & Schiffrin, 2004). Anti-inflammatory type 2 immune responses are instrumental in metabolic homeostasis and preventing T2DM. Hence, limiting the inflammatory cascade may be a reasonable approach to the prevention and management of T2DM, and several novel antidiabetic drugs successfully use manipulation of M1/M2 macrophage phenotypes to manage obesity-induced inflammation (Ni et al., 2020).

1.1.6.2 Metabolic disturbances

Insulin resistance and the link to inflammation

Insulin directs the metabolism of macronutrients by stimulating the uptake of molecules, such as glucose, from the blood into fat, muscle, and liver cells. The hormone is synthesised in and secreted by β cells in the islets of Langerhans in the pancreas, and in healthy subjects, the quantity released is intricately linked to blood glucose concentrations to precisely meet metabolic demands (Schmitz et al., 2008; Wilcox, 2005). In the target cells, insulin binds to its receptor, promoting glucose uptake into the cell by diffusion facilitated by glucose transporters (GLUT). In the brain, the transporter protein GLUT 1 on neuronal cells can extract glucose from the blood at very low levels, such as during fasting without insulin action, thus ensuring a consistent energy supply for these glucose-reliant cells. In contrast, adipocytes and myocytes feature GLUT 4, which is insulin-dependent, allowing the appropriate response to high postprandial glucose levels (Wilcox, 2005). Glucose entry into adipocytes stimulates fatty acid and glycerol synthesis and downregulates lipolysis. In contrast, myocytes and hepatocytes store glucose as glycogen to be utilised as an instant energy source. Low blood glucose and insulin levels promote lipolysis in adipocytes and glycogenolysis in muscle and liver (Wilcox, 2005).

While insulin resistance may sometimes result from excess secretion of counter-regulatory hormones (glucagon, corticosteroids, and catecholamines), post-receptor defects in insulin signalling seem to explain most cases (Wilcox, 2005). Insulin resistance to limit nutrient storage is a necessary, short-term metabolic adaptation to produce an effective immune response during bacterial and viral infections, as the activated host immune cells rely on glycolysis to meet energy demands (Wynn et al., 2013). However, in the context of obesity, prolonged insulin resistance further promotes chronic subacute inflammation (meta-inflammation) in all tissues and becomes the driving force in the development of T2DM (Hotamisligil et al., 1993; Kaur, 2014; Lumeng, Bodzin, et al., 2007; Priest & Tontonoz, 2019; Qatanani & Lazar, 2007; Shoelson et al., 2007). Failure of one or more mechanisms involved in cellular glucose absorption results in hyperglycaemia and dyslipidaemia, causing compensatory intensified pancreatic insulin secretion (Sears & Perry, 2015). Eventually, sustained overstimulation leads to the exhaustion and failure of β cells and T2DM (Orliaguet, Ejlalmanesh, et al., 2020).

The concept of obesity-induced inflammation as the critical factor in the development of insulin resistance is now well established (Kim & Lee, 2021; Saltiel & Olefsky, 2017). Yet the first paper indicating a direct link between inflammation and insulin resistance was published in 1993, not even 30 years ago (Hotamisligil et al., 1993). In a mouse model of obesity and T2DM, the study demonstrated high levels of TNF mRNA in the adipose tissue of mice and neutralising TNF resulted in improved insulin sensitivity. Four years later, Uysal et al. added more weight to these initial findings (Hotamisligil et al., 1997). The authors first reported that deletion of TNF or both its receptors in *ob/ob* or high-fat

induced obese mice improved insulin resistance; one year later, they identified the p55 receptor as instrumental in this mechanism (Uysal et al., 1998). Later mechanistic studies have established that TNF inhibits glucose uptake by interfering with the initial events after insulin binding to its receptors (Kim & Lee, 2021). Chronic TNF exposure also decreases the expression of GLUT 4, which further reduces glucose absorption (Stephens et al., 1997). These initial TNF studies kickstarted a plethora of studies to cement the link between obesity, inflammation, and insulin resistance.

The early 2000s brought the first epidemiological evidence that inflammation was predictive of the future development of CVD and T2DM. Results from the Women's Health Initiative study suggested that elevated circulating levels of IL-6 and high-sensitivity c-reactive protein forecast future T2DM (Pradhan et al., 2001), extending observation from an earlier study that had implicated the same pro-inflammatory cytokines in CVD (Ridker et al., 1997). Although these studies did not provide a causal link, they were nevertheless significant, as they pointed at general inflammation as a causative pathogenic agent in humans. Coinciding with the Women's Health Initiative Study, a mouse model study elucidated the pathway that stimulates insulin resistance in obesity (Kim et al., 2001). The authors showed that obesity activates the IKKβ/NFκB pathway, and inhibition of this pathway with aspirin prevented the development of insulin resistance. Clinical trials in humans using salsalate confirmed the rodent findings, firmly establishing a link between inflammation and insulin resistance (Goldfine et al., 2013; Goldfine et al., 2010; Goldfine et al., 2008). However, as the deletion of IKKβ in different tissue generated conflicting results (Kim & Lee, 2021), the focus soon turned to searching for other immune cells potentially involved in regulating obesity-induced inflammation.

The first immune cells in the line of the investigation were adipose tissue macrophages. Various studies have clearly shown that obesity significantly increases the number of pro-inflammatory M1-like macrophages. Yet, their pro-diabetic function is still not fully clarified, given the upregulation of pro- and anti-inflammatory markers during obesity that does not neatly fit the M1/M2 classification (Kim et al., 2013; Xu et al., 2013). What we do know is that they contribute to many more metabolic processes than inflammation, including adipose tissue fibrosis (Spencer et al., 2010; Tanaka et al., 2014), lipid metabolism (Camell et al., 2017; Xu et al., 2013), and thermogenic regulation via brown fat differentiation/function (Cereijo et al., 2018; Wolf et al., 2017). All of these processes can influence obesity-induced insulin resistance.

The complexity of macrophage differentiation and a 2009 series of *Nature Medicine* papers switched the focus to the role of T cells and mast cells (Feuerer et al., 2009; Liu et al., 2009; Nishimura et al., 2009; Winer et al., 2009) In these knockout mouse models, $CD4^+$ T cells seemed protective against obesity-induced insulin resistance. However, the reconstitution of $CD4^+$ T cells also normalised body weight (Winer et al., 2009). Similarly, mast cell knockout moderated both body mass and insulin resistance (Liu et al., 2009), which opened up the possibility that the improvement in insulin resistance was indirectly mediated via the reduction of body mass rather than via direct regulation of insulin

resistance. In contrast, Treg and CD8 T cells did not modify body mass but protected against obesity-induced inflammation and insulin resistance (Feuerer et al., 2009; Nishimura et al., 2009).

Following on, several studies investigated most other adipose tissue-resident immune cells. Those cells traditionally considered pro-inflammatory, including dendritic cells, neutrophils, natural killer cells, innate lymphoid cells, Th1 and Th17 cells, γδ T cells, and B cells, were able to promote inflammation and insulin resistance (Lee et al., 2016; Mehta et al., 2015; O'Sullivan et al., 2016; Stefanovic-Racic et al., 2012; Talukdar et al., 2012; Winer et al., 2011; Winer et al., 2009; Zuniga et al., 2010). On the other hand, traditionally viewed anti-inflammatory cells, such as eosinophils, ILC2s, and regulatory B cells, inhibited obesity-induced inflammation and insulin resistance (Nishimura et al., 2013; D. Wu et al., 2011; Yang et al., 2010). One cell type, however, did not fit this neat pattern, the invariant natural killer cell (iNKT). Different studies found protective, detrimental, and no effect on obesity-induced inflammation and insulin resistance, which cannot be explained by the use of different mouse models (Lee & Lee, 2014). Further, manipulating the cell types mentioned above in non-obese mice fed standard chow also modified their body fat and mass, which may imply that these cells can directly alter obesity-induced inflammation and insulin resistance or do so via their obesity-regulating effect.

Impaired adipocyte lipid storage function and fatty acid metabolism in tissues

Dysfunctional obese WAT impairs metabolic health and drives the pathogenesis of metabolic diseases (Qatanani & Lazar, 2007; Savage et al., 2007; Shoelson et al., 2006). Intense research spanning several decades has overhauled our view of adipose tissues as inactive tissue destined only for insulation, cushioning, and energy storage (Rosen & Spiegelman, 2014). Instead, we have discovered an exceedingly active organ with homeostatic, paracrine, and endocrine functions (Tchkonia et al., 2006) ideally positioned to communicate the body's nutritional state to other organ systems and regulate metabolic processes via the release of fatty acids (lipokines) peptides, and hormones (adipokines). (Berg & Scherer, 2005; Cao et al., 2008; Ouchi et al., 2003).

Disrupted metabolism in hypertrophied adipocytes contributes to system-wide inflammation. Healthy WAT is highly sensitive to insulin and regulates blood glucose and free fatty acids (FFAs) levels. Post-prandial insulin-stimulated glucose uptake within adipocytes simultaneously increases the uptake of lipids in the form of FFAs liberated from triacylglycerols and curbs the breakdown of triacylglycerols within the adipocytes, thus regulating the levels of circulating FFAs. While WAT generally functions as a buffer for FFAs, this capacity can be blunted in obese inflamed WAT. The enlarged, dysfunctional adipocytes no longer adequately store circulating free fatty acids but rather release them (Faty et al., 2012; Morigny et al., 2016; Tchkonia et al., 2006). Notably, adipose tissue topography can predict detrimental abnormalities in fatty acid metabolism (Jensen et al., 1989).

Specifically, upper body adiposity has been linked to high plasma FFA levels, with FFA delivery to the liver's portal vein increasing in tune with visceral obesity (Guo et al., 1999; Nielsen et al., 2004; Jensen et al., 1989; Roust & Jensen, 1993); however, ethnic and sex differences appear to moderate this association (Guerrero et al., 2009).

Chronic adipose tissue inflammation increases circulating levels of IL-6. This pro-inflammatory cytokine has been suggested to attenuate fatty acid metabolism in multiple tissues, including skeletal muscle, the liver, and the brain (Saltiel & Olefsky, 2017). Although some controversy exists regarding the exact role of IL-6 (Pal et al., 2014), within the adipose tissue, IL-6 released from M1-like macrophages may stimulate lipolysis in surrounding adipocytes and inhibit lipoprotein lipase, impairing adipocyte lipid storage (McLaughlin et al., 2017). Increased flow of FFA's in tissues such as the liver and skeletal muscle initiates lipotoxicity, which fuels insulin resistance. Unlike adipose tissue, these tissues cannot safely store excess fat (Sears & Perry, 2015). Raised portal FFAs may cause non-alcoholic fatty liver disease and mediate hepatic insulin resistance with consequent hepatic inflammation (Boden et al., 2002; Vega et al., 2007). Concurrently, higher plasma concentrations of FFA promote system-wide metabolic disturbances. These disturbances include insulin resistance in skeletal muscle and vascular endothelial cells, abnormal blood lipids (atherogenic dyslipidaemia), and hypertension (Boden, 2008; Grundy, 2015).

Elevated circulating FFA levels also alter immune responses. In macrophages, the accumulation of FFAs stimulates the generation of reactive oxygen species, enhancing NLRP3 inflammasome activation (Guo et al., 2015). Via secretion of IL-1β, inflammasome activation fuels insulin resistance. Further, the inflammasome regulates energy expenditure and adipogenic gene expression during long-term excessive calorie intake (Stienstra et al., 2011).

Mitochondrial dysfunction in adipocytes

White adipose tissue mitochondrial dysfunction is a critical factor in impaired adipocyte fat storage capacity. Appreciation for the importance of mitochondrial function on systemic metabolic health and their therapeutic potential has been growing in recent years (De Pauw et al., 2009; Kusminski et al., 2012; Kusminski & Scherer, 2012; Schottl et al., 2015; Schottl & Klingenspor, 2013; Vernochet et al., 2014). Several studies have identified compromised WAT mitochondrial oxidative metabolism in obesity and diabetes, which may accelerate the development of metabolic disorders (Boudina & Graham, 2014; Choo et al., 2006; De Pauw et al., 2009; Kusminski & Scherer, 2012; Schöttl et al., 2020; Wilson-Fritch et al., 2004). Recent proteomic and metabolite profiling of abdominal adipocytes of obese mice indicated systematic downregulation of OXPHOS capacity and cristae density as part of significant changes in mitochondrial structure (Schöttl et al., 2020). Further supporting a reduced OXPHOS capacity in obesity is the finding of increased oxygen pressure in adipose tissue of obese

individuals that was associated with insulin resistance, indicating lower oxygen consumption (Goossens et al., 2011). Reduced OXPHOS capacity results in decreased ATP production, which is essential for lipogenesis and lipolysis (Heinonen et al., 2019) and may thus contribute to increased circulating FFA and subsequent aggravation of inflammation during obesity.

Disruption of endocrine signalling in metabolic disorders

WAT-secreted factors are essential regulators of systemic metabolism. Some factors signal the body's nutritional status to the central nervous system; others affect distant organs such as muscles and the liver, while the target organs of yet another group of factors with well-known metabolic effects remain unidentified (Priest & Tontonoz, 2019). Ground-breaking for our understanding of AT as an endocrine organ was the 1987 discovery that serum circulating adipsin, an adipocyte-synthesised serine protease homolog, is drastically reduced during obesity. This breakthrough provided the first evidence of a fat cell-derived molecule involved in systemic regulation (Flier et al., 1987).

Consequently, hundreds of adipocyte-secreted pleiotropic molecules (adipokines[1]) linked to obesity, inflammation, T2DM, liver disease, and atherosclerosis have been discovered, firmly establishing visceral WAT as an endocrine organ (Priest & Tontonoz, 2019). These diverse molecules include cytokines, hormones, and growth factors. The two most studied adipokines secreted from adipocytes are leptin and adiponectin, and they dramatically affect whole-body metabolism. Impaired lipid metabolism, increased adipocyte necrosis, and the ensuing influx of macrophages in obesity perturb adipokine secretion, promote adipose tissue inflammation, and dysregulate metabolic homeostasis, thus firmly linking obesity to metabolic disorders (Cinti et al., 2005).

Circulating leptin is increased in obesity

Mature adipocytes produce leptin and adiponectin, but their target organs differ, triggering differential mechanisms (Priest & Tontonoz, 2019). Although leptin's key actions were first described in 1995, a year after the discovery of the obese gene that encodes for this protein, its precise physiologic actions in humans remain somewhat of a mystery due to a dearth of studies. In particular, the term "leptin resistance" has been declared unspecific. In contrast to insulin resistance, which has been the subject of thousands of studies, none have investigated leptin's biological dose responses (Flier & Maratos-Flier, 2017). Leptin acts on the hypothalamus and target tissues to regulate appetite and metabolism (Klok et al., 2007), improves insulin sensitivity (Paz-Filho, Mastronardi, Wong, et al., 2012), and possibly promote inflammatory responses (La Cava, 2017). Several factors regulate leptin

[1] **Adipokine:** The word adipokine or adipocytokine means adipose (adipo-), cell (-cyto-), and movement (-kinos) (Kiliaan, Arnoldussen & Gustafson, 2014)

secretion, including food intake, total body fat, hormones, and insulin (Obradovic et al., 2021). Among these factors, insulin is the primary regulator of leptin production, with prolonged hyperinsulinaemia known to increase plasma leptin concentrations (Nogueiras et al., 2008).

Notably, hyperleptinaemia and resistance to normalising body mass are typical characteristics of obesity (Izquierdo et al., 2019; Liu et al., 2020), reflecting a state of leptin resistance. Several mechanisms have been suggested to explain this phenomenon, including the down-regulation of the leptin-activated signal transduction pathway, a decrease in histone deacetylase activity, or enhanced levels of C-reactive protein (Bjorbaek et al., 1998; Chen et al., 2006; Kabra et al., 2016). Further, modifications in leptin transport through the blood-brain barrier have been suggested to explain leptin resistance (Banks et al., 2004; Banks et al., 1999; El-Haschimi et al., 2000). Leptin must cross the blood-brain barrier to reach its site of action in the brain, particularly the hypothalamus and brainstem, and exert its appetite-suppressing effect (Izquierdo et al., 2019). Obesity and prolonged consumption of a high-fat diet impair the blood-brain-barrier integrity, resulting in a reduced transport of leptin to its target areas and diminished activation of the signalling pathways involved in body mass regulation (Izquierdo et al., 2019).

Adiponectin secretion is down-regulated in obesity

Adiponectin attenuates inflammation, improves lipid profiles, and moderates glycaemic control (Mantzoros et al., 2005). In adipose tissue, adiponectin polarises macrophages to their anti-inflammatory phenotype and reduces reactive oxygen species (Ohashi et al., 2009; Ye & Scherer, 2013). In skeletal muscle, adiponectin reduces triglyceride content, improving GLUT 4 translocation and glucose uptake and likely ameliorating insulin resistance (Yamauchi et al., 2001). In the liver, adiponectin boosts glucose uptake, inhibits gluconeogenesis, and enhances fatty acid oxidation, thus reducing inflammation (Wu et al., 2003). Although predominantly produced by adipocytes, adiponectin is generally down-regulated in obesity and multiple other metabolic disorders (Halleux et al., 2001; Hotta et al., 2000; Stumvoll & Haring, 2002). Notably, adiponectin secretion is lower in men due to inhibiting effects of testosterone (Kadowaki et al., 2006). Processes seen with unhealthy adipose tissue remodelling during obesity, including hypoxia and fibrosis, are implicated in the down-regulation of adiponectin (Sun et al., 2011). Further, insulin lowers circulating adiponectin levels (Motoshima et al., 2002), with hyperinsulinaemia and its adiponectin-reducing action possibly causing insulin resistance. However, establishing the cause and effect in this relationship remains challenging (Yadav et al., 2013).

Omentin-1 (Intelectin-1) - inversely correlated with T2DM and glucose tolerance

Omentin-1 mediates insulin secretion and sensitivity and is inversely related to inflammation and hyperglycaemia (Pan et al., 2019). Studies have reported decreased plasma and adipose tissue concentrations of omentin-1 in obesity. Further, omentin-1 correlated positively with adiponectin and high-density lipoprotein and negatively with BMI and insulin resistance (de Souza Batista et al., 2007; Elsaid et al., 2018; Rothermel et al., 2020). Recent systematic reviews and meta-analyses indicated significantly lower omentin-1 levels in patients with T2DM and impaired glucose tolerance (As Habi et al., 2019; Pan et al., 2019). This adipocyte-secreted hormone boosts insulin signal transduction and promotes insulin-mediated glucose uptake in adipocytes (Yang et al., 2006). Therefore, the observed lower omentin-1 levels in individuals with impaired glucose uptake may partially explain this population's reduced insulin-mediated glucose uptake and subsequent insulin resistance. However, significant heterogeneity among individual publications was evident in both reviews, likely due to diverse study populations and regions, making it difficult to interpret these results. Considering the studied populations' diabetic status, a high proportion of subjects were likely taking prescription medication, which could have interfered with the results. Further, the reviews included several cross-sectional studies, which do not allow a causal link between the variables.

In combination, the adipokines leptin, adiponectin, and omentin-1 contribute to metabolic homeostasis, and their secretion from the adipose tissue is disrupted in obesity. Whereas leptin is generally increased in obese individuals, adiponectin expression is decreased. Omentin-1 appears to be inversely correlated with impaired glucose homeostasis. Yet, study results are conflicting, and the role of omentin-1 in insulin resistance needs further clarification. Together, the studies indicate that metabolic dysfunction is partially mediated by imbalances in the secretion of pro-and anti-inflammatory adipokines, contributing to obesity-induced complications.

1.1.6.3 Dysbiosis of the gut microflora

The gut microbiota communicates with and influences the host's immune, metabolic, and neuro-behavioural systems intricately and reciprocally (Hooper et al., 2012; Valdes et al., 2018). Healthy individuals generally harbour a diverse intestinal microbiota and an intact intestinal barrier, which prevents the translocation of bacteria and their products into the systemic circulation (Cani, 2018; Le Chatelier et al., 2013; Schroeder & Backhed, 2016; Yatsunenko et al., 2012). Contrastingly, individuals with metabolic disorders are characterised by a dysbiotic microbiota with reduced diversity (Lambeth et al., 2015; Larsen et al., 2010; Ley et al., 2006; Ridaura et al., 2013; Turnbaugh, Hamady, et al., 2009; Turnbaugh et al., 2006; Zouiouich et al., 2021) and anatomical and functional alterations of the intestinal barrier (Li et al., 2017). The association between gut microbiota dysbiosis and disease

suggests that greater microbial diversity may allow functionally similar species to take over the function of absent species and, thus, protect against environmental threats.

Gut dysbiosis[2] evoked by a high-fat diet has been implicated as a triggering factor in the onset of inflammation and metabolic disorders (Rastelli et al., 2018). While the isolated occurrence of dysbiosis does not necessarily cause inflammation, it may allow the bloom of certain pathobionts[3] that are generally only present in small numbers but can proliferate during intestinal microflora disturbances (Levy et al., 2017). In metabolic diseases, the composition of the intestinal microbiota is skewed to one that maximises energy harvest and compromises intestinal barrier function, thus furthering adiposity and increasing permeability to bacteria and bacteria-derived products (Everard et al., 2013; Turnbaugh et al., 2006). Mechanisms by which intestinal microbiota dysbiosis likely advances obesity and metabolic complications include dysregulation of the host's immune, energy, and hormonal systems (Valdes et al., 2018).

Early research efforts aimed to identify specific bacterial taxa potentially involved with the development of obesity. While the microbiota composition is unique in individuals, it is dominated by members of the phyla *Bacteroidetes* and *Firmicutes*, with *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, *Cyanobacteria*, and *Verrucomicrobia* playing a lesser role (Li et al., 2017). Alterations in the *Bacteroidetes*/*Firmicutes* ratio were first observed in animal studies. A 2004 mouse study established that colonising germ-free mice with gut microbiota from conventionally-raised animals could induce rapid gain in body and fat mass despite reduced chow consumption (Bäckhed et al., 2004). Colonisation promoted intestinal glucose uptake, resulting in elevated serum glucose and insulin. Based on their observations, the authors suggested that food caloric values are not absolute but microbiota-dependent; they likened the microbiota to a "bioreactor", with a high-efficiency reactor promoting obesity and a lower-efficiency reactor promoting leanness. This study clearly illustrated the role of the microbiota in fat storage. In a follow-up study, the authors demonstrated in a mouse model of obesity that obesity was associated with a 50% reduced abundance of *Bacteroidetes* with a concomitant increase in *Firmicutes* compared to lean mice (Ley et al., 2005).

These initial studies indicated that a higher *Firmicutes/Bacteroidetes* ratio in obese individuals (and animals) might cause a more efficient energy absorption and thus contribute to weight gain. In their 2006 study, Turnbaugh et al. further investigated the *Bacteroidetes*/*Firmicutes* ratio in obese and lean mice via whole-genome shotgun sequencing and microbiota transplant (Turnbaugh et al., 2006). Obese mice had a microbiome enriched in *Firmicutes*, enzymes involved in polysaccharide degradation, and faecal short-chain fatty acids (SCFAs), acetate and butyrate. Further, faecal energy was lower in obese than in lean mice. Their faecal transplant experiment indicated that the increased capacity to

[2] **Dysbiotic:** a gut microbiota with compositional and functional alterations driven by environmental and host-related factors that perturb the microbial ecosystem to an extent that exceeds its resistance and resilience capabilities (Levy et al., 2017)
[3] **Pathobionts:** commensal bacteria with pathogenic potential (Mazmanian & Kasper 2006)

harvest energy in the obese microbiota was transmissible and induced greater body fat increases in the mice receiving the obese microbiota. The group extended their findings to humans in 2006, demonstrating that obese individuals had a lower relative abundance of *Bacteroidetes* and a higher abundance of *Firmicutes* than lean individuals (Ley et al., 2006). Several other studies reported alterations in *Bacteroidetes* and *Firmicutes* in obesity (Armougom et al., 2009; Bervoets et al., 2013; Brignardello et al., 2010; Santacruz et al., 2010; Tomas et al., 2016; Turnbaugh, Hamady, et al., 2009), and some have proposed the *Bacteroidetes/Firmicutes* ratio as a hallmark of obesity. However, loss of body mass by either fat or carbohydrate-reduced diet restored the relative abundance of *Bacteroidetes* and decreased the abundance of *Firmicutes*, implying that their relative abundance in obesity may be primarily diet-shaped.

More recent studies support the influential role of diet on gut microbiota composition. In their 2009 study, Turnbaugh et al. transplanted human faeces into germ-free mice, generating humanised mice (Turnbaugh, Ridaura, et al., 2009). A change in diet from a low-fat, plant polysaccharide-rich diet to a typical western diet high in fat and sugar altered gut microbial composition within a day, with a higher representation of *Firmicutes* and a reduced proportion of *Bacteroidetes.* Similarly, a 2010 study confirmed compositional changes in mice fed a high-fat diet and in obese mice, particularly in response to a high-fat diet (Murphy et al., 2010). Yet, these changes did not correlate with markers of energy harvest, which refutes the proposal that the *Bacteroidetes/Firmicutes* ratio is the sole cause for obesogenic shifts in the microbiota. In the same year, a study compared the faecal microbiota of African children consuming a high-fibre diet and European children consuming a western diet (De Filippo et al., 2010). *Bacteroidetes* were found in higher proportions in African children's microbiota, while *Firmicutes* were twice as abundant in European children than in African children. Some bacteria were exclusively present in African children, including *Prevotella*, *Xylanibacter* (*Bacteroidetes*), and *Treponema* (*Spirochaetes*) and may be instrumental in extracting energy from a high-fibre diet. Nevertheless, not all studies observed these alterations or even reported opposite modifications of *Bacteroidetes* and *Firmicutes.* These contradictory observations may result from differences in cohorts studied, study design, and techniques used in studying microbial composition. On the other hand, they could indicate that other compositional changes, such as changes at the family, genus or species level, may need to be considered.

The gut microbiota is intimately coupled to the development and modulation of the host's immune system. One crucial mechanism in gut microbiota immune modulation is the translocation of bacterial constituents, such as lipopolysaccharides (LPS) produced by Gram-negative bacteria, to metabolic tissue (endotoxemia), where they induce strong inflammatory responses (Cani et al., 2007). The presence of LPS in the systemic circulation is a valuable marker of impaired gut barrier integrity (Turner, 2009). A healthy intestinal barrier function enables the symbiotic relationship between the gut microbiota and its host, preventing the translocation of microbes and their constituents. In particular,

the presence and integrity of the mucus layer formed by mucins secreted from goblet cells determine intestinal permeability (Cornick et al., 2015). As seen in *Clostridium difficile* invasion, perturbation of the microbiota can inhibit mucin production and promote pathogenesis (Cornick et al., 2015). In contrast, *Akkermansia muciniphila* (single-intestinal *Verrucomicrobia*) has been suggested as a health-promoting microbe (Belzer & de Vos, 2012).

Evidence from human and animal studies demonstrates the critical role of *A. muciniphila* in inflammation and metabolic function. A true symbiont and one of the most abundant (1-4%) microbiota species, *A. muciniphila* is localised in the mucus layer and specialises in mucin utilisation (Derrien et al., 2004; Png et al., 2010; Swidsinski et al., 2009). Most of the current knowledge of this microbe's function stems from the study of prebiotics' effects on the gut microbiota (Cani & de Vos, 2017), which revealed that *A. muciniphila* abundance was decreased in many pathological states, including obesity, metabolic syndrome, and T2DM (Dao et al., 2016; Everard et al., 2013; Yassour et al., 2016; Zhang et al., 2013). Conversely, antidiabetic interventions, such as bariatric surgery or metformin administration, restored *A. muciniphila* abundance (de la Cuesta-Zuluaga et al., 2017; Forslund et al., 2015; Shin et al., 2014). These studies suggest that *A. muciniphila* may have great potential in preventing and treating inflammatory and metabolic disorders.

Although the exact mechanisms of protection remain unclear, greater mucus thickness and improved gut barrier integrity may be integral to the beneficial role of *A. muciniphila.* Everard et al. (2013) showed that obesity and a high-fat diet dramatically lowered the abundance of *A. muciniphila* and reduced the mucus layer's thickness by 46%. Prebiotic treatment with *A. muciniphila* restored its abundance as well as the thickness of the mucus layer and reversed metabolic endotoxemia assessed by serum LPS. Further, the treatment normalised body mass, composition, and glucose homeostasis that had been impaired by obesity and diet. Later mouse studies also reported reductions in the abundance of *A. muciniphila* with a high-fat diet in as little as four weeks, which could be reversed by dietary supplementation (Li et al., 2016; Shin et al., 2014). Several human observational and intervention studies in diverse cohorts have noted negative associations of *A. muciniphila* with insulin resistance, fasting glucose, and BMI but a positive association with reduced body mass (Derrien et al., 2017). A recent proof-of-concept human study using daily *A. muciniphila* supplementation found significantly improved insulin sensitivity index (HOMA-IR), decreased total and LDL cholesterol, improved liver function markers, and plasma LPS (Depommier et al., 2019). As compromised barrier function and consequent translocation of LPS have been implicated in the development of metabolic dysfunction (Cani et al., 2007; Lassenius et al., 2011), it is plausible to accept the reduced abundance of *A. muciniphila* as a marker of metabolic diseases.

A recent metagenomic and metaproteomic study (Zhong et al., 2019) described a higher abundance of *Enterobacteriaceae* dominated by *E. coli* in pre-diabetic Chinese adults. Their finding was in agreement with previous studies that also noted an increased abundance of *Enterobacteriaceae*

in individuals with obesity (Peters et al., 2018) and CVD (Jie et al., 2017). *Enterobacteriaceae*-derived molecules, such as LPS, flagellins, and adhesions, can initiate inflammatory pathways, thus linking gut microbial products to systemic inflammation (Zeng et al., 2017).

Further, the gut microbiota and intestinal immune system are intricately related. Intestinal epithelial cells can detect bacterial antigens and signal adjacent immune cells, including dendritic cells, macrophages, and lymphocytes, alerting the immune system to the presence of pathogen-associated molecular patterns present on most microorganisms (Hakansson & Molin, 2011). While tolerant to commensal bacteria, gut epithelial cells are activated by invasive pathogens, triggering an inflammatory cascade involving local and systemic immune responses. Persistently elevated systemic inflammatory mediators contribute to pathological processes seen in multiple chronic disorders, including MetS and T2DM (Cani et al., 2007; Cani et al., 2008), which in turn, shape the gut microbiota.

Gut microbiota metabolites

An important factor concerning gut microbiota composition changes is the subsequent modification of gut microbiota metabolites. Several animal studies have shown the influential role of SCFAs produced by microbial fermentation of undigested carbohydrates in regulating host energy metabolism (den Besten et al., 2013; Liou et al., 2013; Perry et al., 2016). Yet, in humans, the evidence is contradictory, with some reporting a positive correlation between obesity and SCFAs (Fernandes et al., 2014; Rahat-Rozenbloom et al., 2014; Riva et al., 2017) and others reporting a negative association (Barczynska et al., 2018). A 2019 systematic review and meta-analysis found that obesity was associated with higher levels of SCFAs but not microbiota richness at the phylum level (Kim et al., 2019). However, the causal nature of this association and considerable heterogeneity between studies requires careful consideration of these results.

In summary, obesity is a pandemic and closely associated with MetS and the development of metabolic diseases such as T2DM, CVD, and non-alcoholic fatty liver disease. Insulin resistance resulting from inflammatory processes in WAT may be the underlying link between obesity and these devastating metabolic disorders. Further, the gut microbiota and its metabolites regulate inflammation and metabolism and interact reciprocally with other tissues.

1.2 Dialogue between helminths and their human host

Inflammation, metabolic disturbances, and gut dysbiosis are the predominant components of T2DM pathophysiology. Evidence from experimental mouse and human observational studies suggests a protective role of parasitic helminth (worm) infection against metabolic disorders as a bystander effect of infection-induced immunomodulatory events. In the following sections, I have reviewed helminth-mediated immune responses, metabolic improvements, and modification of the gut microbiota. For helminth-induced immune responses, I mainly focused on responses that may explain the metabolic improvements associated with helminth infection rather than responses solely related to worm expulsion.

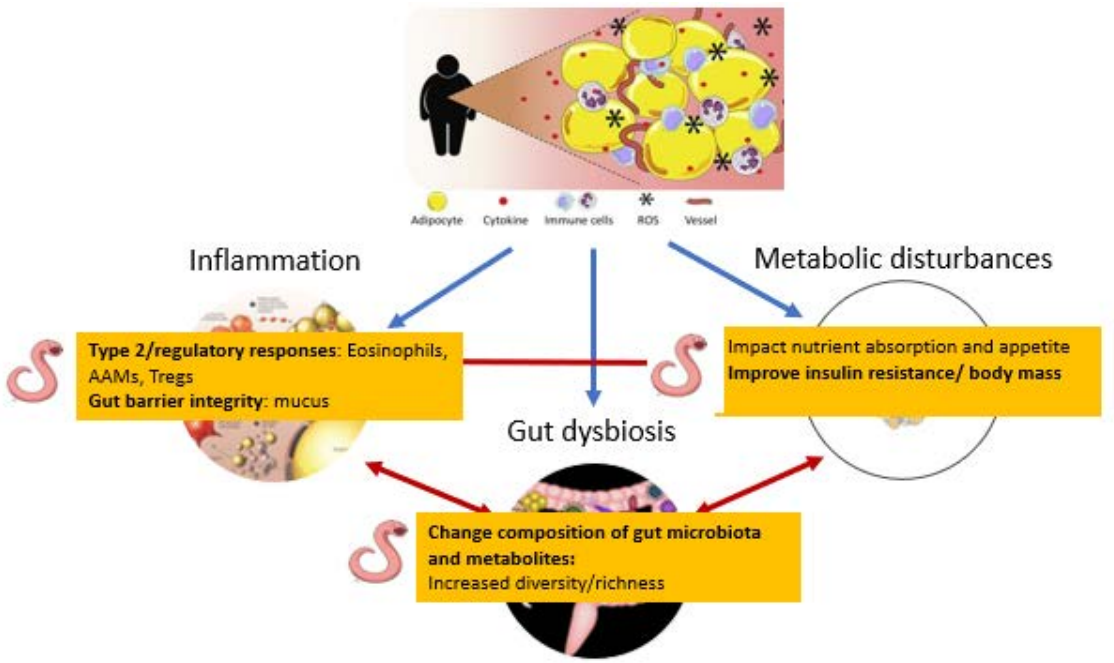


Figure 1-5 Helminth infection and the main pathophysiological features of metabolic diseases
Infection can down-regulate inflammation, improve metabolic disturbances, and change the composition of the gut microbiota.

1.2.1 Immune responses to helminth infections

Helminths and humans have coevolved for millennia. Infections can be associated with significant morbidity, but mounting evidence suggests that helminths also have therapeutic potential (Chapman 2021). These parasites expertly modulate immune responses to ensure life-long survival in the host (Maizels & McSorley, 2016; Nutman, 2015). The resulting immune modulation benefits both parasite and host, protecting the worm from elimination by the host's immune response and the host from excessive inflammation. Findings from studies indicate that helminth infections downregulate pro-inflammatory T helper (Th) type 1 and Th17 immune responses and skew towards a modified Th2

immune response via the release of biologically active excretory-secretory (ES) antigens (Bashi et al., 2015; Croese et al., 2015; Danilowicz-Luebert et al., 2011; Khudhair et al., 2021; Su et al., 2018). The unmodified Th2 immune response is characterised by $CD4^+$ Th2 cells, the cytokines IL-4, IL-5, IL-9, IL-10, and IL-13, and immunoglobulin E. These key players orchestrate the recruitment of macrophages, eosinophils, and mast cells as well as B cells, which contribute to crucial host protective processes against destructive pathogens, such as worms (Allen & Maizels, 2011; Chen et al., 2012; Danilowicz-Luebert et al., 2011; Gause et al., 2013; Wynn & Ramalingam, 2012; Zhou & Liu, 2018). However, in most people, helminth infections lead to a modified Th2 response with high levels of AAMs, Tregs, and IL-10, resulting in an anti-inflammatory and tissue-remodelling environment. The modified immune response elicited by helminths prevents unrelated inflammation, such as allergic immune responses, promotes wound healing, and enables parasite survival (Danilowicz-Luebert et al., 2011), thus establishing a mutually beneficial state for both host and parasite. Significantly, the downregulation of inflammation arising from the type 2 immune response to helminth infections is tightly linked to improved glucose homeostasis and reduced fat mass (Hotamisligil, 2006).

1.2.1.1 Animal models

Much of our current understanding of immune responses to helminth infections stems from experimental animal models, particularly in rodents. The two most widely used models in the context of hookworm infection are *Nippostrongylus brasiliensis* ("rat hookworm") and *Heligmosomoides polygyrus* (Camberis et al., 2003). Lifecycle and Th2 immune responses in *N. brasiliensis* infection are similar to the human hookworm *Na*, which allowed mechanistic insights into the systemic and mucosal immunology of *Na* infection. However, *N. brasiliensis* (Trichostrongyloidea) is phylogenetically different to *Na* (Ancylostomatoidea), and the rat host can resolve infection within ten days, a phenomenon not commonly seen in humans. In contrast, *H. polygyrus*, also a member of the Trichostrongyloidea, has a different lifecycle that does not include skin or lung migration. However, *H. polygyrus* can produce infections more resistant to elimination that mimic subclinical chronic human infections (Fujiwara et al., 2006; Reynolds et al., 2012), enabling the investigation of long-lasting protective immune responses (Nair & Herbert, 2016; Reynolds et al., 2012). Both models induce the modified Th2 immune response defined by eosinophil, type 2 innate lymphoid cell (ILC2), and Th2 lymphocyte expansion, as well as IL-4, IL-10, and IL-13 production. Additionally, *H. polygyrus* infection also includes the expansion of Tregs (McSorley et al., 2013).

Two further hookworm models have been developed in hamsters. Both *Ancylostoma ceylanicum* and *N*a can establish patent infections in this rodent that elicit Th2 responses resembling those seen in human hookworm infections (Mendez et al., 2005). Other commonly used models mimicking human worm infection include *Trichuris muris*, *Schistosoma mansoni*, and *Strongyloides ratti*/*venezuelensis* (Peng & Siracusa, 2021). Together, these animal models provide valuable tools to

analyse host-protective immune mechanisms (Bouchery et al., 2017; Jian et al., 2003), which is of interest in treating inflammatory diseases.

An important consideration in the study of host-parasite interaction is the hookworms' complex lifecycle, which includes traversing several tissues as well as changing morphology and antigenicity. This complexity and the inherent limitations of animal models demand careful consideration of the research question and determining the appropriate time point and tissue included in the investigation to obtain meaningful results (Bouchery et al., 2017; Mourão Dias Magalhães et al., 2020).

1.2.1.2 Type 2 innate lymphoid cells (ILCs)

The early phase of larval migration in hookworm infection triggers a transient Th1 immune response with upregulation of pro-inflammatory cytokines during animal models and human infection (Mourão Dias Magalhães et al., 2020). Tissue destruction and the establishment of adult worms likely initiate this initial inflammatory response (Lawrence et al., 1996). Interestingly, despite the tissue destruction and expression of pro-inflammatory cytokines during lung migration, pulmonary inflammation is rare, and symptoms are generally limited to a sore throat and some coughing (Geiger et al., 2008). During patency (adult hookworm phase), the immune response switches to a Th2 dominant response (Mendez et al., 2005) supported by ILC2 activation in response to tissue damage (Mourão Dias Magalhães et al., 2020).

First identified in 2006, ILC2s were found to be enriched at mucosal sites of *N. brasiliensis*-infected mice (Fallon et al., 2006). Several subsets of ILC2s have since been described, including natural (tissue-resident) ILC2s and inflammatory ILC2s (Huang et al., 2015) that can switch phenotype (K. Zhang et al., 2017) in response to the local microenvironment. For example, Huang et al. reported the expansion of inflammatory ILC2s in response to *N. brasiliensis* infection in mice (Huang et al., 2015). Further, a WAT-resident ILC2 (natural helper cell) population was discovered in 2010 (Moro et al., 2010). As the primary source of IL-5 and IL-13, these cells maintain eosinophils and AAMs associated with a healthy (lean) status of WAT. In contrast, IL-5 deficiency increases susceptibility to diet-induced obesity (Molofsky et al., 2013). While the expansion and activation of ILC2s can be associated with pathologies in the absence of helminth infection, they exert beneficial functions in the context of helminth infection (Bouchery et al., 2019). Alarmins (IL-25, IL-33, and thymic stromal lymphopoietin, TSLP) released from epithelial cells initiate the proliferation and activation of ILC2s, with tuft cells-specific release of IL-25 and TSLP increased in *T. muris* and *N. brasiliensis* infection (Bouchery et al., 2019). Moreover, ILC2 activation can also be neuron-mediated. Released from enteric neurons in response to helminth infection, neuromedin U binds to ILC2s and stimulates their proliferation and expression of IL-5 and IL-13 (Cardoso et al., 2017; Klose et al., 2017). Lastly, cytokines other than alarmins, innate immune cells, and lipid mediators can also activate ILC2s, with little yet known about positive and negative regulators of ILC2 activation (Bouchery et al., 2019).

Expansion of ILC2s seems to promote the clearance of worms; however, their contribution may be attenuated by other Th2 cells (Neill et al., 2010).

Besides their contribution to tissue repair, ILC2s may also be integral to promoting Th 2 cell responses (Oliphant et al., 2014). Although ILC2s seem to produce little IL-4 in murine infections, they can release large quantities during human infections (Mjosberg et al., 2012). Additionally, ILC2s produce IL-13 to stimulate the migration of dendritic cells to the lymph node (Halim et al., 2014) and act as antigen-presenting cells to CD4 T cells (Oliphant et al., 2014).

Human studies characterising ILC2 responses are rare and primarily focused on diseases with pathological ILC2 function, such as asthma and dermatitis (Bernink et al., 2014). Nevertheless, these studies confirmed many of the activation pathways elucidated in murine studies, including the three alarmin and tuft cell pathways. Human studies have presented conflicting results with ILC2 expansion during filarial (*Loa loa, Wuchereria bancrofti* or *Onchocerca volvulus*) (Boyd et al., 2014) and STH infection (de Ruiter et al., 2020), but reductions in young children with *S. haematobiu*m infection that were restored after deworming (Nausch et al., 2015). The latter study indicated that some worms might be able to suppress ILC2s, at least in select cohorts. In support of this, McSorley and colleagues have described that administration of *H. polygyrus* excretory-secretory (ES) products blocked the early release of the alarmin IL-33 from epithelial cells in mice, with a concomitant dramatic decline in ILC2s and suppression of IL-4, IL-5, and IL-13 in allergic airway inflammation (McSorley et al., 2014). In summary, these studies indicate that ILC2s are essential in the immune response to helminth infections. However, we have yet to clarify their exact and likely complex interactions with other immune cells and how these may differ in specific tissues and in response to micro-environmental needs.

1.2.1.3 Eosinophils

The quintessential type 2 cytokines released by ILC2s (IL-4, IL-5, and IL-13) instigate tissue repair via the recruitment of eosinophils and AAMs. A landmark study in 2007 documented that comparable alternative macrophage activation in mice occurred in response to either tissue injury only or surgical implant of *Brugia malayi* (Loke et al., 2007). Still, in the injury-only mice, alternative activation lasted only one week, but it was sustained throughout the three-week experiment in parasite-implant mice and accompanied by a spike in eosinophil numbers within 24 hours. Eosinophil numbers were considerably less and subsided quickly in surgery-only mice, which led the authors to suspect that alternative activation was eosinophil-dependent in an IL-4-dependent manner. The authors also noted that AAMs mediated neutrophil clearance and that $CD4^+$ T cells were required for AAM maintenance. Given their findings, they speculated that AAMs might coordinate cell recruitment during type 2 immune responses via clearance of neutrophils and recruitment of eosinophils, with Th2 cells crucial in later phases.

Intestinal helminths promote adipose tissue eosinophilia in an ILC2-dependent manner. The expansion and activation of ILC2s during helminths infection are stimulated predominantly by IL-25 secreted from tuft cells (Gerbe et al., 2016; Howitt et al., 2016; Hurst et al., 2002; von Moltke et al., 2016). Further, IL-33-mediated activation of ILC2s has been observed in murine helminth infection (Hung et al., 2013; Yasuda et al., 2012). The resulting increased release of IL-5 and IL-13 induces and maintains AAMs directly via IL-13 and indirectly via IL-5-mediated eosinophil recruitment and subsequent IL-4 production. Given the crucial role of eosinophils and AAMs in inflammation and the regulation of glucose homeostasis and fat mass, this helminth infection-initiated cascade may partially explain the protective role of worms in metabolic disorders.

In 2011, Wu et al. shed some more light on Loke's earlier findings. Their seminal study first identified eosinophils as the primary producers of IL-4 in WAT and noted that their presence was essential for maintaining AAMs and insulin sensitivity in a mouse model of obesity (D. Wu et al., 2011). In the same study, infection with *N. brasiliensis* enhanced insulin sensitivity and glucose tolerance, lowered fasting glucose, and reduced perigonadal fat mass. Notably, these improvements were maintained up to 45 days after parasite clearance. As demonstrated one year earlier, IL-4 can improve glucose homeostasis, total cholesterol, and triglycerides via activation of signal transducer and activator of transcription 6 (STAT6) in wild-type obese mice. In turn, STAT6 antagonised the catabolic action of PPAR-γ in the liver and attenuated WAT inflammation (Ricardo-Gonzalez et al., 2010). *N. brasiliensis* infection in RIP2-Opa1KO mice prone to gain body mass also demonstrated that the STAT6 signalling pathway, when activated by Il-13, was crucial in reducing steatosis and played a role in attenuation of body mass gain (Yang et al., 2013). As shown by Molofsky et al., eosinophils and IL-4 appear to influence metabolism by stimulating oxidative metabolism rather than limiting food intake or promoting physical activity (Molofsky et al., 2013). The same study also found that mice with deficient or depleted ILC2s showed significantly reduced adipose eosinophils and AAMs, supporting the concept that ILC2s are essential for the induction of these cells. These findings started to overturn our view of eosinophils, which were traditionally seen as destructive innate immune cells in Th-2 responses to allergic diseases or parasite infections (Jacobsen et al., 2012).

More recent publications proposed novel and diverse eosinophil effector functions. Although eosinophils may participate in worm destruction via the release of toxic effector mediators, such as reactive oxygen species, their absence or presence did not affect worm burden in *S. mansoni*-infected mice (McCormick et al., 1996). New evidence suggests that eosinophils can sometimes promote parasite survival instead of accelerating parasite elimination. Ablation of eosinophils in *Trichinella spiralis*-infected mice resulted in impaired parasite growth and greater parasite death, which correlated with lower eosinophil count and expansion of inducible nitrous oxide synthase (iNOS) secreting neutrophils and inflammatory macrophages. Intravenous reinstatement of eosinophils restored parasite survival, indicating a direct role of eosinophils in parasite survival and inflammation (Gebreselassie et

al., 2012). The exact mechanisms of eosinophil-induced macrophage polarisation need further clarification. Still, sensing by toll-like receptor 4 on eosinophils appears to direct the polarisation in macrophages toward a Th1 or Th2 phenotype (Yoon et al., 2019). Further, eosinophil secretion of IL-4 and IL-13 is required to polarise AAMs (D. Wu et al., 2011), and PPAR-γ activation by IL-4 regulates arginase 1 expression and controls the metabolic processes during alternative activation, such as fatty acid oxidation (Odegaard et al., 2007)

The ability of eosinophils to polarise AAMs seems to play a critical role in maintaining metabolic homeostasis. Eosinophil-deficient mice on a high-fat diet showed a remarkable inability to accommodate excess energy intake by adipose tissue expansion compared to wild-type controls, resulting in systemic insulin resistance and hyperglycaemia. Additionally, eosinophil deficiency increased the expression of Th1 cytokines while significantly reducing levels of Th2 cytokines in adipose tissue, indicating that eosinophils safeguard against insulin resistance and inflammatory changes (Lee et al., 2018). In contrast, their expansion during *N. brasiliensis* infection was associated with enhanced type 2 responses, significantly improved glucose homeostasis, and reduced weight gain (Khudhair et al., 2021).

1.2.1.4 Alternatively activated macrophages

Macrophages take on a central position in the type 2 response against helminth infection. However, their effector functions require careful regulation to avoid pathological type 2 responses resulting in fibrosis or heightened susceptibility to infections (Lechner et al., 2021). During helminth infection, AAMs entrap and/or kill larvae and promote adult worm expulsion (Bouchery et al., 2015; Chen et al., 2014; Coakley et al., 2020; Esser-von Bieren et al., 2013; Krljanac et al., 2019; Obata-Ninomiya et al., 2013). While larval trapping limits injury to infected tissues (Chen et al., 2012; Esser-von Bieren et al., 2013), AAMs also actively promote tissue repair via the production of growth factors, chemokines, and building blocks for collagen synthesis (Bosurgi et al., 2017; Minutti et al., 2017). Further, AAMs coordinate Th2 cell activation to limit type 2 immunopathology during helminth infection through several effector molecules, including arginase 1 and resistin-like protein alpha (RELMα) (Nair et al., 2009; Pesce, Ramalingam, Mentink-Kane, et al., 2009; Pesce, Ramalingam, Wilson, et al., 2009). Helminths, on the one hand, induce AAM activation and type 2 responses; on the other hand, they release immunomodulatory molecules that tightly regulate type 2 immunity (Coakley et al., 2017; de Los Reyes Jimenez et al., 2020; Klotz et al., 2011; Maizels et al., 2018). The exact mechanisms of helminth-directed tissue repair and immune regulation are still incompletely understood, particularly the implication of chronic infections in humans, which is partially due to different AAM activation pathways in mice and humans (Martinez et al., 2013).

Besides their role in parasite elimination, helminth-stimulated AAMs are implicated in protection against diet-induced metabolic disturbances. A 2018 study demonstrated this protective

effect by transferring AAMs induced by *H. polygyrus* into uninfected mice, significantly mitigating obesity, blood glucose and triglyceride levels in obese mice (Su et al., 2018).

1.2.1.5 Basophils

Basophils constitute only about 0.5% of all leukocytes but are expanded during worm infection. Expansion of basophils was reported following helminth-induced expression of IL-3, IL-33 and TSLP in mice (Giacomin et al., 2012; Kondo et al., 2008; Lantz et al., 1998; Lantz et al., 2008; Schwartz et al., 2014), and helminth-derived molecules in humans (Falcone et al., 2009). The presence of basophils in skin lesions during helminth infection indicates their importance in inflammatory processes (Obata-Ninomiya et al., 2020; Shen et al., 2008). These often neglected immune cells promote Th2 cell differentiation in mice by secretion of IL-4 (Giacomin et al., 2012; Min et al., 2004; Schwartz et al., 2014; Torrero et al., 2010). Basophil-secreted IL-4 also promoted AAM differentiation, which could assist in worm expulsion (Obata-Ninomiya et al., 2020). Ground-breaking work in 2005 suggested that basophils convert the neutral IL-3 into IL-4, which, in turn, drove the Th2 polarisation of $CD4^+$ T cells (Hida et al., 2005), a finding later confirmed by other researchers (Otsuka et al., 2013; Tang et al., 2010). Extending this finding, independent groups established the expression of major histocompatibility complex II on murine basophils that induced Th2 cell differentiation (Perrigoue et al., 2009; Sokol et al., 2009; Yoshimoto et al., 2009); this suggested that basophils possibly act as antigen-presenting cells facilitated via passive uptake of MHCII from professional antigen-presenting cells such as dendritic cells (Miyake et al., 2017).

1.2.1.6 Neutrophils

Neutrophils are traditionally viewed as part of the immune response to helminth infections, yet little is known about their exact involvement in type 2 responses. Further, their involvement varies between worm species, depending on the parasites' lifecycles and migration in the affected host (Ajendra, 2021). For example, during *S. mansoni* infection in mice, neutrophils rapidly infiltrate the skin around the penetration site (Paveley et al., 2009). Neutrophils take up ES molecules from cercariae and secrete chemokines, alerting monocytes, macrophages and dendritic cells (Nathan, 2006; Paveley et al., 2009); however, they are almost undetectable during later stages of infection (Reiman et al., 2006). In contrast, neutrophils appear to play a more important role during *S. japonicum* infection, with increases also seen in the blood and spleen (Zheng et al., 2017). In vitro studies suggested neutrophils may collaborate with eosinophils to destroy schistosomulae (Jong et al., 1984). More recent evidence suggests that neutrophils may be able to kill helminth larvae by the production of neutrophil extracellular traps (NETs) (Bouchery et al., 2020), but interestingly some helminths may have evolved sophisticated strategies to evade attack by neutrophils and NETs to ensure their survival (Doolan & Bouchery, 2022). Further, during the lung stage of infection with *N. brasiliensis*, neutrophils constituted

a large portion of immune cells in bronchoalveolar lavage (Sutherland et al., 2014). Their recruitment was IL-17A-dependent, and IL-17A presence was necessary for the subsequent mounting of a complete type 2 response (Ajendra et al., 2020). A further indicator of a link between neutrophils and type 2 immunity is the reported role of efferocytosis of apoptotic cells, specifically neutrophils, in AAM polarisation (Bosurgi et al., 2017). Notably, type 2 cytokines, particularly IL-4, seem to downregulate the neutrophil response, suggesting that neutrophils could be the first responder with "a licence to kill" and bridge the time between invasion and onset of the type 2 response (Egholm et al., 2019).

While neutrophils can release cytokines, it is unclear whether they are a source of type 2 cytokines. One study indicated that distinct stimuli could prime neutrophils to switch their gene expression profile to a more anti-inflammatory type during worm infection, which is different to the LPS-primed profile (Chen et al., 2014). The authors noted upregulation of *Il13*, *Il33*, *Igf1*, *Retnla*, and *Chi313*, which are associated with a type 2 immune response in neutrophils during *N. brasiliensis* infection. In the same study, neutrophil-secreted IL-13 could polarise AAMs, which attached to and destroyed *N. brasiliensis* larvae in vitro. Several other studies also reported the accumulation and cooperation of neutrophils, eosinophils, and macrophages to inhibit parasite migration during *H. polygyrus* and *S. stercoralis* infection, indicating this cooperation as a universal mechanism in several helminth species (Anthony et al., 2006; Bonne-Annee et al., 2013; Galioto et al., 2006; Liu et al., 2004; Morimoto et al., 2004; Sutherland et al., 2014). While the actual release of these cytokines has not been confirmed *in vivo* (Ajendra, 2021), an *in vitro* study demonstrated neutrophil release of IL-4 and IL-13 in response to larval antigen of *Haemonchous contortus* but not to adult antigen (Middleton et al., 2020). Together, these findings provided subtle clues that neutrophils are more than bystanders in the type 2 responses, yet many questions remain regarding their effector functions and involvement in type 2 immune responses.

1.2.1.7 Regulatory T cells

A critical factor in the immunomodulation of hookworm infection could be the induction of regulatory pathways. Regulatory T cells express the transcription factor Foxp3 in mice (or FOXP3 in humans) and a variety of surface markers, depending on function (Sakaguchi et al., 2020). Despite their diverse population, they are commonly referred to as $CD3^+$ $CD4^+$ $CD25^+$ $Foxp3^+$ cells in mouse and human studies. Usually, Tregs coordinate the actions of other T cell populations and innate immune cells (Maloy et al., 2003; Murphy et al., 2005; Nguyen et al., 2007). These regulatory cells are crucial in defending against inappropriate immune responses, such as in autoimmune diseases. In helminth infections, they prevent excessive inflammation that could generate tissue damage. Coincidentally, this also promotes parasite survival (White et al., 2020).

The expansion of Tregs and their protective function during helminth infection have been observed in several studies (Babu et al., 2009; Croese et al., 2015; Matera et al., 2008; Metenou et al.,

2010; Montes et al., 2009; Ricci et al., 2011; Schmiedel et al., 2015; Tanasescu et al., 2020; Wammes et al., 2012; Watanabe et al., 2007). However, not all helminth species cause the expansion of Tregs. Infection with *T. muris*, for example, diminishes Tregs considerably, and the absence of Tregs does not impact parasite survival (Holm et al., 2015; Houlden et al., 2015; Worthington et al., 2013), indicating that different species may rely more or less on Treg-coordinated mechanisms for survival.

The exact drivers of Treg expansion during worm infection are still unknown, but some pathways have been proposed. Helminth-secreted factors seem to drive the conversion of naïve $CD4^+$ T-cells to Tregs and polarise dendritic cells towards a tolerogenic phenotype that induces Tregs. Additionally, the release of the alarmins IL-33 and TSLP in response to helminth-generated epithelial damage drives the production of Tregs, possibly via upregulated IL-10 release from AAMs; however, the mechanism involved has not been clarified. Regulatory B cells, a common feature of worm infections, promote Treg expansion via IL-10 (Hussaarts et al., 2011). Further, it appears that the helminths' proximity to Peyer's patches induces Treg expansion (White et al., 2020).

The therapeutic potential of WAT Treg expansion during obesity-induced inflammation and metabolic disturbances has been shown in loss-of-function and gain-of-function experiments (Feuerer et al., 2009). A later study showed that the unique function of WAT Tregs depended on their higher than usual PPAR-γ expression (Tontonoz & Spiegelman, 2008), enabling them to sustain their FOXP3 status and homeostasis. Endogenous ligands that activate PPAR-γ promote fatty acid metabolism and FOXP3 expression. Notably, leptin seems to curtail Treg proliferation, which may explain the lower Treg levels in obesity compared to lean WAT, thereby aggravating inflammation (Zeng & Chi, 2013). In animal models of diet-induced obesity, Tregs could regulate obesity and glucose homeostasis (Winer et al., 2009). However, many questions about the interplay between Tregs and metabolism remain.

1.2.1.8 Gut barrier integrity

As part of the protective type 2 immune response, helminth infection induces changes in the gut mucus barrier. For example, *N. brasiliensis* promotes mucus hypersecretion and qualitative changes in mucin composition, leading to enhanced mucus production and helminth expulsion (Koninkx et al., 1988; Yamauchi et al., 2006). IL-13 primarily drives the expansion of goblet cells and increased mucus production; however, other immune mediators may also regulate goblet cell function (Sharpe et al., 2018). A thick mucus layer safeguards gut barrier integrity, and increased mucus production during helminth infection may reduce inflammation by inhibiting the translocation of microbial products to systemic circulation. Interestingly, tuft cell expansion triggered by IL-13 release not only contributes to epithelial remodelling but may also regulate food intake or glucose metabolism via chemosensory signalling (Reimann et al., 2012).

Although rare, evidence from epidemiological studies suggests similar immune responses in humans. Hookworm-infected children in Thailand had significantly greater Treg numbers and elevated IL-2 and IL-10 concentrations, which positively correlated with Treg numbers (Phasuk et al., 2022). An Australian study using experimental *Na* infection found a robust Th2 response with increases in IL-4, IL-5 and IL-13 in infected participants that were significant for IL-5 and IL-13 but not IL-4 (Gaze et al., 2012).

In summary, parasitic worms have evolved an array of strategies that enable their long-time survival in the host. The potent anti-inflammatory and regulatory type 2 immune response induced by worms may have emerged to benefit the parasite but could also confer benefits to their host via suppression of inflammation and normalisation of glucose homeostasis. The key players in the worm-orchestrated Th 2 response are ILC2s, eosinophils, basophils, neutrophils, and mucus production, with immune tolerance driven by AAMs, Tregs, and regulatory B cells. This complex interplay between parasite and immune response can be further mediated by the host gut microbiota, which I have discussed in Section 1.2.3.

Worms and metabolism

Prolonged exposure to gastrointestinal parasites may be protective against metabolic disorders. The prevalence of inflammatory disorders such as T2DM is rising dramatically in developed countries and recently urbanised populations in developing countries (International Diabetes Federation, 2022), coincident with the successful elimination of worm infections in these countries (de Ruiter et al., 2017; Guigas & Molofsky, 2015). As worms and mammals, including humans, have co-existed for millions and years, the parasites' lifecycles have evolved to maximise their survival chances while minimising harm to their host (Sanya et al., 2017). For example, during *H. polygyrus* infection, worm-induced damage to the gut epithelial lining is compensated by the secretion of tissue healing and remodelling mediators to curb host morbidity (Allen & Sutherland, 2014). But the worms' most sophisticated survival scheme is the induction of a Th2-type and immune regulatory response, as discussed in the previous section. The regulatory network assists in the control of overt immune responses to support parasite survival while curbing inflammation that could otherwise result in pathology (Elliott & Weinstock, 2017). Given the central role of inflammation in metabolic disorders (Donath, 2016), the accumulating evidence that worm infections could improve obesity and glucose homeostasis via immune modulation is not surprising (Sanya et al., 2017). Numerous cross-sectional and deworming studies in humans, plus experimental studies in animal models, have been conducted to shed some light on this link between worm infections and improved metabolic health.

In the following sections, I have provided an overview of the findings from these studies, starting with human cross-sectional and deworming studies, followed by animal models using live worm infection as well as parasite antigen mixtures and single molecules.

1.2.1.9 Human studies

Since the first cross-sectional study in Turkey in 2001, several others in diverse populations have reported a protective role of worm infection for metabolic health. The Turkish study compared the prevalence of intestinal parasites between diabetic and non-diabetic adults to explore if diabetic patients were more prone to intestinal parasitosis (Nazligul et al., 2001). Their results showed an inverse relationship between worm infection and diabetic status, with a significantly greater parasite burden in the non-diabetic compared to the diabetic group, with the most common intestinal parasite in both groups being *Ascaris lumbricoides*. In contrast, a relatively small Brazilian study found a positive association between *S. stercoralis* diagnosed by serology and diabetic status assessed by glycated haemoglobin (HbA1c) five years later. Interestingly, most patients with positive serology had negative coprology examination results, and except for one, none showed eosinophilia, indicating that most infections were recent rather than present (Mendonca et al., 2006). Similar to the Brazilian study, a 2019 study also noted a positive relationship between *S. stercoralis* infection and T2DM (McGuire et al., 2019). The authors proposed that the greater number of diabetic individuals could be caused by a reduced ability to control or clear strongyloidiasis due to the development of T2DM, resulting in an excess of diabetic individuals with chronic infection over time. In 2010, two epidemiological in southern India undertaken by the same group found a significantly lower prevalence of lymphatic filariasis in both type 1 and type 2 diabetics compared to normal glucose-tolerant individuals (V. Aravindhan et al., 2010; Vivekanandhan Aravindhan et al., 2010). While the Turkish (Nazligul et al., 2001), Brazilian (Mendonca et al., 2006), and one of the Indian studies (V. Aravindhan et al., 2010) reported diabetic status as an exposure rather than an outcome, the focus of the second Indian study investigating type 1 diabetes shifted to treating worm infection as the exposure.

Further cross-sectional studies continued to use worm infection as the exposure and different metabolic parameters as the outcome. Two Indonesian cross-sectional studies on Flores Island reported inverse relationships between current worm infection and risk factors for CVD and T2DM. Individuals with at least one worm infection had lower BMI, waist-to-hip ratio, total cholesterol and LDL in the 2013 study (Wiria et al., 2013) and significantly lower BMI and HOMA-IR in the 2015 study (Wiria et al., 2015). In Australia, Aboriginal adults with *S. stercoralis* infection had a significantly reduced risk of T2DM (Hays et al., 2015). A 2018 Chinese study investigates *S. japonicum* infection in humans and wild-type mice (Q. Duan et al., 2018). Humans with chronic schistosomiasis had lower BMI, fasting blood glucose, total cholesterol, and serum triglycerides compared to non-infected individuals with similar results in the animal model. In contrast, Htun et al. found a positive association between *Taenia spp*. infection and T2DM (HbA1c) in adults in Lao PDR (Htun, Odermatt, Paboriboune, et al., 2018).

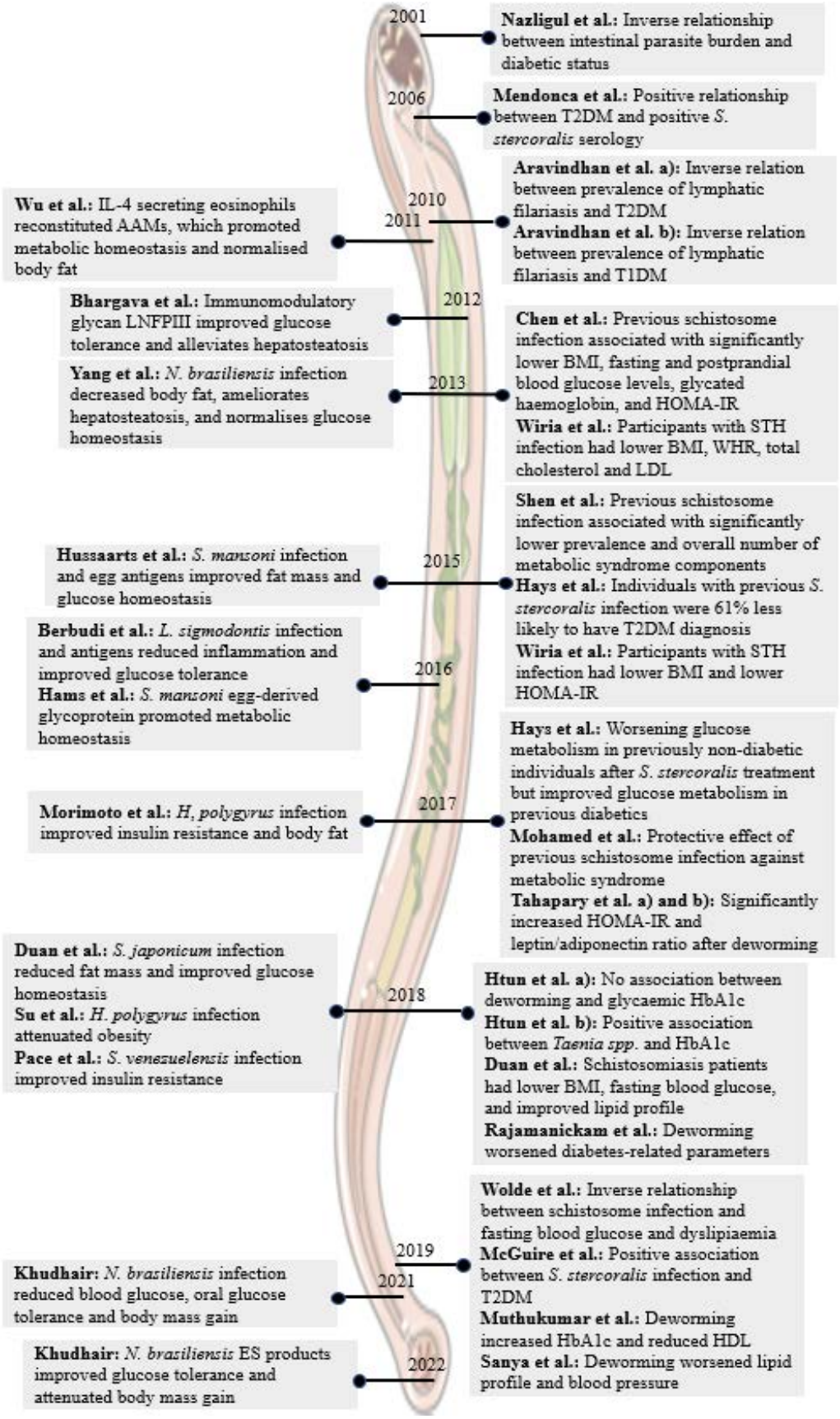


Figure 1-6 Timeline of landmark hookworm infection studies in humans (right) and animal models (left) AAMs, alternatively activated macrophages; ES, excretory-secretory products; STH, soil-transmitted helminth infection; WHR, waist-to-hip ratio. Adapted from (de Ruiter et al., 2017)

Several studies also investigated the potential long-term effects of previous worm infections on the risk of developing MetS components. A 2013 Chines study found that, compared to no infection, previous schistosome infection was associated with significantly lower BMI, fasting and postprandial blood glucose levels, glycated haemoglobin, and HOMA-IR (Chen et al., 2013). In a later Chinese study, prevalence and the overall number of metabolic syndrome components (central obesity, hypertension, elevated triglyceride, LDL, and fasting blood glucose) were significantly lower in adults with previous schistosome infection than in uninfected adults (Shen et al., 2015). In Australia, Aboriginal adults with prior *S. stercoralis* infection had a significantly reduced risk of T2DM (Hays et al., 2015). Further, Egyptian researchers found a significantly lower prevalence of MetS and its components in individuals with previous schistosome infections (Mohamed et al., 2017). Last, an Ethiopian study also reported lower fasting blood glucose levels and dyslipidaemia in currently *S. mansoni*-infected study subjects from an endemic town compared to subjects from a non-endemic town (Wolde et al., 2019).

In combination, these studies provide substantial support for the proposed protective effect of worm infection on metabolic disorders. Considering the multitude of populations and worm species studied and the almost unequivocal findings, these results further indicate that the beneficial effect is not a unique feature of one worm species but rather universal to all worm infections. Nevertheless, the epidemiological nature of the studies does not afford any causal support for the idea that worms could protect humans from metabolic diseases. However, recent deworming studies added some causal evidence to these cross-sectional findings.

Studies in Australia, Indonesia, India, Thailand, Lao PDR, Uganda, and South Africa all found worsening markers of metabolic disease parameters after deworming. In Australian Aboriginals, treatment of *S. stercoralis* infection had differential effects on glucose metabolism in diabetic and non-diabetic individuals (Hays et al., 2017). Infected, non-diabetic individuals showed worsening glucose metabolism (HbA1c) after *S. stercoralis* treatment. Yet, the treatment improved glycaemic control in previously diagnosed diabetics. This differential finding could be explained by the removal of the inflammatory-dampening effect of worm infection, which resulted in heightened inflammation and consequent greater insulin resistance. Infected Indonesian subjects on Flores Island developed significantly increased HOMA-IR following albendazole treatment (Tahapary, de Ruiter, Martin, Brienen, van Lieshout, Cobbaert, et al., 2017). Additionally, deworming significantly increased the leptin/adiponectin ratio. Given that leptin promotes inflammatory immune responses, this increase in leptin/adiponectin ratio may have contributed to the observed increase in insulin resistance in previously infected individuals (Tahapary, de Ruiter, Martin, Brienen, van Lieshout, Djuardi, et al., 2017). In India, *S. stercoralis*-infected individuals exhibited significantly lower initial insulin, glucagon, and adiponectin levels; deworming significantly increased these parameters (Rajamanickam et al., 2018). One year later, a South African found no significant change in HbA1c (%) in school children following

deworming (Htun, Odermatt, Muller, et al., 2018), although the observed trend of increasing HbA1c was in line with previous observations. Praziquantel treatment in Thailand showed that *Opisthorchis viverini* had a protective effect against hyperglycaemia (HbA1c) and improved the lipid profile (HDL) (Muthukumar et al., 2019). Further, Praziquantel treatment in Uganda worsened lipid profile and blood pressure but did not affect insulin resistance (Muthukumar et al., 2019). Overall, these deworming studies support the observations in cross-sectional studies.

1.2.1.10 Animal studies

Mouse studies using experimental infection with diverse worm species or treatment with worm-derived molecules consistently showed improvements in metabolic homeostasis and inflammation. Most of these studies also explored immunomodulatory mechanisms that could clarify the link between worms and metabolic disorders. A landmark 2011 study first demonstrated the role of IL-4 secreting eosinophils in preserving insulin sensitivity, providing the basis for the idea that type 2 immunity is linked to adipose tissue metabolic homeostasis (D. Wu et al., 2011). As worms are known to induce physiological eosinophil elevations, the authors infected mice fed a high-fat diet with *N. brasiliensis.* They reported improved insulin sensitivity and glucose tolerance, lower fasting glucose, and reduced perigonadal fat mass. Notably, these improvements were maintained up to 45 days after parasite clearance. These findings of a protective role of worm infection in metabolic homeostasis have since been replicated and extended in multiple other publications.

Some studies have since confirmed that type 2 immune responses as induced by *N. brasiliensis* (Khudhair et al., 2021; Yang et al., 2013), *S. mansoni* (Hussaarts et al., 2015), the filarial nematode *Litomosoides sigmodontis* (Berbudi, Surendar, et al., 2016), *H. polygyrus* (Morimoto et al., 2017; Su et al., 2018), and *S. venezuelensis* (Pace et al., 2018) promote glucose homeostasis and normalise body fat in diet-induced obese mice. Similar protective effects can also be achieved by treatment with parasite antigen mixtures or ES products (Berbudi, Surendar, et al., 2016; Bhargava et al., 2012; Hussaarts et al., 2015; Khudhair et al., 2022; van der Zande et al., 2021) and treatment with single parasite molecules (Crowe et al., 2020; Hams et al., 2016). Further, Duan et al. (2018) found reduced fat mass, improved glucose tolerance, and decreased fasting blood glucose in *S. japonicum*-infected obese mice that were accompanied by the suppression of inflammatory gene expression.

Further to exploring immunological modifications, a few studies also investigated changes in the gut microbiome as a potential mechanism for the protective role of worms against metabolic disorders. Mice with diet-induced obesity had significantly improved glucose tolerance and insulin sensitivity in the presence of *S. venezuelensis* infection (Pace et al., 2018). Additionally, infection resulted in substantial increases in *Firmicutes*, particularly *Lactobacillus spp*, and decreases in *Bacteroidetes* in the small intestine. These changes in the microbiota reduced inflammation, which improved glucose metabolism. Further, Shimokawa et al. found preventive and therapeutic effects of

H. polygyrus infection against diet-induced obesity in mice (Shimokawa et al., 2019). The authors also noted increases in members of the *Firmicutes* (*Bacillus*) and *Proteobacteria* (*Escherichia*) in infected mice and linked norepinephrine production in these bacteria to body fat reductions via upregulation of uncoupling protein 1. Last, *N. brasiliensis* ES products had prophylactic and therapeutic effects against T2DM in mice fed high-fat or high-glucose diets (Khudhair et al., 2021). Infection also changed the gut microbiota composition, with an increased abundance of Proteobacteria and decreased abundance of *Burkholderiales* in mice fed a high glucose diet and an increased abundance of *Clostridiales* in the high-fat diet mice, which may have mediated the protection against T2DM

Notably, a few studies have also investigated the involvement of adipokines in worm-mediated protection against metabolic disorders. In a mouse model of obesity, helminth-infected obese mice lost significant body and fat mass despite continued high-fat diet consumption (Yang et al., 2013). Furthermore, circulating leptin levels decreased in both obese and lean mice following helminth infection, consistent with improved leptin sensitivity and a decrease in adipose tissue. In the same study, helminth infection also normalised glucose homeostasis and insulin levels in obese mice fed a high-fat Treatment with *L. sigmodontis* antigens increased adiponectin levels in mice fed a high-fat diet, which may have ameliorated glucose tolerance (Surendar et al., 2019). Another study reported that *H. polygyrus*-infected mice on a high-fat diet gained less body mass and showed improved glucose tolerance and triglyceride levels compared to uninfected, which correlated with a marked decrease in leptin gene expression. As leptin is associated with a pro-inflammatory action and is often increased in obesity, leptin downregulation in *H. polygyrus* infection could be a factor in the protective effect of infection (Su et al., 2018)

In summary, results from cross-sectional and deworming studies in humans and experimental animal models established a beneficial role of worm infection against metabolic disorders. The protective effect is mediated by the induction of type 2 immune responses but may also reflect changes in the composition of the gut microbiota and attenuated secretion of adipokines. Emerging evidence suggests that protection can also be achieved by administering worm-secreted antigens and worm-derived products, providing a novel way to identify therapeutic strategies for metabolic disorders that do not rely on live worm infection.

1.2.2 Worm infections and the microbiome

Helminth infections may indirectly modulate immune and metabolic responses via their effect on intestinal microbiota composition (Mishra et al., 2014). Animal studies demonstrated alterations in the gut microbiota following helminth infection (Lee et al., 2014), and evidence from human studies has shown that helminth infections, including hookworms, promote greater microbial species diversity and/or richness (Cantacessi et al., 2014; Giacomin et al., 2015; P. Giacomin et al., 2016; Reynolds et

al., 2014). Several animal and human studies have shown the helminths' capacity to modify the microbiota's diversity, community structure, and function (Jenkins et al., 2017; Peachey et al., 2017).

Intestinal helminths modulate the microbiota through various direct and indirect avenues (Brosschot & Reynolds, 2018). For example, the antimicrobial activity of helminth ES products directly alters microbiota composition (Abner et al., 2001). Additionally, helminth infections alter nutrient availability in the gut. Infection with *H. polygyrus* in mice was shown to impair epithelial glucose absorption in an IL-4-dependent manner (Shea-Donohue et al., 2001), which may skew the microbial microflora towards sugar fermenting commensals. Last, recent studies indicated that helminths could alter the gut metabolomic profile via microbiota compositional changes, intestinal nutrient absorption, or helminth metabolite production (Reynolds 2015). As dysbiotic microbiota play a key role in the development of metabolic diseases (Tilg & Moschen, 2014), helminth-induced alterations of the microbiota could moderate the development of such diseases.

1.2.2.1 Animal studies

Changes in alpha diversity (the average species diversity within one host) are particularly interesting. Greater alpha diversity is commonly indicative of a microbiota favourable to health. Diseases such as obesity and metabolic diseases are generally associated with reduced alpha diversity (Le Chatelier et al., 2013). Most animal studies have found no changes in alpha diversity, with only a few studies showing decreased diversity consistent with the initial stages of helminth infection (Fricke et al., 2015; Khudhair et al., 2021; McKenney et al., 2015; Peachey et al., 2017). In contrast, alpha diversity generally increased in humans following natural or experimental helminth infection (Giacomin et al., 2015; P. Giacomin et al., 2016; Lee et al., 2014). While these findings could have resulted from differences in experimental designs and/or animal and helminth species, it could also be speculated that inflammatory processes during the initial stages of infection induce decreases in diversity which are reversed once the infection has become chronic. In such a scenario, the time point of sampling will significantly influence such studies' findings.

In animal studies, helminths have been shown to alter microbial populations involved in immune regulation. Despite vast differences in study designs, methodologies, helminth and animal species, sampling site, and baseline microbial composition, studies consistently reported distinct changes irrespective of parasite and host species, indicating that these changes are genuinely helminth-related (Peachey et al., 2017). Specifically, populations of *Lactobacillaceae*, a Gram-positive *Firmicute*, reportedly expanded in the murine gastrointestinal tract following infection with *H. polygyrus* (Kreisinger et al., 2015; Rausch et al., 2013; Reynolds et al., 2014; Walk et al., 2010), *T. muris* (Holm et al., 2015), *N. brasiliensis* (Fricke et al., 2015), *S. venezuelensis* (Pace et al., 2018), and in cat faecal microbiota following infection with *Toxocara cati* (Duarte et al., 2016). In hamsters, infection with the carcinogenic liver fluke *Opisthorchis viverrini* increased *Lactobacillaceae* in the

gastrointestinal tract and the biliary ducts (Plieskatt et al., 2013). *Lactobacillaceae* have been shown to protect the mucosal barrier function (Resta-Lenert & Barrett, 2006) and expand regulatory T cell populations in the mesenteric lymph nodes (Kwon et al., 2010), thereby modulating inflammatory diseases. Furthermore, studies have established that the relationship between helminths and *Lactobacillaceae* is mutualistic. Not only do helminth infections increase populations of *Lactobacillaceae*, but these microbes, in turn, promote helminth infections (Dea-Ayuela et al., 2008 14417; Reynolds et al., 2014).

Increased abundance of *Clostridia spp.* with helminth infection appears to moderate both immune and metabolic outcomes. Murine infection studies with *H. polygyrus* and *Trichuris muris* reported attenuated airway inflammation and protection against colitis with a greater abundance of *Clostridia spp.* (Ramanan et al., 2016; Zaiss et al., 2015). *Clostridia spp*. also induce gut Tregs, which critically contribute to gut tissue homeostasis (Cosovanu & Neumann, 2020). Furthermore, via the production of the SCFA butyrate, *Clostridia spp.* provide essential fuel for colonocytes. Butyrate availability orientates colonocyte metabolism towards mitochondrial beta-oxidation of fatty acids and consequently promotes obligate anaerobic bacteria in the microbial community, the signature of a healthy gut microflora (Litvak et al., 2018). On the other hand, T2DM in humans is related to decreased abundance in this group (Karlsson et al., 2013; Larsen et al., 2010; Qin et al., 2010; Sato et al., 2014), which is consistent with a negative correlation between *Clostridia spp.* and insulin resistance in mice (Chen et al., 2018).

Additionally, helminth infections can induce shifts in metabolic potential in the microbiota. *T. suris* infection in piglets did not alter alpha diversity yet significantly suppressed carbohydrate metabolic potential as indicated by the reduction of several bacterial genera and significantly repressed KEGG pathways involved in carbohydrate metabolism (Li et al., 2012), suggesting an impaired potential of the infected pigs to utilise carbohydrates. Similarly, Houlden and colleagues (2015) reported significant shifts in microbial composition with a concomitant decrease in alpha diversity, resulting in reduced breakdown products of plant-derived carbohydrates in the stool of *T. muris*-infected mice and significantly lesser weight gain in infected mice compared to naïve. The authors suggested the significant reduction in *Prevotella* reported in their study as a potential cause for the smaller weight gain in infected mice. *Prevotella* has been related to fibre digestion (G. D. Wu et al., 2011). A further study noted body fat reductions in *H. polygyrus* infection mice fed a high-fat diet (Shimokawa et al., 2019), which was associated with increases in members of the *Firmicutes* (*Bacillus*) and *Proteobacteria* (*Escherichia*). Notably, the administration of *N. brasiliensis* ES products also had prophylactic and therapeutic effects against T2DM in mice fed either a high-fat or high-glucose diet (Khudhair et al., 2021). The helminth-derived products changed the gut microbiota composition, with an increased abundance of *Proteobacteria* and decreased abundance of *Burkholderiales* in mice fed a high glucose diet and an increased abundance of *Clostridiales* in the high-fat diet mice, which may have mediated

the protection against T2DM. These findings show that changes in microbial composition following helminth infections may affect dietary nutrient extraction and, consequently, weight gain.

Helminth-induced alteration of the gut microbiota can also alter the availability of bacterial metabolites, including SCFAs. *H. polygyrus* infection increased *Clostridiales spp.* and significantly elevated SCFAs. Similarly, *A. suum* infection in pigs and *Na* infection in humans could raise SCFAs (Zaiss et al., 2015). Besides providing a source of nutrients for colonocytes (Litvak et al., 2018), SCFAs are known to enter the systemic circulation and contribute to whole-body energy homeostasis via Treg induction and secretion of interleukins (Silva et al., 2020).

1.2.2.2 Human studies

Human studies, albeit sparse, fall into two different categories: studies that evaluated the composition of the microbiota following experimental infection with a controlled number of larvae (Cantacessi et al., 2014; Paul Giacomin et al., 2016; Giacomin et al., 2015; P. Giacomin et al., 2016) and others that evaluated these changes in naturally infected individuals (Cooper et al., 2013; Houlden et al., 2015; Jenkins et al., 2017; Lee et al., 2014; Rosa et al., 2018). While the studies using controlled infections have consistently reported increases in microbial species richness and diversity following experimental helminth infection, studies in naturally infected individuals (Cooper et al., 2013; Jenkins et al., 2017; Lee et al., 2014) reported contrasting results. Cooper and colleagues (2013) reported a decrease in faecal bacterial diversity in Ecuadorean school children infected with two species of helminths (*T. trichiura* and *A. lumbricoides*). Uninfected children or children infected only with *T. trichiura* did not show such reductions. In contrast, Lee and colleagues (2014) found a small but significant increase in faecal alpha diversity in Malaysians infected with multiple gastrointestinal nematodes (a combination of roundworms, hookworms, and whipworms) compared to uninfected Malaysian individuals. The difference was even greater when comparing the infected individuals to uninfected New York individuals. Despite differences in sample processing and age of volunteers, these data supported the influence of helminths on microbial richness and the hypothesis that the ancestral microbiota changes with advancing economic development (Wittebolle et al., 2009). The authors suggested that the effect of natural versus experimental helminth infection also warranted further examination. A later study (Jenkins et al., 2017) compared the gut microbial community profiles of humans from several Sri Lankan communities naturally infected with at least one gastrointestinal microbe to those uninfected and those that had received anthelminthic treatment; the authors reported no significant differences between groups. Differences in baseline gut microbial composition, helminth species and infection stage, and sample size could explain the divergent findings.

Rosa and colleagues (Rosa et al., 2018) recently predicted helminth infection status across distinct geographical regions for the first time based on microbiome taxa consistently associated with helminth infections. They identified *Lachnospiraceae incertae sedis* as the only negatively associated

genus, while positively associated genera included *Olsenella*, *Flavonifractor*, *Enterococcus*, and *Allobaculum*. *Lachnospiraceae* were previously associated with obesity based on their capacity for butyric acid production. At the same time, *Allobaculum* has been linked to gut inflammation, weight reduction, and leptin levels, indicating a key role in gut homeostasis and host energy balance. Decreases in *Lachnospiraceae* and concomitant increases in *Allobaculum* may partially explain reduced BMI and body mass observations in some studies (Wiria et al., 2015; Yang et al., 2013).

Few studies have previously examined the effect of deworming on the microbiota, with inconsistent results. Rosa and colleagues (Rosa et al., 2018) reported that the microbiome of dewormed individuals more closely resembled that of uninfected individuals. Further, Houlden and colleagues (Houlden et al., 2015) showed a gradual yet incomplete restoration of the microbiome to the pre-infective state following anthelminthic treatment. In contrast, Kay et al. (2015) found that anthelmintic treatment to clear *S. haematobium* infection did not change the abundance of operational taxonomic units. Similarly, deworming did not affect microbiome composition in a study investigating the clearance of *T. trichiura* infection in Ecuadorian children (Cooper et al., 2013). Albendazole treatment and clearance of *A. lumbricoides* and *Na* in residents of five rural Kenyan villages did not alter microbial diversity but significantly increased the proportion of *Clostridiales* and significantly reduced *Enterobacteriales* (Easton et al., 2019).

In summary, helminth infection can reshape the gut microbiota composition. Dysbiosis is a crucial factor in the progression of metabolic disorders, as it dysregulates energy homeostasis and host immunity. Dysbiosis further impairs gut barrier function, inducing systemic inflammation and hyperendotoxemia, which eventually drives the development of insulin resistance. Although the results are heterogeneous, animal and human studies have shown the potential of helminth infection to induce beneficial alterations in gut microbial composition that are associated with inflammation, energy absorption, and glucose control. These findings suggest that helminths exert their immunoregulatory function at least in part via their effect on the gut microbiota, a mechanism that could also prove a valuable pathway in the prevention of T2DM.

1.3 Rationale and hypotheses

1.3.1 Current therapies for metabolic disorders

Currently, the prevention and management of T2DM rely heavily on pharmaceutical drugs that primarily target hyperglycaemia without considering the underlying inflammation. The most commonly prescribed drugs include metformin and sulphonylureas, but secondary treatments are often included in the treatment regime to mediate the side effects of these drugs (The Royal Australian College of General Practitioners and Diabetes Australia, 2020). Given that T2DM is primarily a lifestyle disease, lifestyle interventions such as a healthy diet (Thorburn et al., 2014) and regular physical activity (Dubé et al., 2012; Winn et al., 2021) are valuable tools for preventing or improving metabolic disorders. However, increasing public willingness to accept and integrate these lifestyle changes is challenging, and alternative approaches are needed.

As outlined above, data from mouse and human studies support the beneficial role of helminths in metabolic disease. One advantage of helminth infections is that they can attenuate inflammation as well as hyperglycaemia (Moyat et al., 2019; Rennie et al., 2021; Tracey et al., 2016; Wiria et al., 2014). However, the evidence from human studies primarily evolved from cross-sectional studies and cannot infer causality. The authors of cross-sectional studies have called for controlled human helminth infection trials to understand better how helminths can modulate the immune and metabolic response. Interventional clinical studies, particularly randomised, controlled trials, can provide some of the most robust levels of evidence.

The effect of controlled helminth infection on metabolic markers, immune responses, and gut microbiota in humans with metabolic disease has not been investigated in combination. The outcomes of this investigation will improve our understanding of how helminth infections can modify the immune and metabolic response and may result in several unique, translational, and commercial outcomes.

1.3.2 Necator americanus

The choice of *Na* as the investigational product was based on the lack of serious pathology caused by *Na* infection. Common side effects include the so-called “ground itch” at the site of skin penetration and various degrees of abdominal discomfort during the initial stages of worm attachment to the intestinal lining (P. R. Chapman et al., 2021; Croese et al., 2015; Feary et al., 2010; McSorley et al., 2011). High worm burden may result in iron deficiency anaemia and intestinal haemorrhage (Hotez et al., 2004), but the low doses used in human clinical trials, including the present trial, generally have no pathological consequences. Even though *S. mansoni* appears to have greater therapeutic potential than *Na* (Rennie et al., 2021), the possible severe and life-threatening pathology associated with this parasite has previously precluded the use of *S. mansoni* in clinical trials. However, a more recent clinical

safety trial using male-only *Schistosoma mansoni* cercariae, which circumnavigates the pathology mediated by egg deposition, has reported detectable and well-tolerated infection with 20 *S. mansoni* in 82% of volunteers, paving the way for a new *S. mansoni* infection model (Langenberg et al., 2020)

Human clinical trials investigating treatment for inflammatory diseases have been using the pig whipworm *T. suis* and *Na* for nearly 20 years. Although orally administered *T. suis* ova showed great promise in early trials, the results from subsequent trials were disappointing (Ryan et al., 2020). Besides, *T. suis* ova do not establish chronic infection in humans and require repeated dosing. In contrast, *Na* is very efficient in establishing chronic infection in humans (Paul R. Chapman et al., 2021) and has a life expectancy of 3-10 years in the human gut (Hoagland & Schad, 1978). Further, if required, *Na* infection can be quickly cleared by administering anthelminthic medication.

In countries with high sanitation standards, such as Australia, *Na* infection in clinical trials is unlikely to pose a risk to the general population. Further, its lifecycle (Figure 1-7) prevents the uncontrolled expansion of the initial worm burden. Soil-transmitted helminths, including *Na*, owe their name to the crucial role of parasite egg-contaminated soil in transmission. Adult worms reside in the intestine, and the females produce thousands of eggs daily, passed in the faeces. In some species, faecal-oral transmission occurs via ingestion of eggs or even larvae (*S. stercoralis*) that may be attached to vegetables or be obscured in contaminated water and soil.

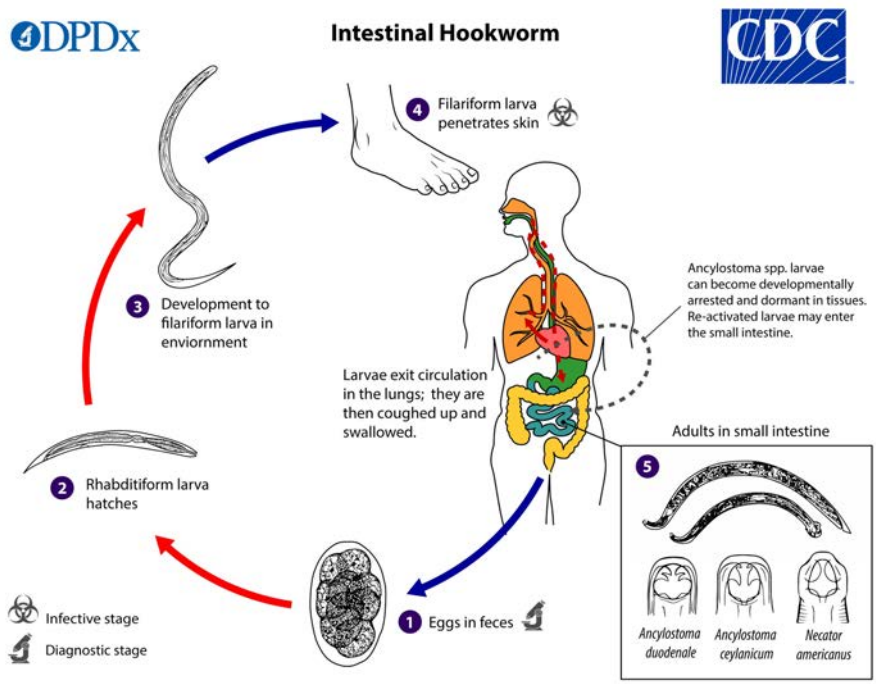


Figure 1-7 Necator americanus lifecycle (Centre for Disease Control and Prevention, 2019)

In contrast, hookworm eggs are not infective. In warm and moist conditions, they hatch and mature into infective larvae in the soil and can penetrate the skin of individuals walking barefoot on contaminated ground. For *A. duodenale*, oral transmission of larvae is also possible (Jourdan et al., 2018). After skin penetration, the larvae migrate passively in the circulation to the lungs, infiltrate the alveoli, travel up the bronchial tree, and then down the intestinal tract. After arrival in the duodenum, they use their buccal plates to attach to the intestinal mucosa and feed on blood (Loukas et al., 2016). Given the need for eggs to hatch in a warm and moist environment in the soil, the use of modern toilets eliminates the risk of eggs hatching in the soil and prevents uncontrolled transmission of the infection.

In summary, experimental *Na* infection is safe at low doses, long-lasting, easily controlled and does not pose a threat to the wider community. These characteristics make *Na* a preferred choice for human helminth infection trials.

1.4 Hypotheses and aims

My hypotheses were that controlled, low-dose infections with the human hookworm *Na:*

1. Will be safe, tolerable, and acceptable in otherwise healthy adults at risk of T2DM.
2. Can stabilise or improve determinants of metabolic disease.
3. Can induce a biased Type 2 and immune regulatory response.

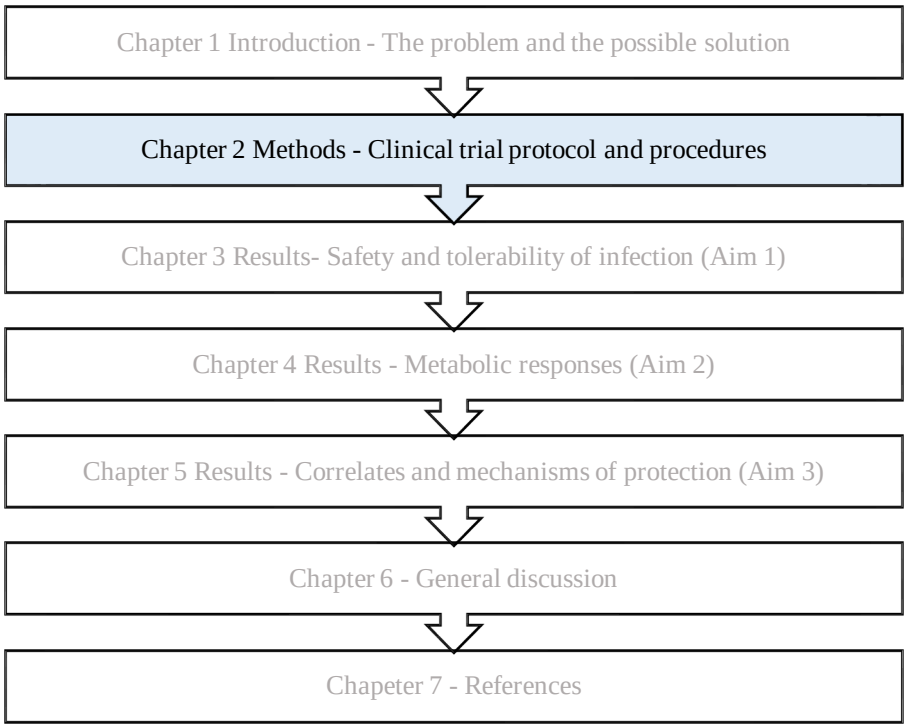
Thus, the aims of this project are as follows:

AIM1: Establish the acceptability, tolerability, and safety of experimental infection with the human hookworm *Na* in otherwise healthy adults with metabolic syndrome.

AIM 2: Determine the influence of infection with *Na* on key metabolic and physical parameters.

AIM 3: Investigate potential correlates of protection against metabolic disease.

2 METHODS - CLINICAL TRIAL PROTOCOL AND PROCEDURES



This chapter details all protocols, procedures, and materials used during the clinical trial and consequent analysis of the results. The trial protocol titled *Safety and Tolerability of Experimental Hookworm Infection in Humans with Metabolic Disease: Study Protocol for a Phase 1b Randomised Controlled Clinical* Trial was published in BMC Endocrine in 2019 (Pierce et al., 2019)

2.1 Clinical trial protocol

The protocol was registered on ANZCTR.org.au on 05 June 2017 with identifier ACTRN12617000818336 (https://anzctr.org.au/Trial/Registration/TrialReview.aspx?id=372957&isReview=true).

2.1.1 Design

The randomised, double-blind, placebo-controlled Phase 1b safety and tolerability trial assessed the effect of inoculation with 20 or 40 infective stage-three larvae (L3) of *Na* on metabolism, inflammation, and immune response in otherwise healthy women and men aged 18-50 with central obesity and features of MetS over 24 months. An initial recruitment target of 54 allowed for a drop-out rate of 20%, leaving 45 volunteers (15 in each treatment group) to participate in the trial. Volunteers who met the eligibility criteria, had been approved following the screening, and had given informed consent were randomised into three trial groups. Participants in the L3-10 and L3-20 groups received 20 (2 x 10) and 40 (2 x 20) L3 of *Na*, respectively. Participants in the placebo group were administered Tabasco® sauce in a solution identical in appearance to the L3 solutions. All groups were monitored over 24 months, followed by an optional extension to 36 months to assess the impact of eliminating the worm infection following anti-worm medication (Figure 2-1).

2.1.2 Setting

We recruited participants from the Cairns and Townsville Region on the southeast coast of Far North Queensland, Australia. The study sites were the James Cook University (JCU) campuses in Cairns and Townsville. Participant recruitment initiatives included advertising in local media, poster notices around Cairns, including the JCU Cairns campus, student email lists (with permission from the university), and social media. The human hookworm *Na* is not endemic to these metropolitan areas.

2.1.3 Ethics approval and consent to participate

The study (Trial Protocol Version 5.0 dated 03/04/2018) was approved by JCU's Human Research Ethics Committee (Approval number C26), and trial conduct was subject to the sponsor's (JCU) Research Governance. Necessary protocol modifications were communicated to all investigators, JCU's Human Ethics Research Committee, and trial registries. These modifications did not impact already recruited participants.

Before any eligibility assessment, the study doctor obtained written informed consent from potential participants after clarifying all questions, and the volunteer agreed to participate. Participants could withdraw from the trial at any time without providing reasons. In case of withdrawal, participants

were asked to complete an Early Termination Visit for final health assessments. Data collected prior to and during the Early Termination visit formed part of the study results.

The study was not expected to involve any substantial risk or cost to the participants; therefore, volunteers did not receive financial compensation for participation in this trial.

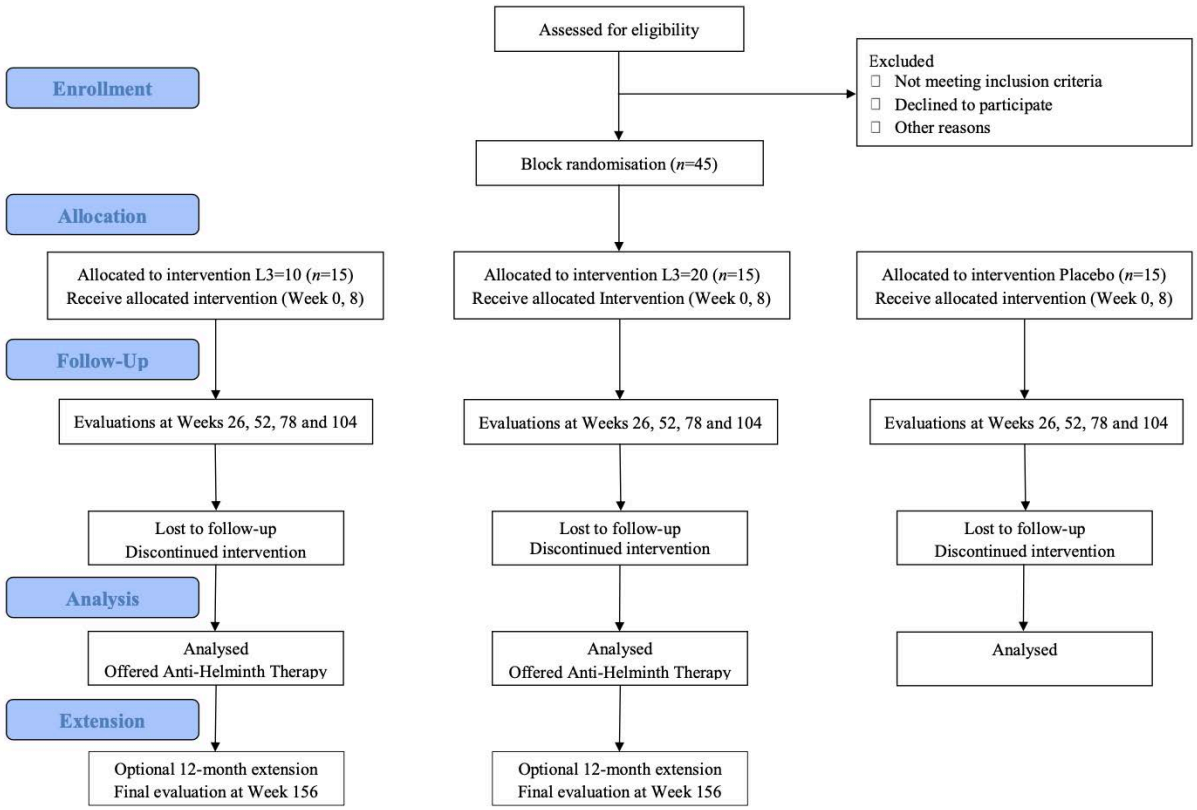


Figure 2-1 The WAM trial flow diagram

2.1.4 Participant characteristics

2.1.4.1 Inclusion criteria

Eligible for inclusion in this study were otherwise healthy women and men aged 18-50 years, the primary age window for progression to type 2 diabetes mellitus (T2DM) (Kautzky-Willer et al., 2016). Criteria included central obesity (waist circumference, WC > 90 cm for women and >102cm for men) and increased insulin resistance as assessed via abnormal homeostatic model assessment of insulin resistance (HOMA-IR) > 2.12 (Gayoso-Diz et al., 2013**)** or at least two other features of MetS. These included elevated blood pressure (> 135/85 mmHg), dyslipidaemia[4], elevated fasting blood sugar concentration, or abnormal liver function tests[5] suggesting fatty liver disease as per the screening

[4] **Dyslipidaemia:** elevated total cholesterol triglycerides, LDL, or total cholesterol/HDL ratio; low HDL
[5] **Abnormal liver function tests:** abnormal alanine transaminase, aspartate transaminase, alkaline phosphatase, albumin and total protein, globulin, bilirubin, and gamma-glutamyl transferase

pathology report. (Figure 2-2). Volunteers had to be willing to comply with all protocol scheduled visits and provide written informed consent before study entry with the option to withdraw at any time. Women of child-bearing potential agreed to use acceptable methods of contraception.

2.1.5 Exclusion Criteria

Exclusion criteria included pregnancy, established chronic disease, historical or current substance abuse, major allergies, known immunodeficiency disorder, unstable asthma requiring significant maintenance treatment, taking oral prescription medications or nutritional supplements likely to interfere with study outcomes, and inability to give informed consent (Figure 2-2)

2.1.6 Initial clinical assessment and sample collection/Week -6

During the first visit, the trial doctor interviewed and examined consenting participants. Eligibility was determined based on inclusion criteria, medical history, pre-existing conditions, current medications, recent travel to worm-endemic countries, known worm infections, and deworming treatments. The trial doctor also conducted a complete physical examination during initial screening, including measurement of height and body mass, examination of respiratory, cardiovascular, and nervous systems, and a pregnancy test for females. Participants were asked to attend Sullivan Nicolaides Pathology (SNP) for a fasting blood test the next day, assessing lipid profile, liver function, fasting glucose and insulin-s (Figure 2-3). After the screening visit, the study team contacted participants to inform them of their eligibility to proceed. If eligible, the study team booked the next visit according to the study schedule. Clinical assessments were completed during subsequent visits to ensure patient safety and monitoring. Pathology sampling (blood and urine) was repeated throughout the evaluation period.

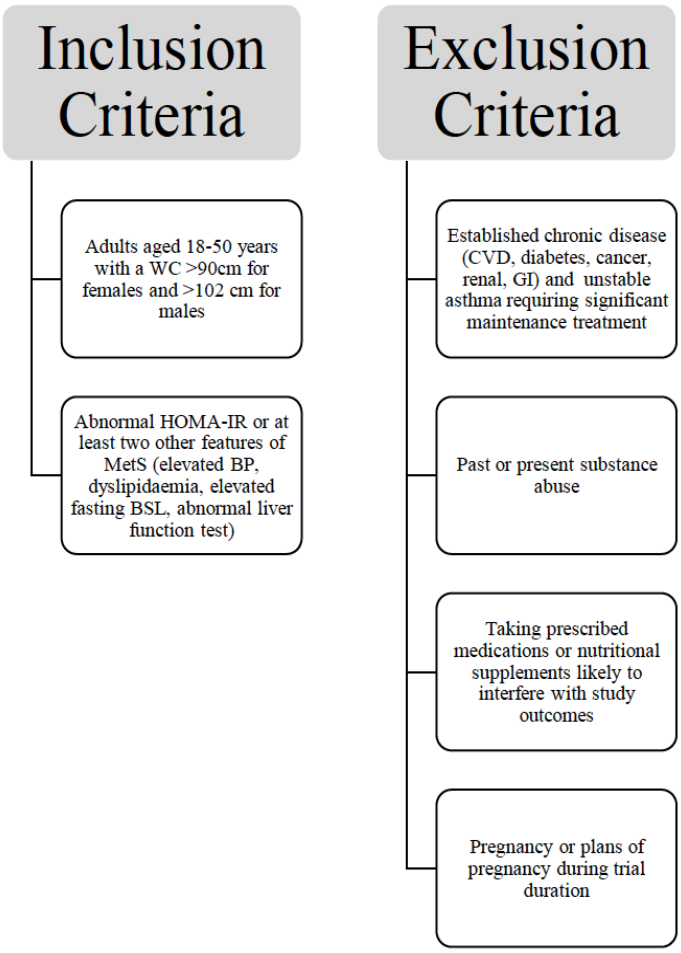


Figure 2-2 Inclusion and exclusion criteria

2.1.7 Processes, interventions, and comparisons

2.1.7.1 Baseline visit/Week -4

During this visit, the study doctor reviewed the laboratory test results, confirmed continued eligibility for participation, performed a complete physical examination, and administered diet, health, and activity questionnaires, which had been previously validated (Kroenke et al., 2010; Martínez-González et al., 2012; Papadaki et al., 2018; Schroder et al., 2011). Blood samples were collected, and the participants were asked to undergo a body composition scan via dual-energy X-ray absorptiometry (DEXA) and deliver a faecal sample. The study doctor obtained written informed consent to store health information and blood or tissue samples in the university's Biobank.

2.1.7.2 Treatment period/Week 0 to Week 104

The treatment period lasted 104 weeks, during which participants were required to attend four evaluation visits approximately six months apart. To promote retention, participants could opt for telephone and Zoom appointments with the study doctor that accommodated personal circumstances and/or COVID-19 restrictions. Participants were told their study allocation during their final exit consultation and examination with the study doctor. As a mandatory requirement of the study, participants in the worm treatment groups were provided with the anthelmintic treatment mebendazole at no cost to clear the hookworm infection; however, participants didn't need to take this medication. Participants electing to refrain from taking the anthelmintic treatment (choosing to keep their helminths) following the 104-week evaluation period were advised that their future medical care would be passed onto their general practitioner. Willing participants could opt into a 52-week extension to the study to examine the continued effects of infection or the potential impact of deworming medication on safety, immunological, and metabolic parameters.

2.1.7.1 Randomisation and inoculation (Week 0 and Week 8)

Previous studies have established the safety of experimental inoculation with *Na* in dose-controlled conditions (Croese et al., 2015; Mortimer et al., 2006). Their results indicated that *Na* is well tolerated in human trial subjects up to a dose of 25 L3, beyond which adverse effects such as abdominal pain become more problematic (Feary et al., 2010). Doses > 50 L3 have been associated with more severe adverse reactions, including gastrointestinal reactions (Paul R. Chapman et al., 2021). Based on these findings, doses of 20 and 40 L3 were selected for the current study, consistent with doses from a parallel study in people with coeliac disease (Trial identifier NCT02754609). The allocation sequence for the three cohorts was generated by random block number generation (block size of 6). The unblinded researcher subsequently implemented allocation using sequentially numbered, opaque, sealed envelopes and containers. The inocula, 10 x L3, 20 x L3, or Tabasco solution (placebo), were prepared by the unblinded research assistant freshly on-site following an established protocol that has been used for similar previous studies (Croese et al., 2015) and is detailed in Section 2.2.2 below. Larvae were decontaminated using iodine disinfectant and individually selected by an experienced researcher based on morphological integrity and active motility to ascertain optimal larval viability. All inocula appeared identical, and study doctors, investigators, participants, and data analysts were blinded until participants left the study. Depending on randomised group allocation, consenting participants were inoculated with either 10 or 20 *Na* L3 in 300µL of deionised water or the placebo. Two doses were administered eight weeks apart (Figure 2-3) by applying the solution to a dressing pad placed on the participant's forearm. Participants were advised to expect localised itch and irritation at the inoculation site, much the same as would be experienced if chilli pepper was rubbed into the skin. Following inoculation, participants were observed for one hour in case of an adverse response. Unblinding occurred if participants

experienced an adverse event that led to them leaving the trial. Participants were reminded of the importance of maintaining their standard hygiene practices (using a toilet) during the study. This reminder was essential for preventing parasite eggs shed in the faeces from developing in the environment into the infective stage larvae, a process that takes 7-10 days.

	STUDY PERIOD									
	Enrolment		Allocation		Post-allocation					Early termination
Week	-6	-4	0	8	26	52	78	104	156	
ENROLMENT:										
Eligibility criteria review	X									
Informed consent	X									
Demographics	X									
Medical history	X									
Full physical examination	X	X	X	X	X	X	X	X	X	X
Vital signs (BP, HR, Temp, Resp)	X	X	X	X	X	X	X	X	X	X
Height	X									
Body mass	X	X	X	X	X	X	X	X	X	X
Screening pathology (fasting blood glucose, fasting insulin, lipid profile)	X				X	X	X	X	X	X
Urine dipstick pregnancy test	X									
INTERVENTIONS:										
First Inoculation with experimental treatment			X							
Second inoculation with experimental treatment				X						
ASSESSMENTS:										
Assessment pathology HIV, HepB & C		X								
FBC, U&E, LFT, CRP, adiponectin, omega 3 PUFA profile, Hb1Ac, LPSBP, UACR)		X			X	X	X	X	X	X
DEXA scan		X				X		X	X	
Lifestyle and activity questionnaires		X			X	X	X	X	X	X
PBMC storage		X	X	X	X	X	X	X	X	X
Serum storage		X			X	X	X	X	X	X
Faecal sample		X			X	X	X	X	X	X
Adverse events	←	—	—	—	—	—	—	—	—	→
Serious adverse events	←	—	—	—	—	—	—	—	—	→
Provision of anthelminthic treatment								X	X	X

Figure 2-3 Schedule of investigations
BP, blood pressure; HR, heart rate; Temp, temperature; Resp, respiratory rate; HIV, human immunodeficiency virus; Hep B & C, hepatitis B and hepatitis C; FBC, full blood count; U & E, urea and electrolytes; LFT, liver function

2.1.7.2 Safety and clinical monitoring

During the initial screening and every subsequent visit (or as required in an adverse event), the study doctor conducted a general health assessment and physical examination and obtained vital signs (blood pressure, heart rate, temperature, and respiratory rate) to monitor participant safety. A serum pregnancy test was performed for female participants at the beginning of the study to exclude pregnancy; a positive test would have excluded the participant from the trial.

Incidence and severity of adverse events (AEs) and serious adverse events (SAEs) were evaluated formally via structured questionnaires reviewed by the investigating clinician and informally via participant-initiated contact. The most significant side effects of infection were expected to occur during the first 4-6 weeks following inoculation as the worms anchor themselves to the intestinal mucosa to facilitate feeding and avoid ejection by gut peristalsis (Loukas et al., 2016). Common side effects during this initial colonisation include abdominal pain, increased flatulence, nausea, and bloating (Daveson et al., 2011). Eight weeks after the first inoculation, the second inoculation visit provided an ideal opportunity to identify/monitor any side effects. Before informed consent, potential side effects had been outlined in an extensive participant information sheet and were reiterated during the first inoculation visit. Participants were encouraged to contact the trial doctor if they noticed any unusual signs and symptoms after the first inoculation visit. If deemed necessary by the trial doctor, participants were called in for an additional examination, during which they could elect to discontinue the trial. The participant was unblinded and offered anthelmintic medication if symptoms were consistent with hookworm infection's potential side effects.

Pathology samples (blood) were monitored every six months for the incidence and severity of laboratory abnormalities. In the case of an SAE, the participant would have been referred to their general practitioner and the case to an independent safety committee.

2.1.7.3 Outcomes

The primary outcome measure was the safety of experimental hookworm infection of otherwise healthy women and men with 20 *Na* L3 as evaluated by the number of reported AEs and SAEs, assessment of general health, and successful completion of the 24-month trial.

Secondary outcomes included:

1. safety and tolerability of the higher dose of 40 *Na* L3
2. longitudinal and inter-cohort changes in metabolic parameters (HOMA-IR, fasting insulin and blood glucose, body mass, central obesity, glycated haemoglobin, blood lipids)
3. longitudinal and intercohort changes in correlates of protection (diet, physical activity, type 2 immune responses, adipokines)
4. longitudinal and inter-cohort changes in the composition of the faecal microbiome

5. impact of de-worming medication on safety, immunological, and metabolic markers

2.1.8 Data collection, management, and statistical analyses

2.1.8.1 Sample size

This phase 1b safety and dose-ranging study aimed to include 45 participants with 15 infected with 20 L3, 15 with 40 L3, and 15 in the placebo control group. In the absence of other effect size data from human trials upon which to base a power calculation, and considering HOMA-IR change from baseline was the primary metabolic outcome, we adapted the SUGARSPIN HOMA-IR result (Tahapary et al., 2015) and assumed an effect size of 1.06 over two years. A total of 15 participants in each group reflected 80% power to detect an effect size of 1.06 using the T-statistic and 1.023 using the Z-statistic. The aim to recruit 54 participants allowed for a potential drop-out rate of 20%.

2.1.8.2 Data collection and management

All eligible participants were assigned a unique identification number at their first screening visit. Participant confidential data, including anthropometric measures, lifestyle, and medical history, were recorded on a hardcopy Case Report Form (CRF). I then entered all CRF, pathology, and DEXA data into a password-protected electronic database explicitly developed for this trial. Hard copy CRFs, pathology, and DEXA reports were stored in participant trial folders in a locked cabinet in a secure environment at the JCU Cairns campus. Access to the folder containing the databases was restricted to study personnel only, who had signed a confidentiality agreement as a condition of their involvement in the study.

Data from the final trial data set was provided to investigators and statisticians in de-identified electronic format. This data was linked to the Case Report Form and pathology data using the unique identification number, initials, and collection date. While a data management team was not formed due to minimal risks associated with the study, independent monitors audited data regularly for independent source data verification. The electronic database was set up in Microsoft Excel, and I used GraphPad Prism for all statistical analyses and data presentations.

At the trial conclusion, participants were given an individual report, including their baseline test results and any changes over the study period. They were also informed of study allocation (20 L3, 40 L3, Placebo). Upon publication of the results, the participants can opt to receive a copy of the manuscript.

2.1.8.3 Safety and efficacy of infection

To assess the primary outcome, the safety of infection with 20 *Na* L3, I used the chi-squared test for trend to determine any differences in the proportions of AEs between the Placebo, L3-10, and

L3-20 groups. I applied the Kaplan Meir model to illustrate progression through the study. Successful *Na* infection was confirmed using qPCR detection of parasite eggs in faecal samples taken at wk26 (see Section 2.6.3). The faecal sample collected at baseline (wk0) was used to assess current or previous helminth infections that could have interfered with trial outcomes. Standard descriptive statistics were performed on all baseline characteristics (categorical variables: absolute and relative frequencies; numerical variables: mean and standard deviation or median and interquartile range, dependent on data distribution).

2.1.8.4 Impact of helminth infection on metabolic parameters

To determine HOMA-IR, I multiplied fasting blood glucose (mmol/L) with insulin (mU/L) values and divided the product by 22.5 ((Zajak, 2020). Changes in metabolic determinants, particularly insulin resistance (HOMA-IR), body mass, visceral adiposity (WC and DEXA), and blood lipids, were analysed via comparison of baseline and follow-up data and visually checking the outcome measures moved in the expected direction for each allocation group. Standard descriptive statistics were performed on all outcome measures at baseline and each of the four evaluation visits (categorical variables: absolute and relative frequencies; numerical variables: mean and standard deviation or median and interquartile range, dependent on data distribution).

Normality testing for most data showed a non-parametric distribution. Therefore, I used Kruskal-Wallis with Dunn's multiple comparisons tests to detect inter-cohort differences in absolute values and changes. Mann-Whitney tests were applied to find differences between the cohorts for analyses comparing the combined worm treatment groups with Placebo, as the outcome variable distributions were nonparametric. Given several missing values, I applied the mixed-effects model for longitudinal analyses (which allows missing values) with Tukey's multiple comparisons tests.

To calculate effect sizes (Cohen's *d*) for HOMA-IR changes, I used the following equation (http://www.bwgriffin.com/gsu/courses/edur9131/content/EffectSizeBecker.pdf):

$d = M_1 - M_2/\delta_{pooled}$ (δ_{pooled}, pooled standard deviation)

with

$\delta_{pooled} = \sqrt{[(\delta_1^2 + \delta_2^2)/2]}$

2.1.8.5 Impact of helminth infection on immune responses

Eosinophils and basophils

As with metabolic parameters, I used Kruskal-Wallis with Dunn's multiple comparisons tests to explore inter-cohort differences in absolute values and changes. Given several missing values, I

applied the mixed-effects model for longitudinal analyses (which allows missing values) with Tukey's multiple comparisons tests.

Cytokine responses

Serum samples collected at each scheduled visit were batch-analysed using the Biolegend® LEGENDplex™ Human Th Cytokine Panel of 12 cytokines (IL-2, 4, 5, 6, 9, 10, 13, 17A, 17F, 22, IFN-γ and TNF) and the LEGENDplex™ Data analysis software suite. Final data presentation and statistical analyses were performed in GraphPad Prism. I used Mann-Whitney tests to analyse the response difference between Placebo and infected at each time point and Friedman's test for longitudinal analyses, as the data were not normally distributed.

2.1.8.6 Impact of helminth infection on microbiome composition

Faecal specimens collected in anaerobic sample collection containers were stored at -80ºC and DNA was extracted (described in Section 2.6) before genome-wide shotgun sequencing by the Ramaciotti Centre in Sydney for future analysis of microbiota composition and metabolic potential.

2.1.8.7 Missing data and early termination

Two research team members reviewed data from each participant to identify and address missing information. To account for missing data at the analysis stage, this study followed the method prescribed by Jakobsen et al. (2017). Specifically, as missing data exceeded 40%, no imputation was undertaken, and results were interpreted in the context of this limitation. (Jakobsen et al., 2017). Early termination and withdrawals from the trial were recorded throughout the intervention and evaluation periods.

2.2 Procedures

Note: I have collated all materials used in a table in Section 2.7.

2.2.1 Larval culture

2.2.1.1 Donors

Volunteers from the Cairns, QLD, metropolitan area, who had given informed consent, were inoculated with infectious *Na* larvae according to ongoing JCU Human Research Ethics Committee approval (H5936). All donors were negative (screened pre-infection and yearly after that) for HIV and hepatitis. When required, donors were asked to provide a faecal sample in a sealed container approximately ten days prior to inoculation of the trial participants. Samples were maintained at room temperature (20-30°C) during transport and in AITHM laboratories at the JCU Nguma-bada campus.

2.2.1.2 Premises

Production of L3 for inoculation took place in a suite of laboratories within the AITHM facility that requires a swipe card and physical key access; the area is restricted to students, postdoctoral fellows, and technical staff of the Loukas and Giacomin laboratories. All procedures were carried out in a laboratory designated for the exclusive cultivation of *Na* L3, and only faecal samples intended for this purpose were contained in this facility.

All cleaning and maintenance of this room were performed by trained AITHM staff, with no general cleaning staff permitted in this facility. All equipment used in L3 production was decontaminated with 80% ethanol and the liquid disinfectant F10SC (1:125) and autoclaved where appropriate. Liquid waste was disinfected using F10SC, followed by autoclaving and disposal. Consumables such as filter papers, Petri dishes, pipettes, and wooden applicators were disposed of in PC2 waste bins. All production areas, including benchtops, microscopes, and incubators, were routinely wiped with 80% ethanol and paper towels, which is highly effective in killing L3 (Speare et al., 2008).

2.2.1.3 Safety precautions

N. americanus L3 are infectious and will penetrate skin and mucus membranes. Gloves had to be worn at all times. Containers with L3 remained closed unless L3 were transferred. I did not work in the same environment simultaneously with people unaware of the risk of L3. Gloves needed to be changed if they became contaminated with water drops, and the contaminated glove was sprayed with 80% ethanol before disposal. For spills of potentially contaminated liquids onto surfaces, the area was flooded with 80% ethanol.

2.2.1.4 Documentation

For each sample, I recorded the following:

- the date of receipt
- donor ID
- time and date of sample origin
- consistency of sample
- faecal parasite egg burden/gram (microscopic determination)
- date of larval harvest
- number of plates harvested
- condition of plates (e.g., the presence of mould)
- batch number
- motility of larvae

2.2.1.5 Methodology of production

Equipment and materials

- Large glass bowl
- Stainless steel slotted spoon
- Bench coat
- 150x15mm Petri dishes with cams
- Chux Superwipes
- Disposable pipettes
- Large zip lock bags
- Permanent marker
- Scissors
- 50mL Falcon Tubes
- Whatman filter paper 110mm in diameter
- Wooden applicator sticks 150mm
- Paper towels
- Deionised water
- Granulated (Figure 2-5A) and fine charcoal (Figure 2-5 B)

Preparation

1. Covered all working space with bench coat.

2. Prepared sterile Petri dishes with four layers of approximately 5cmx5cm squares of Chux Superwipes fanned out in the centre of the dish.
3. Using a disposable pipette, moistened the Chux squares with deionised water until wet but still held the water (~ 10 squirts per dish).
4. Added one layer of round filter paper to cover the Chux squares and waited until all the filter paper was wet.
5. Transferred the faecal sample from the collection container into the glass bowl (Figure 2-5 C), noting the consistency as classified in the Bristol stool chart (https://www.continence.org.au/bristol-stool-chart).
6. Using two wooden applicators, transferred approximal 2 g of the faecal sample to a 50mL Falcon tube for egg count (see 2.2.3).

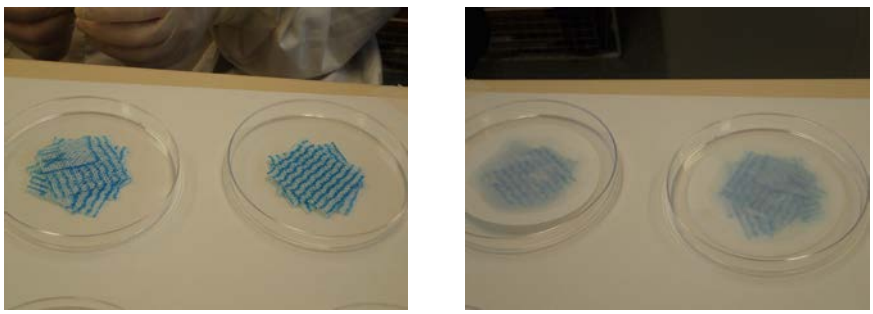


Figure 2-4 Preparation of Petri dishes with Chux squares and moistened filter paper

Culturing faecal samples to generate L3

1. Added a granulated charcoal volume similar to the faeces' (Figure 2-5 D).
2. Stirred this mixture with the stainless-steel cooking spoon until well homogenised (Figure 2-5 E and F).
3. Added fine charcoal at approximately half the volume of the granulated charcoal (Figure 2-5 G).
4. Stirred for several minutes until the mixture was black and grainy throughout and had a glossy appearance. The texture of the mix should be a dry paste that just holds together without crumbling. I adjusted the texture by adding extra water if necessary (Figure 2-5H)
5. Plated a heaped spoon of faeces onto the filter paper in the Petri dishes and spread it carefully to leave a ring of filter paper clear of faeces (Figure 2-6).
6. Closed the lid and labelled the dishes with date, donor initials, and batch number.

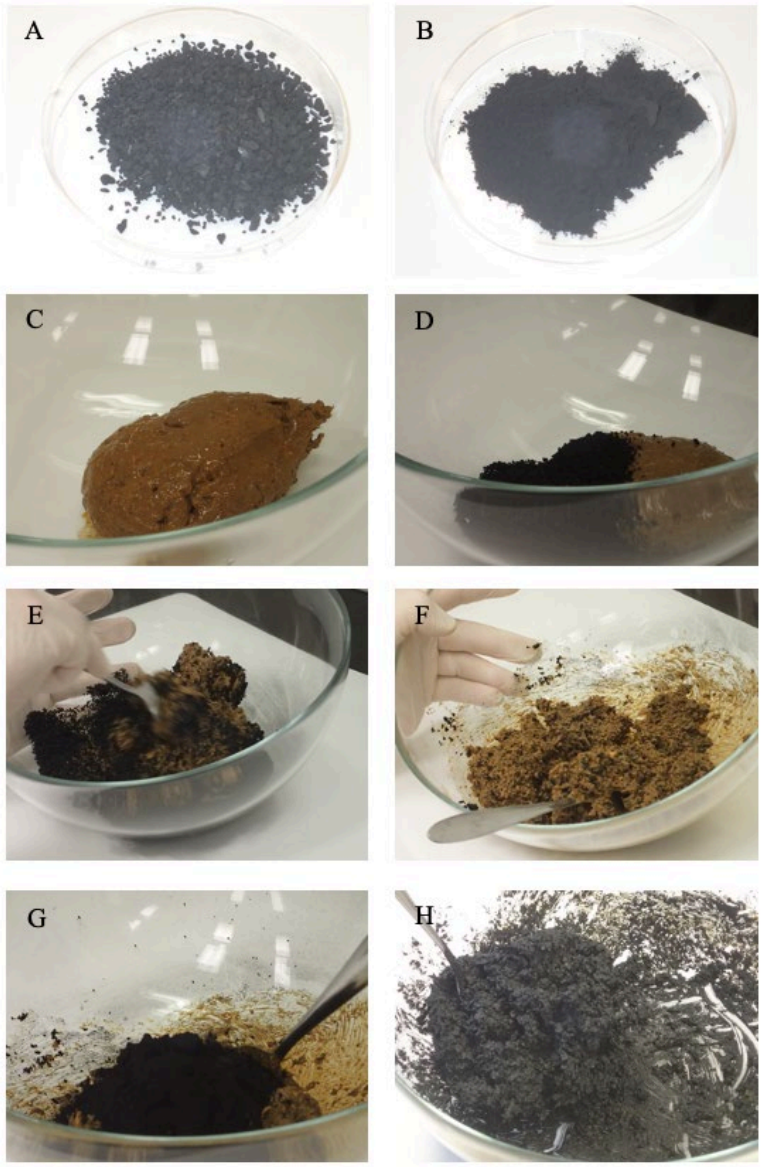


Figure 2-5 Faecal culture procedure
Granulated (A) and fine (B) charcoal; faecal sample transferred to a glass bowl (C); granulated charcoal added to sample (D); mixing of granulated charcoal and sample and homogenised sample (E and F); addition of fine charcoal (G); desired consistency and appearance (H). Photos Doris Pierce

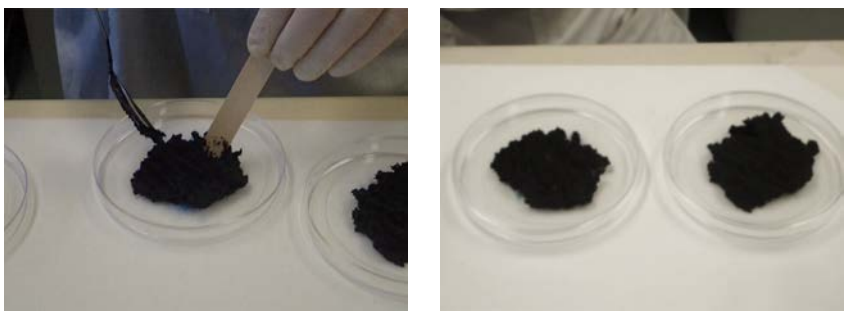


Figure 2-6 Plating the faecal mixture. Photos Doris Pierce

Humidity chamber

1. Inserted three layers of paper towels into large zip lock bags and added water until saturated (Figure 2-7). Poured off excess water into the sink.
2. Placed the Petri dishes (2 x 3) inside each bag and sealed the bags carefully (Figure 2-7).
3. Placed the bags into incubator at 25°C for at least 7 days, checking daily for the presence of fungal growth. Added 1mL of deionised water if the faecal mix showed signs of drying out.

Figure 2-7 Preparation of the humidity chamber and storage of plates. Photos Doris Pierce

Harvest and disinfection

1. By day 7-10, L3 had evaporated from the faecal mix into water droplets that could be harvested.
2. To harvest, added a little tap water to the droplets with a disposable pipette by carefully holding the plate at an angle.
3. Slowly turned the plate in a circular motion to allow the water to “collect” all the droplets containing the larvae without touching the faecal mix.
4. Aspirated all the water from the plastic rim with the pipette and transferred all collected water into a 50mL Falcon tube.
5. Once larvae from all plates were harvested, topped up the Falcon tube to 50mL with deionised water.
6. Centrifuged the Falcon tube containing the larvae for 5 min at 25°C at 2500 rpm with brakes off (brake = 0). Checked a pellet had formed and aspirated the fluid using a pipette boy to leave app. 1mL.
7. Added 100µL of Betadine 10% w/v antiseptic liquid to the fluid and left for 5 min at room temperature.
8. Topped up the tube with deionised water and then centrifuged at the same settings as above.
9. After centrifuging, aspirated fluid again, leaving app. 2mL, and topped up with deionised water again.
10. Performed a total of three washes in this manner.

11. After the third wash, aspirated the fluid to leave app. 2mL and topped up with deionised water to 25mL. Labelled (Na, coded ID of donor and batch number, date of sample production) and stored the Falcon tube at room temperature, protected from light until inocula preparation.

2.2.1.6 Quality control

Cultured plates were monitored daily during the culture process to check for fungal growth. Plates with fungal growth were excluded from harvest. Virus and bacterial removal were performed during harvest by iodine disinfectant. The finished product was a thoroughly washed and centrifuged sample of the disinfected product, retaining its disinfection level.

Following disinfection and washing, the harvested larvae were inspected using a dissection microscope to examine their integrity. L3 should be curved and motile. If larvae were found to be straight or non-motile, the sample was warmed to 37°C in an incubator to encourage motility and then re-inspected. If the larvae remained non-motile following this process, they were not used for infection but retained in the laboratory for potential future in vitro or gene sequencing projects.

2.2.2 L3 and placebo preparation for injection

I prepared one Eppendorf tube with Placebo, 10 x L3 or 20 x L3 for each participant. The tubes remained unlabelled and were given to the research assistant in charge of randomisation to assign the correct tube for each participant.

2.2.2.1 L3 inocula

1. Poured the 25mL L3 solution into a six-well plate for collection.
2. Using a dissection microscope and a 200μL pipette tip, harvested visibly motile worms individually and transferred them into 1.5mL Eppendorf tubes (10 or 20), specifying numbers in each tube with a small piece of tape.
3. All tubes were levelled to 300μL by either aspirating fluid or adding water and stored at room temperature and protected from light.

2.2.2.2 Placebo

Added 300µL of water to 1.5mL Eppendorf tube, followed by approximately 2µL of Tabasco sauce. Fluid should not be discoloured after adding the Tabasco sauce to maintain the same appearance as the tubes containing the larvae.

2.2.3 Faecal egg count

I used the McMaster egg counting technique (University of Saskatchewan, 2021) to estimate the sample's parasite infection intensity. This quantitative faecal flotation technique uses a special counting chamber that allows the examination of a known volume of faecal suspension. Given the known mass of faeces and volume of flotation solution, the number of eggs per gram of faeces can be approximated.

1. Weighed an empty 50mL Falcon tube and recorded the mass (13.0g).
2. Using two wooden applicator sticks, added a small amount (~ 2g) of raw faeces from the donor sample (see Step 6 during Preparation) and weighed the tube again, recording the net mass of the sample.
3. Added saturated NaCl made in-house to make a total volume of 30mL.
4. Mixed well by shaking the tubes until faeces were dissolved and the mixture was homogeneous.
5. Filtered the mixture through a strainer and collected the filtrate in a new Falcon tube.
6. Added 0.3mL of the diluted faeces to one-third of a Whitlock egg-counting chamber (Figure 2-8) and counted all visible eggs (see Figure 2-9) under the microscope.
7. Example calculation to determine eggs per gram for seven eggs counted in a dilution of 2g of faeces:

7 eggs/0.3mL= 700 eggs/30mL

2g of faeces in 30mL. therefore:

700 eggs/2g = 350 eggs/g

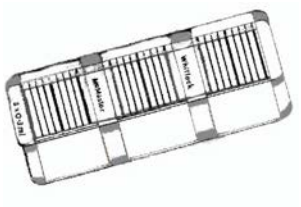


Figure 2-8 Whitlock McMaster 3 chamber slide

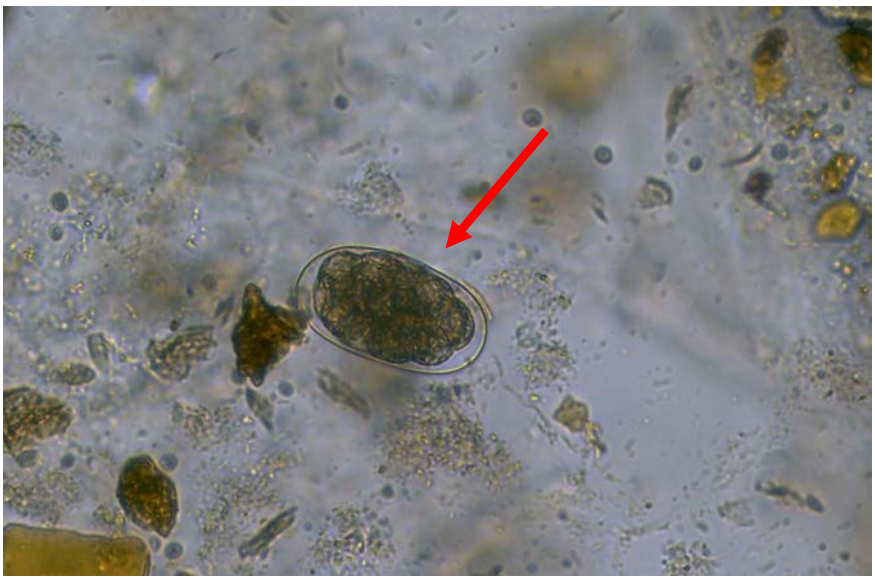


Figure 2-9 Hookworm egg in faecal suspension (red arrow). Photo courtesy of Luke Becker

2.2.4 Inoculation

Following randomisation, participants were infected with either 2 x 10 L3, 2 x 20 L3, or 2 x Placebo during Visits 3 and 4 at wk0 and wk8. Before inoculation, the trial team detailed the potential side effects of hookworm infection and addressed any remaining participant questions or concerns. Wearing gloves, I transferred the prepared inoculum to a dressing pad using a pipette and rinsed the empty tube with 300μL of deionised water to ensure all larvae were collected. The rinsing water was added to the dressing pad, and the dressing pad was then applied to the inner surface of the participant's forearm with the instruction to leave it in place for at least one hour. All materials and equipment were disinfected with 80% ethanol to prevent cross-infection and disposed of for autoclaving.

2.3 Case Report Form

During each visit, the trial doctor recorded the participants' anthropometric (waist circumference, height, body mass) and physiological measurements (blood pressure, heart rate, temperature, respiratory rate) as detailed below. The doctor reviewed the participants' recent history and recorded any health or other life events and medication changes since the last visit. Additionally, the participants completed the self-administered diet, mood, and physical activity questionnaires. After each visit, I entered all measurements, questionnaire responses, and comments into the trial database.

2.3.1 Anthropometric measurements

2.3.1.1 Waist circumference

We used a flexible measuring tape to measure WC. Participants were asked to remove any bulky clothing and stand with both feet flat on the ground. The tape measure was placed halfway between the bottom of the lowest rib and the top of the hipbones, roughly in line with the belly button, and the circumference was recorded in centimetres.

2.3.1.2 Height

We used a measuring tape fixed to the trial room's wall (Cairns) or a stadiometer (Townsville) to measure height. The trial team member asked the participant to remove their shoes and stand straight with both feet on the floor and their back against the scale to read their height in centimetres (one decimal place) level with the top of the scalp.

2.3.1.3 Body mass

Digital scales with a maximum capacity of 250kg were used to determine body mass in kilograms (one decimal place). The participants stepped on the scales with their shoes removed and stood flat on the scale with both feet. Using height and body mass measurements, I calculated body mass index (BMI) as mass/height in metres squared for each participant at each visit.

2.3.2 Physiological measurements

2.3.2.1 Blood pressure and heart rate

Automated blood pressure devices were used to monitor the participants' blood pressure and heart rate. However, these automated devices could not take an accurate reading for some participants due to inadequate cuff length or participant discomfort. In these cases, the study doctor used a manual

blood pressure device to obtain a reading and the radial pulse to determine heart rate. Where possible, three readings for blood pressure were taken, and their average was calculated and recorded.

2.3.2.2 Body temperature and respiratory rate

The participants' body temperature was measured in Celsius on their forehead via a contactless infrared thermometer. Respiratory rate, the number of breaths per minute at rest, was measured by counting the number of chest rises for one minute with the participant seated in a chair.

2.3.3 Questionnaires

2.3.3.1 Diet – The PREDIMED

To monitor notable changes in our participants' diet, we adopted the14-item self-administered Prevención con Dieta Mediterránea (PREDIMED) diet questionnaire. This questionnaire assesses adherence to a Mediterranean diet, with greater adherence reported to reduce rates of CVD, cancer, and neurodegenerative disorders (Estruch & Ros, 2020). High adherence to a Mediterranean diet may also improve glycaemic control and lower the risk of future T2DM independent of weight loss, which renders the questionnaire relevant to the present study (Esposito et al., 2015). Curiously, a large and very influential 2012 Spanish study (Martínez-González et al., 2012), which reported substantial beneficial effects of increased adherence to a Mediterranean diet on cardiovascular death, stroke or myocardial infarction, was recently retracted and republished with amended effect sizes due to concerns about serious protocol deviations (Agarwal & Ioannidis, 2019). Nevertheless, support for the association between adherence to a Mediterranean diet and a reduction in cardiometabolic risk factors and inflammation remains strong (Estruch & Ros, 2020). Table 2-1 details the questions and scoring method for the PREDIMED questionnaire (Martínez-González et al., 2012). Most questions allow considerable heterogeneity of answer formats, prohibiting an automated scoring procedure. Therefore, I manually scored all questions for each participant and visit and calculated a total score during data analysis.

Table 2-1 Scoring of the PREDIMED diet questionnaire

Questions	Criteria for 1 point
1. Do you use olive oil as main culinary fat?	Yes
2. How much olive oil do you consume in a given day (including oil used for frying, salads, out-of-house meals, etc.)?	≥4 tbsp
3. How many vegetable servings do you consume per day? (1 serving: 200 g [consider side dishes as half a serving])	≥2 (≥1 portion raw or as a salad)
4. How many fruit units (including natural fruit juices) do you consume per day?	≥3
5. How many servings of red meat, hamburger, or meat products (ham, sausage, etc.) do you consume per day? (1 serving: 100–150 g)	<1
6. How many servings of butter, margarine, or cream do you consume per day? (1 serving: 12 g)	<1
7. How many sweet or carbonated beverages do you drink per day?	<1
8. How much wine do you drink per week?	≥7 glasses
9. How many servings of legumes do you consume per week? (1 serving: 150 g)	≥3
10. How many servings of fish or shellfish do you consume per week? (1 serving 100–150 g of fish or 4–5 units or 200 g of shellfish)	≥3
11. How many times per week do you consume commercial sweets or pastries (not homemade), such as cakes, cookies, biscuits, or custard?	<3
12. How many servings of nuts (including peanuts) do you consume per week? (1 serving 30 g)	≥3
13. Do you preferentially consume chicken, turkey, or rabbit meat instead of veal, pork, hamburger, or sausage?	Yes
14. How many times per week do you consume vegetables, pasta, rice, or other dishes seasoned with sofrito (sauce made with tomato and onion, leek, or garlic and simmered with olive oil)?	≥2

2.3.4 Depressive state – The PHQ-9

As an additional safety measure, we observed and tracked potential changes in our participants' mood and depressive state using the validated Patient Health Questionnaire (PHQ)-9. This 9-item self-administered depression module of the more comprehensive 3-page PHQ includes nine depressive symptom criteria and is widely used in primary healthcare settings worldwide to diagnose depression and monitor treatment. Two recent studies (El-Den et al., 2018; Levis et al., 2019) have identified the PHQ-9 as the most reliable screening tool for depression. Scores can range from 0 to 27, as each of the nine items can attract scores from 0 (not at all) to 3 (nearly every day). An additional functional health assessment at the bottom of the test asks the responder to appraise the impact of any of the rated criteria on their daily life (Figure 2-10) (Kroenke et al., 2001). While a cut-off score of ten may provide optimal test sensitivity and specificity, with scores of ten and higher indicative of moderate severity of depression symptoms, factors such as age or educational level may modify the optimal cut-off score (Costantini et al., 2021). Figure 2-10 details the questions included in the PHQ-9 and their scoring. The candidates circled the relevant response to each question and rated the overall impact of the problems on their lives. To score the tests, I added the column results and recorded the sum of the column totals as the final score.

Over the last 2 weeks, how often have you been bothered by any of the following problems?	Not at all	Several days	More than half the days	Nearly every day
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself – or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed? Or the opposite – being so fidgety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead or of hurting yourself in some way	0	1	2	3

For office coding: Total Score _____ = _____ + _____ + _____

Total Score _____

If you checked off any problems, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

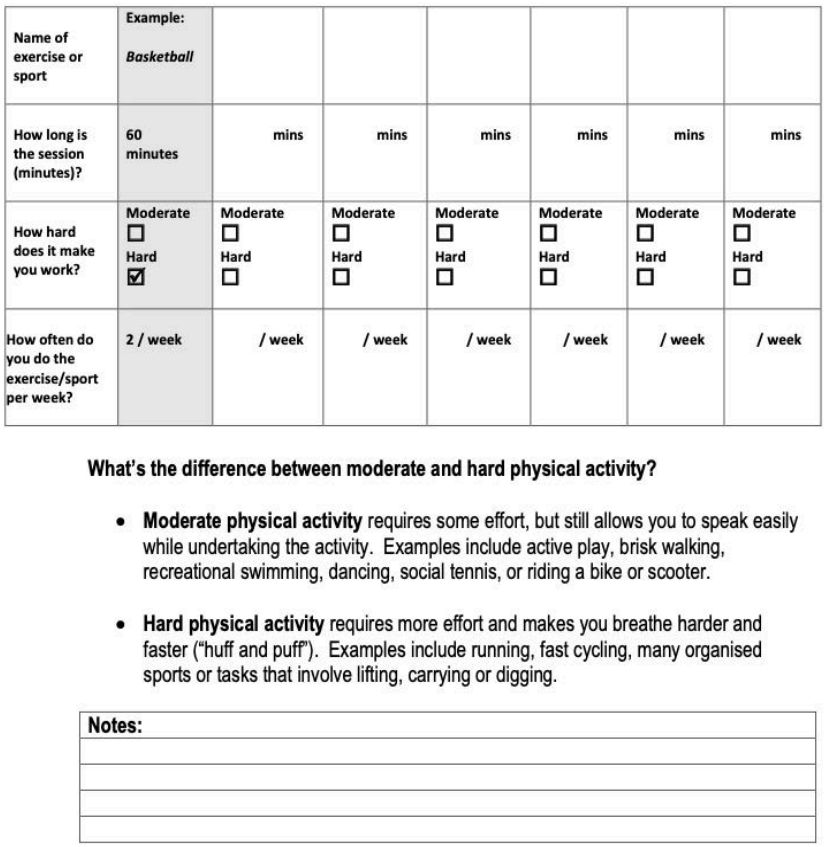
☐ Not difficult at all ☐ Somewhat difficult ☐ Very difficult ☐ Extremely difficult

Figure 2-10 The nine-item Patient Health Questionnaire (PHQ)-9
The maximum score was 27, with a lower score indicating improved mental well-being.

2.3.4.1 Physical activity

The physical activity questionnaire

Finally, the participants reported their weekly physical activity. For any exercise or physical activity they regularly undertake, participants listed the type of activity, the typical duration in minutes, the intensity (moderate or hard), and the frequency per week. Participants could list up to six different activities. We provided an explanation and examples to assist with the differentiation between moderate and hard exercise (Figure 2-11). Participants could also add any comments regarding their activity, such as reasons for not exercising or further details about their activities



Name of exercise or sport	Example: *Basketball*						
How long is the session (minutes)?	60 minutes	mins	mins	mins	mins	mins	mins
How hard does it make you work?	Moderate ☐ Hard ☑	Moderate ☐ Hard ☐	Moderate ☐ Hard ☐	Moderate ☐ Hard ☐	Moderate ☐ Hard ☐	Moderate ☐ Hard ☐	Moderate ☐ Hard ☐
How often do you do the exercise/sport per week?	2 / week	/ week	/ week	/ week	/ week	/ week	/ week

What's the difference between moderate and hard physical activity?

- **Moderate physical activity** requires some effort, but still allows you to speak easily while undertaking the activity. Examples include active play, brisk walking, recreational swimming, dancing, social tennis, or riding a bike or scooter.
- **Hard physical activity** requires more effort and makes you breathe harder and faster ("huff and puff"). Examples include running, fast cycling, many organised sports or tasks that involve lifting, carrying or digging.

Notes:

Figure 2-11 Physical activity questionnaire

Soring the physical activity questionnaire

To combine type, duration, frequency, and intensity into a single metric, I used the 2011 Adult Compendium of Physical Activities (Ainsworth et al., 2011) and calculated the metabolic equivalent of task (MET) for each activity. One MET is approximately equivalent to an oxygen uptake of 3.5mL/kg/min, or 1 kcal/kg/hour, which approximates the energy cost of sitting still (or resting metabolic rate). The term MET indicates the mass-specific energy cost of individual activities as a ratio of the work metabolic rate to resting metabolic rate, that is, exercise oxygen volume in $mL/kg^{-1}min^{-1}$ divided by $3.5mL/kg^{-1}min^{-1}$. Initially developed in the 1980s, the Compendium aimed to be a convenient tool to determine the energy cost of activities reported in surveillance and epidemiological studies by standardising the MET intensities of various physical activities.

Notably, the Compendium's intention is not to determine the exact cost of physical activities in individuals, which would require consideration of factors such as age, sex, body mass and environmental conditions. Instead, the compendium provides a standardised classification system to estimate the MET intensities of the reported activities (Ainsworth et al., 2011)

I generalised the MET values for some activities due to limited details regarding the exact intensity of the listed exercise/physical activity. For example, for walking, the most commonly reported activity in our study, the Compendium distinguishes between more than 50 different intensities depending on speed, incline, load carried, et cetera. Our participant responses rarely allowed such detailed classification, as most only indicated walking at moderate intensity. As a generalisation, I categorised walking at moderate intensity as "walking, 2.8 to 3.2mph, level, moderate pace, firm surface" (4.5 to 5.1 km/h), which the Compendium equates to 3.5 METS and corresponds to the reported average walking speeds in our participant age group (Schimpl et al., 2011). Where an activity seemed to fit two types listed in the Compendium, I selected a value midway between the two associated MET values.

After determining the relevant MET for each reported activity (listed in Table 2-2), I used the following calculation to determine a summary metric for each activity:

MET x duration x frequency = METs/week

In the case of multiple activities per participant, the sum of METs for all activities determined the final score.

Table 2-2 MET scores for physical activity reported on the Case Report Form

Activity	METs	Compendium code
Aquafit	5.5	18355
Archery	4.3	15010
Basketball	6.5	15055
Bike riding moderate	4	01010
Body attack/HIIT	7.8	02062
Bootcamp	8	02040
Cleaning hard	4.3	05027
Cleaning moderate	3.5	05021
Cycling hard	10	01040
Dancing	4	03030 & 03040
Fencing	6	15200
Gardening hard	6	08262
Gardening moderate	4.3	08140
Gym general	5	02061
Hiking hard	7	17230
Hiking moderate	5.3	17082
Jogging/running moderate	7	12020
Kayak	9	18050 & 18060
Ninjitsu	4	no code
Rowing moderate	4.8	02071
Shooting	2.5	4145
Skateboarding	5	15580
Sprints	12.3	12100
Stretching/Pilates/biomechanics	3	02105
Swimming	5.5	18265 & 18240
Walking	3.5	17160
Walking the dog	3	17165
Walking uphill/castle hill	6	17210 &17211
Weights hard	6	02050
Weights moderate	4	02052 & 02054
Yoga	3.5	02180 & 02160

MET, metabolic equivalent of task (Ainsworth et al., 2011). If two codes were deemed applicable, a value approximately half-way between these was selected.

2.4 Blood and urine collection and processing

The study doctor collected blood from the trial participants during each visit except the initial screening visit. The baseline and all evaluation visits required the collection of one each of the green- (Lithium heparin) and red-capped BD vacutainer tubes (Silica Clot Activator) for peripheral blood mononuclear cells (PBMCs) isolation and serum separation, respectively, at the JCU Nguma-bada (Smithfield) campus.

At baseline and evaluation visits, during which participants needed to attend fasting, the doctor also collected one each of the gold- (SST) and purple-capped BD vacutainer tubes (EDTA). Participants provided a urine sample in a yellow-capped specimen container during these visits. A trial team member delivered the blood and urine samples to the nearest Sullivan Nicolaides Pathology (SNP) laboratory immediately after the visit for pathology testing by haematology analyser. If participants were not fasting during their baseline or evaluation visits, we only collected blood in the green- and red-capped tubes and the urine sample. The participant received a pathology request form to attend their nearest SNP collection centre in a fasting state at their convenience.

2.4.1 In-vitro isolation of PBMCs using SepMate ™-50

After collection in BD Vacutainer® Plastic Serum tube with green BD Hemogard™ closure, I inverted the tube gently 5-6 times and stored the samples at room temperature (lab) protected from light until processing.

2.4.1.1 Preparation

Ensured sample, DPBS(1x) + 2% Fetal Bovine Serum (FBS), density gradient medium (Lymphoprep™), FBS and FBS +20% dimethyl sulfoxide (DMSO) were brought to room temperature. Decanted PBS + 2% FBS in biosafety cabinet into small flask/bottle before bringing to room temperature.

2.4.1.2 Procedure

Work completed in biosafety cabinet:

1. Poured blood into a 15mL Falcon tube (if several vials of blood, combine all in one Falcon tube).
2. To collect as many cells as possible, I used an equal volume of DPBS + 2% FBS to “rinse” the blood collection tube, which was then added to the blood for 1:1 dilution. Blood and DPBS + 2% FBS should make at least 8mL for the Sepmate™-50 protocol. For blood volumes < 4m, I used the Sepmate™-15 protocol. (https://www.google.com/search?client=firefox-b-d&q=sepmate+protocols)

3. Added 15mL of density gradient medium (Lymphoprep™) to SepMate™ tube by carefully pipetting it through the central hole in the insert; it reached above the insert once all was added.
4. Keeping the SepMate™ tube vertical, I added the diluted blood sample from Step 3 by slowly and carefully pipetting it down the side of the SepMate™ tube (used a 10mL stripette).
5. Centrifuged the tubes for 10 min. at 1200 x g at room temperature with brakes on (if the sample was older than 24 hours, increased time to 20 min.).
6. Poured off top layer (= enriched PBMCs) into new 50mL Falcon tube. SepMate™ tube should only be inverted up to a max. of 2 seconds.
7. Washed enriched PBMCs with DPBS + 2% FBS twice (topping up to 40mL each time) and centrifuged at 300 x g with brakes on for 8 min. at room temperature after adding DPBS. Aspirated supernatant after each wash. (Resuspended cells gently by pipetting up and down several times).
8. **For Cairns samples:** After the second wash, I resuspended the cell pellet to a volume of 2000µL, adding app. 1900µL of FBS, as some liquid was still around the pellet.
9. Added 2000µL of 20% DMSO in FBS to FBS containing the cells DROP BY DROP while gently swirling the tube to mix DMSO and cells.
10. Transferred suspended cells to labelled (WAM MNCS, ID, visit #, date) 4 x cryovials, transferred to Mr Frosty ASAP, and placed in -80°C freezer. Ensured all slots in the Mr Frosty were filled by water-containing tubes.
11. **For Townsville samples:** After the second wash, resuspended the cell pellet to a volume of 1800µL, adding app. 1700µL of FBS, as some liquid was still around the pellet.
12. Added 1800µL of 20% DMSO in FBS to FBS containing the cells DROP BY DROP while gently swirling the tube to mix DMSO and cells.
13. Transferred suspended cells to 6 x FluidX tubes and recorded each participant's rack ID and tube numbers.
14. Transferred to Mr Frosty ASAP and placed in the -80°C freezer. Ensured all spare slots in Mr Frosty were filled by water-containing tubes.
15. Disposed of all blood-contaminated products in the clinical waste bin.
16. After 24h, transferred tubes to a storage location in the -80°C freezer.

2.4.2 Serum preparation

After collecting blood in BD Vacutainer® Plastic Serum tube with red BD Hemogard™ closure, I inverted the tube gently 5-6 times and stored it upright for 45 min (30-60 min recommended) at room temperature and protected from light.

1. 45 min after collection, centrifuged the tube at 1300 x g at room temperature (lab) for 10 min and stored in the cold room overnight if serum couldn't be processed immediately.
2. If stored overnight**,** centrifuged tube again at 1500rpm for 3 min at room temperature before aliquoting.
3. **For Cairns:** In the biosafety cabinet, aliquoted a minimum of 100µL into screw cap tubes with black rubber O-ring), labelled with WAM serum, ID, visit#, and date.
4. **For Townsville**: In the biosafety cabinet, aliquoted a minimum of 100µL into FluidX tubes and recorded each participant's rack ID and tube numbers.
5. Stored in the -80C freezer.
6. All blood-contaminated products were disposed of in the clinical waste bin.

2.4.3 Pathology testing

Blood in gold- (SST) and purple-capped BD vacutainer tubes (EDTA) underwent pathology testing by haematology analyser at Sullivan Nicolaides Pathology. Immediately after collection, I inverted the tubes gently five times and stored them protected from light before delivery with the relevant laboratory request form to the closest pathology centre. The requested blood tests included fasting blood glucose and insulin, blood lipids (cholesterol, triglycerides, LDL, HDL), liver function test, full blood count, UEC, and HbA1c. Urine pathology included albumin, creatinine, and the albumin-creatinine ratio.

2.4.4 Poly-unsaturated fatty acids (PUFA) cards

At baseline and each evaluation visit, we collected blood via a PUFA coat Dried Blood Spot Collection Card for Fatty Acid Analysis. Immediately following the blood draw, the study doctor applied a couple of drops of blood remaining in the collection kit to the windows of absorbent paper on the card (Figure 2-12), taking care not to touch the windows with the skin or latex gloves. The cards were left to dry for at least one hour before being resealed in their foil zip-lock bags with the completed participant's details. I stored all cards in the -80°C freezer until trial completion, and they will be sent to the University of Adelaide for analysis.

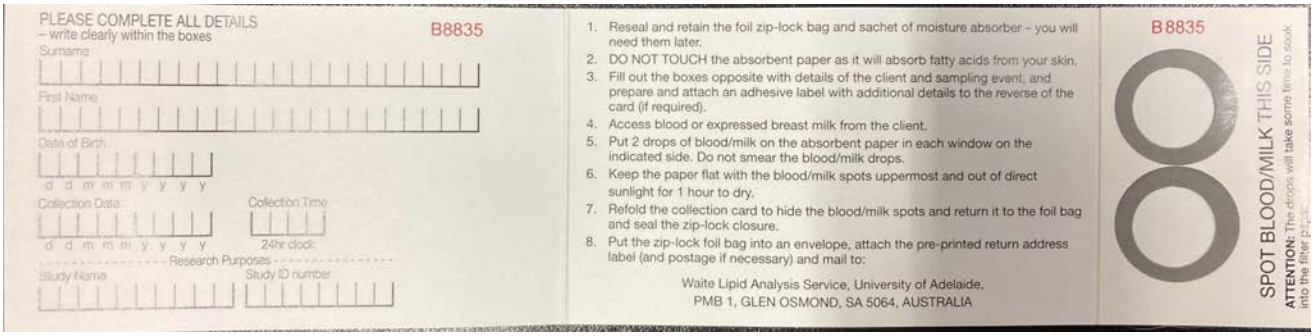


Figure 2-12 Blood spot collection card for fatty acid analysis

2.5 Faecal sample collection and processing

2.5.1 Collection and storage

Participants were asked to provide a faecal sample for microbiome analysis at baseline and each evaluation visit. I supplied each participant with a collection kit, which included step-by-step instructions on the correct process to collect and store the sample. Specifically, the aim was to generate optimal atmospheric conditions for anaerobic microorganisms as present in the gut following collection. For this purpose, we instructed participants to store their loosely closed sample container in an airtight zip-lock bag with an AnaeroGen™ Compact Sachet at room temperature for at least one hour before securing the lid tightly. We encouraged participants to contact us on collection day to organise delivery or pick-up by a trial team member. If hand-over was delayed by more than a day, participants froze the sample until a delivery date was arranged. I instructed participants to transport a frozen sample in a chiller bag with freezer bricks to ensure the sample remained frozen. Once a trial team member received, I stored the sample in the -80°C freezer.

2.5.2 DNA extraction for microbiome analysis

I extracted DNA for microbiome analysis using a commercially available kit recommended by the sequencing Ramaciotti Centre. The complete extraction protocol is available on the supplier's website (https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/qiaamp-fast-dna-stool-mini-kit/). Following extraction, I used nano photometry for quality control of the samples and diluted the samples as required for sequencing.

2.5.2.1 Preparation

1. Removed all samples of interest from the -80°C freezer at least 2 hours before DNA extraction.
2. Set a heat block to 80°C for use in Steps 3 and 8.

3. Where necessary, redissolved any precipitates in Buffer AL and InhibitEX® Buffer by heating and mixing.
4. Added ethanol to Buffer AW1 and Buffer AW2 concentrates.
5. Mixed all buffers before use.
6. Homogenised thawed faecal sample using wooden applicator sticks until smooth and well mixed.

Note: The supplier recommended centrifugation at room temperature (15–25°C) and 20 000 x g; however, the maximum setting on our centrifuge was 17 400 x g. To compensate for the lower g forces, I increased the time for each centrifuge step by 25%.

2.5.2.2 Procedure

1. Weighed 180–220mg stool for each sample into a 2mL microcentrifuge tube and placed tubes on ice.
2. Added 1mL InhibitEX Buffer to each stool sample. Vortexed continuously for 1 min or until the stool sample was thoroughly homogenised.
3. Heated the suspension for 5 min at 80°C.
4. Vortexed for 15 s and centrifuged sample for 1:15 min to pellet stool particles.
5. Pipetted 15µL of Proteinase K into a new 1.5mL microcentrifuge tube.
6. Pipetted 200µL of supernatant from Step 9 into a 1.5mL microcentrifuge tube containing Proteinase K.
7. Added 200µL Buffer AL and vortexed for a minimum of 15s. (Note: Sample and buffer needed to be thoroughly mixed to form a homogenous solution.)
8. Incubated at 70°C for 10 min.
9. Added 200µL ethanol (100%) to the lysate and mixed by vortexing.
10. Carefully applied 600µL of lysate from Step 9to the QIAamp spin column. Closed the cap and centrifuged for 1:15 min. Placed the QIAamp spin column in a new 2mL collection tube and discarded the tube containing the filtrate.
11. Carefully opened the QIAamp spin column and added 500µL of Buffer AW1. Centrifuged for 1:15 min. Placed the QIAamp spin column in a new 2mL collection tube and discarded the tube containing the filtrate.
12. Carefully opened the QIAamp spin column and added 500µL of Buffer AW2. Centrifuged for 3:45 min.
13. Placed the QIAamp spin column in a new 2mL collection tube and discarded the collection tube containing the filtrate. Centrifuged for 3:45 min.
14. Transferred the QIAamp spin column into a new, labelled (ID, visit number) 1.5mL microcentrifuge tube and pipetted 200µL of Buffer ATE directly onto the QIAamp membrane. Incubated for 1 min at room temperature, then centrifuged for 1:15 min to elute DNA.

2.5.2.3 Quality control

Once extracted, I tested each sample's DNA concentration and nucleic acid ratios using the Implen NanoPhotometer® N60/N50.

2.5.3 Quantitative PCR to confirm successful infection

Frozen aliquots of faecal samples were sent to the University of Melbourne for qPCR testing to confirm infection success and exclude potential pre-existing hookworm infection. I defrosted each participant's baseline and wk26 samples and homogenised them. Using wooden applicator sticks, I transferred approximately 1g of each into 2mL Eppendorf tubes, labelled with participant ID and visit. The aliquots remained in the -80°C freezer until ready for shipment. Extractions and quantitative PCR (qPCR) testing were performed at the University of Melbourne as previously prescribed (Hii et al., 2018).

2.6 Adipokine and cytokine analyses

2.6.1 Adipokines

Leptin

The entire protocol ab222508 Human Adiponectin SimpleStep ELISA® Kit is available at https://www.abcam.com/ps/products/179/ab179884/documents/Human-Leptin-elisa-kit-protocol-book-v1a-ab179884%20(website).pdf.

Protocol Summary

1. Prepared all reagents, samples, and standards as instructed
2. Added standard or sample to appropriate wells
3. Added Antibody Cocktail to all wells
4. Incubated at room temperature for 1 hour
5. Wash each well three times with 350µL 1X Wash Buffer PT
6. Added 100µL TMB Development Solution to each well and incubated for 10 minutes.
7. Added 100µL Stop Solution and read OD at 450 nm

Reagent Preparation

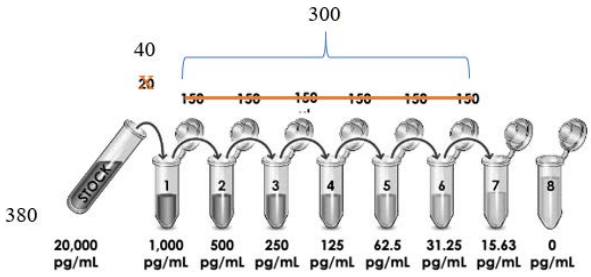
Equilibrated all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjusted volumes as needed for the number of strips in the experiment.

1. **1X Wash Buffer PT:** Prepared 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionised water. To make 50mL 1X Wash Buffer PT, combine 5mL Wash Buffer PT 10X with 45 mL deionised water. Mix thoroughly and gently. Made 200mL by combining 20mL Wash Buffer PT 10X with 180mL deionised water.
2. **Antibody Cocktail:** Prepared Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 3mL of the Antibody Cocktail, combine 300µL 10X Capture Antibody and 300µL 10X Detector Antibody with 2.4mL. Made 8mL of the Antibody Cocktail by combining 800µL 10X Capture Antibody and 800µL 10X Detector Antibody with 6.4mL Antibody Diluent 4BI. Mixed thoroughly and gently.

Standard and Sample Preparation

Reconstituted the Leptin standard by adding 500µL Sample Diluent NS. Held at room temperature for 10 minutes and mixed thoroughly and gently. This was the 20,000pg/mL Stock Standard Solution.

1. Labelled eight tubes Standards1–8.
2. Added 380µL Sample Diluent NS into tube number 1 and 150µL of Sample Diluent NS into numbers 2-8.
3. Used the Stock Standard to prepare the following dilution series. Standard #8 contained no protein and was the Blank control:



Diluted samples 1:40 into Sample Diluent

Assay procedure

Equilibrated all materials and prepared reagents to room temperature prior to use.

1. Prepared all reagents, working standards, and samples as described above.
2. Removed excess microplate strips from the plate frame, returned them to the foil pouch containing the desiccant pack, resealed and returned to 4ºC storage.
3. Added 50µL of all standards to appropriate wells.
4. Added 100µL of all samples to appropriate wells. (Pipetting error: should have been 50µL)
5. Added 100µL of the Antibody Cocktail to each well. (Pipetting error: should have been 50µL)
6. Sealed the plate and incubated for 1 hour at room temperature on a rocking platform (no appropriate plate shaker available).
7. Washed each well with 3 x 350µL 1X Wash Buffer PT. Washed by decanting from wells then dispensing 350µL 1X Wash Buffer PT into each well. Completely removed all liquid at each step. After the last wash, inverted the plate and blotted it against clean paper towels to remove excess liquid.

8. Added 100µL of TMB Development Solution to each well and incubated for app, 10 minutes. (Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes).
9. Added 100µL of Stop Solution to each well. Recorded the OD at 450 nm using the FLUOstar Omega microplate reader.
10. Used the MARS data analysis software to plot a standard curve and determined the target protein concentration by multiplying individual results by the dilution factor (Dilution was 1:40).
    Note: Multiply standards by 0.67 for entry in the software due to pipetting error in Step 5, which meant standard – antibody ratio was not 1:1 as intended but 1:2

Adiponectin

The entire protocol ab222508 Human Adiponectin SimpleStep ELISA® Kit is available at https://www.abcam.com/ps/products/222/ab222508/documents/Human-Adiponectin-ELISA-kit-protocol-book-v2-ab222508%20(website).pdf.

Protocol Summary

1. Prepared all reagents, samples, and standards as instructed
2. Added standard or sample to appropriate wells
3. Added Antibody Cocktail to all wells
4. Incubated at room temperature for 1 hour
5. Wash each well three times with 350µL 1X Wash Buffer PT
6. Added 100µL TMB Development Solution to each well and incubated for 10 minutes.
7. Added 100µL Stop Solution and read OD at 450 nm

Reagent Preparation

Equilibrated all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjusted volumes as needed for the number of strips in the experiment.

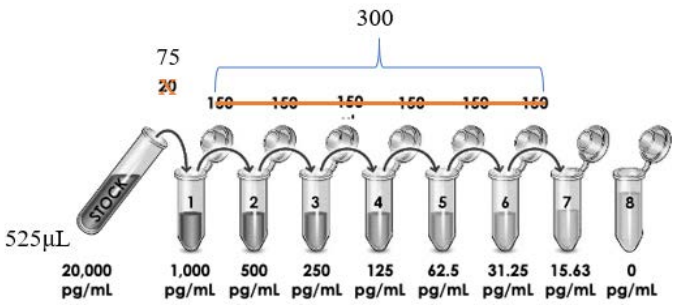
1. **Sample Diluent NS + Enhancer:** Prepared Sample Diluent NS + Enhancer by diluting 50X Cell Extraction Enhancer Solution to 1X with Sample Diluent NS. To make 10mL Sample Diluent NS + Enhancer, combined 9.8mL Sample Diluent NS and 200µL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently. Made 22mL Sample Diluent NS + Enhancer, combined 21.56mL Sample Diluent NS and 440µL Cell Extraction Enhancer.

2. **40X Denaturant:** Prepared 40X Denaturant by diluting Denaturant with Sample Diluent NS. To make 1mL 40X Denaturant. combine 111uL Denaturant with 889uL Sample Diluent NS. Mix thoroughly and gently. Made 5.2mL by combining 577.2uL Denaturant with 4622.8uL Sample Diluent NS
3. **1X Wash Buffer PT:** Prepared 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionised water. To make 50mL 1X Wash Buffer PT, combine 5mL Wash Buffer PT 10X with 45 mL deionised water. Mix thoroughly and gently. Made 200mL by combining 20mL Wash Buffer PT 10X with 180mL deionised water.
4. **Antibody Cocktail:** Prepared Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 3mL of the Antibody Cocktail, combine 300µL 10X Capture Antibody and 300µL 10X Detector Antibody with 2.4mL. Made 8mL of the Antibody Cocktail by combining 800µL 10X Capture Antibody and 800µL 10X Detector Antibody with 6.4mL Antibody Diluent 4BI. Mixed thoroughly and gently.

Standard and Sample Preparation

Reconstituted the Adiponectin standard by adding 500µL Sample Diluent NS + Enhancer. Held at room temperature for 10 minutes and mixed gently. This was the 100 ng/mL Stock Standard Solution.

1. Labelled eight tubes Standards1–8.
2. Added 525µL sample diluent NS with 1Xc cell extraction enhancer into tube number 1 and 300µL of sample diluent NS with 1X cell extraction enhancer into numbers 2-8.
3. Used the Stock Standard to prepare the following dilution series. Standard #8 contained no protein and was the Blank control:



Diluted samples 1:2000 as follows:

1. Added 10µL neat sample to 490µL Sample Diluent NS in 1.5mL Eppendorf tubes to generate a 2% sample solution.

2. Combined 50µL 2% sample with 50µL 40X Denaturant in 96-well round-bottom plates to generate a 1% sample with 20X Denaturant.
3. Heated samples on a heat block at 75°C for 10 minutes.
4. Cooled samples to room temperature (15 minutes).
5. Mixed 12.5µL treated sample with 237.5µL Sample Diluent NS to generate a 0.05% sample with 1X Denaturant.

Assay procedure

Equilibrated all materials and prepared reagents to room temperature prior to use.

1. Prepared all reagents, working standards, and samples as described above.
2. Removed excess microplate strips from the plate frame, returned them to the foil pouch containing the desiccant pack, resealed and returned to 4ºC storage.
3. Added 50µL of all standards and samples to appropriate wells.
4. Added 50µL of the Antibody Cocktail to each well.
5. Sealed the plate and incubated for 1 hour at room temperature on a rocking platform (no appropriate plate shaker available).
6. Washed each well with 3 x 350µL 1X Wash Buffer PT. Washed by decanting from wells, then dispensing 350µL 1X Wash Buffer PT into each well and completely removing all liquid at each step. After the last wash, inverted the plate and blotted it against clean paper towels to remove excess liquid.
7. Added 100µL of TMB Development Solution to each well and incubated for app. 10 minutes. (Given the variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes).
8. Added 100µL of Stop Solution to each well. Recorded the OD at 450 nm using the FLUOstar Omega microplate reader.
9. Used the MARS data analysis software to plot a standard curve and determined the target protein concentration by multiplying individual results by the dilution factor (Dilution was 1:40).

Omentin

The entire protocol ab269545 Human Omentin SimpleStep ELISA® Kit (ITLN1) is available at https://www.abcam.com/ps/products/269/ab269545/documents/Human-Omentin-ELISA-Kit-protocol-book-v2-ab269545%20(website).pdf.

Protocol Summary

1. Prepared all reagents, samples, and standards as instructed
2. Added standard or sample to appropriate wells
3. Added Antibody Cocktail to all wells
4. Incubated at room temperature for 1 hour
5. Washed each well three times with 350µL 1X Wash Buffer PT
6. Added 100µL TMB Development Solution to each well and incubated for 10 minutes
7. Added 100µL Stop Solution and read OD at 450nm

Reagent Preparation

Equilibrated all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjusted volumes as needed for the number of strips in the experiment.

1. **Sample Diluent NS + Enhancer:** Prepared Sample Diluent NS + Enhancer by diluting 50X Cell Extraction Enhancer Solution to 1X with Sample Diluent NS. To make 10mL Sample Diluent NS + Enhancer, combine 9.8mL Sample Diluent NS and 200µL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently. Made 22mL Sample Diluent NS + Enhancer combining 21.56mL Sample Diluent NS and 440µL Cell Extraction Enhancer.

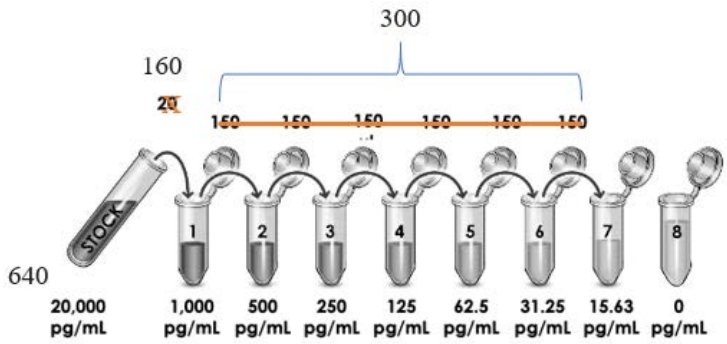
1. **1X Wash Buffer PT:** Prepared 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionised water. Made 200mL by combining 20mL Wash Buffer PT 10X with 180mL deionised water.
2. **Antibody Cocktail:** Prepared Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 3mL of the Antibody Cocktail, combine 300µL 10X Capture Antibody and 300µL 10X Detector Antibody with 2.4mL. Made 8mL of the Antibody Cocktail by combining 800µL 10X Capture Antibody and 800µL 10X Detector Antibody with 6.4mL Antibody Diluent 4BI. Mixed thoroughly and gently.

Standard and Sample Preparation

Reconstituted the Omentin standard sample by adding 500µL of Sample Diluent NS with 1X cell extraction enhancer. Mixed thoroughly and gently. Hold at room temperature for 10 minutes and mixed gently. This was the 100 ng/mL Stock Standard Solution.

1. Heated the reconstituted protein stock, along with any serum samples, by placing them on a heat block set to 65°C for 10 minutes. Cooled tubes to room temperature, mixed gently and spun down any liquid condensed on the walls of the tubes.

2. Labelled eight tubes Standards 1–8.
3. Added 640µL sample diluent NS with 1Xc cell extraction enhancer into tube number 1 and 300µL of sample diluent NS with 1X cell extraction enhancer into numbers 2-8.
4. Used the Stock Standard to prepare the following dilution series. Standard #8 contained no protein and was the Blank control:



Diluted samples 1:10 into Sample Diluent with 1Xc cell extraction enhancer

Assay procedure

Equilibrated all materials and prepared reagents to room temperature prior to use.

Prepared all reagents, working standards, and samples as described above.

1. Removed excess microplate strips from the plate frame, returned them to the foil pouch containing the desiccant pack, resealed and returned to 4ºC storage.
2. Added 50µL of all standards and samples to appropriate wells.
3. Added 50µL of the Antibody Cocktail to each well.
4. Sealed the plate and incubated for 1 hour at room temperature on a rocking platform (no appropriate plate shaker available).
5. Washed each well with 3 x 350µL 1X Wash Buffer PT. Washed by decanting from wells then dispensing 350µL 1X Wash Buffer PT into each well. Completely removed all liquid at each step. After the last wash, inverted the plate and blotted it against clean paper towels to remove excess liquid.
6. Added 100µL of TMB Development Solution to each well and incubated for app, 10 minutes. (Given the variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes).
7. Added 100µL of Stop Solution to each well. Recorded the OD at 450 nm using the FLUOstar Omega microplate reader.

8. Used the MARS data analysis software to plot a standard curve and determined the target protein concentration by multiplying individual results by the dilution factor (Dilution was 1:40).

2.6.2 Cytokines

Serum samples collected at baseline, wk26, and wk52 were batch-analysed using the Biolegend® LEGENDplex™ Human Th Cytokine Panel of 12 cytokines (IL-2, 4, 5, 6, 9, 10, 13, 17A, 17F, 22, IFN-γ and TNF) for circulating inflammatory biomarkers associated with Th1, Th2, Th9, Th17, and Th22 immune responses. The bead-based multiplex assay panel uses fluorescence-encoded beads suitable for use in flow-cytometry and allows simultaneous quantification of 12 human cytokines. The panel provides higher sensitivities and a broader dynamic range than ELISA methods. Impact of helminth infection on faecal microbiota. The complete protocol is available on https://www.biolegend.com/Files/Images/media_assets/pro_detail/datasheets/75475-Hu-Th-Cytokine-Panel-Replacement_R5.pdf.

For this cytokine analysis, I used samples from baseline, wk26, and wk52 to explore if immune responses relating to inflammatory processes had occurred with *Na* infection.

2.6.2.1 Preparation

1. Thawed the frozen serum samples
2. Mixed and centrifuged them to remove particulates before use
3. Vortexed the pre-mixed beads bottle for 1 minute to completely resuspend the beads
4. Brought the Wash Buffer to room temperature and mixed to bring all salts into solution
5. Diluted 25mL of 20X Wash Buffer with 475mL deionised water
6. Added 5.0mL LEGENDplex Assay Buffer to the bottle containing lyophilised Matrix B. Allowed at least 15 minutes for complete reconstitution. Vortexed to mix well.

Standard and sample preparation

1. Reconstituted lyophilised Standard Cocktail with 250µL Assay Buffer.
2. Mixed and allowed to sit at room temperature for 10 minutes. This was the top standard C7 (10 000pg/mL).
3. Added 75 µL of C7 to the designated wells on two 96-well plates
4. On the same 96-well plate, added 75µL of Assay Buffer to the designated wells for C1 to C6
5. Prepared a 1:4 dilution of the top standard by adding 25 µL of C7 to the C6 well and mixed thoroughly to make the C6 standard.

6. In the same manner, performed a 1:4 serial dilution to obtain C5, C4, C3, C2, and C1 standards. Assay Buffer was used as the C0 (0pg/mL).
7. Diluted serum samples in the same 96-well plates, adding 30µL of sample and 30µL of Assay buffer
8. Ran both standards and samples as duplicates.

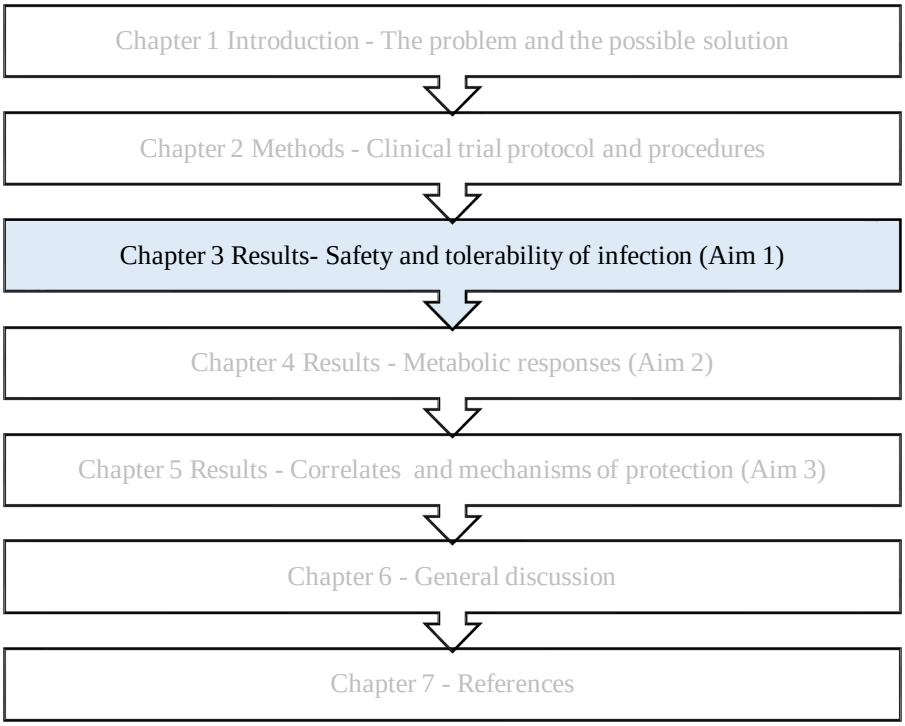
Assay procedure

1. Allowed all reagents to warm to room temperature before use
2. Loaded the assay plate with 17µL of each of the standards and Matrix B in the standard wells and 17µL each of sample and Assay Buffer for the sample wells
3. Vortexed mixed beads for 30 seconds. Added 16µL of mixed beads to each well of the assay plate.
4. Covered the plates with their lids and wrapped entire plates with aluminium foil to protect the plates from light. Placed on plate shaker set at 800rpm for 1 hour at room temperature
5. Refrigerated overnight and placed on plate shaker again for another hour at the same setting the next day
6. Centrifuged the plate at 1050 rpm for 5 minutes, using a swinging rotor bucket
7. Immediately after centrifugation, dumped the supernatant into a sink by quickly inverting and flicking the plate in one continuous and forceful motion
8. Blotted the plate on a stack of clean paper towels and drained the remaining liquid from the wells as much as possible, being careful not to disturb the bead pellet
9. Washed the plate by dispensing 200µLof 1X Wash Buffer into each well and incubated for one minute. Repeated Steps 6 to 8 above
10. Added 16µL of detection antibodies to each well
11. Covered the plates with their lids and wrapped entire plates with aluminium foil to protect the plates from light. Placed on plate shaker set at 800rpm for 1 hour at room temperature
12. Added 16µL of SA-PE to each well
13. Covered the plates with their lids and wrapped entire plates with aluminium foil to protect the plates from light. Placed on plate shaker set at 800rpm for 1 hour at room temperature
14. Washed the plate by dispensing 200µLof 1X Wash Buffer into each well and incubated for one minute. Repeated Steps 6 to 8 above
15. Added 85µL of filtered 1X Wash Buffer to each well. Resuspended the beads by pipetting
16. Read samples on a flow cytometer (The setup instructions for the flow cytometer are available at www.biolegend.com/legendplex under the **Instrument Setup** tab. The assay file was analysed using Biolegend's LEGENDplex data analysis software.

2.7 Materials

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, Peptides, and Recombinant Proteins		
80% v/v ethanol Ajax Finechem	ThermoFisher	AJA726-20 also known as AJA726-20
F10SC	ThermoFisher	Cat#111TS
Betadine 10% w/v (Povidone-Iodine 10%w/v,)	Chemist Warehosue	Product ID 2505692
Critical Commercial Assays/Kits		
QIAamp®Fast DNA Stool Mini Kit	Qiagen	Cat#51604
Human Leptin SimpleStep ELISA®Kit	Abcam	ab179884
Human Omentin SimpleStep ELISA®Kit	Abcam	ab269545
Human Adiponectin SimpleStep ELISA®Kit	Abcam	ab222508
LEGENDplex™ HU Th Cytokine Panel (12-plex) w/FP V02	Biolegend	Cat# 74102
Software and Algorithms		
Excel	Microsoft	Version 16.56
Prism	GraphPad	Version 9.3.1
FluidX Intellicode decoding software	BioTools	
LEGENDplex™ Data analysis software suite	Biolegend	
Other		
BD Vacutainer® with red BD Hemogard™, 10mL	BD	Cat#367895
BD Vacutainer® with lavender BD Hemogard™, 10mL	BD	Cat#367525
BD Vacutainer® with gold BD Hemogard™, 8.5mL	BD	Cat#367958
BD Vacutainer® with green BD Hemogard™, 10mL	BD	Cat#367526
BD Vacutainer® Safety-Lok™ blood collection set, 23G x 3.4" x 7"	BD	Cat#367288
BD Vacutainer® one-use holder	BD	Cat#364815
DPBS (1x), 500mL	ThermoFisher	Cat#14190144
FBS		
Lymphoprep™ Density Gradient Medium, 500mL	Stemcell	Cat#07801
Sepmate™-15 and Sepmate™50 tubes	Stemcell	Cat#15415 and 15460
Falcon® Conical Centrifuge Tubes, 15mL	Corning	Cat#352095
Falcon® Conical Centrifuge tubes, 50mL	Corning	Cat#352070
FluidX 0.75mL barcoded tubes	BioTools™	Cat#67-0750-21
PUFAcoat Dried Blood Spot Collection Card for Fatty Acid Analysis	FOODplus Research Centre, University of Adelaide	
Oxoid™ AnaeroGen™ Compact Sachet	ThermoFisher	Cat#AN0020D
Petri dish 150x15mm with cams	Sarstedt	Cat#82.1184.001
Whatman filter paper, 110mm Ø	Merck	Cat#1001-110
Wooden applicators 150mm	Livingstone	Item No WAPPSTP
Activated charcoal powder	Sigma	Cat#4386-500
Activated charcoal granular	Sigma	Cat#2889500
Chux gauze	Chux®	NA
Zip-lock bags	Glad® or equivalent	NA
NanoPhotometer®	Implen	N60/N50
Professional Intellisense ® Blood Pressure Monitor	OMRON® Healthcare, Inc.	Model HEM-907XL
FLUOstar®Omega Plate Reader	BMG LABTECH	

3 SAFETY AND TOLERABILITY OF INFECTION (AIM 1)



3.1 Introduction

Soil-transmitted helminths (STH) account for a significant burden of disease worldwide. An estimated 1.5 billion people are currently infected with STH (World Health Organization, 2022), with infections most prominent in the tropics and subtropics. The species most commonly infecting humans include the roundworm *Ascaris lumbricoides*, the whipworm *Trichuris trichiura*, the threadworm *Strongyloides stercoralis*, and hookworms (predominantly *Ancylostoma duodenale*, *Ancylostoma ceylanicum*, and *Necator americanus, Na*). Recent control and elimination efforts have reduced the disease burden in terms of disease-associated life years worldwide. However, the absolute and relative decline was disproportionately greater in upper-middle-income countries, shifting the burden mainly to middle and low-income countries (Stolk et al., 2016). Morbidity in STH infections positively relates to worm burden (World Health Organization, 2022). While light infections generally do not cause any suffering, heavy infections can result in various health problems such as anaemia, asthenia (weakness, lack of energy), lack of appetite and weight loss, abdominal pain, and growth retardation (Jourdan et al., 2018; World Health Organization, 2022).

Among STH, hookworms are the greatest contributor to the global disease burden. Hookworm infections affect nearly 500 million people worldwide, particularly the poorest individuals in tropical and subtropical regions. Although *Ancylostoma spp.* and *Na* exist in all regions, *Na* is the main culprit in human infections worldwide, while *A. duodenale* infections predominantly occur in China, northern India, and the Mediterranean (Clements & Addis Alene, 2022). However, a lack of large-scale, population-based studies investigating hookworm-associated morbidity hampers the quantification of the relative pathogenicity and disease burden of individual hookworm species (Clements & Addis Alene, 2022). Hookworm infections are often asymptomatic but may include “ground itch” (localised erythematous reaction) at the skin penetration site, respiratory symptoms during the lung stage, and abdominal discomfort when the worms attach to the intestinal lining (Hotez et al., 2004).

While these transient symptoms may be irritating, hookworm-associated disease burden stems primarily from iron deficiency anaemia in heavily infected adults. Furthermore, anaemia can occur with much lower worm burdens in young children and women due to lower iron stores in these populations (Loukas et al., 2016; Parija et al., 2017; Stoltzfus et al., 1997). In children, anaemia may impair growth, especially during puberty, leading to cognitive and intellectual delays (Brooker et al., 2004). In pregnant women, anaemia can result in pre-term labour, low birth weight, and increased maternal mortality (Brooker et al., 2008; Christian et al., 2004; Hotez & Whitham, 2014). The degree of anaemia appears to depend not only on worm burden but also on worm species. Among the hookworm species commonly infecting humans, *Na* causes the least blood loss per day (0.01-0.03mL), with blood loss in *A. duodenale* infection estimated to be more than ten times that of *Na* (0.14-0.4mL) (Roche & Layrisse, 1966).

Despite these potentially adverse outcomes of helminth infection on human health and development, eliminating worms from our environment may also have unexpected detrimental consequences.

Epidemiological studies first invoked the notion of an inverse relationship between helminth infections and inflammatory disorders. These early and more recent studies highlighted a possible protective role of helminth infection in less developed populations against the soaring prevalence of metabolic diseases such as T2DM seen in industrialised countries (Cooper et al., 2003; Greenwood, 1968; Hays et al., 2015; Shen et al., 2015; Su et al., 2020; Wiria et al., 2015; Wolde et al., 2019; Yazdanbakhsh et al., 2002). Experimental mouse studies have added causal support to these observations, demonstrating reduced body mass, improved glucose tolerance, and reduced insulin resistance in helminth-infected mice in mouse models of obesity (Berbudi, Ajendra, et al., 2016; Q. Duan et al., 2018; Hussaarts et al., 2015; Su et al., 2018; D. Wu et al., 2011; Yang et al., 2013). Deworming studies in humans indicating worsening glucose control and increased pro-inflammatory markers after administration of anthelmintics added some causal evidence for a beneficial role of helminths in metabolic diseases (Chen et al., 2013; Q. Duan et al., 2018; Muthukumar et al., 2019; Rajamanickam et al., 2018; Rajamanickam, Munisankar, Dolla, et al., 2020; Rajamanickam, Munisankar, Menon, et al., 2020; Tahapary, de Ruiter, Martin, Brienen, van Lieshout, Cobbaert, et al., 2017). However, experimental therapeutic helminth infection was essential to investigate these associations.

Therapeutic hookworm infection dates back to 1939 when Brumpt and Gujar (1948)successfully used *A. duodenale* to normalise erythrocyte count in a patient suffering from polycythemia[6]. Building on this initial success, they proceeded to use *A. duodenale* in a further 53 patients with polycythemia and hypertension. However, more recent hookworm infection trials favoured *Na* over *A. duodenale*, possibly due to the lesser blood loss with *Na* infection. Since 1984, over 300 participants in diverse cohorts have been safely infected with up to 100 L3 of *Na* in therapeutic hookworm trials in Australia, the USA, the Netherlands, and the United Kingdom (P. R. Chapman et al., 2021; Croese et al., 2015; Croese et al., 2020; Croese et al., 2006; Daveson et al., 2011; Feary et al., 2009; Feary et al., 2010; Hoogerwerf et al., 2019; Hoogerwerf et al., 2021; McSorley et al., 2011; Mortimer et al., 2006; Tanasescu et al., 2020). None of the infected individuals in the contemporary studies experienced a serious adverse event or died. While doses of up to 100 L3 have been administered, most recent trials limited the maximum dose to 50 larvae based on the results from a safe dose-evaluation study (Mortimer et al., 2006).

Experimental *Na* infection was well-tolerated in diverse cohorts, including subjects with coeliac disease (Daveson et al., 2011), asthma (Feary et al., 2010), and multiple sclerosis (Tanasescu et

[6] **Polycythemia:** a rare chronic disorder involving the overproduction of blood cells (myeloproliferation) with the overproduction of red blood cells the most dramatic consequence (National Organization for Rare Disorders, 2018. Accessed June 10 2022)

al., 2020). Transient side effects commonly reported were mostly mild to moderate severity and self-limiting, with dermal reactions at the inoculation site, including itching and a pruritic, erythematous rash ubiquitous in all infected individuals. Similarly, gastrointestinal symptoms such as bloating, diarrhoea, abdominal pain, and nausea were regularly observed (P. R. Chapman et al., 2021; Daveson et al., 2011; Feary et al., 2010; Hoogerwerf et al., 2021; Loukas et al., 2016). Surprisingly, hookworm infection also appears to afford unanticipated positive side effects, with previous studies noting overall improved mood and well-being that may be clinically relevant (Croese et al., 2015; Croese et al., 2020; Daveson et al., 2011). While this observation has not been further explored, a possible explanation could be increased intestinal serotonin content with gastrointestinal helminth infection (Wang et al., 2018). Considering that low serotonin and noradrenaline levels are a critical mechanism in mood disorders, such as depression and anxiety (Hindmarch, 2002), increases in these neurotransmitters could potentially mediate the observed improvements. Despite findings from numerous human and murine studies indicating a protective role of helminth infection in metabolic disease, no study has previously experimentally infected individuals in this cohort to assess these findings.

The primary aim of this phase 1b clinical trial was to establish the safety, tolerability, and acceptability of experimental infection with the human hookworm *N. americanus* in otherwise healthy adults with metabolic syndrome. I hypothesised that infection in this cohort would be safe, tolerable and acceptable

3.2 Results

Normality testing indicated that the majority of data were not normally distributed. Not normally distributed data are presented as median and interquartile range (IQR), while normally distributed data are presented as mean and 95% confidence interval. For test results stated as not significant, *p* was greater than 0.05. For significant results in tests with post hoc analyses, I have reported the test-specific omnibus *p*-value followed by the adjusted *p*-value for transparency.

3.2.1 Recruitment and participant characteristics

3.2.1.1 Recruitment

We started recruiting in January 2018 and proceeded until June 2020. Initial recruitment targeted only female participants in the Cairns and Hinterland region, later expanded to males and the Townsville region. Media outlets took a keen interest in the trial, and we fielded over 200 enquiries from interested individuals. Of these, 85 qualified for a screening visit, with 41 volunteers meeting the inclusion/exclusion criteria to proceed to the baseline visit. Despite prolonged efforts, the recruitment result fell short of our goal of 54 participants, based on an *a priori* power calculation described in 2.1.6.1.

3.2.1.2 Participant characteristics

The majority of recruits were women of Caucasian descent. Of the 41 individuals that qualified for and completed the baseline visit, one did not proceed to the first inoculation visit and was not randomised. Another participant, who was randomised into the placebo arm, unintentionally received the L3-10 treatment during the second inoculation due to a labelling error. I included this individual in the Placebo group in intention-to-treat (ITT) analyses and the L3-10 group in per-protocol (PP) analyses, which resulted in a slight imbalance in group numbers in PP baseline characteristics. Table 3-1 and Table 3-2 present ITT and PP baseline characteristics for the 40 randomised individuals, respectively. For the ITT data, Kruskal-Wallis (KW) tests showed no significant baseline differences between groups for age, body mass, waist circumference, and HOMA-IR.

In contrast, a significant baseline difference between the L3-10 and Placebo groups existed in the PP data for age (KW $p = 0.0276$, adjusted $p = 0.0358$) and HOMA-IR (KW $p = 0.0492$, adjusted $p = 0.0486$). No significant differences between groups were evident for body mass and waist circumference.

3.2.1.3 Prescription medication for chronic conditions at baseline

Twenty-one of the 40 randomised participants reported the use of prescription medication at baseline. One participant disclosed three different types of medication, a further seven disclosed two,

and 13 listed one. Antidepressants were the most commonly prescribed medication (13 participants), followed by antihypertensives (6 participants) (Table 3-3). Both participants taking metformin agreed to discontinue the medication to meet the inclusion criteria. Similarly, the two individuals using statins reported no longer using the medication during follow-up visits, suggesting that these medications did not impact trial outcomes.

Table 3-1 Intention-to-treat baseline characteristics for all randomised participants (n = 40)

	Placebo ***(n = 13)***	**L3-10** ***(n = 14)***	**L3-20** ***(n = 13)***
Sex			
Female	10 (75%)	10 (73%)	10 (77%)
Male	3 (25%)	4 (27%)	3 (23%)
Age (years)	35 27-50	43 30-45	36 27-49
Ethnicity			
Caucasian	11	13	10
Indigenous	0	1	2
Pacific Islander	0	0	1
Body mass (kg)	105.7 90.0-120.6	103.5 88.5-126.1	101.5 90-129.7
Waist circumference (cm)	111.3 101.9-122.0	110.9 103.3-116.2	110.7 105.2-122.7
HOMA-IR (units)	2.18 2.01-2.82	3.04 2.28-3.51	2.36 2.02-3.24

Data are presented as median and range for age and median and IQR for body mass, waist circumference, and HOMA-IR. IQR, inter-quartile range. No significant differences were found.

Table 3-2 Per-protocol baseline participant characteristics for all randomised participants (n = 40)

	Placebo ***(n = 12)***	**L3-10** ***(n = 15)***	**L3-20** ***(n = 13)***
Sex			
Female	9 (75%)	11 (73%)	10 (77%)
Male	3 (25%)	4 (27%)	3 (23%)
Age (years)	34 27-50	**43*** 30-45	36 27-49
Ethnicity			
Caucasian	12	14	10
Indigenous	0	1	2
Pacific Islander	0	0	1
Body mass (kg)	104.5 89.5-115.1	104.1 90.6-140.9	101.5 90.0-129.7
Waist circumference (cm)	109.5 101.6-119.6	111 104.0-119.7	110.7 105.2-122.7
HOMA-IR (units)	2.18 1.97-2.62	**2.99*** 2.29-3.48	2.36 2.04-3.24

Data are presented as median and range for age and median and IQR for body mass, waist circumference, and HOMA-IR. IQR, inter-quartile range. *significant difference to Placebo

Table 3-3 Prescription medication for chronic conditions at baseline by treatment (ITT)

	Placebo ***(n = 13)***	**L3-10** ***(n = 14)***	**L3-20** ***(n = 13)***
Antidepressants	6	5	2
Antihypertensives	0	3	3
Statins	0	1	1
Antiasthmatics	1	1	0
Anticoagulants	0	0	1
Analgesics (opioids)	0	1	1
Immunosuppressants	1	0	0
Antiarthtritics	1	0	1
Antacids	0	0	1
Metformin	1	1	0

3.2.2 Skin reactions and adverse events

3.2.2.1 Dermal reactions

Although the trial protocol did not ask to record skin reactions to inoculations, several participants in the worm treatment groups mentioned such reactions. During the initial monitoring period after inoculation, participants routinely noted itching at the inoculation site that they described as moderate to intense; no participant found the itch problematic or challenging to manage. During the consecutive visit's interview, many participants also reported the onset of dermal reactions at the inoculation site within hours to days. Consistent with the hookworm inoculation rash, some participants experienced a transient erythematous rash with distinct eruptions, as seen in Figure 3-1. None of these reactions was classified as an adverse event.

3.2.2.2 Patency of infection

Baseline and wk26 faecal samples were tested for the presence of hookworm by human hookworm multiplex qPCR to determine the prevalence and egg intensity of three human hookworm species (*Na*, *A. duodenale*, A. *ceylanicum*). I used peak eosinophilia at wk26 and qPCR results in combination to assess the patency of infection. All infected participants developed varying degrees of eosinophilia (Table 3-4). Males tended to have a greater eosinophil response than females (Figure 3-2), but this difference was not significant (Welch's t-test).

Unexpectedly, one participant (ID 016) showed eosinophilia and tested positive for *Na* infection by qPCR at baseline; all others had low eosinophil counts and tested negative for the three hookworm species assessed (Table 3-4). This particular participant had dropped out after the first inoculation due to a GI-related AE during their initial enlistment (ID 006). The participant was unblinded as an L3-10 recipient, provided with anthelminthic medication, and re-recruited on the participant's request after the GI symptoms had settled. The surprisingly high eosinophil levels and positive qPCR result at their second baseline visit suggest that deworming was only partially successful, possibly due to inappropriate dosing given the participant's large body mass.

Of the 28 participants randomised into either L3 treatment group, four wk26 samples were unavailable for qPCR testing due to early dropout. A further wk26 sample was unavailable, as the participant had moved away, and all evaluation visits were conducted remotely with no opportunity to deliver a faecal sample. Two of the remaining 23 samples (017 and 205) tested negative for all hookworm species by qPCR despite increased eosinophil counts. Although the hookworm multiplex qPCR is very sensitive and more capable of detecting low infection intensities than conventional microscopy and PCR-based methods, a false-negative result is not impossible.

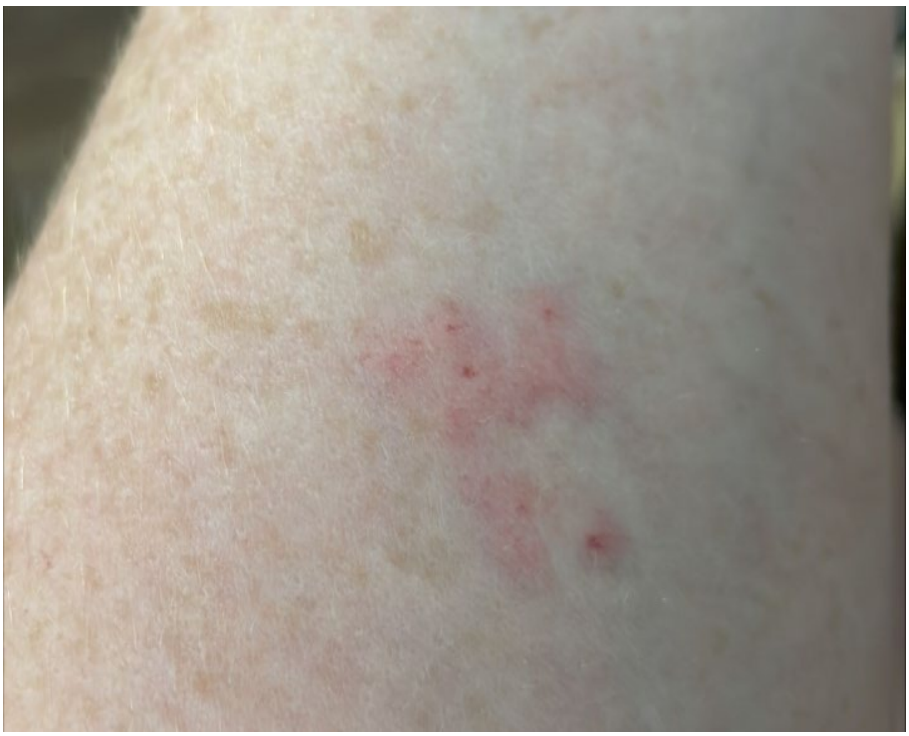
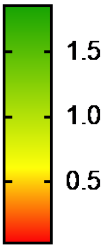


Figure 3-1 Representative photograph of dermal reactions at the inoculation site

Table 3-4 Patency of infection for all randomised participants assessed by qPCR and peak eosinophil count at wk26

ID	Allocation	baseline qPCR	wk26 qPCR	baseline eosinophils	Week 26 eosinophils
211	L3-10		n/a	0.39	
029	L3-10			0.38	1.19
013	L3-10			0.18	0.58
213	L3-10			0.14	0.80
016	L3-10			1.86	0.99
006	L3-10		n/a	0.34	
049	L3-10			0.13	1.10
230	L3-10		n/a	0.26	
205	L3-10			0.05	0.48
008	L3-10			0.49	1.01
019	L3-10			0.13	0.25
017	L3-10			0.15	0.77
215	L3-10			0.35	0.98
203	L3-10			0.09	0.37
026	L3-10			0.02	0.58
027	L3-20		n/a	0.11	
210	L3-20		n/a	0.31	0.54
028	L3-20			0.11	0.65
056	L3-20			0.06	0.52
015	L3-20			0.07	1.26
057	L3-20			0.10	1.85
018	L3-20			0.09	0.39
208	L3-20			0.06	0.54
021	L3-20		n/a	0.19	
005	L3-20	n/a		0.13	0.24
227	L3-20		n/a	0.06	0.98
218	L3-20			0.70	1.43
209	L3-20			0.07	0.41
023	P			0.12	0.15
221	P			0.23	0.19
030	P			0.12	0.33
224	P			0.16	0.18
010	P			0.19	0.15
212	P			0.13	0.15
223	P			0.08	0.13
214	P			0.24	0.14
217	P			0.12	0.18
011	P			0.17	0.12
048	P			0.54	0.12
201	P			0.19	0.15



ID, participant ID number. Participants highlighted in yellow dropped out of the trial before wk26. Participant highlighted in turquoise missed wk26 visit, and no sample was available for comparison. Two other participants failed to deliver a sample for wk26 (210, 227) Red shading indicates negative qPCR result, i. e. no hookworm presence; green shading indicates positive qPCR result. The reference scale reflects the magnitude of eosinophilia.

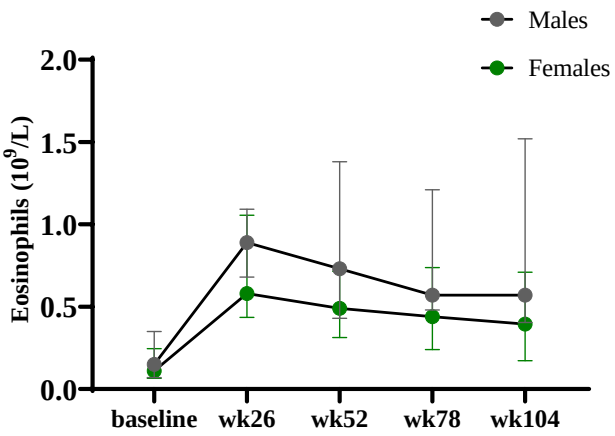


Figure 3-2 Eosinophil count in males versus females throughout the trial.
No significant differences were found (Welch's t-test)

3.2.2.3 Adverse events

Note: Adverse events and study progression reporting are based on ITT analyses.

The trial's primary safety outcome was the number of AEs and SAEs. We recorded 20 AEs and no SAEs throughout the two-year trial duration. As expected, several adverse gastrointestinal (GI) events occurred in the L3-10 and L3-20 treatment groups; no treatment-related GI event occurred in the Placebo group (Table 3-5). Participants reported the onset of treatment-related GI events between four and eight weeks after the first inoculation, consistent with the worms' arrival and attachment to the intestinal wall. Symptoms varied considerably among participants and included bloating, nausea, vomiting, constipation, diarrhoea, epigastric upset, hungry feeling, stomach cramps and abdominal pain. Most AEs were *mild* or *moderate*, with only three (15%) classified as *severe*, including one (5%) GI event (Table 3-6). I performed a Chi-square test for trend to determine differences in AEs between groups. The test result suggested a significant linear trend for AEs, χ^2 (1, N = 40) = 3.846, p = 0.0499, with more AEs occurring in the worm treatment groups compared to the Placebo group.

All 20 AES were spread across 15 participants, with five experiencing two AEs each (Table 3-7). Three of the 12 GI events lead to dropouts before the first evaluation visit (wk260. One of the dropouts was consequently re-enlisted and completed the 2-year trial. All other GI events resolved after 2-3 weeks without medical intervention.

Adverse events considered unrelated to worm infection included one case each of concussion, laryngitis, appendicectomy, gynaecological procedure, salmonella infection acquired overseas, cancer, and a bout of dermatitis.

In summary, eosinophil counts and qPCR test results indicate successful infection of all L3-10 and L3-20 participants. While AEs were more prevalent in the worm groups, helminth-related AEs were mostly mild to moderate, and infection was well tolerated in most individual

Table 3-5 Adverse events by group

	Total ***n* = 20**	**Placebo** ***n* = 13**	**L3-10** ***n* = 14**	**L3-20** ***n* = 13**
Any AEs	20 (100%)	3 (15%)	9 (45%)	8 (40%)
Related (GI)	12 (60%)	0 (0%)	5 (25%)	7 (35%)
Unrelated	8 (40%)	3 (15%)	4 (20%)	1 (5%)

AE, adverse event; GI, gastrointestinal

Table 3-6 Severity and nature of adverse events by treatment group

	Placebo ***n = 13***			**L3-10** ***n = 14***			**L3-20** ***n = 13***		
	Mild	**Moderate**	**Severe**	**Mild**	**Moderate**	**Severe**	**Mild**	**Moderate**	**Severe**
Any AEs	1	1	1	2	6	1	3	4	1
Related (GI)	0	0	0	1	4	0	2	4	1
Unrelated	1	1	1	1	2	1	1	0	0

AE, adverse event; GI, gastrointestinal

Table 3-7 Adverse event distribution across groups and participants

	All groups ***(n = 40)***	**Placebo** ***(n = 13)***	**L3-10** ***(n = 14)***	**L3-20** ***(n = 13)***
Participants with **any** AEs *n* (%)	15 (37.5)	2 (15.4)	7 (50.0)	6 (46.2)
Participants with **one** AE *n* (%)	10 (25.0)	1 (7.7)	5 (35.7)	4 (30.8)
Participants with **two** AEs *n* (%)	5 (12.5)	1 (7.7)	2 (14.3)	2 (15.4)

AE, adverse event

3.2.3 Study progression and dropouts

3.2.3.1 Study progression

Completion of the two-year trial was a secondary safety outcome. The CONSORT Flow Diagram in Figure 3-3 and Kaplan-Meir curve in Figure 3-4 detail progression through the study. Twenty-four (60%) of the forty randomised participants completed the trial, including eight (61.54%) in the Placebo, seven (50%) in the L3-10, and nine (69.23%) in the L3-20 group. The difference in study completion between groups was not significant (Log-rank test for trend, $p = 0.8321$). Bar one, all participants who received the worms and completed the study rejected deworming and opted to retain their "little friends" or "family" as they had affectionately labelled them. The sole person who dewormed did so in preparation for a medical procedure.

3.2.3.2 Missed visits

Four participants missed one evaluation visit each. One L3-10 participant missed the wk26 visit, as he had moved away for family reasons and could not commit to a timely visit. However, this individual completed the remaining evaluation visits. Two participants, one Placebo and one L3-10, missed the wk52 visit. Both did not respond to any form of communication in the required time frame but returned for their wk78 visit. A fourth person missed the wk78 visit due to COVID restrictions but consequently completed the wk104 visit.

3.2.3.3 Dropouts and early terminations

Reasons for leaving the trial before completion of the two-year trial period varied and were only associated with worm infection during the early trial stage (Table 3-8). Three early dropouts due to gastrointestinal symptoms were likely worm-related. The infected participants accepted deworming medication and were treated as early terminations. Two individuals recovered promptly after deworming. However, symptoms in one individual persisted for some time following deworming, indicating another cause may have contributed to their symptoms. A fourth infected individual was dewormed and terminated early due to a cancer diagnosis. Reasons for dropouts and early terminations later in the trial included participants moving away, gastric sleeve surgery, undisclosed personal reasons, failure to respond to communication, and starting medication likely to interfere with the trial outcomes. All participants leaving the trial during the later stages decided to keep their worms.

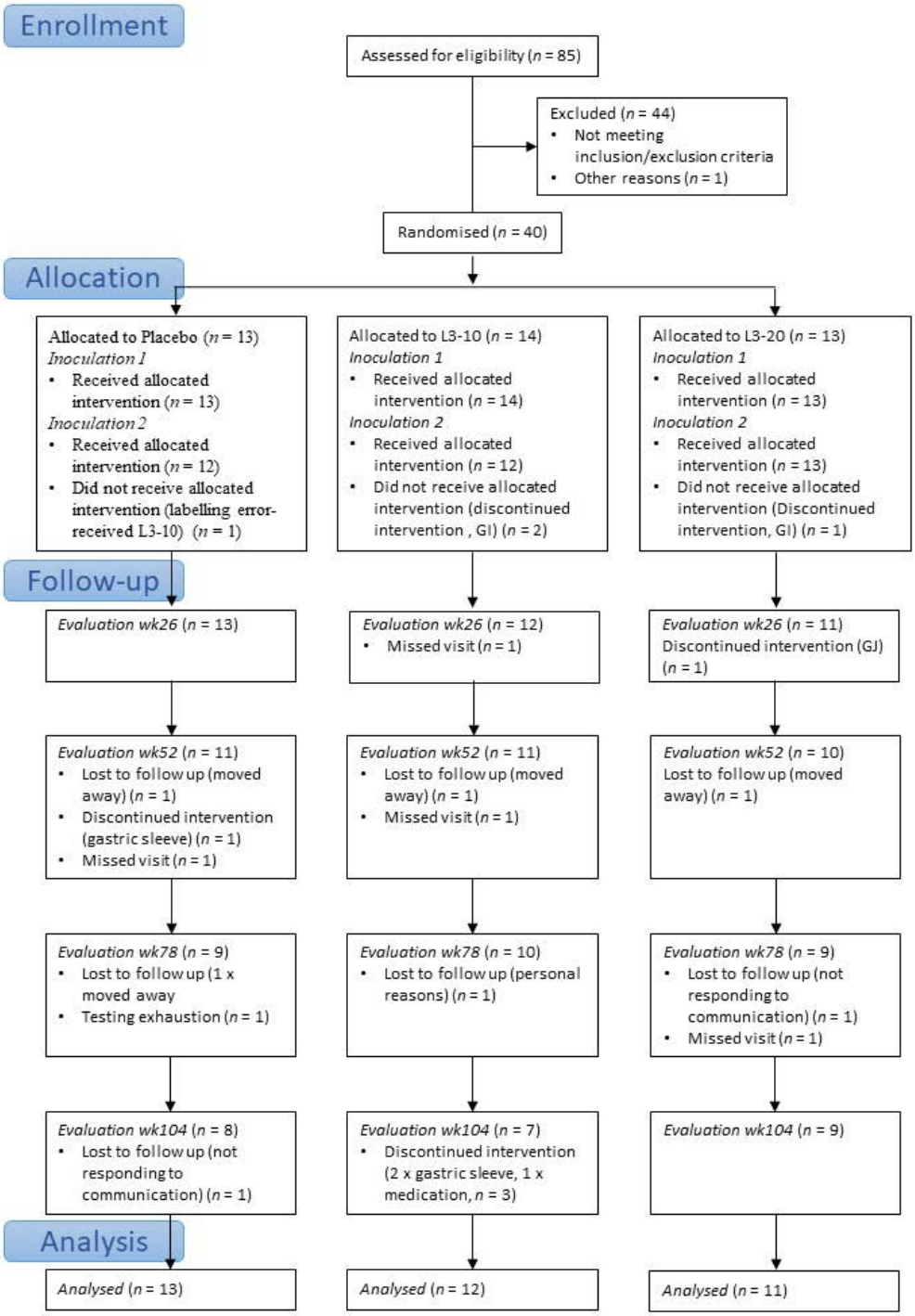


Figure 3-3 CONSORT Flow Diagram of trial progression

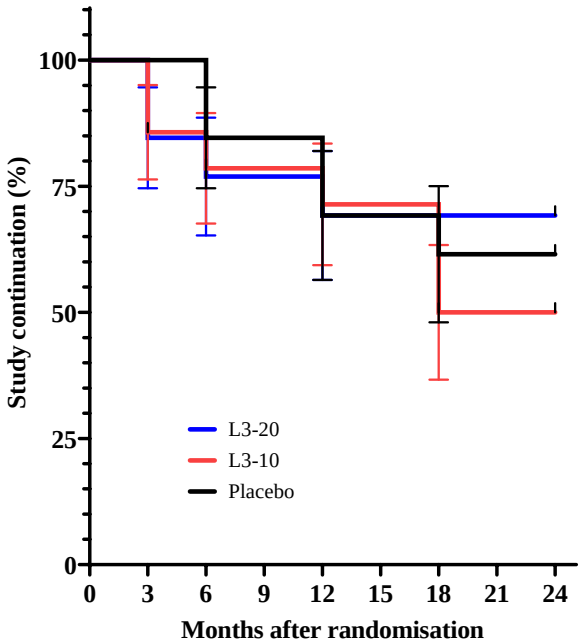


Figure 3-4 Kaplan-Meir curve of study progression. Data are presented as the mean and standard error of the mean.

Baseline differences of potential covariates between dropouts and others

Table 3-8 provides an overview of dropout time points, causes, age, and sex. To investigate if specific covariates possibly shaped participants' decisions to leave the trial early, I compared baseline differences in age, sex, body mass, WC, fasting blood glucose, and serum insulin between dropouts and those that completed the two-year trial (Table 3-9). Mann-Whitney tests showed no significant differences for age ($p = 0.0623$), body mass ($p = 0.8807$), waist circumference ($p = 0.9185$), HOMA-IR ($p = 0.6672$), fasting blood glucose ($p = 0.3186$), and fasting serum insulin ($p = 0.8748$). Similarly, contingency analysis via Fisher's exact test indicated no significant difference in sex distribution ($p = 0.7110$).

Baseline differences of potential covariates between early and later dropouts

Baseline differences for the same covariates also did not differ significantly between early (≤ wk26) and later dropouts (Table 3-9). Mann-Whitney test results for age ($p = 0.5894$), body mass ($p = 0.2345$), WC ($p = 0.3972$), HOMA-IR ($p = 0.7428$). fasting blood glucose ($p = 0.8936$), and fasting serum insulin ($p = 0.6263$) suggested no significant differences between early and late dropouts. Also, Fisher's exact test indicated that sex distribution was not significantly different ($p > 0.9999$). Together, the results implied that significant baseline differences in these covariates did not influence participants' decisions to leave the trial or the timing of their withdrawal.

Table 3-8 Overview of dropout time points, reasons, age, and sex

Timepoint	Placebo	L3-10	L3-20
≤ wk12	***n = 0***	***n = 2***	***n = 2***
Reason		1 x GI 1 x cancer	2 x GI
Age (years)		44/40	34/47
Sex		F/F	F/F
wk26	***n = 2***	***n = 1***	***n = 1***
Reason	gastric sleeve moved away	moved away	moved away
Age (years)	33/32	45	49
Sex	M/F	F	F
wk52	***n = 2***	***n = 1***	***n = 1***
Reason	1 x moved away 1 x testing exhaustion	personal reasons	NRC
Age (years)	50/27	44	28
Sex	F/F	F	F
wk78	***n = 1***	***n = 3***	***n = 0***
Reason	NRC	1 x medication 2 x gastric sleeve	
Age (years)	38	41/ 44/ 44	
Sex	F	M/ M/ F	

GI, gastrointestinal; F, female; M, male; NRC, not responding to communication. No significant differences were found.

Table 3-9 Comparison of baseline measures between dropouts and non-dropouts

	Non-dropouts *n = 24*	Dropouts *n = 16*	Early dropouts *n = 8*	Late dropouts *n = 8*
Age, years	35.5 30.5-39.5	41.5 33.3-44.8	42.0 33.3-46.5	41.5 29.5-43.0
Sex, female/male	17/7	13/3	7/1	6/2
Body mass, kg	103.1 90.2-124.2	104.3 90.5-130.7	116.6 94.7-153.1	95.45 89.1-105.1
Waist circumference, cm	111.0 102.9-119.6	110.0 102.9-125.8	116.8 98.8-135.9	107.5 102.9-111.5
HOMA-IR, units	2.38 2.14-2.99	2.84 2.12-3.46	2.62 1.99-3.46	2.89 2.18-3.4
Fasting blood glucose, mmol/L	5.0 4.5-5.3	5.1 4.8-5.5	5.1 4.7-5.8	5.1 4.9-5.5
Fasting serum insulin, mU/L	11.5 10.0-13.8	11.5 9.0-15.5	11.0 7.5-15.5	11.5 10.3-15.5

Data are presented as median and IQR. No significant differences existed between dropouts and non-dropouts or early and late dropouts

3.2.4 Mood and anaemia

Note: Mood and haemoglobin levels analyses are based on participants that completed at least one evaluation visit (n = 36). As the Placebo participant who received an unintentional dose of worms was effectively infected, I based the analyses on PP data, including the individual in the L3-10 group.

Throughout the trial, participants' mood and depressive state were assessed via the PHQ-9 as a further secondary safety outcome. Table 3-10 details median absolute scores and changes by treatment group at each visit, with a lower score indicating a less depressive state. The median total score in the Placebo group remained relatively stable over time but was slightly higher than baseline at wk104, hinting at a marginally worsened mood. In contrast, I noticed a trend of lower median absolute scores compared to baseline in the worm treatment groups at all evaluation visits. However, Mixed-effects analyses did not detect any significant changes over time in any treatment group. Similarly, Kruskal-Wallis tests showed no significant difference in absolute scores or changes between groups at any time point.

The trend for lower median scores in the infected groups indicated that worm infection might be associated with improved mood regardless of infection magnitude. Therefore, I also explored the differences between Placebo and infected participants, combining the L3-10 and L3-20 groups into infected (Table 3-11). Mann-Whitney test results showed no significant differences in median scores between Placebo and infected groups at any time point. Similarly, no significant differences in median change scores were evident.

While the median changes in each treatment group were similar, I found considerable heterogeneity in individual changes (Figure 3-5). Some participants consistently reported increased PHQ-9 scores, indicating worse mood, while others reported improvements; however, no similarities, such as changes in life circumstances or medication, were evident among these participants. Furthermore, the consequences of COVID lockdowns and restrictions possibly also distorted participant responses. Together, these findings indicate that worm infection does not seem to worsen mood and may even lead to a slight improvement, but other factors likely also play a role.

Responses to Questions 1, 3, 4, and 5 were the most consistently improved in all treatment groups (Figure 3-6). These questions relate to taking pleasure in doing things, sleep disturbances, energy levels, and appetite. Worm-infected participants also reported improvements to most other questions except Question 9, for which initial scores were already low.

Table 3-10 PHQ-9 scores by treatment group throughout the trial

	Placebo	L3-10	L3-20
baseline	7 3.5-13.5 *n = 12*	8 2.0-11.0 *n = 13*	10 5.0-12.0 *n = 11*
wk26	5 3.3-7.5 *n = 12*	4 2-6.8 *n = 12*	9 3.0-12.0 *n = 11*
wk52	6 1.0-13.0 *n = 9*	4 2.0-7.0 *n = 11*	5 2.5-7.5 *n = 10*
wk78	6 2.8-9.0 *n = 8*	3 1.0-9.0 *n = 11*	6.5 1.8-9.8 *n = 8*
wk104	9 2.0-16.0 *n = 7*	3 0.5-5.5 *n = 8*	4 2.0-9.0 *n = 9*

Data are presented as median and IQR.
No significant differences were found.

Table 3-11 PHQ-9 scores in Placebo and combined worm treatment groups

	Placebo	Na
baseline	7 3.5-13.5 *n = 12*	8 3.0-12.0 *n = 24*
wk26	5 3.3-7.5 *n = 12*	5 2.0-11.0 *n =23*
wk52	6 1.0-13.0 *n = 9*	4 2.0-6.5 *n = 21*
wk78	6 2.8-9.0 *n = 8*	6 1.0-9.0 *n = 19*
wk104	9 2.0-16.0 *n = 7*	4 2.0-7.5 *n = 17*

Na, combined worm treatment groups. Data are presented as median and IQR. No significant differences were found.

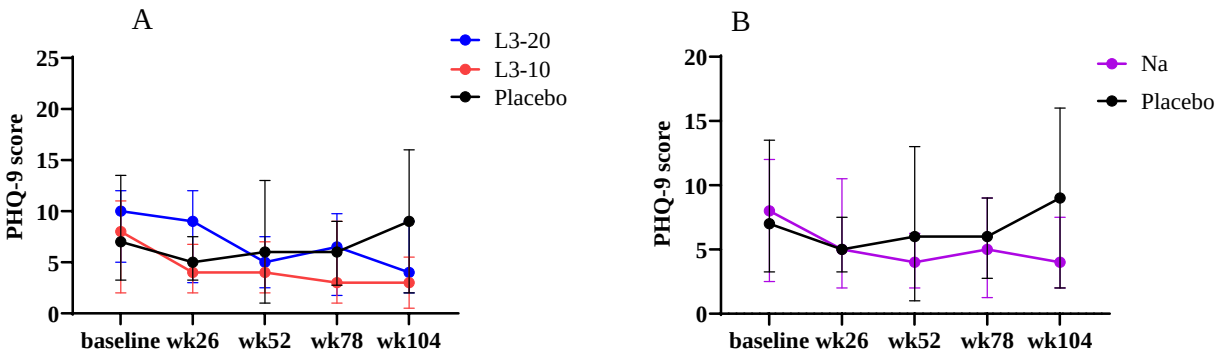


Figure 3-5 PHQ-9 scores
A) PHQ-9 total scores by treatment group throughout the trial.
B) PHQ-9 total scores for Placebo and combined worm treatment groups (Na) throughout the trial.
Data are presented as median and IQR.No significant differences were found (Mixed effects model)

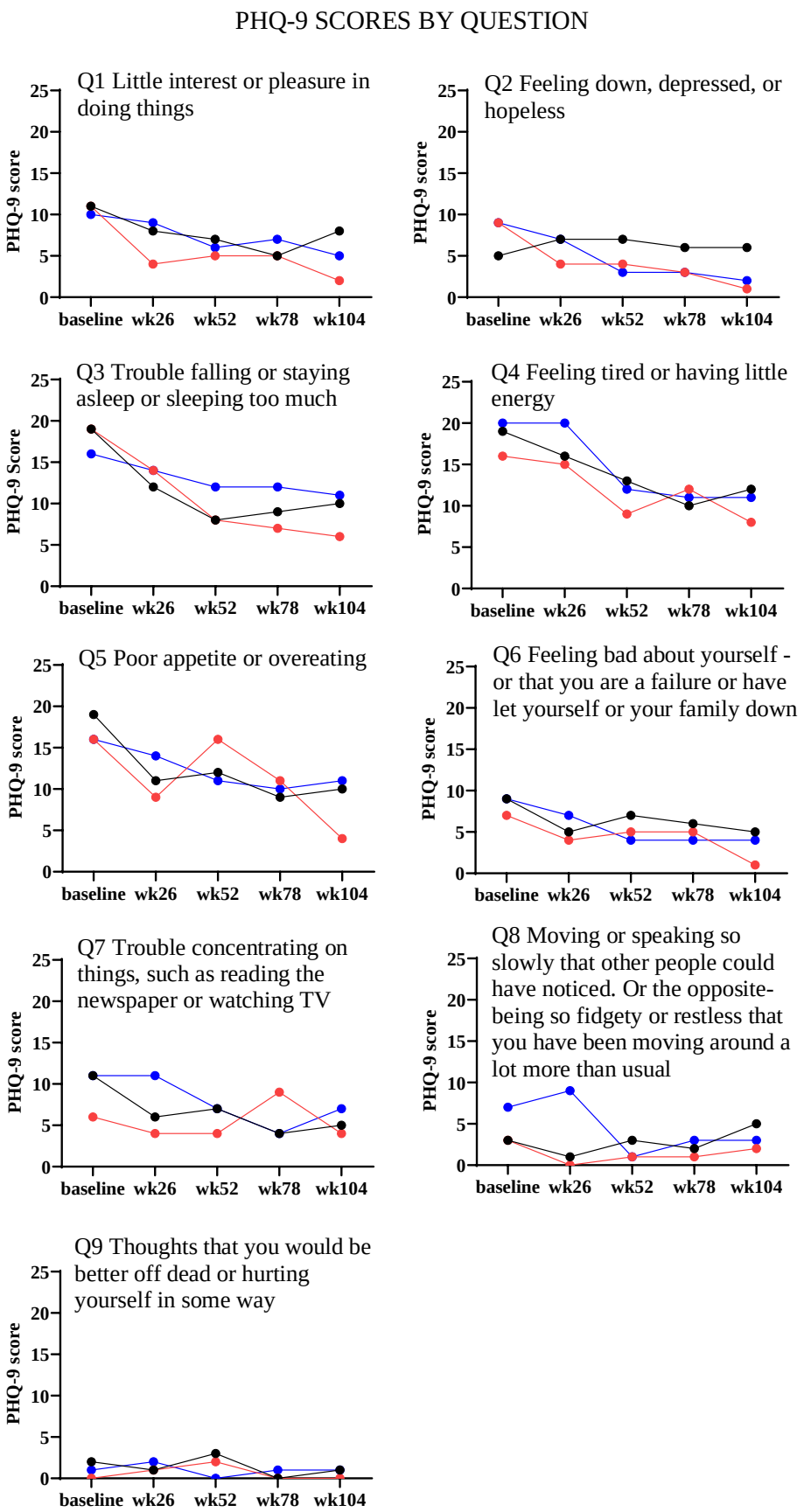


Figure 3-6 Median PHQ-9 scores for each question by treatment group throughout the trial

3.2.5 Haemoglobin

Adult *Na* attach to the intestinal lining and feed on blood, and iron-deficiency anaemia as a side effect of infection is a slight possibility; therefore, haemoglobin measures were an additional safety outcome of the trial. Kruskal-Wallis tests did not show significant differences in absolute values or changes between groups at any time point. Similarly, longitudinal analyses (Mixed-effect model) did not show significant differences between time points. Furthermore, irrespective of worm infection, haemoglobin levels remained well in the healthy range at all time points (Figure 3-7). The data imply that infection with 20 or 40 L3 larvae does not lower haemoglobin levels in adults with metabolic disease.

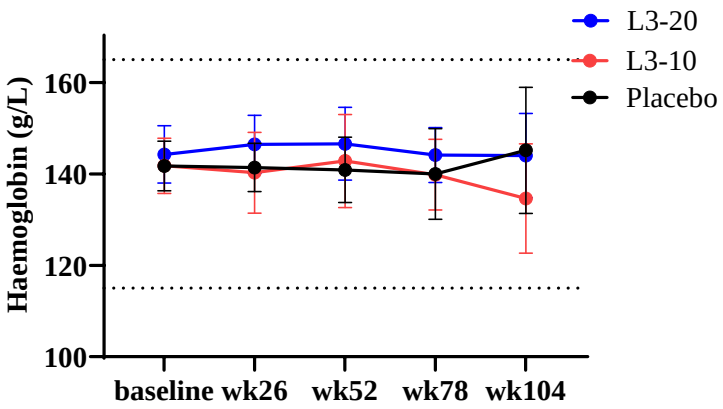


Figure 3-7 Haemoglobin (g/L) by treatment group throughout the trial.
Data are presented as median and IQR. No significant differences were found and haemoglobin remained in the healthy range in all participants (Mixed effects model)

3.3 Discussion

The primary aim of this world-first phase 1b clinical trial using experimental hookworm infection in humans with metabolic disease was to establish the safety, tolerability and acceptability of experimental infection with the human hookworm *N. americanus* in otherwise healthy adults with metabolic syndrome. The results supported my hypothesis that infection in this cohort is safe, tolerable and acceptable.

The number of AEs and SAEs events was the primary outcome measure of infection safety. Throughout the two-year trial, 15 participants experienced a total of 20 mainly mild to moderate AEs, with only three (one in each group) classified as severe. More importantly, no SAE occurred. The significant difference in the number of AEs between the Placebo and worm groups was primarily driven by the incidence of GI events exclusive to the worm treatment groups. Despite the significantly higher number of AEs in the worm groups compared to the Placebo group, each group's proportion of participants completing the two-year trial was similar and highest in the L3-20, indicating that infection-related AEs did not disproportionally impact trial completion.

Sixty per cent (12) of all AEs were GI and occurred early in the trial at a time consistent with worm settlement in the intestine; these were deemed hookworm-related. Despite representing a high proportion of total AEs, a mere three GI events, equalling 19 per cent of all dropouts, led to early withdrawals before the first evaluation visit at wk26. One participant with abdominal discomfort after the first inoculation decided to deworm at home before consulting the trial doctor and reported immediate relief following the administration of the medication. The participant was subsequently unblinded as an L3-20 recipient and excluded. The other two participants completed an early termination visit after notifying the trial doctor of abdominal symptoms, were unblinded (1x L3-10 and 1x L3-20), and received deworming medication on the doctor's advice; the L3-10 participant recovered promptly. However, the L3-20 participant was still unwell two weeks after deworming and booked in with their doctor to further investigate the cause of the abdominal symptoms. Interestingly, this participant's faecal examination two days before deworming found no ova, cysts, or parasites. These findings indicate that the infection may have been unsuccessful, and the cause of gastric upset was possibly unrelated to *Na* infection. All other participants with GI events tolerated the transient GI symptoms without requiring medical intervention and remained in the trial.

Completion of the two-year trial was a secondary safety outcome. Sixteen of the forty randomised participants left the study before completion, with the majority of dropouts and withdrawals (13/16) spurred by reasons unrelated to worm infection. A dominant reason for discontinuation was participant relocation; five individuals moved to a distant location within Australia. Although all agreed initially to participate remotely, only one achieved completion of the trial, leaving four to drop out early. A repeatedly cited hurdle to remote participation was the challenge of finding a new local general

practitioner, which can generally be difficult after relocation. COVID restrictions and lockdowns further exacerbated the issue, making it near impossible for participants to arrange an appointment. Another recurrent reason for discontinuation was gastric sleeve surgery. Three participants were disqualified from further participation following the surgery, as the surgery's effects significantly impact metabolic outcomes (Stenberg & Thorell, 2020). The remaining reasons included personal reasons, testing exhaustion, unsuccessful communication efforts, diagnosis of cancer, and starting metformin. Baseline characteristics that may have influenced participants' decision to withdraw (sex, age, body mass, WC, insulin resistance, fasting blood glucose, and serum insulin) did not significantly differ between dropouts and non-dropouts or between early and late dropouts. Together, these findings indicated that AEs contributed minimally to study withdrawals. Dropouts occurred primarily for reasons unrelated to the intervention and appeared to have occurred at random.

To evaluate the patency of infection, I used a composite definition of hookworm infection that included eosinophil count and positive hookworm qPCR. Although the magnitude of eosinophil response varied widely between individuals (see Section 5.2.5) and tended to be higher in males at all time points, increased eosinophil count was universal in all infected participants. Additionally, 90% of available wk26 faecal samples were egg positive. While qPCR indicated one person had no faecal eggs, their eosinophil count reached clinical eosinophilia and remained elevated throughout the trial, so the treatment could be considered successful. The negative result could suggest an imbalance of male and female worms, causing egg deficiency. Further, egg output can be variable, with no output for up to two weeks documented previously (Mortimer et al., 2006), so this snapshot of egg presence may not accurately reflect worm presence. My results indicate an excellent infection succession rate that compares favourably with other experimental infection studies (Croese et al., 2015; Croese et al., 2020; Daveson et al., 2011; Feary et al., 2010; Mortimer et al., 2006).

As hookworm infection carries a small risk of anaemia, we monitored the participants' haemoglobin levels during the trial as an additional safety outcome. Anaemia is primarily a concern in adults with a high worm burden, young children, and pregnant women. Given the trial's low worm doses and exclusion criteria, we did not expect to see any negative impact of infection on haemoglobin levels, which was substantiated by the pathology results. Haemoglobin levels were similar between groups and well within the healthy range in all participants and throughout the trial.

To complement the physical health safety evaluations, we also assessed our participants' mental well-being throughout the trial via the PHQ-9, in which a lower score indicates improvements in depressive state. Independent of allocation, most participants reported improved mood and well-being throughout the trial, with only a small number consistently scoring higher than baseline at evaluation visits. I found no evidence of similarities, such as accounts of challenging life circumstances, that might explain the scores in individuals with worsening mood. In all groups, scores declined the most for Questions 1. 3, 4, and 5, hinting at positive changes regarding pleasure in doing things, sleep

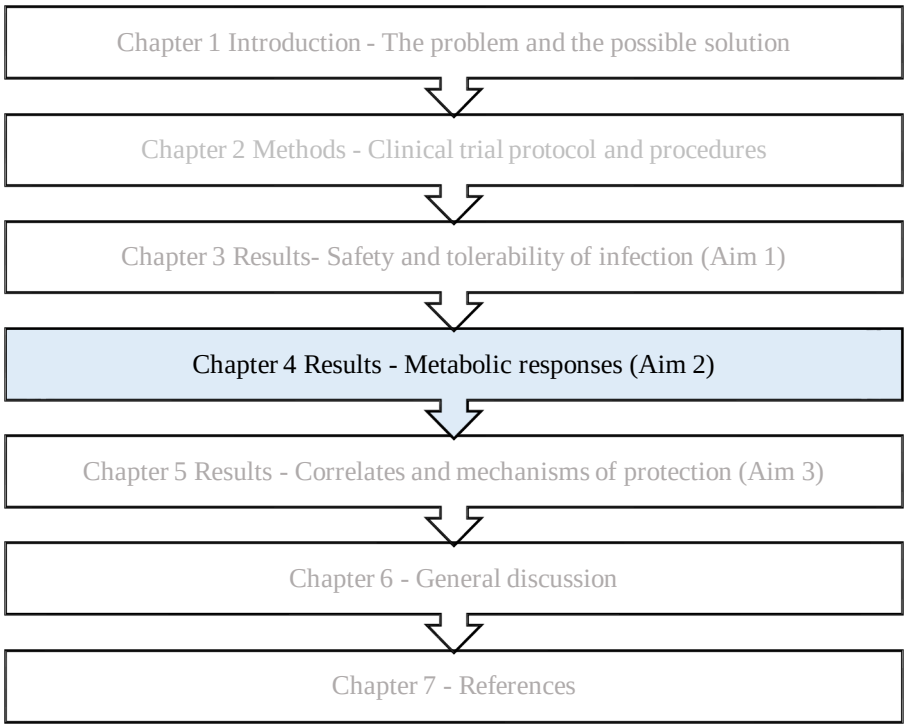
disturbances, energy levels, and appetite. Additionally, helminth-infected participants scored lower in most other questions, except for Question 9, which related to thoughts about death and hurting yourself and already scored low at baseline. Overall, helminth-infected participants seemed to enjoy better mental well-being than the Placebo group. However, this difference was not statistically significant, possibly due to the low numbers in each group, particularly towards the later trial stages. Our finding is consistent with those from previous hookworm trials that also described improved mood and sense of well-being in infected participants (Croese et al., 2015; Croese et al., 2020; Daveson et al., 2011).

Other factors could have influenced participants' mental state. The anticipation of a desired physical effect of helminth infection, such as reduced body mass or simply the optimism of taking a positive step towards a healthy change, may have initiated mood improvements. In contrast, the COVID pandemic unfolded during the trial. Anxiety and uncertainty resulting from repeated lockdowns and restrictions could have triggered significantly worse mental well-being in our participants and possibly distorted participant responses. Still, our participants appeared to cope well overall. Together, these findings indicated that worm infection certainly does not seem to worsen mental well-being and may even lead to a slight improvement, but other factors likely also play a role.

Despite the persisting negative perception of germs and parasites, controlled, low-dose hookworm infection appears to be an acceptable preventative intervention for humans with metabolic diseases. The trial announcement generated great public interest, and over 200 individuals were eager to join. Based on comments from screened volunteers, the potential for infection to initiate weight loss was a strong motivator for many candidates with central obesity an inclusion criterion of the trial. Others were genuinely fascinated by the rationale for the trial and keen to participate in an alternative approach to targeting metabolic disease. Specifically, women were more strongly represented among all applicants. As divulged during trial visits, many participants promoted the trial by excitedly sharing news of their participation and clarifying some misconceptions about worms with friends, colleagues, and their general practitioners. After unblinding, all bar one infected participants refused deworming medication and decided to retain their worms, or "friends", as some affectionately named them. This refusal to deworm appeared as a consistent theme and was noted in previous hookworm infection trials (Croese et al., 2015; Croese et al., 2020; Daveson et al., 2011).

In conclusion, in the current trial, experimental infection with the human hookworm *Na* as a novel strategy to improve metabolic disorders was safe, well-tolerated, and acceptable in humans with metabolic disease. Adverse events were mainly mild to moderate in nature and largely unrelated to early withdrawal from the trial. Discomfort in the earl trial stage stemming from worm migration to the intestine was well-tolerated. Public interest in the trial was strong, and infected participants became very attached to their new cohabitants.

4 METABOLIC RESPONSES (AIM 2)



4.1 Introduction

The increasing prevalence of obesity poses significant challenges for health systems worldwide. Not only is the presence of obesity linked to mental health disorders such as anxiety disorders, depression, and neurodegenerative diseases, but it also markedly exacerbates the risk of chronic metabolic diseases, including CVD, MetS and T2DM (Golia et al., 2014; Himmerich et al., 2009; Luppino et al., 2010; Trayhurn & Wood, 2005). Visceral adiposity, in particular, appears to be the main culprit in the development of T2DM (Liu et al., 2018). Global numbers of individuals living with T2DM are spiralling out of control, with more than half a billion adults already diagnosed and one person dying of diabetes every five seconds (International Diabetes Federation, 2022). The hardest hit are low and middle-income countries, where an estimated 75% of all people with diabetes reside, inflicting catastrophic economic and personal strain on the affected countries (International Diabetes Federation, 2022). Once established, T2DM is costly to manage and cannot be cured due to enduring damage to the pancreatic β-islets; however, caloric restrictions and weight loss may promote reversal (remission) (Hallberg et al., 2019). When poorly managed, the disease almost inevitably progresses to complications such as retinopathy, neuropathy, peripheral arterial disease, kidney disease, CVD, stroke, and foot ulcers (International Diabetes Federation, 2022). The soaring prevalence of T2DM is undoubtedly related to lifestyle factors such as an energy-dense diet and a sedentary lifestyle. However, emerging evidence supports the idea that removing worms and other pathogens from our environment has contributed to the increased prevalence of diabetes and its critical drivers, inflammation and insulin resistance.

Insulin resistance, triggered by inflammatory processes in abdominal adipose tissue, is the foremost pathophysiological aspect of T2DM (Berbudi, Ajendra, et al., 2016; Heilbronn & Campbell, 2008). Insulin release from cells in pancreatic β-islets is tightly linked to blood glucose concentrations and precisely meets metabolic demands in healthy subjects (Schmitz et al., 2008). In skeletal muscle and adipocytes, insulin mainly promotes glucose uptake, whereas it suppresses gluconeogenesis and glycogenolysis in the liver (Ormazabal et al., 2018; Shulman, 2000). Further, insulin stimulates lipid storage in adipocytes by inducing triacylglycerol synthesis and impeding lipolysis (Ormazabal et al., 2018). Pathological remodelling of WAT during obesity with concomitant increases in FFAs and inflammatory cytokines promotes system-wide inflammation, which is recognised as the primary mechanism in insulin resistance (Hotamisligil et al., 1993; Kahn et al., 2006; Luft et al., 2013; Weisberg et al., 2003; Xu et al., 2003). During insulin resistance, the main insulin-sensitive tissues (liver, skeletal muscle, and adipose tissue) become desensitised to insulin action and progressively fail to uptake glucose and synthesis glycogen, resulting in hyperglycaemia and hyperlipidaemia (Ormazabal et al., 2018). Consequently, higher insulin concentrations are required to elicit a normal response (Wang et al., 2004). Pancreatic β-cells respond to insulin resistance with increased secretion, ultimately leading to β-cell exhaustion and enduring hyperglycaemia if insulin resistance persists (Shulman, 2000).

Evidence from human epidemiological studies suggests that helminth infections can downregulate inflammatory immune responses and promote glucose homeostasis (de Ruiter et al., 2017).

Helminth infection has significant and long-lasting consequences on metabolic function in the host. A systematic review and meta-analysis of four early studies showed a 50% reduced likelihood of metabolic dysfunction in adults with previous or current worm infections (Tracey et al., 2016). In a later study, Mohamed et al. analysed anthropometric and metabolic measurements in adults with and without previous schistosome infection (Mohamed et al., 2017). Infected individuals had a significantly lower prevalence of MetS and its components (central obesity, hypertension, and abnormal lipid profile). A 2017 Chinese study also described improved markers of MetS and reduced body mass in adults with schistosomiasis compared to uninfected controls (Q. Duan et al., 2018). Similarly, a 2019 cross-sectional study in Ethiopia compared schistosome-infected adults with non-infected and reported lower fasting blood sugar and more favourable lipid profiles in infected compared to uninfected participants (Wolde et al., 2019). While these cross-sectional studies supported the idea of a protective role of helminths in metabolic disease, they did not provide evidence of a causal relationship between helminth infection and improved metabolic health.

Deworming studies delivered the first fragments of causal evidence in humans to advance our understanding of worms and metabolism. The enduring co-evolution of worms and humans has led to an intricate relationship between worms and the human immune system (Loukas et al., 2016), which has even inspired claims that the entire Th2 response may have essentially evolved to control the parasites and remedy the damage caused by their migration and feeding (Allen & Sutherland, 2014). Recent mass deworming programmes offered a unique opportunity to study the effects of removing one part of this intimate relationship on immune and metabolic responses. A cluster-randomised double-blind clinical trial in Indonesia noted significantly increased insulin resistance (Tahapary, de Ruiter, Martin, Brienen, van Lieshout, Cobbaert, et al., 2017) and significantly increased leptin-adiponectin ratio in previously infected individuals that may have contributed to the increases in insulin resistance after deworming (Tahapary, de Ruiter, Martin, Brienen, van Lieshout, Djuardi, et al., 2017). Subsequent studies in India, Thailand, and Uganda described that worm removal increased levels of insulin and glucagon (Rajamanickam et al., 2018), enhanced hyperglycaemia, HbA1c, and risk of metabolic disease (Muthukumar et al., 2019), and worsened blood lipid profiles (Sanya et al., 2019). A 2019 systematic review and meta-analysis of all previous cross-sectional and deworming studies investigating metabolic function concluded that helminth infection was typically associated with improved metabolic function; however, improvements in individual parameters may be helminth species-specific (Rennie et al., 2021). Experimental evidence from several mouse studies corroborates these findings from human studies.

Mouse studies using experimental infection with various helminth species or treatment with helminth-derived molecules consistently showed improvements in metabolic homeostasis and

inflammation. A landmark 2011 study first demonstrated the role of IL-4 secreting eosinophils in preserving insulin sensitivity, inspiring the idea that type 2 immunity is linked to adipose tissue metabolic homeostasis (D. Wu et al., 2011). As helminths are known to induce physiological eosinophil elevations, the authors infected mice on a high-fat diet with *N. brasiliensis.* They reported improved insulin sensitivity and glucose tolerance, lower fasting glucose, and reduced perigonadal fat mass. Notably, these improvements were maintained up to 45 days after parasite clearance. These findings of a protective role of helminth infection in metabolic homeostasis have since been replicated in multiple other publications. Whole worm infection studies (Berbudi, Surendar, et al., 2016; Q. Duan et al., 2018; Hussaarts et al., 2015; Khudhair et al., 2021; Morimoto et al., 2017; Pace et al., 2018; Shimokawa et al., 2019; Su et al., 2018; Yang et al., 2013), treatment with parasite antigen mixtures or ES products (Berbudi, Surendar, et al., 2016; Bhargava et al., 2012; Hussaarts et al., 2015; Khudhair et al., 2022; van den Berg et al., 2017), and treatment with single parasite molecules (Crowe et al., 2020; Hams et al., 2016; van der Zande et al., 2021) all resulted in marked improvements in glucose control and reduced body mass. In combination, these findings suggest that worm therapy could present a potentially beneficial treatment for human metabolic disorders.

The current clinical trial aimed to determine the influence of infection with *Na* on key metabolic and physical parameters, including insulin resistance assessed by HOMA-IR, body mass, central adiposity, and blood lipid profile; the primary metabolic outcome was HOMA-IR. I hypothesised that infection would improve these parameters.

4.2 Results

Notes: For one male L3-10 participant, wk78 data were excluded from all metabolic analyses, as he had undergone gastric sleeve surgery five months earlier, as this procedure affects all metabolic outcomes investigated in this chapter (Lechea et al., 2019). Similarly, wk104 data for one female Placebo who had undergone the same surgery nine weeks prior to the visit were excluded. One outlier with abnormally high values was removed from wk26 glucose, insulin, and HOMA-IR analyses. For the same participant, wk78 metabolic data except blood lipids were excluded, as he had started taking metformin some weeks before the visit. A further L3-10 participant was retrospectively discovered to have taken metformin at wk78 and wk104, excluding these visits from glucose metabolism, body mass, and central adiposity, but not blood lipid analyses.

4.2.1 Glucose homeostasis

4.2.1.1 Insulin resistance

Insulin resistance, assessed by HOMA-IR, was the trial's primary metabolic outcome. For HOMA-IR, I have presented absolute values and changes for both ITT and PP-based analyses but focused on PP results only for all other metabolic outcomes, as the participant in question (see Section 3.2.1.2) was effectively worm-infected. For ITT analyses (Table 4-1), Kruskal Wallis (KW) tests did not identify any significant differences in absolute values or changes between groups at any time point. Longitudinal analyses (Mixed-effects model) did not reveal significant differences between time points in any group. Although not statistically significant, median HOMA-IR improved considerably in the L3-10 group from 3.01 units at baseline to 1.67 units at wk78, with smaller improvements in the L3-20 group.

For PP analyses (Table 4-2) KW did not find significant intercohort differences in absolute values but showed significantly different changes between Placebo and L3-10 at wk52 (KW $p = 0.0448$, adjusted $p = 0.0387$) and between L3-10 and L3-20 at wk78 (KW $p = 0.0344$, adjusted $p = 0.0486$). These differences translated to a reduction in median HOMA-IR of 1.09 units in the L3-10 group compared to a median increase of 0.80 units in the Placebo group. By wk78, median HOMA-IR in the L3-10 group was further reduced by 1.48 units, significantly greater than the median reduction of 0.25 in the L3-20 group. The Mixed-effects model omnibus results were not significant in either group, but in the L3-10 group, Tukey's posthoc tests found significantly ($p = 0.0275$) reduced HOMA-IR in the L3-10 group at wk78 (1.57 units) compared to baseline (2.95 units). No other significant inter-cohort or longitudinal differences were evident.

As significance tests depend greatly on sample size, and numbers in all groups were low, I also calculated the effect sizes of treatment versus control for a measure of the treatment effect independent of participant numbers (Table 4-3). Effect sizes for HOMA-IR changes in the L3-10 versus Placebo

group were medium at wk26 (d = 0.76) and large at wk52 (d = 1.24), wk78 (d = 1.01) and wk104 (d = 1.03), indicating a considerable treatment effect in the L3-10 group. For the L3-20 group, effect sizes were moderate at wk26 (d = 0.55) and wk78 (d = 0.75) and small at wk104 (d = 0.25).

Longitudinal median changes in the Placebo group did not demonstrate a consistent trend in either direction, fluctuating between improvements and worsening throughout the trial. Also, changes among participants in the Placebo group were heterogeneous (Figure 4-1), with some individuals experiencing worsening HOMA-IR while others showed improvements. In contrast, longitudinal median HOMA-IR changes in the worm treatment groups were more consistent with improvements at every evaluation time point. Likewise, changes in worm-infected participants were more homogenous, with nearly all individuals showing improvements in HOMA-IR. I have explored possible correlates for protection in Chapter 5 to shed some light on these observations.

In summary, *Na* infection improved insulin resistance in the worm treatment groups. Notably, the lower dose of 20 worms in the L3-10 group was more effective than the higher dose of 40 in the L3-20 group. Insulin resistance in the Placebo group worsened initially but returned to near baseline values by wk104.

Table 4-1 Intention-to-treat fasting HOMA-IR (units) absolute values and changes from baseline throughout the trial

	Placebo	L3-10	L3-20
Baseline	2.18 2.01-2.82 *n = 13*	3.01 2.25-3.58 *n = 12*	2.36 2.12-3.00 *n = 11*
wk26	2.35 2.08-3.04 *n = 13*	2.21 1.33-3.38 *n = 10*	2.05 1.78-2.58 *n = 11*
Δ wk26	-0.23 -0.61-0.94 *n = 13*	-0.91 -1.91-0.16 *n = 10*	-0.48 -1.21-0.16 *n = 11*
wk52	2.45 1.60-3.95 *n = 10*	2.01 1.68 - 2.86 *n = 10*	2.40 1.65-3.22 *n = 10*
Δ wk52	0.38 -0.96-2.32 *n = 10*	-1.05 -1.49- (-0.35) *n = 10*	-0.44 -1.37-0.87 *n = 10*
wk78	1.71 1.52-3.52 *n = 9*	1.67 0.73-3.13 *n = 7*	2.54 1.91-3.14 *n = 8*
Δ wk78	-0.50 -1.13-1.80 *n = 9*	-1.47 -1.80-0.20 *n = 7*	-0.25 -0.39-0.37 *n = 8*
wk104	2.43 1.38-2.54 *n = 7*	2.01 1.41-2.17 *n = 6*	1.53 1.03-4.83 *n = 9*
Δ wk104	-0.52 -0.80-0.44 *n = 7*	-0.98 -1.89 - (-0.33) *n = 6*	-0.48 -1.23-1.12 *n = 9*

Data are presented as median and IQR. No significant differences were found.

Table 4-2 Per-protocol fasting HOMA-IR (units) absolute values and changes from baseline throughout the trial

	Placebo	L3-10	L3-20
baseline	2.18 1.97-2.62 *n = 12*	2.95 2.27-3.55 *n = 13*	2.36 2.12-3.00 *n = 11*
wk26	2.55 2.06-3.09 *n = 12*	2.27 1.47-3.17 *n = 11*	2.05 1.78-2.58 *n = 11*
Δ wk26	-0.19 -0.58-0.98 *n = 12*	-0.60 -1.87- (-0.09) *n = 11*	-0.48 -1.21-0.16 *n = 11*
wk52	2.93 1.76-4.20 *n = 9*	1.78 1.68-2.49 *n = 11*	2.40 1.65-3.22 *n = 10*
Δ wk52	0.80 -0.74-2.34 *n = 9*	**-1.09*** -1.47-(-0.48) *n = 11*	-0.44 -1.37-0.87 *n = 10*
wk78	2.00 1.59-3.84 *n = 8*	**1.57**[&] 0.91-2.98 *n = 8*	2.54 1.91-3.14 *n = 8*
Δ wk78	-0.16 -0.91-2.33 *n = 8*	**-1.48†** -1.77-(-0.05) *n = 8*	-0.25 -0.39-0.37 *n = 8*
wk104	1.95 1.36-2.74 *n = 6*	2.09 1.49-2.27 *n = 7*	1.5 1.03-4.83 *n = 9*
Δ wk104	-0.26 -0.81-0.56 *n = 6*	-0.8 -1.34-(-0.37) *n = 7*	-0.48 -1.23-1.12 *n = 9*

Data are presented as median and IQR; *significant difference to Placebo; [&] significant difference to baseline; † significant difference to L3-20

Table 4-3 HOMA-IR change effect sizes at each evaluation visit

	Placebo	L3-10	L3-10 vs Placebo	L3-20	L3-20 vs Placebo
wk26	0.180 ± 1.128 *n* = 12	-0.742 ± 1.285 *n* = 11	*d* = 0.76[M]	-0.417 ± 1.048 *n* = 11	*d* = 0.55[M]
wk52	0.987 ± 2.002 *n* = 9	-0.915 ± 0.852 *n* = 11	*d* = 1.24[L]	-0.259 ± 1.258 *n* = 10	*d* = 0.75[M]
wk78	0.794 ± 2.474 *n* = 8	-1.090 ± 0.880 *n* = 8	*d* = 1.01[L]	0.294 ± 1.314 *n* = 8	*d* = 0.25[S]
wk104	-0.133 ± 0.768 *n* = 6	-1.133 ± 1.142 *n* = 7	*d* = 1.03[L]	-0.042 ± 1.790 *n* = 9	*d* = -0.15

M, medium effect size; L, large effect size; S, small effect size.

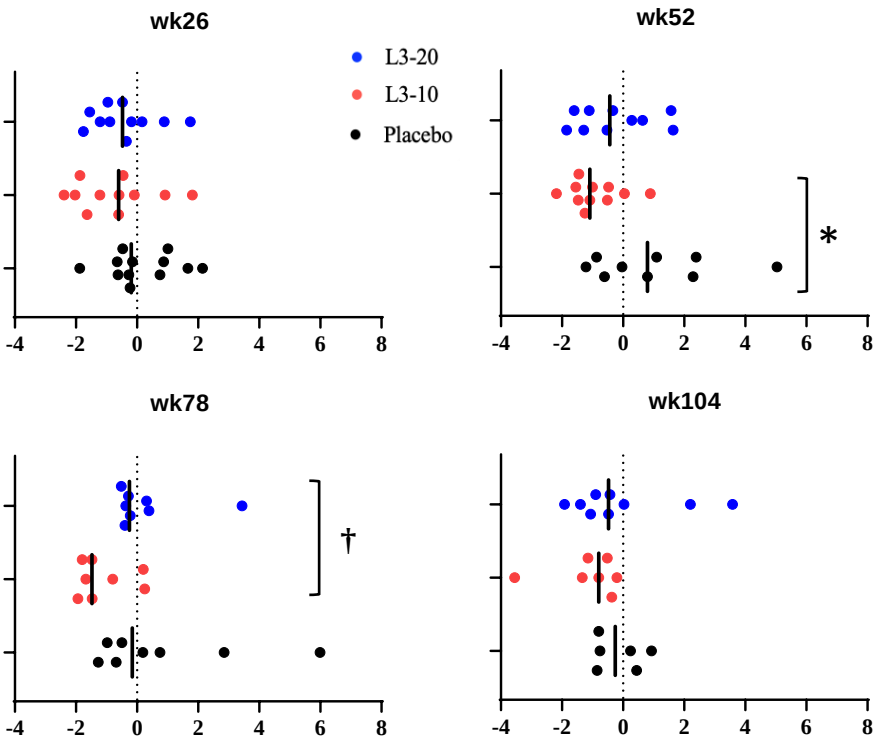


Figure 4-1 HOMA-IR changes (units) by treatment group at each evaluation visit
Individual data points are shown for each participant, and median values in each treatment group are indicated by the vertical lines.
* significant difference between Placebo and L3-10; † significant difference between L3-10 and L3-20 (Kruskal-Wallis test)

HOMA-IR is calculated from blood glucose and insulin values. To investigate if either of these components was the driving factor in HOMA-IR changes, I separately analysed changes in fasting blood glucose and serum insulin (Table 4-4, Figure 4-2, and Figure 4-3). I also examined glycated haemoglobin levels (HbA1c IFCC) to understand the potential effects on average blood sugar levels over three months (Table 4-4 and Figure 4-4).

4.2.1.2 Fasting blood glucose

Kruskal-Wallis tests showed no significant differences in absolute median values between groups at all time points. In the Placebo group, median values remained stable or increased until wk52 but were reduced slightly at wk78 and wk104, consistent with the HOMA-IR changes at these time points. In contrast, both worm treatment groups experienced reductions in fasting glucose at all evaluation time points, reflecting the HOMA-IR improvements in these groups (Table 4-4). Median changes (Figure 4-2) in the L3-20 treatment group were significantly different to the Placebo group at wk26 (KW p = 0.0244, adjusted p = 0.0265) and wk52 (KW p = 0.0290, adjusted p = 0.0243). Longitudinal analyses by Mixed-effects models revealed no significant treatment effect in the Placebo group. In the L3-10 group, the omnibus result was insignificant, but Tukey's multiple comparisons showed significantly lower than baseline median fasting glucose (adjusted p = 0.0454) at wk26. In the L3-20 group, significantly lower (Mixed-effects model p = 0.0015) median values compared to baseline were evident at wk26 (adjusted p = 0.0158), wk52 (adjusted p = 0.0068), and wk104 (adjusted p = 0.0151) (Table 4-4).

4.2.1.3 Insulin

As for fasting glucose, KW tests found no significant differences in absolute values between groups at any time point. Median Placebo values were mainly stable or slightly elevated compared to baseline at evaluation time points. In the L3-10 group, median values were consistently lower than baseline at all evaluation visits, whereas results for the L3-20 group were more inconsistent (Table 4-4). Change in the L3-10 group was opposite and significantly different to Placebo at wk52 (KW p = 0.0187, adjusted p = 0.0195) and significantly different to L3-20 at wk78 (p = 0.0142) (Figure 4-3). Omnibus results for longitudinal Mixed-effects model analyses were not significant in any group; however, Tukey's multiple comparisons identified significantly lower insulin compared to baseline in the L3-10 group at wk78 (adjusted p = 0.0431). Together these results indicate that both infected groups experienced similar blood glucose reductions. However, insulin only declined in the L3-10 group, driving the more significant HOMA-IR reduction in this group.

4.2.1.4 HbA1c IFCC

Median absolute HbA1cIFCC values, as assessed by KW tests, were not significantly different between groups at any time point. Values in the Placebo group showed an upward trend throughout the trial, while values in the L3-10 group experienced a modest decline before returning to baseline by wk104 (Table 4-4). At wk78, the median change in the L3-10 group was significantly different (KW, $p = 0.0353$) from the change in the L3-20 group (Figure 4-4). In the L3-20 group, no consistent trend was evident, but at wk78, mixed-effects models found a significant difference to baseline ($p = 0.0273$) and wk26 ($p = 0.0067$); values at wk104 equalled baseline values (Table 4-4). These results imply that hookworm infection did not alter HbA1c levels but may have prevented worsening, as seen in the Placebo group.

Table 4-4 Fasting blood glucose (mmol/L), serum insulin (mU/L), and glycated haemoglobin (mmol/L) absolute values and changes from baseline by treatment group throughout the trial

	baseline	wk26	Δ wk26	wk52	Δ wk52	wk78	Δ wk78	wk104	Δ wk104
Fbg (mmol/L)									
Placebo	4.8 4.5-5.1 *n = 12*	5.0 4.0-5.3 *n = 12*	0.0 -0.5-0.5 *n = 12*	4.8 4.4-5.4 *n = 9*	0.3 -0.4-0.8 *n = 9*	4.6 4.2-5.3 *n = 8*	-0.1 -0.7-0.6 *n = 8*	4.0 3.1-5.1 *n = 6*	-0.8 -1.9-0.6 *n = 6*
L3-10	5.2 4.7-5.6 *n = 13*	**4.5**[&] 4.0-5.1 *n = 11*	-0.7 -1.0- (-0.3) *n = 11*	4.2 3.6-5.6 *n = 11*	-0.9 -1.1-0.6 *n =11*	4.5 4.2-4.7 *n = 8*	-0.5 -1.0-0.1 *n = 8*	4.7 3.9-4.8 *n = 7*	-0.6 -1.2- (-0.4) *n = 7*
L3-20	5.3 4.9-5.4 *n = 11*	**4.3**[&] 4.0-5.3 *n = 11*	**-0.7*** -1.0- (-0.6) *n = 11*	**4.1**[&] 3.7-4.9 *n = 10*	**-1.0*** -1.4- (-0.7) *n = 10*	4.5 4.1-5.1 *n = 8*	-0.4 -1.2- (-0.0) *n = 8*	**4.3**[&] 4.0-4.9 *n = 9*	-0.6 -1.1- (-0.4) *n = 9*
Insulin (mU/L)									
Placebo	10.5 9.3-11.8 *n = 12*	12.0 10.0-14.5 *n = 12*	1.0 -1.8-3.8 *n = 12*	12.0 8.0-19.0 *n = 9*	2.0 -3.0-9.5 *n = 9*	10.5 8.0-15.8 *n = 8*	0.0 -3.0-8.0 *n = 8*	11.0 9.5-13.5 *n = 6*	0.5 -0.5-2.3 *n = 6*
L3-10	13.0 11.5-15.5 *n = 13*	10.0 7.0-14.0 *n = 11*	-2.0 -7.0-1.0 *n = 11*	10.0 9.0-14.0 *n = 11*	**-4.0*** -5.0- (-3.0) *n = 11*	**9.0**[&] 4.8-13.8 *n = 8*	**-4.5†** -7.0- (-0.8) *n = 8*	10.0 7.0-12.0 *n = 7*	-3.0 -4.0-1.0 *n = 7*
L3-20	10.0 9.0-13.0 *n = 11*	10.0 9.0-15.0 *n = 11*	-1.0 -3.0-2.0 *n = 11*	11.5 9.3-17.5 *n = 10*	1.0 -4.0- 6.0 *n = 10*	12.0 9.8-16.8 *n = 8*	2.0 -1.0-3.0 *n = 8*	8.0 6.0-21.5 *n = 9*	-1.0 -4.3-10.8 *n = 9*
HbA1c IFCC (mmol/mol)									
Placebo	31.5 30.0-33.0 *n = 12*	33.5 30.0-35.8 *n = 12*	1.0 0.0-3.0 *n = 12*	34.0 29.5-36.5 *n = 9*	2.0 0.0-4.0 *n = 9*	32.5 30.3-35.8 *n = 8*	2.5 0.0-4.0 *n = 8*	35.5 33.3-37.0 *n = 6*	3.5 1.0-5.3 *n = 6*
L3-10	33.0 30.5-35.0 *n = 13*	32.0 30.0-34.0 *n = 11*	0.0 -1.0-1.0 *n = 11*	32.0 31.0-35.0 *n = 11*	0.0 -1.0-1.0 *n = 11*	32.5 32.0-36.0 *n = 8*	**1.0†** 0.0-2.0 *n = 8*	33.0 32.0-37.0 *n = 7*	2.0 1.0-4.0 *n = 7*
L3-20	32.0 29.0-34.0 *n = 11*	31.0 30.0-35.0 *n = 11*	1.0 0.0-3.0 *n = 11*	33.0 31.8-35.0 *n = 10*	2.5 0.8-4.0 *n = 10*	**33.5**[&#] 33.0-36.5 *n = 8*	4.0 2.3-6.5 *n = 8*	32.0 31.0-37.0 *n = 9*	2.0 2.0-4.5 *n = 9*

Data are presented as median and IQR. Fbg, fasting blood glucose; HbA1c IFCC, IFCC standardised glycated haemoglobin. [&]significant difference to baseline; * significant difference to Placebo; † significant difference to L3-20; # significant difference to wk26

FASTING BLOOD GLUCOSE

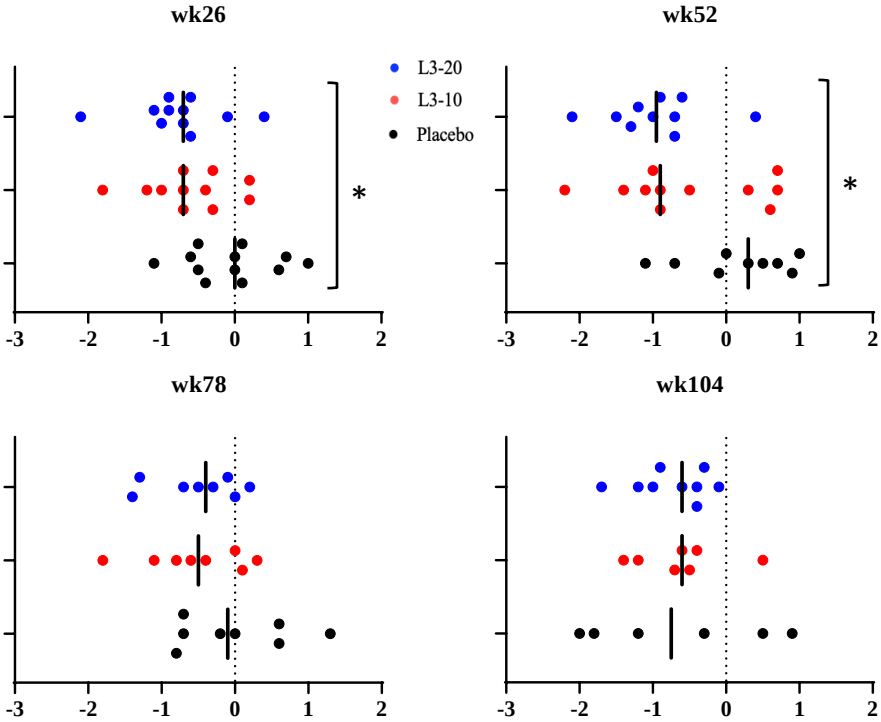


Figure 4-2 Fasting blood glucose changes (mmol/L) from baseline by treatment group at each evaluation visit
Individual data points are shown for each participant, and median values in each treatment group are indicated by the vertical lines. *significant difference between Placebo and L3-20 (Kruskal-Wallis test)

FASTING INSULIN

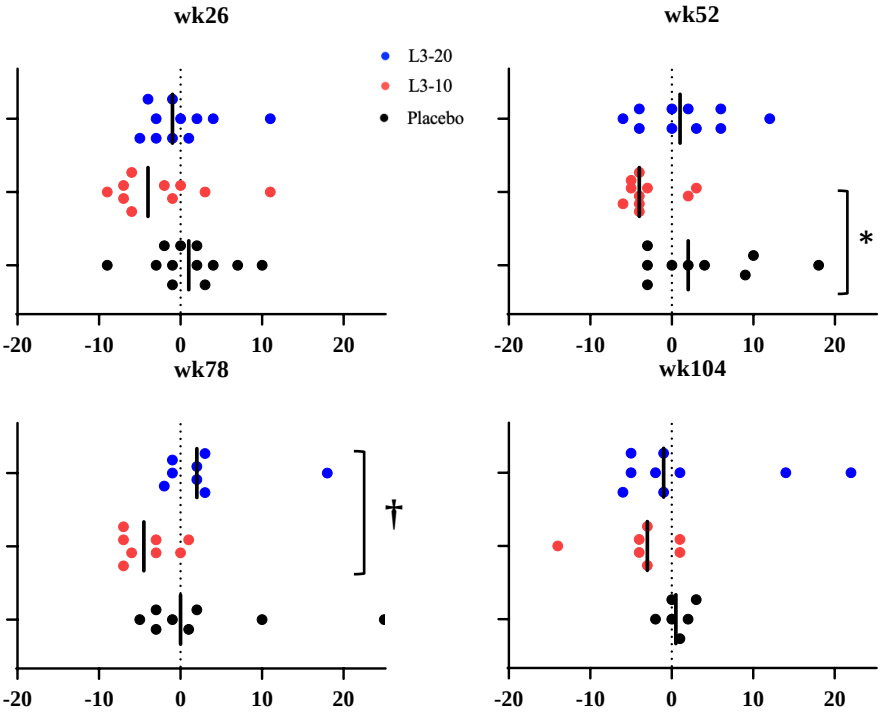


Figure 4-3 Fasting insulin changes (mU/L) from baseline by treatment group at each evaluation visit.
Individual data points are shown for each participant, and median values in each treatment group are indicated by the vertical lines. * significant difference between Placebo and L3-10; † significant difference between L3-10 and L3-20 (Kruskal-Wallis test)

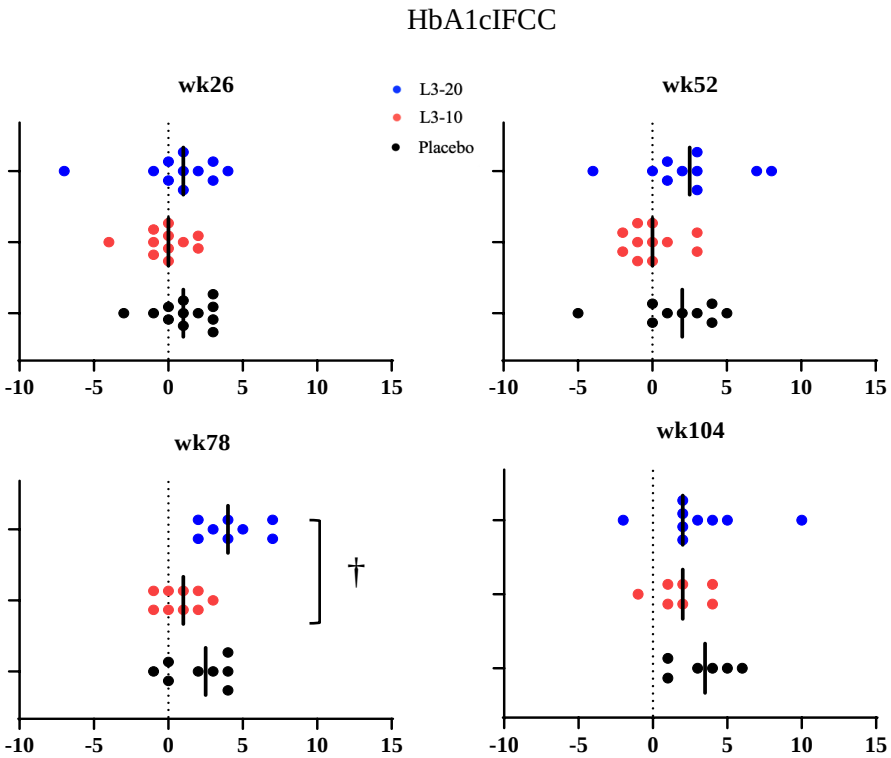


Figure 4-4 HbA1c IFCC changes (mmol/mol) from baseline by treatment group at each evaluation visit.
Individual data points are shown for each participant, and median values in each treatment group are indicated by the vertical lines. † significant difference between L3-10 and L3-20 (Kruskal-Wallis test)

4.2.2 Body mass

Median body mass was similar in all groups at baseline and not significantly different (KW) between groups at any time point (Table 4-5). The Placebo group experienced a small median loss at wk52 but had returned to baseline by wk104. In the L3-10 group, median body mass remained largely stable until wk52 but had dropped by approximately 10kg by wk78 and wk104. In contrast, median body mass in the L3-20 group was increased after wk26. At wk104, the change in body mass was opposite and significantly different between L3-10 and L3-20 (KW $p = 0.0169$, adjusted $p = 0.0243$) (Figure 4-5). Mixed-effects model omnibus test results were not significant in any group, but in the L3-10 group, Tukey's multiple comparisons suggested a significant difference to baseline at wk78 (adjusted $p = 0.0192$) and wk104 (adjusted $p = 0.0313$). Together these results suggest that worm infection did not induce consistent body mass loss in humans with metabolic disease.

Table 4-5 Body mass absolute values and changes (kg) from baseline by treatment group throughout the trial

	Placebo	L3-10	L3-20
Baseline	104.5 89.5-115.1 *n = 12*	102.8 86.4-131.0 *n = 13*	101.5 90.0-130.5 *n = 11*
wk26	106.7 90.9-115.3 *n = 12*	101.4 81.6-133.8 *n = 12*	101.5 86.2-130.0 *n = 11*
Δ wk26	2.2 -3.7-3.8 *n = 12*	-2.5 -3.3-1.0 *n = 12*	-0.5 -2.3-1.2 *n = 11*
wk52	99.2 89.8-113.0 *n = 8*	104.0 90.0-133.0 *n = 11*	106.4 91.3-129.3 *n = 10*
Δ wk52	0.4 -4.3-3.9 *n = 8*	-0.6 -5.2-1.4 *n = 11*	0.9 -2.3-5.6 *n = 10*
wk78	105.0 88.8-119.3 *n = 8*	**92.8**[&] 76.4-120.0 *n = 8*	109.2 100.0-136.3 *n = 8*
Δ wk78	-0.8 -8.0-8.6 *n = 8*	-5.0 -12.1- (-2.7) *n = 8*	1.7 -0.9-8.2 *n = 8*
wk104	105.5 90.7-117.8 *n = 6*	**93.3**[&] 77.2-124.0 *n = 7*	107.8 94.5-132.6 *n = 9*
Δ wk104	2.3 -3.6-4.9 *n = 6*	**-5.0†** -9.5- (-1.3) *n = 7*	3.7 -1.8-5.9 *n = 9*

Data are presented as median and IQR. [&] significant difference to baseline; † significant difference to L3-20

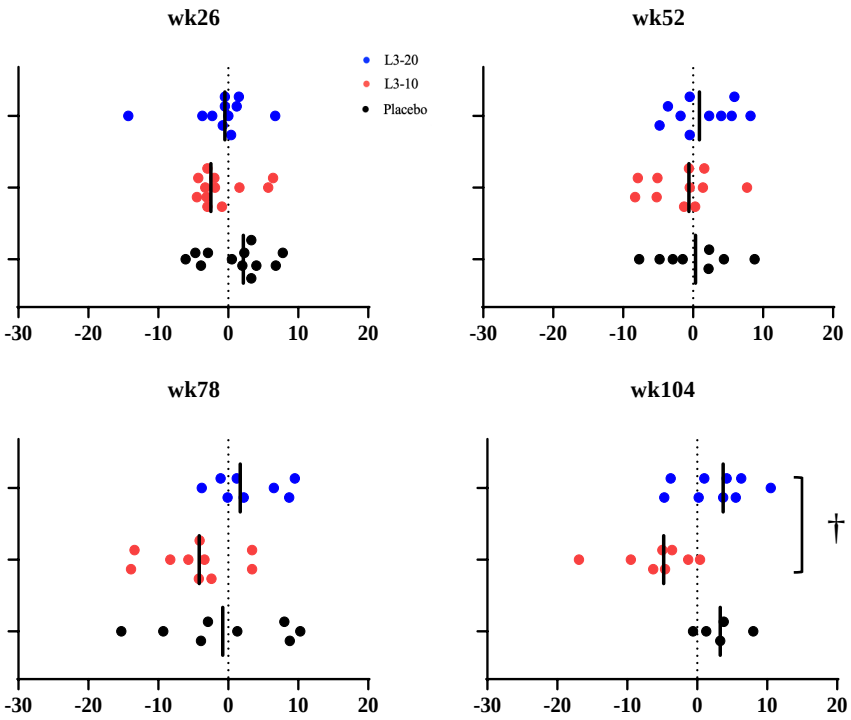


Figure 4-5 Body mass changes (kg) from baseline
Individual data points are shown for each participant, and median values in each treatment group are indicated by the vertical lines. † significant difference between L3-10 and L3-20 (Kruskal-Wallis test)

4.2.3 Central adiposity - waist circumference and body fat distribution

4.2.3.1 Waist circumference

Mean waist circumference was similar at baseline and remained largely stable in all treatment groups throughout the trial (Table 4-6). One-way ANOVA tests showed no significant differences in mean absolute values between treatment groups at any time point. Also, Mixed-effects analyses detected no significant differences in absolute values over time in any treatment group. The mean change at wk104 was opposite and significantly different (ANOVA $p = 0.0488$, adjusted $p = 0.0464$) between L3-10 and L3-20 (Figure 4-6), reflecting the changes in body mass at this time point. As seen with body mass, changes in each treatment group were heterogeneous, with reductions in some individuals contrasting expansions in others

Table 4-6 Waist circumference totals and changes (cm) from baseline by treatment group throughout the trial

	Placebo	L3-10	L3-20
Baseline	112.7 104.2, 121.1 *n = 12*	113.1 102.8, 123.5 *n = 13*	112.2 106.1, 118.3 *n = 11*
wk26	111.0 102.0, 119.9 *n = 12*	113.0 101.3, 124.7 *n = 12*	109.5 102.4-116.5 *n = 11*
Δ wk26	-1.7 -5.4, 2.0 *n = 12*	-0.3 -4.4, 3.8 *n = 12*	-2.7 -5.7, 0.3 *n = 11*
wk52	106.5 97.5, 115.5 *n = 8*	115.5 106.0, 124.9 *n = 11*	110.6 105.1, 116.1 *n = 10*
Δ wk52	-3.2 -8.5, 2.1 *n = 8*	-1.4 -5.3, 2.4 *n = 11*	-0.7, -3.1-1.8 *n = 10*
wk78	108.9 98.4, 119.4 *n = 8*	107.4 46.2, 120.5 *n = 8*	111.6 104.1, 119.2 *n = 8*
Δ wk78	-3.2 -9.9, 3.5 *n = 8*	-5.2 -9.4, -0.9 *n = 8*	-1.0 -3.3, 1.4 *n = 8*
wk104	111.0 100.5, 121.5 *n = 5*	107.0 91.4-122.6 *n = 7*	112.1 106.3, 117.9 *n = 9*
Δ wk104	-0.2, -8.5, 8.1 *n = 5*	**-5.8**† -10.1, -1.4 *n = 7*	0.9 -2.5, 4.3 *n = 9*

Data are presented as mean and 95% confidence interval. † significant difference to L3-20

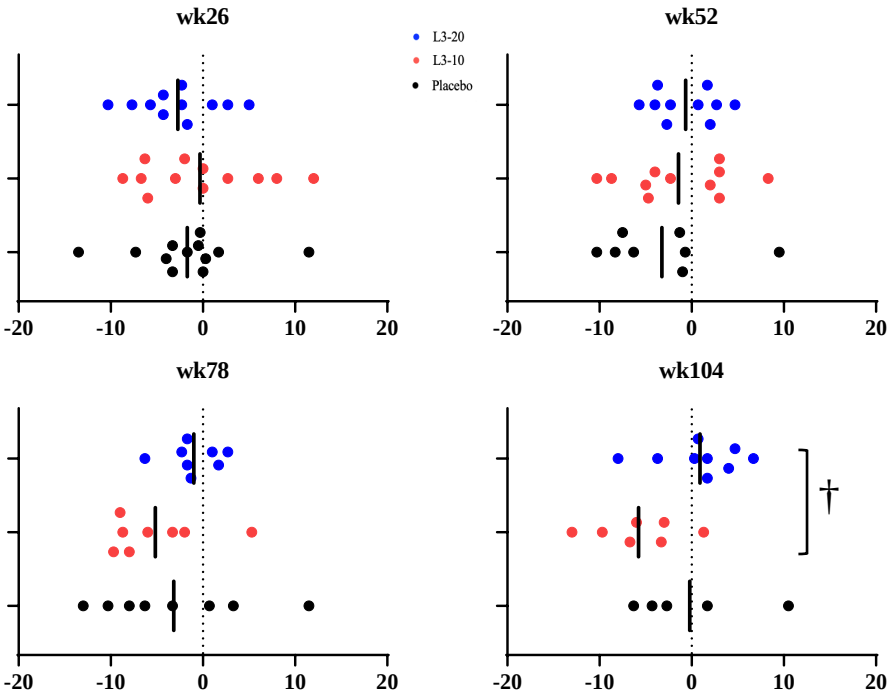


Figure 4-6 Waist circumference changes (cm) from baseline by treatment group at each evaluation visit.
Individual data points are shown for each participant, and mean values in each treatment group are indicated by the vertical lines. † significant difference between L3-10 and L3-20 (One-way ANOVA)

4.2.3.2 Body fat distribution

The trial protocol asked participants to attend a local X-ray service at baseline, wk52, and wk104 for a body composition scan by dual-energy X-ray absorptiometry (DEXA). DEXA is one of the most accurate measurements of body fat distribution and less subject to measurement variation than waist circumference assessment. In particular, I was interested in android fat changes as a complementary measure of central adiposity, but the report also included gynoid fat, total fat percentage, and android-gynoid fat ratio (Table 4-7).

Thirty-four participants attended the baseline scan, with a further two individuals unable to obtain a reading as they exceeded the equipment's weight limit. Only 18 participants completed their wk52 scan, and six completed the wk104 scan. The poor compliance indicates that this activity may have demanded undue time commitment, or the baseline body scan image and report may have been confronting, leading to avoidance of later scans.

Kruskal-Wallis tests found no significant differences in absolute values or changes between groups at baseline and wk52. The low compliance at wk104 does not support statistical analysis or conclusions regarding differences between groups or over time.

Table 4-7 Body fat distribution assessed by dual-energy X-ray absorptiometry (DEXA)

	baseline	wk52	Δ wk52	wk104	Δ wk104
Android fat (%)					
Placebo	54.4 50.7-57.6 *n = 11*	51.2 46.4-56 *n = 2*	-3.5 -4.3- (-2.7) *n = 2*	53.0 50.1-55.8 *n = 2*	-0.6 -2.9-1.8 *n = 2*
L3-10	54.1 50.3-60.0 *n = 12*	54.5 47.6-60.0 *n = 8*	-0.4 -3.6-1.1 *n = 8*	57.3 53.7-65.4 *n = 3*	-0.6 -7.0-3.9 *n = 3*
L3-20	54.0 49.8-58.8 *n = 11*	52.8 51.3-60.5 *n = 8*	0.5 -0.8-2.4 *n = 8*	52.2 52.2-52.2 *n = 1*	4.4 4.4-4.4 *n = 1*
Gynoid fat (%)					
Placebo	45.8 42.4-53.6 *n = 11*	48.6 45.4-51.7 *n = 2*	-2.5 -4.5- -0.4 *n = 2*	51.6 47.4-55.7 *n = 2*	0.6 -0.5-1.7 *n = 2*
L3-10	53.5 43.5-56.2 *n = 12*	49.6 39.3-58.4 *n = 8*	-0.6 -1.6-0.5 *n = 8*	54.3 52.6-60.3 *n = 3*	-1.7 -3.7- (-1.0) *n = 3*
L3-20	47.0 42.3-54.0 *n = 11*	48.1 41.8-54.6 *n = 8*	-0.7 -3.4-2.8 *n = 8*	39.6 39.6-39.6 *n = 1*	-2.2 -2.2- -2.2 *n = 1*
Android-gynoid ratio					
Placebo	1.11 1.06-1.19 *n = 11*	1.05 1.02-1.08 *n = 2*	-0.02 -0.09-0.04 *n = 2*	1.03 1.00-1.06 *n =2*	-0.02 -0.04-0.00 *n = 2*
L3-10	1.06 0.99-1.25 *n = 12*	1.10 1.00-1.37 *n = 8*	0.01 -0.04-0.04 *n = 8*	1.06 1.02-1.09 *n = 3*	0.03 -0.06-0.09 *n = 3*
L3-20	1.14 1.07-1.22 *n = 11*	1.16 1.06-1.23 *n = 8*	0.02 -0.03-0.09 *n = 8*	1.32 1.32-1.32 *n = 1*	0.18 0.18-0.18 *n = 1*
Total fat					
Placebo	45.1 43.8-52. *n = 11*	46.5 43.3-49.7 *n = 2*	-2.3 -2.7- (-1.8) *n = 2*	48.5 45.2-51.7 *n = 2*	0.4 -0.7-1.4 *n = 2*
L3-10	47.4 43.4-52.7 *n = 12*	46.2 43.0-54.2 *n = 8*	-0.8 -3.0-0.3 *n = 8*	52.1 49.1-57.1 *n = 3*	-0.7 -3.4-0.4 *n = 2*
L3-20	44.4 41.5-50.8 *n = 11*	45.9 42.6-51.8 *n = 8*	-0.5 -0.9-1.4 *n = 8*	41.5 41.5-41.5 *n = 1*	0.0 0.0-0.0 *n = 1*

Data are presented as median and IQR. No significant differences were found.

4.2.4 Blood lipids

4.2.4.1 Total cholesterol

Median total cholesterol declined moderately in the Placebo and L3-10 groups throughout the trial. In contrast, levels rose in the L3-20 group before returning to baseline values by wk104, with a modest reduction in the L3-10 group (Table 4-8). Kruskal-Wallis tests did not reveal significant differences in absolute values between groups at any time point. However, at wk26, the change in the L3-20 group was opposite and significantly different to Placebo (KW $p = 0.0164$, adjusted $p = 0.0410$) and to L3-10 (adjusted $p = 0.0338$). Change in the L3-20 group was also opposite and significantly different to Placebo at wk78 (KW $p = 0.0427$, adjusted $p = 0.0382$). Mixed-effects model omnibus results were not significant, but Tukey's multiple comparisons indicated significantly lower than baseline levels in the Placebo group at wk78 (adjusted $p = 0.0368$).

4.2.4.2 Triglycerides

Median triglyceride levels remained largely stable in the Placebo and L3-10 groups but increased towards wk104 in the Placebo group. In the L3-20 group, levels rose slightly during the trial before returning to baseline at wk104 (Table 4-8). Like total cholesterol, KW tests showed no significant differences in absolute values between groups at any time point. The KW omnibus result suggested a significant difference in changes between groups at 104 (KW $p = 0.0443$), but post hoc comparisons were not significant. Longitudinal analyses by Mixed-effects model did not reveal significant differences between time points in any group.

4.2.4.3 High-density lipoprotein (HDL)

In all groups, median HDL levels fluctuated only marginally. No significant inter-cohort differences in absolute values or changes (KW) were evident. Likewise, longitudinal analyses (Mixed-effects model) found no significant differences between time points in any group (Table 4-8).

4.2.4.4 Low-density lipoprotein (LDL)

In contrast to HDL, median LDL values decreased in all groups throughout the trial despite a small surge in the L3-20 group at wk78 (Table 4-8). Kruskal-Wallis tests did not identify significant inter-cohort differences in absolute values. However, change in the L3-10 group at wk26 was opposite and significantly different to the L3-20 group (KW $p = 0.0219$, adjusted $p = 0.0414$). Similarly, change in the L3-20 group was opposite and significantly different to the Placebo group at wk78 (KW $p = 0.0241$, adjusted $p = 0.0193$). While Mixed-effects model omnibus results were not significant in any group, Tukey's multiple comparisons suggested a significantly lower LDL than baseline at wk78 in the Placebo group (adjusted $p = 0.0090$).

4.2.4.5 Total cholesterol/HDL ratio

Median ratio values decreased modestly in the Placebo group throughout the trial and remained stable in the L3-10 group at wk78. In the L3-20 group, the ratio was elevated at wk78 but had returned to near baseline level by wk104 (Table 4-8). Kruskal-Wallis analyses found no significant inter-cohort differences in absolute values at any time point. The omnibus test result for wk52 suggested significantly different changes between groups (KW p = 0.0311); however, Dunn's multiple comparisons did not identify significant differences between specific groups. Longitudinal analyses (Mixed-effects model) did not show significant differences over time in any group.

Table 4-8 Blood lipid absolute values and changes from baseline by treatment group throughout the trial

	baseline	wk26	Δ wk26	wk52	Δ wk52	wk78	Δ wk78	wk104	Δ wk104
Cholesterol (mmol/L)									
Placebo	5.4 4.9-6.3 *n = 12*	4.9 4.4-6.0 *n = 12*	-0.4 -0.7- (-0.0) *n = 12*	4.8 4.4-5.4 *n = 9*	-0.4 -0.6-(-0.1) *n = 9*	4.9 4.3-5.8 *n = 8*	**-0.6**[&] -1.1- (-0.1) *n =8*	5.0 4.5-5.3 *n = 6*	-0.2 -0.6-0.1 *n = 6*
L3-10	5.2 4.6-6.3 *n = 13*	5.1 3.9-5.9 *n = 12*	**-0.3**† -0.6- (-0.2) *n = 12*	5.0 4.2-5.6 *n = 11*	-0.2 -0.7-0.5 *n = 11*	5.1 4.5-5.7 *n = 10*	-0.2 -0.4-0.4 *n = 10*	4.9 3.9-6.8 *n = 8*	-0.2 -0.5-0.6 *n = 8*
L3-20	5.4 4.9-5.5 *n = 11*	6.0 4.9-6.3 *n = 11*	**0.7*** -0.2-0.9 *n = 11*	5.6 4.8-5.7 *n = 10*	0.2 -0.2-0.3 *n = 10*	5.7 4.6-6.1 *n = 8*	**0.4*** -0.2-0.6 *n = 8*	5.3 4.3-5.8 *n = 9*	-0.1 -0.8-0.6 *n = 9*
Triglycerides (mmol/L)									
Placebo	1.4 0.9-2.0 *n = 12*	1.4 1.0-2.0 *n = 12*	0.15 -0.4-0.6 *n = 12*	1.3 0.9-1.6 *n = 9*	0.0 -0.2-0.3 *n = 9*	1.4 1.0-1.9 *n = 8*	0.3 -0.2-0.5 *n = 8*	1.8 1.4-2.9 *n = 6*	0.7 0.4-1.4 *n = 6*
L3-10	1.3 0.9-1.8 *n = 13*	1.3 0.8-1.7 *n = 12*	0.0 -0.2-0.2 *n = 12*	1.2 1.0-1.5 *n = 11*	-0.1 -0.4-0.2 *n = 11*	1.4 0.8-2.5 *n = 10*	0.0 -0.2-0.8 *n = 10*	1.1 0.9-1.9 *n = 8*	0.1 -0.3-0.3 *n = 8*
L3-20	1.4 0.8-2.0 *n = 11*	1.3 1.0-2.7 *n = 11*	-0.2 -0.4-0.9 *n = 11*	1.8 1.0-2.6 *n = 10*	0.1 -0.4-0.6 *n = 10*	1.7 1.1-1.9 *n = 8*	-0.1 -0.2-0.7 *n = 8*	1.3 0.9-2.1 *n = 9*	0.1 -0.3-0.3 *n = 9*
HDL (mmol/L)									
Placebo	1.2 1.1-1.4 *n = 12*	1.1 1.0-1.3 *n = 12*	-0.1 -0.1-0.0 *n = 12*	1.2 1.1-1.3 *n = 9*	0.0 -0.0-0.1 *n = 9*	1.2 1.1-1.6 *n = 8*	-0.1 -0.2-0.2 *n = 8*	1.2 1.0-1.7 *n = 6*	0.0 -0.3-0.2 *n = 6*
L3-10	1.2 1.0-1.5 *n = 13*	1.7 1.0-1.4 *n = 12*	-0.1 -0.2- (-0.1) *n = 12*	1.1 1.0-1.4 *n = 11*	-0.0 -0.1-0.0 *n = 11*	1.2 1.0-1.3 *n = 10*	-0.2 -0.2-0.0 *n = 10*	1.1 1.0-1.7 *n = 8*	0.1 -0.1-0.2 *n = 8*
L3-20	1.3 1.1-1.5 *n = 11*	1.3 1.1-1.5 *n = 11*	0.0 -0.2-0.2 *n = 11*	1.3 1.1-1.3 *n = 10*	-0.0 -0.1-0.1 *n = 10*	1.2 1.1-1.3 *n = 8*	-0.1 -0.2- (-0.1) *n = 8*	1.1 1.0-1.4 *n = 9*	-0.1 -0.3-0.0 *n = 9*

	baseline	wk26	Δ wk26	wk52	Δ wk52	wk78	Δ wk78	wk104	Δ wk104
LDL (mmol/L)									
Placebo	3.5 3.0-4.4 *n = 12*	3.1 2.6-3.9 *n = 12*	-0.2 -0.7- (-0.0) *n = 12*	2.9 2.6-3.4 *n = 9*	-0.3 -0.8- (-0.1) *n = 9*	**2.9**[&] 2.5-3.5 *n = 8*	-0.6 -0.8- (-0.3) *n = 8*	2.7 2.6-3.2 *n = 6*	-0.6 -0.7-0.2 *n = 6*
L3-10	3.3 2.9-4.3 *n = 13*	3.4 2.5-3.8 *n = 12*	**-0.4**† -0.6- (-0.0) *n = 12*	3.2 2.7-3.7 *n = 11*	-0.1 -0.6-0.6 *n = 11*	3.1 2.6-3.9 *n = 10*	-0.3 -0.5-0.5 *n = 10*	3.1 2.4-4.1 *n = 8*	-0.2 -0.4-0.3 *n = 8*
L3-20	3.4 2.6-3.5 *n = 11*	3.4 3.0-4.0 *n = 11*	0.3 -0.3-1.3 *n = 11*	3.2 3.0-3.8 *n = 10*	0.2 -0.3-0.4 *n = 10*	3.8 2.9-4.0 *n = 8*	**0.5*** 0.0-0.6 *n = 8*	3.1 2.7-3.7 *n = 9*	0.1 -0.5-0.5 *n = 9*
Chol/HDL ratio									
Placebo	4.4 3.8-5.2 *n = 12*	4.0 3.8-4.9 *n = 12*	-0.1 -0.3-0.3 *n = 12*	4.0 3.5-4.3 *n = 9*	-0.4 -0.6- (-0.2) *n = 9*	3.9 3.3-4.6 *n = 8*	-0.2 -0.7-0.3 *n = 8*	3.9 3.3-5.1 *n = 6*	0.1 -0.6-0.6 *n = 6*
L3-10	4.1 3.8-4.9 *n = 13*	4.2 3.7-4.9 *n = 12*	0.2 0.0-0.3 *n = 12*	4.2 3.7-5.3 *n = 11*	-0.1 -0.2-0.5 *n = 11*	4.3 3.6-5.6 *n = 10*	0.3 -0.3-1.1 *n = 10*	4.2 3.3-5.0 *n = 8*	-0.2 -0.6-0.4 *n = 8*
L3-20	4.0 3.7-4.9 *n = 11*	4.4 4.0-4.7 *n = 11*	0.5 -0.2-0.8 *n = 11*	4.5 3.9-5.1 *n = 10*	0.1 -0.4-0.8 *n = 10*	4.8 4.1-5.0 *n = 8*	0.5 0.0-0.9 *n = 8*	4.3 4.0-5.4 *n = 9*	0.2 -0.1-0.7 *n = 9*

Data are presented as median and IQR. [&] significant difference to baseline; †significant difference to L3-20; *significant difference to Placebo.

4.3 Discussion

With safety, tolerability, and acceptability of *Na* infection in humans with metabolic diseases as the primary overall outcome, the current trial also aimed to determine the influence of infection on key metabolic and physical parameters. The primary metabolic outcome was a change in insulin resistance assessed by HOMA-IR. Further, the trial evaluated body mass, central adiposity, and blood lipid profile changes. I hypothesised that infection would improve these parameters. The trial results partially support my hypothesis, indicating a significant beneficial effect of *Na* infection on insulin resistance but not body mass, central adiposity, or lipid profile.

Experimental *Na* infection consistently improved insulin resistance, particularly in the L3-10 group (20 worms). In this low-dose group, HOMA-IR improvement was most pronounced at wk78, with a median change of -1.48 units, translating to a significant reduction in median HOMA-IR from baseline (2.95 units) to wk78 (1.57 units). In contrast, the change in the L3-20 group (40 worms) was less dramatic, ranging from -0.25 to -0.48, indicating that a higher worm burden did not yield a superior improvement in insulin resistance. Generally, individuals with the greatest potential to improve, that is, with the highest baseline HOMA-IR, experienced the most substantial reductions. The considerably higher median baseline value in the L3-10 group with a greater potential for improvement could explain the enhanced response in this group compared to L3-20. Notably, the median baseline age in the L3-10 group was also significantly higher, which may have allowed more time for insulin resistance to worsen and contributed to the group's higher median baseline HOMA-IR.

The curiously more remarkable improvement in the low-dose group certainly evokes further speculation. It could be hypothesised that a strong type 2 immune response induced by high worm burden might generate opposite immunometabolic effects at an organ-specific level, e. g. a beneficial impact in AT but a detrimental effect in the liver. For example, Gieseck et al. (2016) have shown that type 2 immune responses can trigger hepatic fibrosis, which has also been observed when obese mice are treated with helminth-derived-single molecules (van der Zande et al., 2021). Further, in this trial's worm-naïve cohorts, a small worm burden may have provided sufficient stimulus to promote beneficial responses, whereas a larger burden may have provoked an excessive immune response, weakening favourable responses. Last, the difference between the two worm-treatment groups may be an artefact of the low participant numbers and may lessen with higher participant numbers.

The overall improvement in insulin resistance in the worm treatment groups is consistent with findings from human and mouse studies that suggested a protective role of worm infection in glucose homeostasis (Chen et al., 2013; Hussaarts et al., 2015; Morimoto et al., 2017; Wiria et al., 2015). Interestingly, a 2019 systematic review and meta-analysis of human cross-sectional and deworming studies (Rennie et al., 2021) that investigated the impact of worm infection on the incidence of MetS found that, compared to other infections, active schistosomiasis was most effective at improving fasting

blood glucose. In contrast, infection with soil-transmitted worms had no effect. However, the review included only one study involving hookworm infection (Sanya et al., 2019) that was subsequently excluded from the meta-analysis due to insufficient data but also demonstrated lower fasting blood glucose in chronically infected individuals. Further, the excluded study was one of only three that reported HOMA-IR as an outcome, which was not analysed in the review. This dearth of previous studies made it difficult to draw conclusions about the specific association between hookworm infection and insulin resistance in humans.

A small number of outliers in the worm treatment groups did not see consistent improvements. One L3-10 participant had a baseline HbA1c at the upper limit of the healthy range at baseline, which indicated suboptimal glucose control and the potential onset of β-cell damage. The immune-regulatory effect of *Na* infection is likely insufficient to revert detrimental changes in individuals with advanced dysregulated glucose metabolism. This participant was subsequently excluded from the trial after wk78, as he had started to take metformin on his general practitioner's advice. One L3-20 participant had a considerable HOMA-IR reduction of 1.55 units at wk26, which gradually became less substantial until wk78 (-0.40) and turned into an increase of 2.2 at wk104. This turnaround in HOMA-IR change was entirely insulin driven, with fasting blood glucose remaining well within the normal range. Notably, this person's eosinophil count was already elevated at baseline (0.70 x 10^9/L) and still exceeded double the baseline value (1.56 x 10^9/L) at wk104. Further, liver function tests and high bilirubin values suggested ongoing comorbidity, which may have interfered with the worm infection. Similarly, another L3-20 participant with abnormal liver function tests experienced a reduction at wk26 with subsequent increases in HOMA-IR driven by rising insulin with normal fasting blood glucose. Coinciding, this participant also experienced rising glycated haemoglobin levels throughout the trial.

As the numbers in our groups were relatively low, I also calculated HOMA-IR change effect sizes to understand the effect magnitude independent of participant numbers. Large effect sizes from wk52 to wk104 in the L3-10 group substantiated the statistical findings and further supported the beneficial role of hookworm infection in glucose homeostasis. Notably, the observed change in HOMA-IR was also clinically relevant. In general, determining optimal HOMA-IR cut-off values to classify individuals into healthy and compromised glucose control is challenging. Covariates such as age, sex, and the existence of other MetS criteria can affect the discriminatory capacity of HOMA-IR as a biomarker. Therefore, cut-off values should be adapted to the population studied (Gayoso-Diz et al., 2013). Previous reports have estimated HOMA-IR cut-off values to classify MetS and suggested a value of 2.11 and 2.05 for 30 and 50-year-old women, respectively. The recommended cut-off for men of any age was 2.25 (Gayoso-Diz et al., 2013). In the current trial, the median baseline HOMA-IR of 2.95 units in the L3-10 group was within the compromised glucose control range. Therefore, a median change of -1.09 at wk52 and -1.48 at wk78 shifted the median absolute value into the healthy range. While the

median reduction in the L3-20 group was lower, the median value at wk104 was also in the healthy range.

HOMA-IR changes were more heterogeneous in the Placebo group, with minor improvements in some individuals and considerable increases in others. In two participants, sizable body mass reductions may explain their HOMA-IR improvements. One individual stopped taking antidepressant medication and lost >10kg during the trial, while another lost >15kg. A third individual with improved HOMA-IR seems to have made lifestyle changes, likely in preparation for planned gastric sleeve surgery following the wk26 visit. This participant's PREDIMED score rose from 0 at baseline to five at wk26, and body mass dropped by close to 5 kg, which could have induced improvements in glycaemic control (Sleiman et al., 2015). It is also plausible that the placebo effect led to subtle lifestyle changes that were not captured by the trial's assessment schedule. This powerful placebo effect of worm infection was previously described in a randomised placebo-controlled trial in coeliacs, where more than half of all placebo patients completed a 42-week gluten challenge with duodenal histology and tissue transglutaminase assessments remaining in the normal range (Croese et al., 2020). To shed more light on these findings, I have explored other potential correlates that could have influenced HOMA-IR changes in Chapter 5, including age, sex, diet, physical activity, immune responses, and adipokines. Importantly, wk104 values should be interpreted cautiously. The Placebo group included only six data points in this final value due to dropouts and exclusions.

Given that HOMA-IR is a composite parameter dependent on insulin and blood glucose, I also wanted to explore if any parameter was more influential in the observed HOMA-IR changes. Glucose responses mostly reflected HOMA-IR responses, with sustained reductions in the worm groups and almost stable values in the Placebo group. Yet only the L3-10 group also showed consistently lower median insulin, whereas the Placebo and L3-20 groups experienced fluctuating small increases. Thus, it appears that the more significant improvements in HOMA-IR in the L3-10 compared to the L3-20 group were mediated by insulin reductions not evident in the L3-20 group. These results support a beneficial effect of both doses of *Na* infection on blood glucose levels, but curiously, only the lower dose also improved insulin levels.

As HOMA-IR can only present a snapshot of blood glucose levels, we also assessed glycated haemoglobin (HbA1c IFCC) levels as a long-term measure of glycaemic control. In the Placebo group, median HbA1c IFCC levels trended upwards throughout the trial but remained stable in the infected groups, indicating a protective role of infection against future increases. Except for the one L3-10 participant with borderline high baseline HbA1c IFCC values who was later excluded from the trial, all values remained below 48 mmol/mol, which is the accepted cut-off for established diabetes (Mawson & Tien-Ming, 2021). Further, as baseline levels were mostly in the healthy range, considerable improvements were not expected.

Experimental mouse studies (Q. Duan et al., 2018; Hussaarts et al., 2015; D. Wu et al., 2011; Yang et al., 2013) and some human studies (Chen et al., 2013; Q. Duan et al., 2018; Wiria et al., 2015; Wiria et al., 2013) have noted lower body mass in infected individuals. In the current trial, the median body mass in the Placebo group did not change, which was anticipated. In agreement with previous studies, median body mass was significantly lower by about 10kg in the L3-10 group at wk78 and wk104. In contrast, median levels increased in the L3-20 group, which was unexpected and peculiar but can provide some insight into the different dynamics of HOMA-IR changes in the two worm groups. As increases in fat and body mass worsen insulin resistance, this development in the L3-20 group may have contravened and somewhat blunted the beneficial effect of worm infection. Overall, body mass changes were heterogeneous in all three groups, particularly during the later stages of the trial, and I have explored a possible correlation between body mass changes and HOMA-IR changes in Chapter 5.

While mouse studies have demonstrated a protective action of worm infection against body fat gains, this was not the case in the L3-20 group. Baseline body mass was nearly identical in all groups, excluding different starting masses as a causal factor. The different number of worms is also an unlikely factor. Mouse studies routinely use a much higher worm burden than the current trial, such as 500 L3 (Yang et al., 2013), which may significantly reduce the mice's available energy, thereby reducing body mass gains. Naturally infected humans often also harbour a large number of worms, so the low worm burden in the current trial may have been insufficient to induce dramatic body mass changes. However, the difference between the two doses was negligible compared to the much higher numbers in natural human or experimental mouse infection, so we should not have seen a change in either worm group. Further, if body mass changes were indeed dependent on worm numbers, the changes in the worm groups should have been reversed, with L3-20 losing mass and L3-10 stabilising or gaining mass. Excluding baseline differences and worm burden as causative factors in the differential body mass changes, potential other explanations could include distinct immune responses or gut microbial compositional changes.

During conversations with participants, it became evident that one powerful motivator for individuals to join the trial was the prospect of considerable body mass improvements with helminth infection, as seen in previous studies. When these losses did not eventuate, some participants expressed their disappointment and those that had already contemplated gastric sleeve surgery possibly based their decision to proceed with surgery before trial completion on this disappointment. Interestingly, this was not Placebo-specific but also occurred in the worm treatment cohort. Most others were less concerned and continued with unabated enthusiasm.

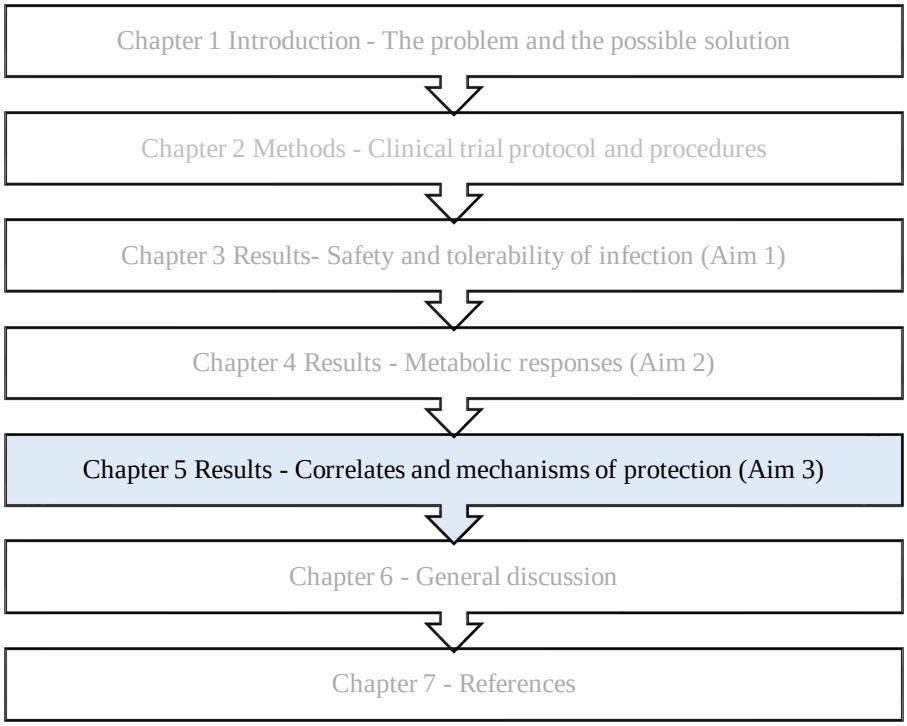
Given the critical role of central adiposity in the development of T2DM, we also assessed WC and body fat distribution. Not surprisingly, WC measurements varied only marginally over time and remained very similar between groups. The changes in body mass were not substantial enough to translate to noticeable changes in WC. While WC assessment could be prone to measurement error,

DEXA scans confirmed only minor shifts in body fat distribution and total fat mass. In combination, these results suggest that worm infection could induce considerable improvements in HOMA-IR that were enhanced in the presence of modest body mass reductions.

Infection with *Na* neither improved nor worsened blood lipid profiles in the current trial. My findings contrast previous studies that described a beneficial effect of helminth infection on blood lipid markers (Q. Duan et al., 2018; Mohamed et al., 2017; Sanya et al., 2019; Shen et al., 2015; Wiria et al., 2013; Wolde et al., 2019; Zinsou et al., 2020). A potential explanation for this lack of effect could lie in the different helminth species used in the current trial, which was the first to use experimental infection with *Na*. Previous studies noting improved blood lipid markers included cross-sectional and deworming studies of infections with multiple soil-transmitted helminths (Wiria et al., 2013) and previous and current schistosomiasis (Q. Duan et al., 2018; Mohamed et al., 2017; Shen et al., 2015; Wolde et al., 2019) but not hookworm infection alone. Results from a 2019 systematic review and meta-analysis suggested that different helminth species may have different efficacy (Rennie et al., 2021). The authors postulated that the liver-specific residence of *Schistosoma spp*. compared to intestinal helminths could facilitate greater metabolic improvements in schistosomiasis.

The “old friends” hypothesis is a persuasive explanation for the increasing prevalence of inflammatory and metabolic disorders in populations with a low prevalence of helminth infections. Our world-first proof-of-principle study demonstrated that infection with a low dose of *Na* improved glucose homeostasis significantly and could be a preventative intervention in humans at risk of T2DM.

5 CORRELATES AND MECHANISMS OF PROTECTION (AIM 3)



5.1 Introduction

Obesity is a pandemic and was the fourth-leading cause of death worldwide in 2017. The worldwide prevalence of obesity nearly tripled between 1975 and 2016, and estimates suggest that nearly 2 billion adults were overweight or obese in 2016. Obesity is also becoming a global concern for children, with close to 400 million children classified as overweight or obese. Traditionally mainly a burden in developed countries, obesity now has a strong foothold in low to middle-income countries, and globally, more individuals are currently obese than underweight (World Health Organization, 2021. Accessed July 3 2022). Primarily a consequence of lifestyle factors, including excess energy intake and an inactive lifestyle (World Health Organization, 2021. Accessed July 3 2022), obesity is a leading cardiometabolic risk factor in Mets (Grundy, 2015).

Metabolic syndrome describes a cluster of metabolic risk factors that precede the development of non-communicable diseases such as CVD and T2DM (O'Neill & O'Driscoll, 2015). Besides obesity, specifically central obesity, the cluster includes impaired lipid profile, elevated blood pressure, and hyperglycaemia. Concurrently exceeding the thresholds for at least three of these factors suffices to diagnose MetS (Alberti et al., 2009). Although global estimates of MetS prevalence are hard to measure (Saklayen, 2018), MetS prevalence was estimated as almost one-quarter of the global population in 2006 (International Diabetes Federation, 2006. Accessed July 3 2022). The pathogenesis of MetS is complex and not fully understood, yet dysregulated lipid metabolism, chronic inflammation and subsequent insulin resistance are recognised as critical elements (McCracken et al., 2018; Rochlani et al., 2017). Additionally, lifestyle factors, including inappropriate dietary intake and physical inactivity, are strongly implicated in the pathogenesis of MetS (World Health Organization, 2021. Accessed July 3 2022). These factors, in combination, shape the growing waistline in central obesity and are independently associated with chronic inflammation and insulin resistance (Y. Duan et al., 2018; Fischer et al., 2007; Hamburg et al., 2007; Hancock et al., 2008). As MetS generally precedes T2DM, lifestyle programmes targeting individuals with MetS present an effective strategy in preventing T2DM (Gillies et al., 2007) but are poorly adopted.

The previous chapters have shown that hookworm infection in humans with metabolic disease was safe, well-tolerated, and improved glucose homeostasis. Yet, questions remain as to how and why. Is the observed improvement an actual biological response initiated by immune and hormonal mechanisms, or did potential confounders such as sex, age, diet, physical activity, or body mass reduction regulate inflammation and glycaemic control?

Interestingly, host-specific differences, such as sex and age, may moderate the immune responses to helminth infection. Animal and human studies have confirmed distinct immunophysiology in males and females that may explain their diverging susceptibility to certain diseases, including parasites (vom Steeg & Klein, 2016). For example, innate immune cells, including dendritic cells and

macrophages, are present in higher numbers and exhibit greater activity in females than males (Boissier et al., 2003; Melgert et al., 2010; Xia et al., 2009). Clinical studies found lower $CD3^+$ and $CD4^+$ T cell counts, $CD4^+$ to $CD8^+$ cell ratios, and Th1 cytokines in men than women (Amadori et al., 1995; Villacres et al., 2004). Also, IL-4 deficient male mice developed chronic *T. muris* infection, whereas deficient females could expel the worms, a difference that appeared to be IL-13 dependent (Bancroft et al., 2000). Sex steroid hormones could be critical mediators of this differential immune response in males and females (Hepworth et al., 2010; vom Steeg & Klein, 2016).

Besides the influence of sex on immune responses, the host's immune system experiences complex changes throughout an individual's lifespan; however, we still know very little about how age affects the immune response to worms. Babayan et al. (2018) used supervised statistical learning techniques to unravel the processes involved. They found that ageing in mice infected with *L. sigmodontis* impaired adaptive immunity, specifically naive T cells, T cell responsiveness to parasites, and antibody production. In humans, an ageing innate immune system manifests as a heightened pro-inflammatory environment due to dysregulated inflammatory responses (Shaw et al., 2013). Chronic inflammation can impair the innate immune response to pathogens. Notably, ageing dysregulates cytokine release in WAT and induces NLRP3-dependent inflammation (Shaw et al., 2013). Together, sex- and age-related differences in immune responses could modify the response to experimental hookworm infection.

Further, lifestyle interventions to reverse central obesity can reduce metabolically triggered inflammation and insulin resistance (Kirwan et al., 2017). Adipose tissue is a dynamic organ that responds remarkably to diet-induced weight loss. A moderate 5 -10% reduction in body mass is commonly recommended to improve metabolic function and health outcomes (Jensen et al., 2014). Magkos et al. demonstrated the therapeutic effect of a 6-month lifestyle intervention using individual behaviour education sessions and dietary counselling to achieve 5%, 11%, and 16% body mass reductions in obese adults with evidence of insulin resistance (HOMA-IR >2.0) (Magkos et al., 2016). A 5% reduction promoted system-wide insulin sensitivity and β-cell function. The authors also noted decreases in plasma concentrations of glucose, triglycerides, alanine transaminase, and leptin that continued with progressive body mass reductions. While the 5% loss did not lessen inflammation, 11-16% loss decreased circulating inflammatory markers (IL-6, CRP, MCP1). Further, all levels of body mass reduction down-regulated biological pathways and genes involved in lipid synthesis, oxidative stress, and extracellular matrix remodelling but upregulated those involved in cholesterol flux. These findings indicated that reduced inflammation did not mediate the improvement in insulin sensitivity observed with a 5% loss. However, the authors speculated that an early modest increase in inflammation might be a beneficial adaptive response that assists tissue remodelling in response to caloric restrictions (Rutkowski et al., 2015). Beyond caloric restriction, diet composition may also moderate glycaemic control.

In particular, adherence to a Mediterranean diet may benefit glucose homeostasis. A 2015 systematic review examining the effect of adherence to a Mediterranean diet on diabetes control and cardiovascular risk modification found favourable effects on glycaemic control and cardiovascular disease (Sleiman et al., 2015). Consumption of a Mediterranean diet appears to lower HbA1c, insulin resistance, and mortality. Proposed mechanisms for this effect are decreased oxidative stress and inflammation, possibly mediated by the high intake of olive oil, fibre, fruit and vegetables and the moderate consumption of wine (Barona et al., 2012; Esposito et al., 2004; Estruch et al., 2006; Mena et al., 2009; Ryan et al., 2000; Sanchez-Moreno et al., 2006; Sierksma et al., 2004; Soriguer et al., 2004). A second 2015 systematic review of five randomised controlled trials and eight meta-analyses showed similar results (Esposito et al., 2015). Adherence to a Mediterranean diet compared favourably to control diets regarding glycaemic control and cardiovascular risk factors, including body mass, lipid profile, and remission from MetS. Parallel to caloric restriction and dietary composition, physical activity can reverse components of MetS.

Beyond diet, evidence from interventional and observational studies suggests that increasing physical activity and higher cardiorespiratory fitness alleviate MetS. The steadily climbing prevalence of MetS components in the last four decades has coincided with declining physical activity levels in Western societies (Chau et al., 2017; Hallal et al., 2012). Significantly, cardiorespiratory fitness is inversely related to the development of MetS (Church, 2011; Duncan, 2006). Together, low physical activity levels and cardiorespiratory fitness are closely linked to MetS (Strasser, 2013; D. Zhang et al., 2017). Numerous studies have demonstrated the capacity of physical activity and higher cardiorespiratory fitness to favourably modify each MetS component (Church, 2011; Dubé et al., 2012; Duncan, 2006; Heiston et al., 2021; Snowling & Hopkins, 2006; Strasser, 2013; D. Zhang et al., 2017) and attenuate inflammation (Gleeson et al., 2011; You et al., 2013). The benefits of physical activity and improved cardiorespiratory fitness include normalising body mass, lipid profile, and insulin resistance (Myers et al., 2019). These beneficial effects of physical activity may partly arise from the exercise-induced alteration in the adipose tissue secretome, with increases in anti-inflammatory and decreases in pro-inflammatory adipokines (Golbidi & Laher, 2014). Mouse studies also indicated that exercise could correct high-fat diet-induced dysbiosis (Campbell et al., 2016; Feng et al., 2017), further repealing pathological processes emanating from the dysfunctional adipose tissue.

Similar to lifestyle changes, intestinal helminth infection can mediate inflammation and insulin resistance. These parasites increase their chances of survival in the host by expertly modulating immune responses, which ultimately benefit both parasite and the host. The worm is protected from elimination by the host's immune response and the host from excessive inflammation. A large body of evidence demonstrates that worm infections downregulate pro-inflammatory T helper (Th) type 1 and Th17 immune responses and skew towards a modified Th2 immune response via the release of biologically active ES antigens (Bashi et al., 2015; Croese et al., 2015; Danilowicz-Luebert et al., 2011; Khudhair

et al., 2021; Su et al., 2018). The unmodified Th2 immune response is characterised by $CD4^+$ Th2 cells, the cytokines IL-4, IL-5, IL-9, IL10, and IL-13, and immunoglobulin E. These key players orchestrate the recruitment of macrophages, eosinophils, and mast cells as well as B cells, which contribute to crucial host protective processes against worms (Allen & Maizels, 2011; Chen et al., 2012; Danilowicz-Luebert et al., 2011; Gause et al., 2013; Wynn & Ramalingam, 2012). However, in most people, helminth infections lead to a modified Th2 response with high levels of AAMs, Tregs, and IL-10, resulting in an anti-inflammatory and tissue-remodelling environment. This modified immune response prevents unrelated inflammation, such as allergic immune responses, promotes wound healing, and enables parasite survival (Danilowicz-Luebert et al., 2011), thus establishing a mutually beneficial state for both the host and parasite. Significantly, the downregulation of inflammation arising from the type 2 immune response to worm infections is tightly linked to improved glucose homeostasis and reduced fat mass (Hotamisligil, 2006).

Obesity, inflammation, and insulin resistance also alter the adipose tissue secretome. Adipose tissue function is not limited to energy storage, insulation, and cushioning but also encompasses the regulation of metabolic processes via the release of adipokines (Berg & Scherer, 2005; Ouchi et al., 2003). Cellular changes in expanding visceral WAT deregulate the synthesis and secretion of these biologically active proteins, which alters immune responses and is associated with the pathogenesis of various diseases (Ouchi et al., 2011). Some of the most studied adipokines include leptin, adiponectin, and omentin-1. Leptin, probably the best-characterised adipokine, is an essential hormone regulating food intake and energy homeostasis (Klok et al., 2007), improving insulin sensitivity (Paz-Filho, Mastronardi, Franco, et al., 2012) and promoting inflammatory responses (La Cava, 2017). Obesity induces a leptin-resistant state, and hyperleptinaemia, common in obese individuals, may act as a compensatory mechanism to overcome leptin resistance (Landecho et al., 2019). In contrast, adiponectin, which increases insulin sensitivity and curbs inflammation (Mantzoros et al., 2005), is down-regulated in obesity (Stumvoll & Haring, 2002). Further, omentin-1, a hormone involved in the regulation of insulin secretion and sensitivity, is inversely related to inflammation and hyperglycaemia (Pan et al., 2019) and levels are commonly significantly lower in patients with T2DM and impaired glucose tolerance (As Habi et al., 2019; Pan et al., 2019). Altered expression of these adipokines initiates and progresses impaired metabolic homeostasis and obesity-induced metabolic complications.

The current trial primarily aimed to establish the safety, tolerability, and acceptability of *Na* infection in humans with metabolic disease. Given the proposed protective role of worm infection on key metabolic and physical parameters, the trial also aimed to explore potential correlates that could mediate protection against metabolic disease. These included worm-related correlates such as immune responses and alterations in adipose tissue-secreted hormones, plus worm-unrelated factors, including age, sex, diet, and physical activity.

I hypothesised that *Na* infection would induce a biased Type 2 immune response and suppress pro-inflammatory immune responses. Further, I hypothesised that infection would modify the adipokine profile to promote metabolic homeostasis. Finally, I speculated that age, sex, diet, and physical activity would moderate improvements in metabolic parameters.

5.2 Results

5.2.1 Sex, age, and HOMA-IR changes

As sex and age can attenuate immune responses to helminth infection and could thus also moderate metabolic outcomes, I investigated the association of these parameters with HOMA-IR. Kruskal-Wallis tests did not show significant differences in HOMA-IR changes from baseline between infected males, infected females, or the combined worm treatment group at any time point (Figure 5-1). Further, simple linear regression analyses between age and changes in HOMA-IR were not significant at any time point (Figure 5-2 and Figure 5-3). While not significant, at wk78 and wk104, a trend for older individuals to see greater HOMA-IR improvements existed in the combined worm groups. Together, these results indicated that sex and age differences likely did not cause the heterogeneity in HOMA-IR responses observed in both the Placebo and combined worm treatment groups and that improvements in HOMA-IR were seen in both males and females, irrespective of age.

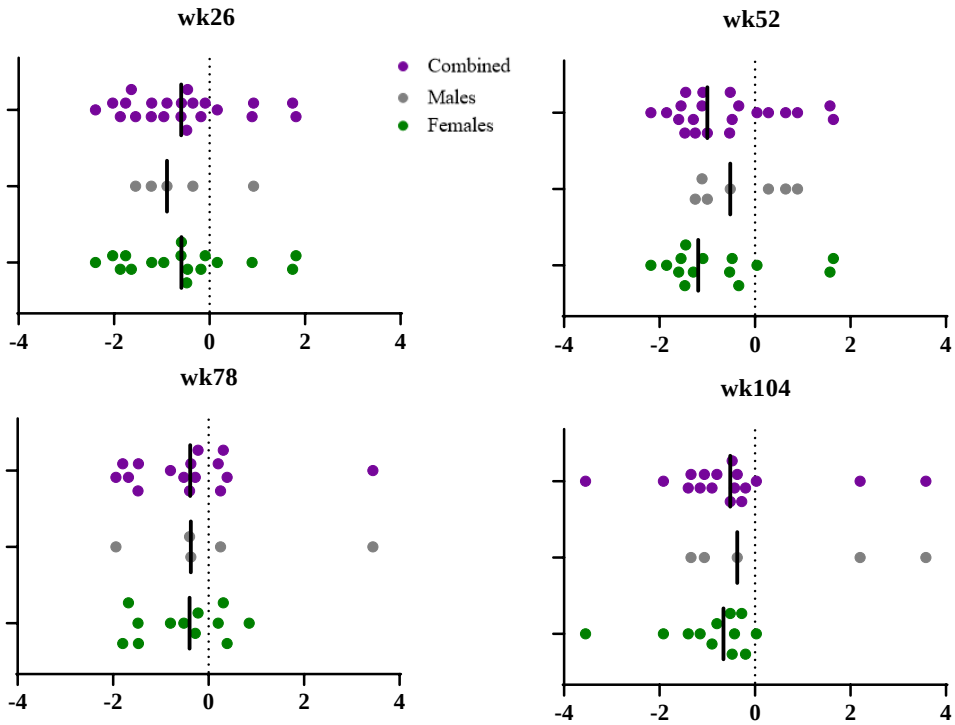


Figure 5-1 HOMA-IR changes (units) from baseline in worm treatment groups combined and separated by sex at each evaluation visit.
Individual data points are shown for each participant, and median values in each group are indicated by the vertical lines.
No significant differences were found (Kruskal-Wallis test)

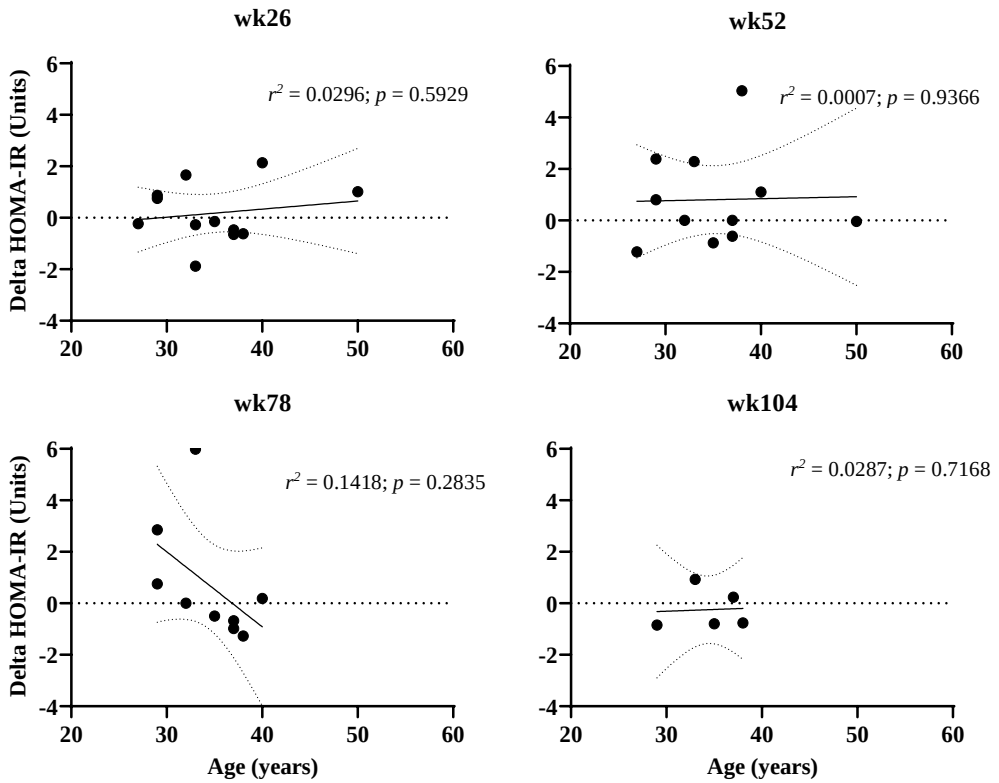


Figure 5-2 Simple linear regression between age and HOMA-IR change (units) from baseline in Placebo group at each evaluation visit.
No significant correlation was found.

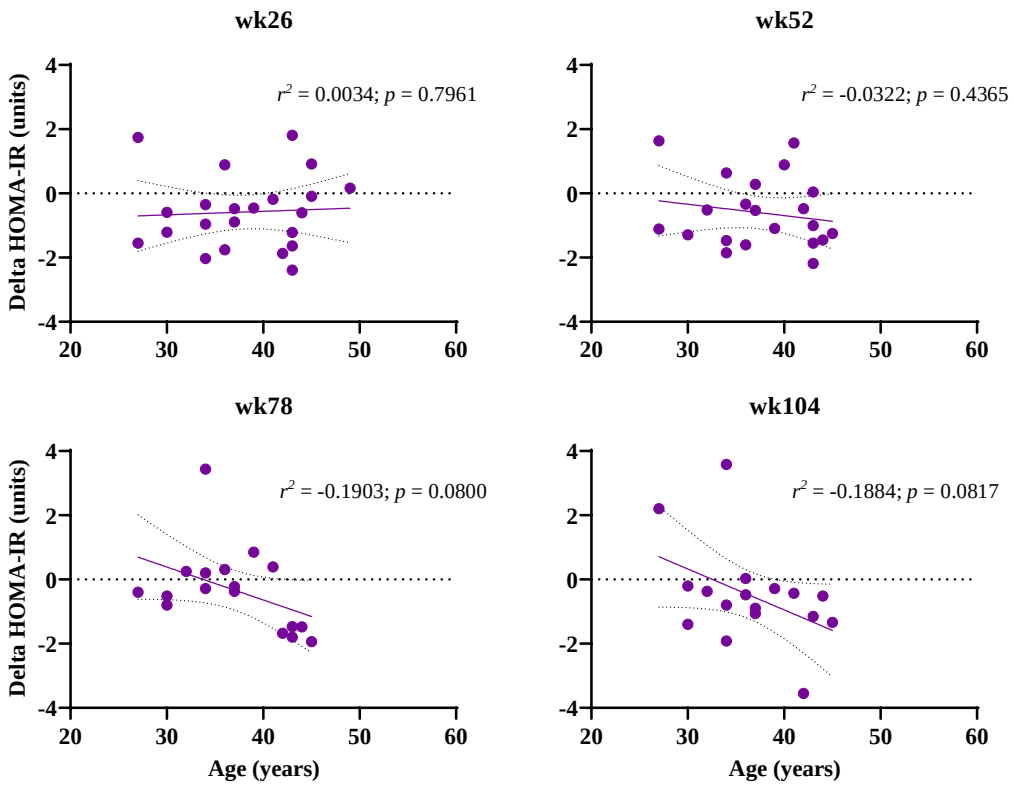


Figure 5-3 Simple linear regression between age and HOMA-IR changes (units) from baseline in combined worm treatment groups at each evaluation visit.
No significant correlation was found.

5.2.2 Diet and HOMA-IR changes

As diet can remarkably influence inflammation and insulin resistance, the trial protocol included a diet questionnaire to assess our participants' adherence to a Mediterranean diet throughout the trial. Median PREDIMED scores were low (possible maximum was 14) and similar in all treatment groups at baseline and all evaluation visits (Figure 5-4). The scores indicated an overall low adherence to a Mediterranean diet that persisted throughout the trial. Kruskal-Wallis tests did not identify significant differences between groups in total scores or changes at any time point. Similarly, Mixed-effects models showed no significant differences between time points in any group (Table 5-1). These results suggested that a change in adherence to the Mediterranean diet did not cause changes in HOMA-IR. To understand a potential relationship between diet and HOMA-IR changes on a more individual level, I investigated correlations between these measures in the Placebo and combined worm groups (Figure 5-5 and Figure 5-6). No significant correlation was evident in the Placebo group. In contrast, at wk26, the correlation between PREDIMED change and HOMA-IR change was significant in the worm treatment groups (rs = -0.4379, p = 0.0415), pointing to a possible influence of a Mediterranean diet on HOMA-IR changes.

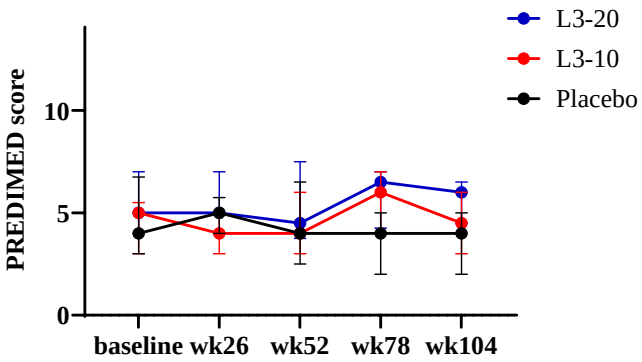


Figure 5-4 PREDIMED total scores by treatment group throughout the trial (median and IQR).
The possible maximum was 14 with a higher score indicating greater adherence to a Mediterranean diet. No significant difference was found (Mixed effects model).

Table 5-1 PREDIMED scores and changes by treatment group throughout the trial

	Placebo	L3-10	L3-20
Baseline	4.0 *3.0-6.8* *n = 12*	5.0 3.0-5.5 *n = 13*	5.0 4.0-7.0 *n = 11*
wk26	5.0 4.0-5.8 *n = 12*	4.0 3.3-5.0 *n = 12*	5.0 5.0-7.0 *n = 11*
Δ wk26	0.5 -0.8-1.0 *n = 12*	-0.5 -1.0-1.0 *n = 12*	1.0 -1.0-2.0 *n = 11*
wk52	4.0 2.5-6.5 *n = 9*	4.0 3.0-6.0 *n = 11*	4.5 3.8-7.5 *n = 10*
Δ wk52	0.0 0.0-1.0 *n = 9*	0.0 -1.0-0.0 *n = 11*	0.0 -1.3-2.3 *n = 10*
wk78	4.0 2.0-5.0 *n = 8*	6.0 4.0-7.0 *n = 11*	6.5 4.3-7.0 *n = 8*
Δ wk78	-0.5 -1.8-1.0 *n = 8*	1.0 -1.0-3.0 *n = 11*	0.0 -0.8-0.8 *n = 8*
wk104	4.0 2.0-5.0 *n = 7*	4.5 3.0-6.0 *n = 8*	6.0 4.5-6.5 *n = 9*
Δ wk104	1.0 -3.0-1.0 *n = 7*	0.0 -1.0-1.0 *n = 8*	0.0 -1.5-3.0 *n = 9*

Data are presented as median and IQR. No significant differences were found.

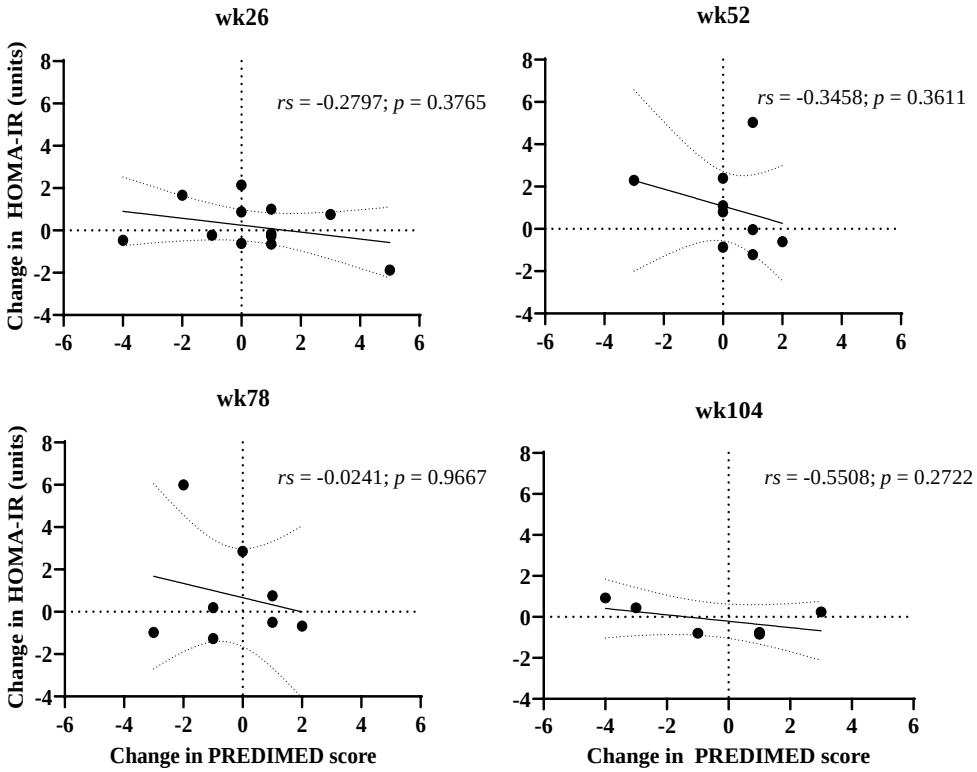


Figure 5-5 Correlation between PREDIMED score changes and HOMA-IR changes in the Placebo group at each evaluation visit.
No significant correlation was found.

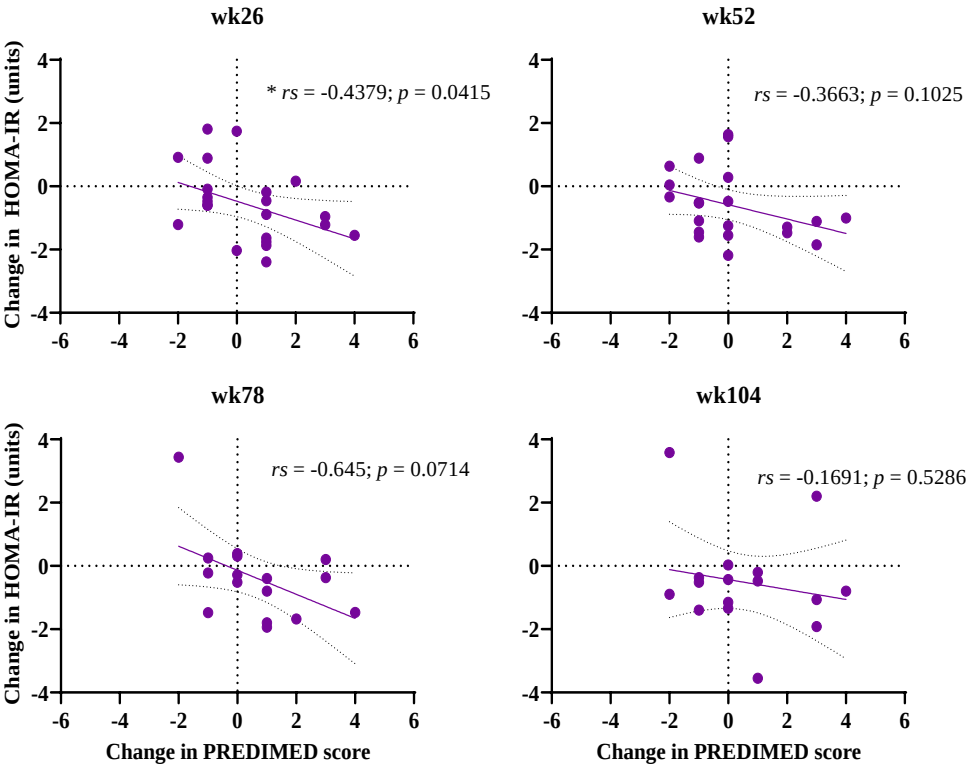


Figure 5-6 Correlation between PREDIMED score changes and HOMA-IR changes (units) in the worm treatment groups at each evaluation visit.
* significant correlation

5.2.3 Physical activity and HOMA-IR changes

Physical activity and higher cardiorespiratory fitness can positively modify MetS components. Therefore, altered activity levels could have influenced HOMA-IR changes observed in the current trial. Walking was the most popular activity reported by more than half of all participants. Other activities included swimming, gym/resistance training, yoga, and other unusual choices such as fencing, archery, and ninjitsu. No significant differences in absolute METs/week and changes in METs/week between groups were evident at any time point (KW) (Table 5-2). Longitudinal analyses via Mixed-effects models also did not show significant differences throughout the trial for any treatment group (Figure 5-7). A positive correlation between physical activity change and HOMA-IR change was found in the Placebo group at wk26, but no other significant correlation was evident in Placebo (Figure 5-8) and worm treatment groups (Figure 5-9). These findings indicate that increased physical activity did not induce improvements in HOMA-IR.

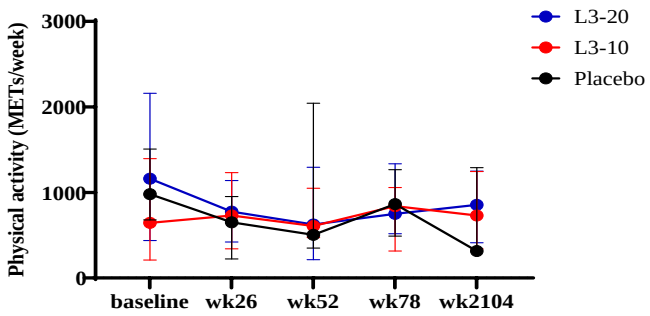


Figure 5-7 Physical activity (METs/week) by treatment group throughout the trial (median and IQR)
No significant differences were found (Mixed effects model).

Table 5-2 Physical activity totals and changes (METs/week) by treatment group throughout the trial

	Placebo	L3-10	L3-20
Baseline	981 679-1508 *n = 12*	645 210-1395 *n = 13*	1160 438-2160 *n = 11*
wk26	650 222-953 *n = 12*	732 341-1232 *n = 12*	775 420-1140 *n = 11*
Δ wk26	-358 -887-57 *n = 12*	35 -728-300 *n = 12*	-18 -1020-135 *n = 11*
wk52	505 350-2043 *n = 9*	608 473-1050 *n = 11*	625 214-1296 *n = 10*
Δ wk52	-353 -986-503 *n = 9*	158 -270-520 *n = 11*	-623 -1316-154 *n = 10*
wk78	864 491-1268 *n = 8*	840 315-1058 *n = 11*	750 518-1336 *n = 8*
Δ wk78	-266 -497-465 *n = 8*	60 -645-615 *n = 11*	-45 -785-205 *n = 8*
wk104	315 270-1290 *n = 7*	730 341-1245 *n = 8*	855 413-1249 *n = 9*
Δ wk104	-588 -803- (-234) *n = 7*	-68 -596-751 *n = 8*	-295 -795-65 *n = 9*

Data are presented as median and IQR. MET, metabolic equivalent of task. No significant differences were found.

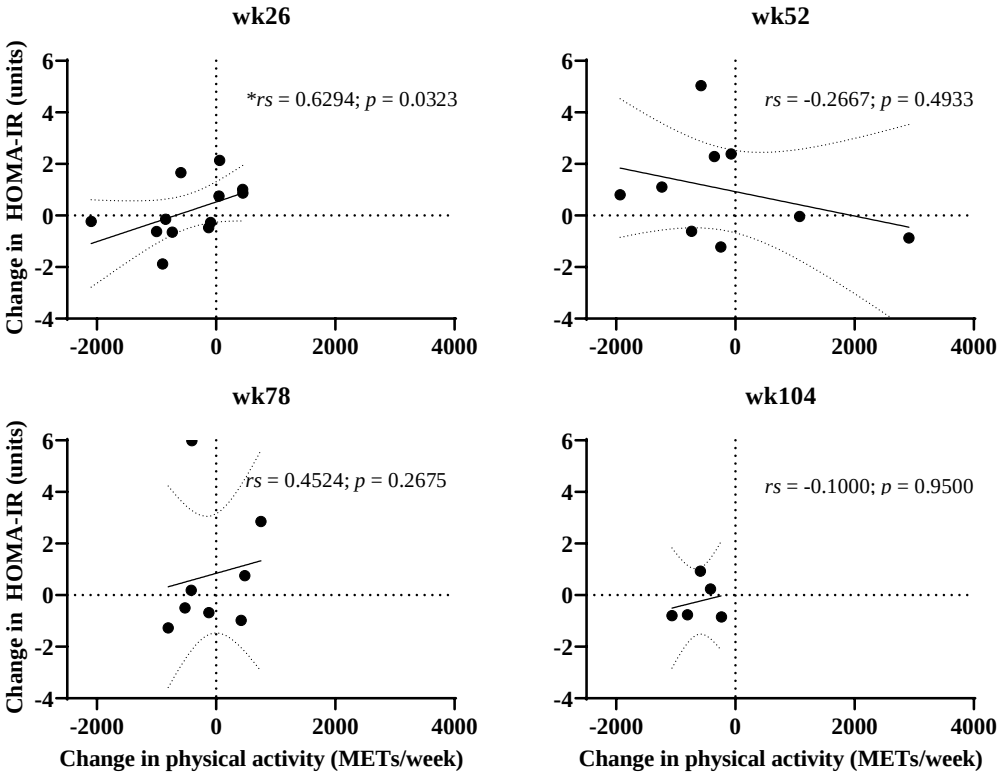


Figure 5-8 Correlation between physical activity changes (METs/week) and HOMA-IR changes (units) from baseline in the Placebo group at each evaluation visit.
*significant correlation

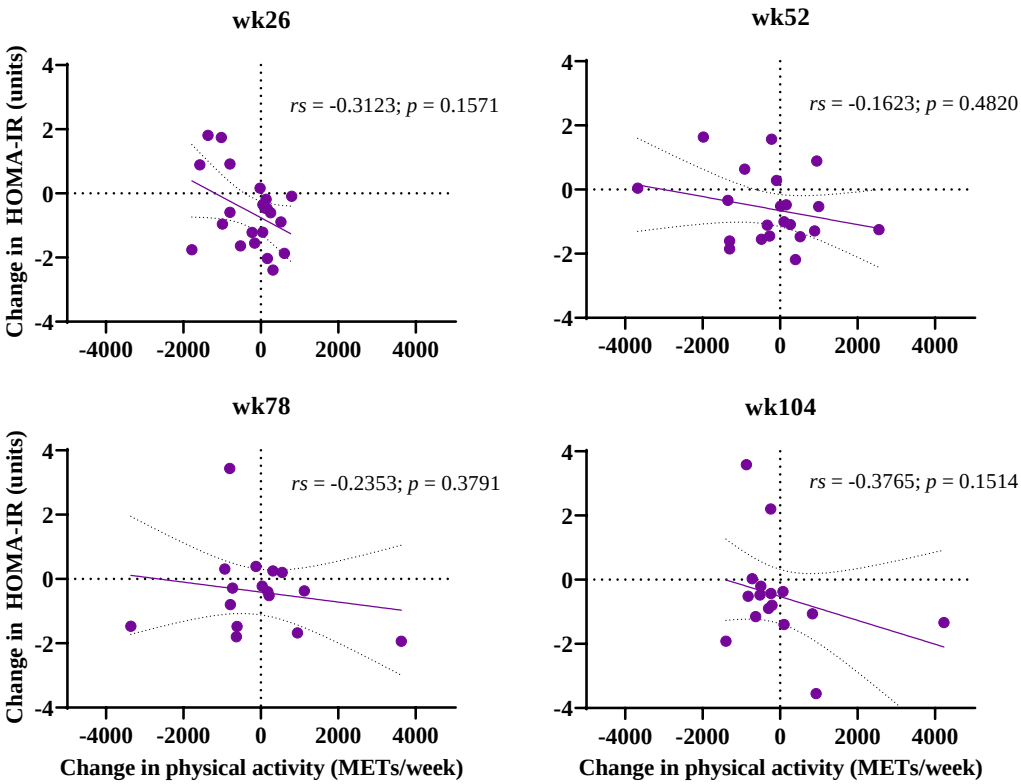


Figure 5-9 Correlation between physical activity changes (METs/week) and HOMA-IR changes (units) from baseline in the worm treatment groups at each evaluation visit.
No significant correlation was found.

5.2.4 Body mass and HOMA-IR changes

As I noted considerable heterogeneity in body mass changes, I wanted to discern the association between body mass changes and HOMA-IR changes. In the Placebo group, correlations between body mass changes and HOMA-IR were not significant (Figure 5-10). However, in the combined worm treatment group (Figure 5-11), this correlation was significant in wk26 (rs = 0.4967, p = 0.0220), wk52 (rs = 0.5324, p = 0.0360), and wk78 (rs = 0.5176, p = 0.0423). These results provide strong evidence for a positive association between improved HOMA-IR and reduced body mass in the infected groups that was not evident in the Placebo group.

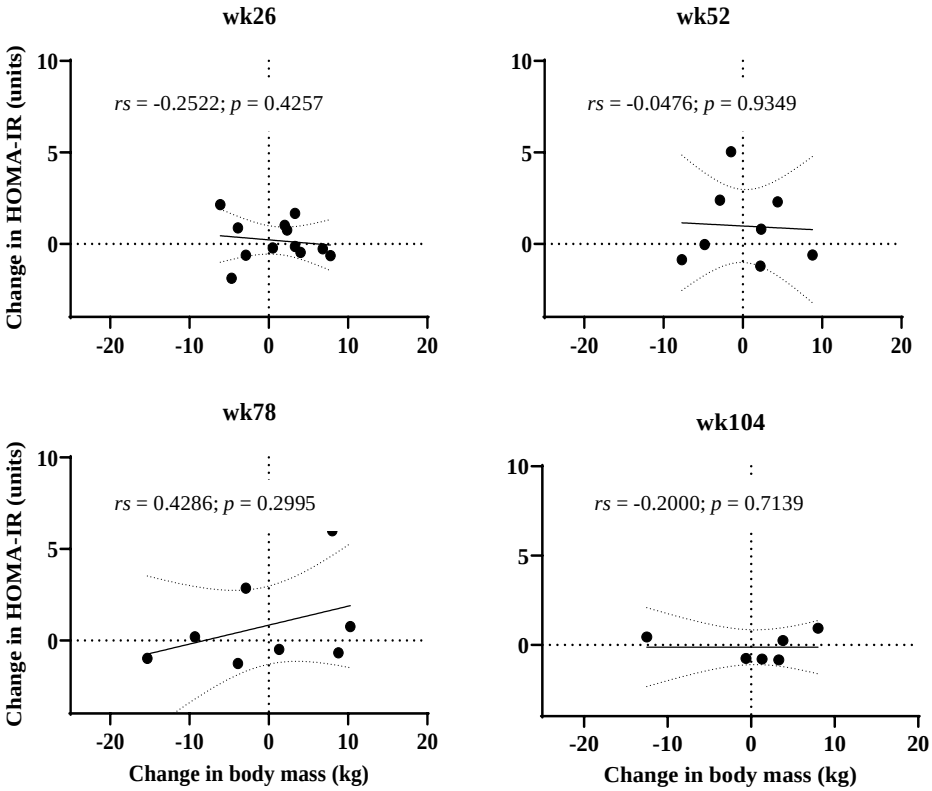


Figure 5-10 Correlation between body mass changes (kg) and HOMA-IR changes (units) in the Placebo group at each evaluation visit
No significant correlation was found.

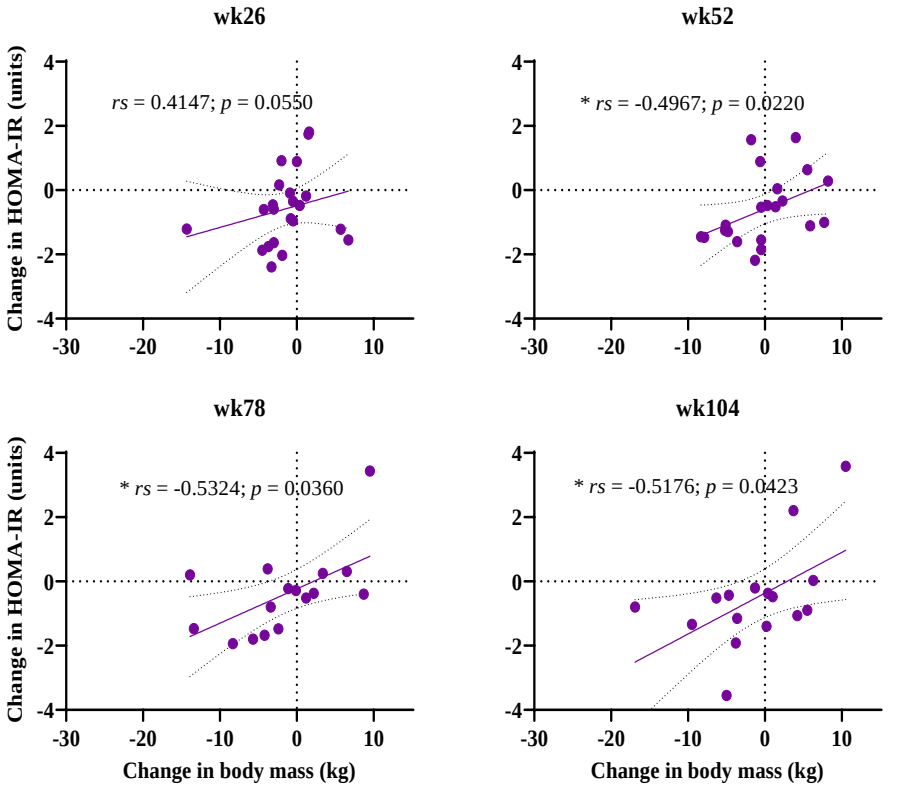


Figure 5-11 Correlation between body mass changes (kg) and HOMA-IR changes (units) in the worm treatment groups at each evaluation visit
* significant correlation

5.2.5 Eosinophils

As a measure of established infection and initiation of a type 2 immune response, I analysed the eosinophil response in all groups. Counts in the Placebo group remained close to the baseline value, whereas all participants in the worm treatment groups experienced an increase in peripheral blood eosinophil count, suggesting successful infection. At wk26, the maximum counts were 1.19 x 10^9/L in the L3-10 group and 1.85 x 10^9/L in the L3-20 group. Median absolute values in both worm groups were significantly different to Placebo at wk26 (KW $p < 0.0001$; L3-10 adjusted $p < 0.0001$; L3-20 adjusted $p = 0.0002$), wk52 (KW $p = 0.0009$; L3-10 adjusted $p = 0.0032$; L3-20 adjusted $p = 0.0027$), and wk78 (KW $p = 0.0047$; L3-10 adjusted $p = 0.0121$; L3-20 adjusted $p = 0.0124$) (Table 5-3). Correspondingly, eosinophil count changes were significantly different to Placebo in both worm groups at wk26 (KW $p < 0.0001$; L3-10 adjusted $p = 0.0011$; L3-20 adjusted $p = 0.0003$) and wk52 (KW $p = 0.0011$; L3-10 adjusted $p = 0.0201$; L3-20 adjusted $p = 0.0011$) but only for L3-20 at wk78 (KW $p = 0.0076$; adjusted $p = 0.0067$). Mixed-effects model analyses showed significantly greater ($p = 0.0011$) eosinophil counts compared to baseline in the L3-20 group at wk26 (adjusted $p = 0.0023$), wk52 (adjusted $p = 0.0070$), and wk78 (adjusted $p = 0.0233$). For L3-10, the omnibus *p*-value for the Mixed-effects model was not significant; however, Tukey's posthoc test showed a significant difference to baseline at wk26 (adjusted $p = 0.0468$) (Figure 5-12). As expected, hookworm treatment caused eosinophilia in both infected groups that peaked at six months and remained above baseline for most of the two-year trial.

Eosinophil responses were heterogeneous, and I wanted to investigate if HOMA-IR changes were associated with the magnitude of the eosinophile increase. The correlation was significant at wk52 ($rs = 0.4631$, $p = 0.0345$), wk78 ($rs = 0.6313$, $p = 0.0103$), and wk104 ($rs = 0.5489$, $p = 00297$) (Figure 5-13). Interestingly, this indicated that induction of lower levels of eosinophilia correlated with greater HOMA-IR improvement. These results were somewhat unexpected since I hypothesised that a greater type 2 immune response (including eosinophilia) may have promoted superior insulin improvements in HOMA-IR.

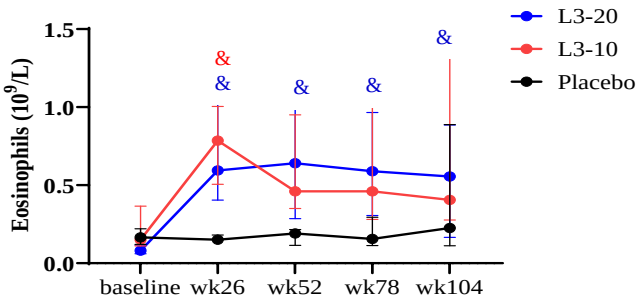


Figure 5-12 Eosinophil counts by treatment group throughout the trial (median and IQR)
[&]significant difference to baseline (Mixed effects model)

Table 5-3 Eosinophil counts and changes (10^9/L) from baseline by treatment group throughout the trial

	Placebo	L3-10	L3-20
Baseline	0.17 0.12-0.22 *n = 12*	0.15 0.11-0.37 *n = 13*	0.09 0.06-0.13 *n = 11*
wk26	0.15 0.13-0.18 *n = 12*	**0.79*[&]** 0.50-1.01 *n = 12*	**0.54*[&]** 0.41-1.26 *n = 11*
Δ wk26	-0.01 -0.05-0.05 *n = 12*	**0.54*** 0.31-0.65 *n = 12*	**0.48*** 0.30-0.92 *n = 11*
wk52	0.19 0.12-0.22 *n = 9*	**0.46*** 0.35-0.95 *n = 11*	**0.66*[&]** 0.29-0.92 *n = 10*
Δ wk52	0.01 0.01-0.06 *n = 9*	**0.29*** 0.20-0.46 *n = 11*	**0.48*** 0.23-0.84 *n = 10*
wk78	0.16 0.11-0.30 *n = 8*	**0.46*** 0.28-1.06 *n = 11*	**0.59*[&]** 0.31-0.97 *n = 8*
Δ wk78	0.01 -0.07-0.14 *n = 8*	0.30 0.08-0.46 *n = 11*	**0.47*** 0.20-0.66 *n = 8*
wk104	0.22 0.11-0.34 *n = 7*	0.41 0.28-1.33 *n = 8*	0.57 0.17-0.83 *n = 9*
Δ wk104	0.04 -0.05-0.11 *n = 7*	0.18 0.05-0.27 *n = 8*	0.51 0.09-0.75 *n = 9*

Data are presented as median and IQR. *significant difference to Placebo; [&] significant difference to baseline

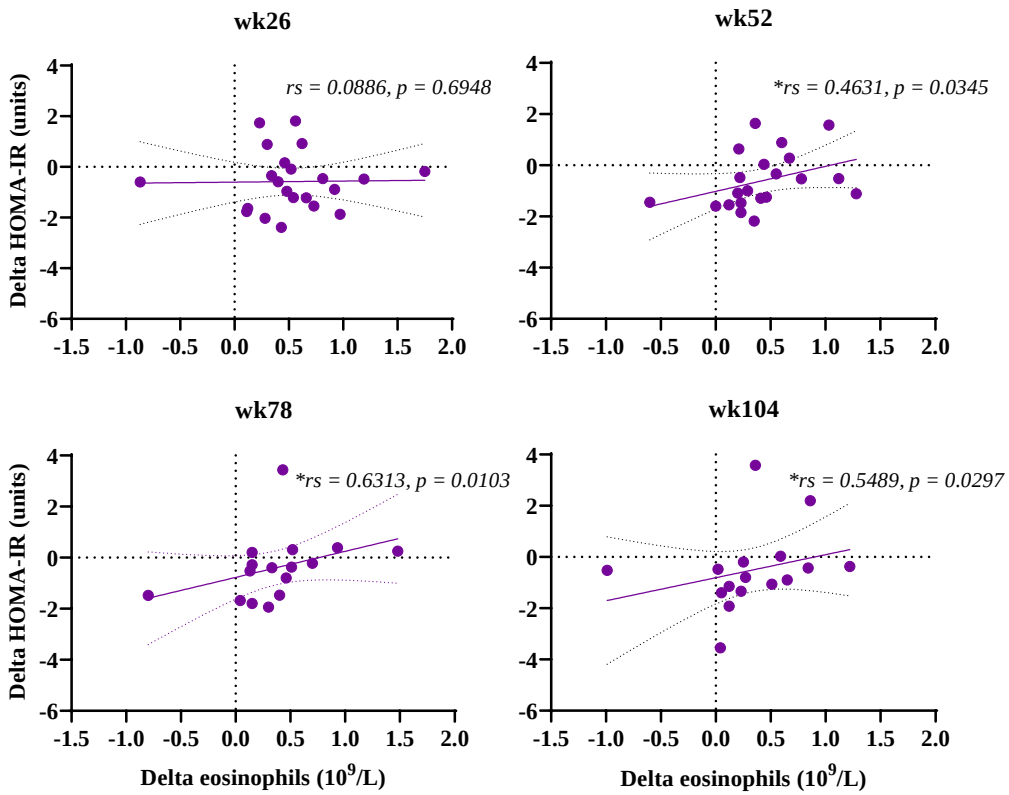


Figure 5-13 Correlation between eosinophil count changes (10^9/L) and HOMA-IR changes (units) in the combined worm treatment groups at each evaluation visit
* significant correlation

5.2.6 Basophils

Given their important role in IL-4 production to support antigen-presenting cells and Th2 cell differentiation, I investigated potential changes in these innate immune cells (Table 5-4). Kruskal-Wallis tests showed significantly greater absolute values in the L3-20 group compared to Placebo at wk26 (KW $p = 0.0479$, $p = 0.0482$) and wk78 (KW $p = 0.0087$, $p = 0.0062$). As expected, basophil counts increased in the worm treatment groups, with similar increases in both groups at wk26 (Table 5-4). Change in the L3-20 group at wk26 was significantly different to Placebo (KW $p = 0.0207$, $p = 0.0432$). At wk78, the omnibus test result also indicated a significant difference (KW $p = 0.0410$) in changes between groups; however, Dunn's multiple comparisons did not determine a significant difference in individual comparisons. Mixed-effects model omnibus results were not significant for any group. Still, in the L3-10 group, Tukey's multiple comparisons suggested a significant difference to baseline at wk52 (adjusted $p = 0.0106$) and wk78 (adjusted $p = 0.0056$) (Figure 5-14).

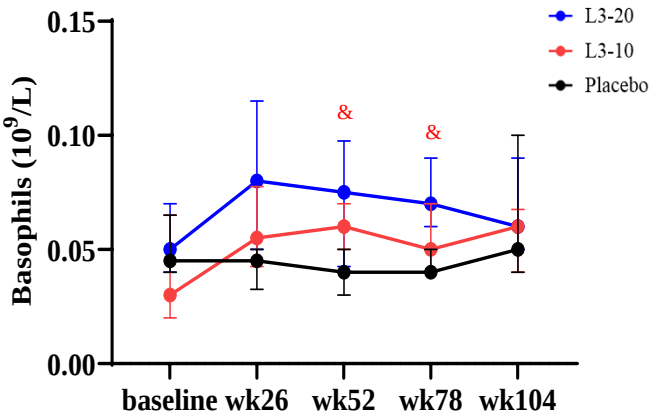


Figure 5-15 Basophil absolute counts by treatment group throughout the trial (median and IQR)
[&] significant difference to baseline (Mixed effects model)

Table 5-4 Basophil counts and changes (10^9/L)from baseline by treatment group throughout the trial

	Placebo	L3-10	L3-20
Baseline	0.05 0.04-0.07 *n = 12*	0.03 0.04-0.06 *n = 13*	0.04 0.04-0.07 *n = 11*
wk26	0.05 0.03-0.05 *n = 12*	0.06 0.04-0.08 *n = 12*	**0.08*** 0.05-0.10 *n = 11*
Δ wk26	0.00 -0.02-0.01 *n = 12*	0.03 0.01-0.04 *n = 12*	**0.03*** 0.00-0.06 *n = 11*
wk52	0.04 0.03-0.05 *n = 9*	**0.06**[&] 0.05-0.07 *n = 11*	0.07 0.04-0.09 *n = 10*
Δ wk52	0.00 -0.01-0.02 *n = 9*	0.01 0.01-0.03 *n = 11*	0.01 0.00-0.03 *n = 10*
wk78	0.04 0.04-0.05 *n = 8*	**0.05**[&] 0.04-0.07 *n = 11*	**0.07*** 0.05-0.09 *n = 8*
Δ wk78	0.00 -0.03-0.01 *n = 8*	0.01 0.01-0.02 *n = 11*	0.02 0.00-0.04 *n = 8*
wk104	0.04 0.04-0.07 *n = 7*	0.06 0.04-0.07 *n = 8*	0.06 0.05-0.09 *n = 9*
Δ wk104	0.00 0.00-0.01 *n = 7*	0.01 0.00-0.02 *n = 8*	0.01 0.00-0.03 *n = 9*

Data are presented as median and IQR. *significant difference to Placebo; [&]significant difference to baseline

5.2.7 Inflammatory markers

To further explore immune responses relating to inflammatory processes associated with Th1, Th2, Th9, Th17, and Th22 immune responses, serum samples from baseline, wk26, and wk52 were batch-analysed using the Biolegend® LEGENDplex™ Human Th Cytokine Panel of 12 cytokines (IL-2, 4, 5, 6, 9, 10, 13, 17A, 17F, 22, IFN-γ and TNF).In particular, I was interested in the differences between Placebo and the infected groups and have therefore combined the worm groups to compare against the Placebo group.

No significant differences between Placebo and the combined worm treatment groups (Mann-Whitney test) or over time (Friedman test) were evident for IL-2, 4, 6, 9, 10, 13, 22, IFN-γ and TNF. However, IL-5 was significantly increased at wk26 compared to baseline in the combined worm treatment groups (Friedman test $p = 0.0006$, adjusted $p = 0.0004$). Further, IL-5 was significantly greater in the worm groups compared to Placebo at wk26 ($p = 0.0292$) and wk52 ($p = 0.0141$). Similar, IL-17-A was significantly increased at wk26 compared to baseline in the combined worm treatment groups (Friedman test $p = 0.0129$, adjusted $p = 0.0179$), but I found no other significant differences.

As IL-5 induces expansion and activation of eosinophils and basophils, I also investigated correlations between this cytokine and the peak fold changes in these leukocytes at wk26. IL-5 correlated positively and significantly with both eosinophils ($rs = 0.6235$, $p = 0.0115$) and basophils ($rs = 0.7619$, $p = 0.0009$). Additionally, simple linear regression indicated that IL-5 significantly predicted eosinophil ($r^2 = 0.4890$, $p = 0.0026$) but not basophil fold change (Figure 5-16).

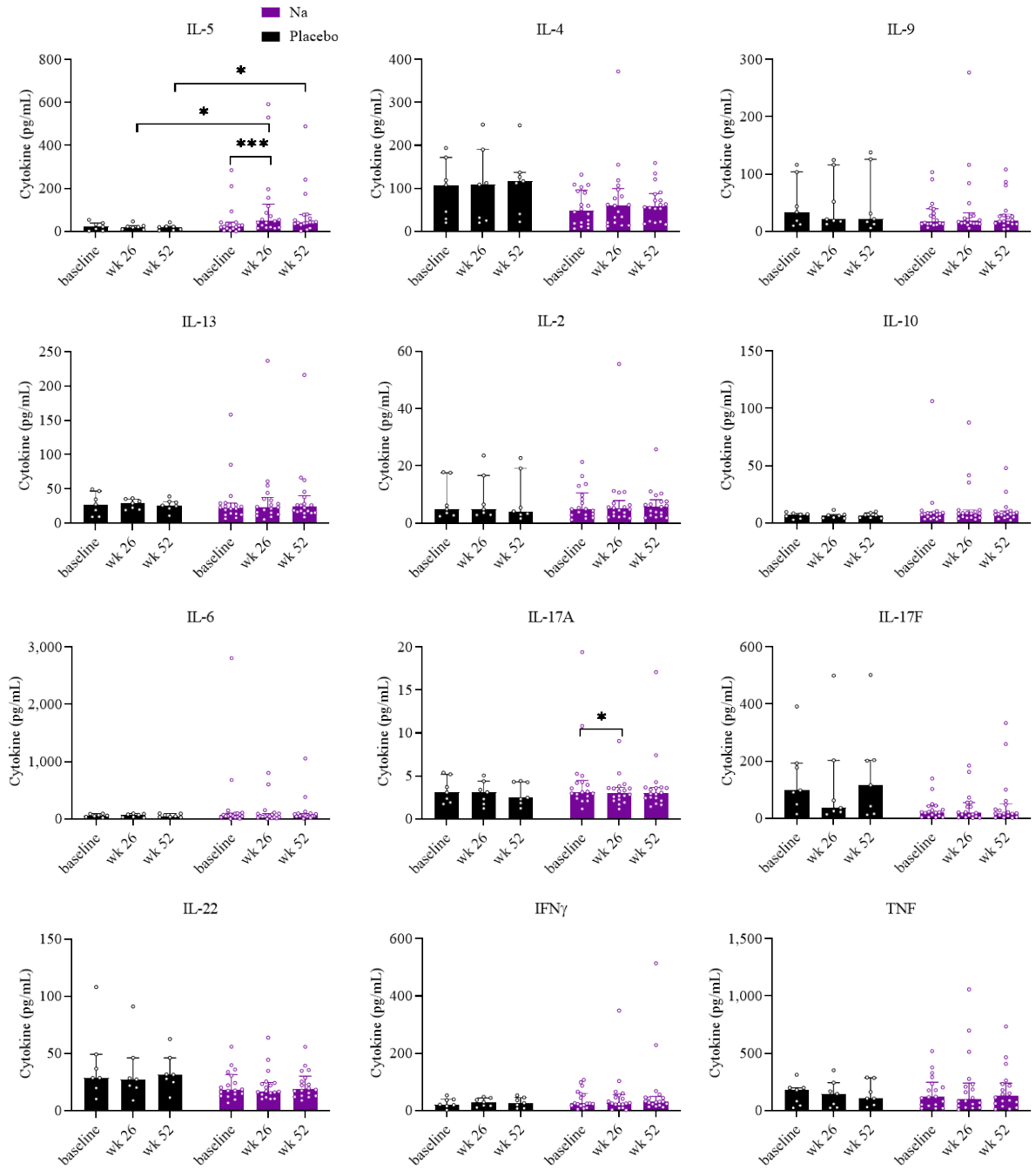


Figure 5-16 Cytokine concentrations (pg/mL) at baseline, wk26, and wk52 in Placebo and combined worm treatment groups (Na)
* $p \leq 0.05$; *** $p \leq 0.001$ (Mann-Whitney test of differences between groups and Friedman test for longitudinal differences)

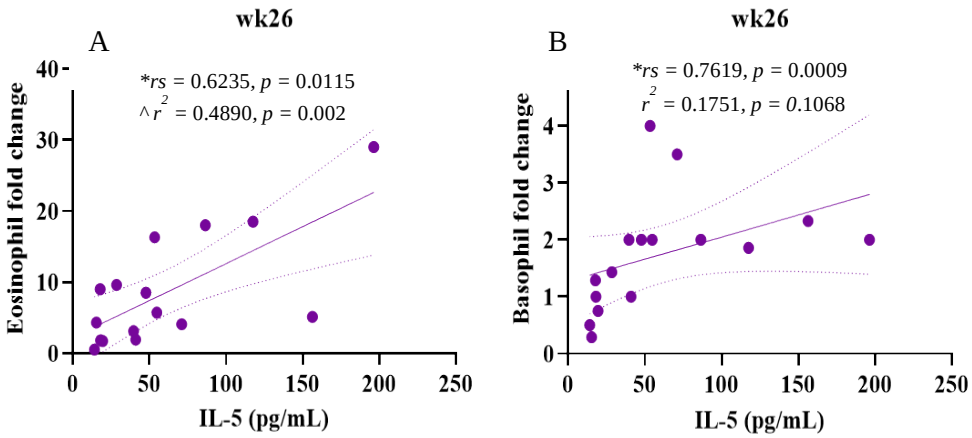


Figure 5-17 Correlation and linear regression of IL-5 (pg/mL) with A) eosinophil and B) basophil fold changes at wk26
* significant correlation; ^ significant regression

5.2.8 Adipokines

Adipokines play an important role in metabolic homeostasis, and their secretion is disturbed in obesity. Evidence from animal and human studies suggests that helminth infection can modify adipokine secretion. As an exploratory component of the trial, I investigated hormonal changes frequently highlighted in metabolic disturbance: leptin, adiponectin (including leptin/adiponectin ratio), and omentin. I was particularly interested in exploring if infection per se would result in differential concentrations of these hormones and have therefore combined the worm groups to compare against the Placebo group.

5.2.8.1 Leptin

Mann-Whitney tests showed no significant differences in fold changes from baseline between Placebo and the combined worm groups (Table 5-5 and Figure 5-17). Given the complementary roles of insulin and leptin in regulating blood glucose, I also investigated the association between leptin fold changes and HOMA-IR changes. No significant correlations between leptin fold changes and HOMA-IR changes were evident in any group (Figure 5-18).

5.2.8.2 Adiponectin

As for leptin, Mann-Whitney tests showed no significant difference in median fold changes from baseline between Placebo and combined worm groups (Table 5-5 and Figure 5-17). As adiponectin levels positively correlate with insulin resistance, I also explored the association between adiponectin fold changes and HOMA-IR changes (Figure 5-20). While there was a trend in the combined worm treatment groups for greater HOMA-IR improvement with higher adiponectin increases at wk52 (Figure 5-19), the correlation was not significant ($p = 0.0895$).

5.2.8.3 Omentin-1

Altered omentin-1 secretion appears to modulate insulin sensitivity, with a reported inverse relationship between omentin-1 serum levels and insulin resistance. Median omentin levels were near-identical between groups at baseline and did not vary greatly throughout the trial or between groups (Figure 5-17). Fold changes from baseline were not significantly different between Placebo and the combined worm groups (Table 5-5. Like leptin and adiponectin, individual responses in all groups were heterogeneous (Figure 5-17), with some individuals showing increases and others showing decreases over time. Therefore, I explored if individual changes in omentin-1 were associated with changes in HOMA-IR. A significantly positive correlation between fold changes from baseline and HOMA-IR changes was evident in the Placebo group at wk26 ($rs = 0.6364$, $p = 0.0299$) (Figure 5-20).

5.2.8.4 Leptin/adiponectin ratio

A lower leptin /adiponectin ratio observed with helminth infection may improve insulin resistance. In the present trial, Mann-Whitney tests showed no significant differences between groups in absolute leptin/adiponectin ratio and ratio changes (Table 5-6). Further, Mixed-effect models found no significant difference between baseline, wk26, and wk52 (Figure 5-21), indicating that *Na* infection did not improve the leptin/adiponectin ratio.

In summary, *Na* infection did not change adipokine concentrations significantly. Higher adiponectin fold change trended to be associated with greater improvement in HOMA-IR.

Table 5-5 Adipokine fold changes in Placebo and combined worm treatment groups at wk26 and wk52

		Placebo	Na
wk26	Leptin	1.00 0.85-1.08 *n = 12*	0.97 0.73-1.23 *n = 23*
	Adiponectin	1.30 0.40-2.08 *n = 10*	1.07 0.56-1.92 *n = 19*
	Omentin	0.74 0.53-1.24 *n = 12*	0.95 0.75-1.55 *n = 23*
wk52	Leptin	1.07 0.79-1.35 *n = 8*	0.89 0.76-1.13 *n = 20*
	Adiponectin	1.19 0.60-4.16 *n = 7*	1.51 0.65-2.72 *n = 18*
	Omentin	0.95 0.55-1.41 *n = 8*	1.07 0.57-1.92 *n = 20*

Data are presented as median and IQR. Na, worm treatment groups combined. No significant differences were found.

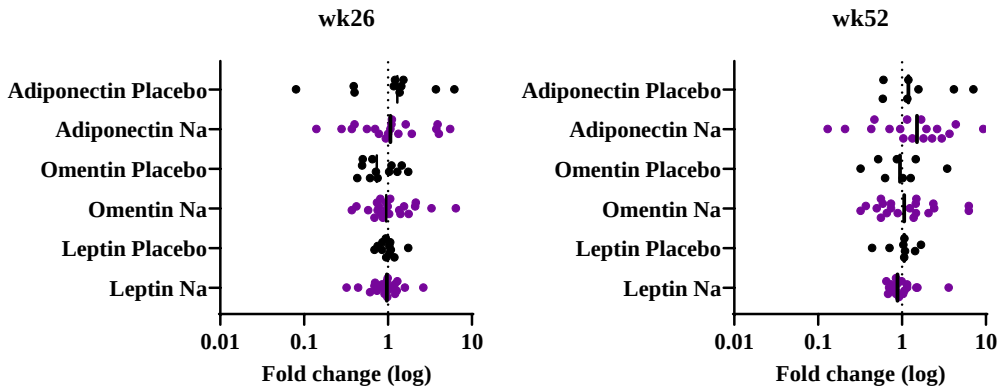


Figure 5-18 Median adipokine fold changes (log) in Placebo and combined worm treatment groups (Na) at wk26 and wk52.
No significant differences were found.(Mann-Whitney test)

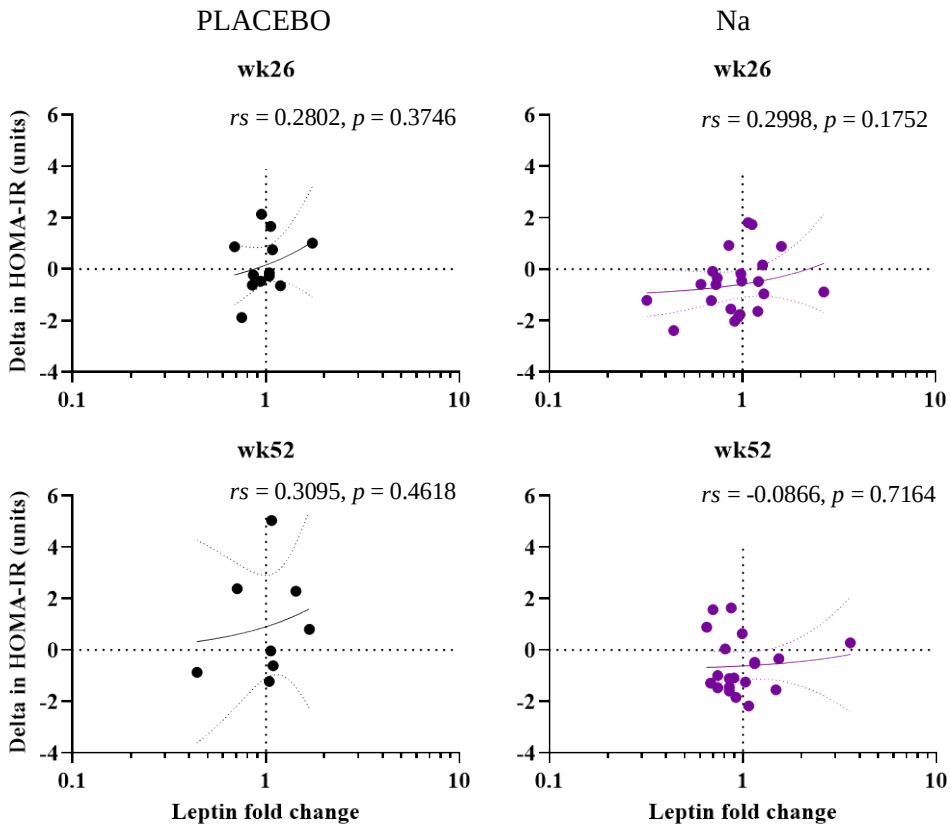


Figure 5-19 Correlation between leptin (log fold change) and HOMA-IR changes (units) from baseline in Placebo and worm treatment groups (Na) at wk26 and wk52
No significant correlation was found.

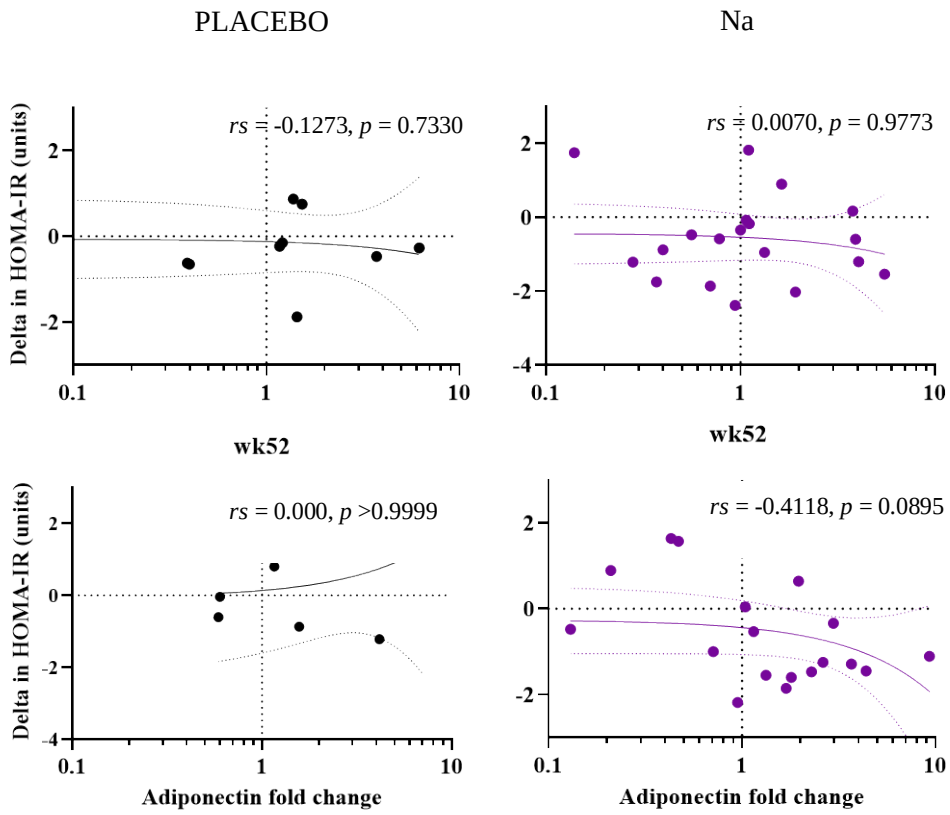


Figure 5-20 Correlation between adiponectin (log fold change) and HOMA-IR changes (units) from baseline in Placebo and worm treatment groups (Na) at wk26 and wk52
No significant correlation was found.

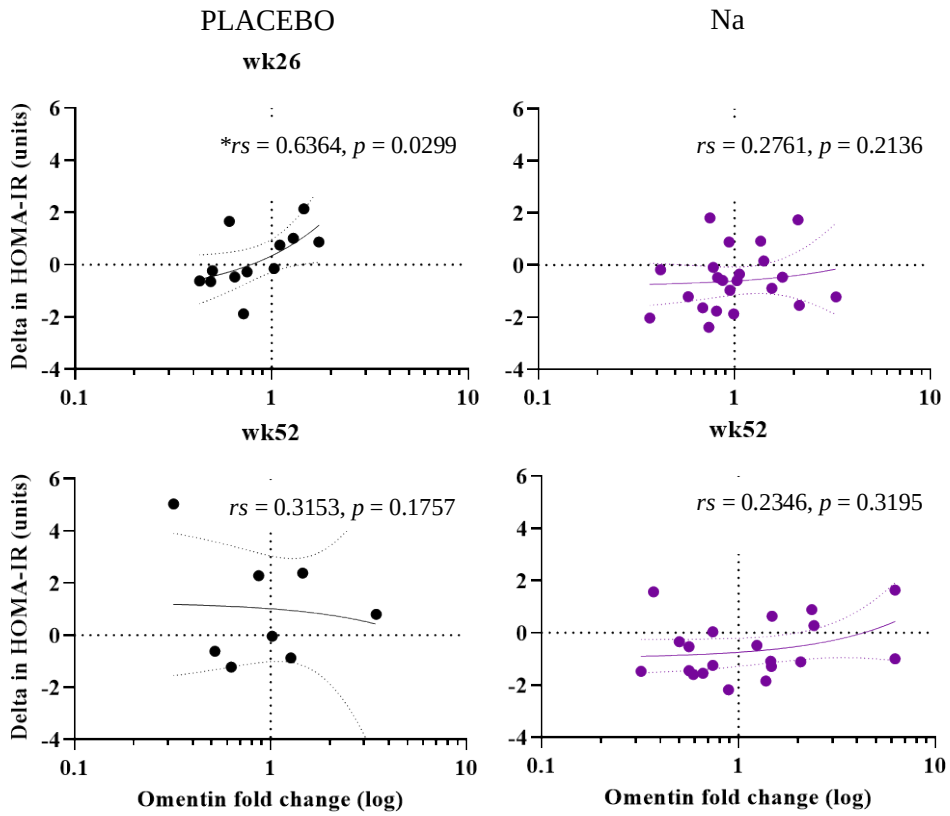


Figure 5-21 Correlation between omentin-1 (log fold change) and HOMA-IR changes (units) from baseline in Placebo and worm treatment groups (Na) at wk26 and wk52.
* significant correlation

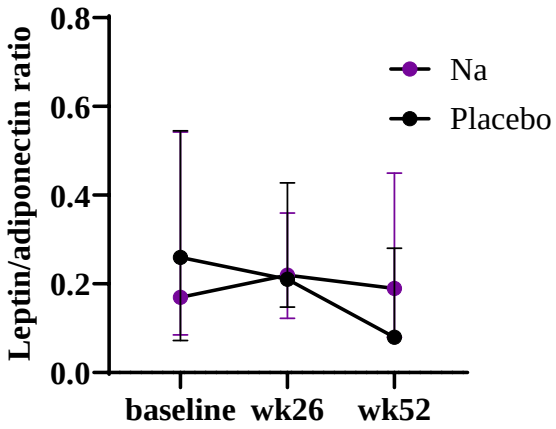


Figure 5-22 Leptin/adiponectin ratio in Placebo and combined worm treatment groups (Na) from baseline to week52 (median and IQR).
No significant difference was found (Mixed effects model)

Table 5-6 Leptin/adiponectin ratio by treatment group from baseline to wk52

	Placebo	**Na**
Baseline	0.26 0.07-0.56 *n = 10*	0.17 0.09-0.54 *n = 22*
wk26	0.21 0.15-0.43 *n = 10*	0.22 0.12-0.36 *n = 20*
Δ wk26	-0.06 -0.27-0.15 *n = 10*	-0.01 -0.30-0.16 *n = 20*
wk52	0.08 0.07-0.28 *n = 7*	0.19 0.08-0.45 *n = 19*
Δ wk52	-0.17 -0.22-0.03 *n = 7*	-0.02 -0.18-0.14 *n = 19*

Data are presented as median and IQR.
Na, combined worm treatment groups

5.3 Discussion

The current trial has shown that *Na* infection is safe, tolerable, and acceptable in humans with metabolic disease. We also found significant improvements in insulin resistance with low-dose infection (20 larvae) that were more modest with the higher dose (40 larvae). To shed some more light on these findings, the trial aimed to explore potential correlates that could mediate protection against metabolic disease. These included worm-related correlates such as immune responses and alterations in adipose tissue-secreted hormones, plus worm-unrelated factors, including age, sex, diet, and physical activity. I hypothesised that age, sex, diet, and physical activity would moderate improvements in metabolic parameters. Further, I hypothesised that *Na* infection would induce a biased Type 2 immune response and suppress pro-inflammatory immune responses. Finally, I speculated that infection would modify the adipokine profile to promote metabolic homeostasis.

Several publications have reported differential immune landscapes and responses to parasite infections between males and females in mice and humans (Bancroft et al., 2000; vom Steeg & Klein, 2016), possibly driven by sex steroid hormones. Given the central role of inflammation in the development of insulin resistance, these pre-existing differences could have biased our findings towards one sex. However, I did not find any significant differences in HOMA-IR responses between males and females or between either sex and the combined worm treatment group. A possible explanation for this lack of difference may be the low number of males in this trial and females outnumbering males by 3:1. Seven of the ten males in the trial were in the infected groups, but due to dropouts and some exclusions, data for only five were included in the analyses, which complicates interpretation of any potential trends.

Similarly, we did not observe a significant correlation between age and changes in HOMA-IR in the Placebo or the combined worm treatment groups. Ageing is associated with considerable changes in the competence and distribution of immune cells, which are thought to start in the sixth decade (Weyand & Goronzy, 2016). Most of our understanding of the ageing effect on our immune system stems from studies of the adaptive response, while changes in the innate response are not as well understood (Weyand & Goronzy, 2016). Still, studies in mice older mice (>20 months) and humans aged 65 and over have shown a dysregulated inflammatory response of the old innate immune system, impairing an efficient response to newly encountered invaders (Shaw et al., 2013). The term “inflamm-ageing” describes the ageing-associated basal inflammation characterised by elevated levels of pro-inflammatory cytokines, clotting factors, and acute phase reactants in the steady state. Given that most of our participants were not in their sixth decade, these changes presumably had not affected them.

Dietary intake can mediate inflammation and insulin resistance directly via the consumption of foods with pro-inflammatory or anti-inflammatory potential or indirect via the regulation of body mass. The current trial’s long duration prohibited detailed documentation of the participants’ food intake.

While food diaries could have provided more detailed insights into dietary behaviours, food journaling faces considerable challenges despite recent advances facilitating more convenient and even automatic entries (Cordeiro et al., 2015). These challenges include reliable food entry due to uncertainty about the proportions of ingredients and portion sizes, missing food in databases or the opposite, too many choices, and eating out. Further, feelings of shame and judgement can lead to incorrect entries (Cordeiro et al., 2015). Journalers also easily miss entries, which can easily lead to a breakdown in journaling habits. Considering all the challenges associated with food journaling and the two-year duration of the current trial, asking the participants to maintain a food diary was not feasible. The PREDIMED questionnaire provided an attractive and easily administered alternative to allow some observations regarding dietary habits. The simple format facilitates honest reporting, mainly as the responder is unfamiliar with the scoring criteria and does not try to match their answer to the desired score.

Overall, the self-reported adherence to a Mediterranean diet was low in all cohorts, remaining mostly stable throughout the trial. Three questions attracting the lowest scores in all groups related to olive oil, fish, and wine consumption. While the median absolute scores did not seem to worsen or improve in any group, there was some heterogeneity within each group, and I wanted to understand if changes in diet were associated with changes in HOMA-IR. No significant association was evident in the Placebo group, possibly due to the low numbers. However, in the combined worm groups, an increase in PREDIMED score correlated significantly with improved HOMA-IR at wk26, with a similar trend apparent for other time points. Consumption of a Mediterranean diet favourably affects glycaemic control (Ryan et al., 2000; Soriguer et al., 2004), possibly via downregulation of inflammatory markers (Esposito et al., 2004; Mena et al., 2009) and increases in adiponectin (Sierksma et al., 2004). Our results indicate that changed adherence to a Mediterranean diet may have contributed to positive and negative HOMA-IR changes.

The association between an improvement in feeding behaviour (adherence to a Mediterranean diet) and improved HOMA-IR at six months post-hookworm infection was intriguing. It is unlikely that the hookworm infection has changed feeding behaviour directly. A more likely explanation could be the enrolment in the trial and the requirement to complete the PREDIMED itself. Even though participants were unfamiliar with the scoring criteria, individual questions may have stimulated subtle subconscious changes in feeding behaviour, such as switching to olive oil or reducing soft drink consumption. We did find a trend towards a positive correlation between improved diet and HOMA-IR, even in the Placebo group. However, due to the small sample size, this did not reach statistical significance but reiterates this might be a trial-specific artefact.

Baseline self-reported physical activity, another potential factor in altered glycaemic control and inflammation, was unexpectedly high in all groups. The Placebo and L3-20 group reported a median of approximately 1000 METs per week, double the recommended 500 METs for cardiovascular health, and the L3-10 group reported around 650 METs per week. Evaluating whether these numbers reflected

actual activity levels is challenging, given the self-reported nature. Further, COVID-19 restrictions and lockdowns influenced individual behaviours, with several participants reporting reduced activity. More importantly, though, we were interested in any activity increases that may have been associated with the observed HOMA-IR improvements. Although median levels fluctuated moderately in all groups, no significant differences between groups or over time were evident. In the Placebo group, more participants reported lower activity at each evaluation visit, whereas responses in the worm treatment group were more heterogeneous. However, I did not find any significant correlation between physical activity and HOMA-IR changes in either group, suggesting that increased activity levels did not mediate HOMA-IR changes.

The influential role of body mass reduction on insulin resistance and inflammation is well established. Even a 5-10% reduction can improve metabolic function and health outcomes (Jensen et al., 2014). Median body mass was only significantly lower by about 10kg (~10%) at wk78 and wk104 in the L3-10 group, which also saw the biggest improvement in HOMA-IR. However, overall, responses were heterogeneous; therefore, I further explored the association between body mass changes and HOMA-IR changes. No significant association was evident in the Placebo group, possibly due to the low numbers. In contrast, body mass changes significantly correlated with HOMA-IR changes in the combined worm treatment groups from wk52 to wk104, where a greater improvement in insulin resistance was associated with a larger body mass reduction. While the association is compelling, a causal relationship is difficult to establish, and several possible mechanisms could explain this chicken-and-egg relationship. Worm-induced dampening of inflammation may have altered adipose tissue function, which enhanced metabolic control, including glycaemic control and body mass regulation. Alternatively, worm- or diet-induced body mass reduction may have improved glucose homeostasis. Or, it could have been a combination of these or some other unexplored mechanism.

Further delving into the mechanistic aspects of protection, I investigated selected Th 2 cellular and molecular responses to helminth infection. As expected, median eosinophil and basophil counts spiked at wk26 in both worm treatment groups and remained elevated throughout the trial, with no changes in the Placebo group. Responses varied considerably among participants, ranging from a 1.7 to a 29-fold increase, and eosinophil changes correlated significantly with HOMA-IR changes at all evaluation visits from wk52. This finding was not surprising given the previously highlighted importance of eosinophils in insulin resistance and glucose homeostasis (D. Wu et al., 2011). However, the correlation unexpectedly showed that smaller eosinophil increases correlated with greater HOMA-IR improvement. Within the confines of the current trial, it is impossible to interpret the consequences and significance of this finding, but it could indicate an optimal range of eosinophil counts beyond which eosinophil contribution to glucose homeostasis stagnates or even reverses. Of note, previous mouse models generally assessed eosinophil levels in liver and fat tissue (D. Wu et al., 2011), while my results are based on blood concentrations, which may have some bearing on my findings. No significant

association between basophil changes and HOMA-IR changes was evident, and a potential contribution of basophils to HOMA-IR changes could not be further clarified in the current trial.

Concurrent with the eosinophil and basophil peaks at wk26, IL-5 levels were significantly increased in the worm treatment groups. This highly specific Th-2 cytokine is pivotal in eosinophil and basophil biology and induces their expansion and activation (Roufosse, 2018). Supporting the crucial role of IL-5 in eosinophil and basophil expansion, IL-5 correlated significantly with the expansions of these leukocytes in the present trial. To the best of my knowledge, this is the first study to provide cellular and molecular validation of a long-lasting type 2 immune response during *Na* infection that was previously only demonstrated in animal models and circumstantially described in human cross-sectional and deworming studies.

Interestingly, IL-17A was also transiently elevated at wk26 in the worm treatment group. Although traditionally linked to inflammatory processes during bacterial and fungal infection, this cytokine is also a major regulator of type 2 immune responses via activation of neutrophils and AAMs (Ajendra et al., 2020; Allen et al., 2015). Helminth-induced tissue injury results in the production of chitinase-like proteins, leading to increased IL-17A release from δγT cells following activation by IL-1. Together with IL-25, IL-33, and TSLP, which activate dendritic cells and suppress INF-γ, IL-17A promotes type 2 responses. The subsequent release of IL-4 and IL-13, along with IL-17-mediated complement secretion from neutrophils, polarises macrophages to their alternatively activated phenotype, promoting tissue repair (Allen et al., 2015). The presence of both IL-17 and type immune responses may lead to pathologic complications during helminth infection. However, type 2 cytokines can also suppress IL-17A production in a critical feedback loop to prevent IL-17-driven excess pathology (Ajendra et al., 2020). Thus, this early but transient rise in IL-17A was likely associated with worm-induced tissue damage to the intestinal lining, which was repressed by the ensuing type 2 response.

Dysregulation of adipose tissue secreted hormones during obesity contributes to system-wide inflammation, insulin resistance, and disturbed energy homeostasis. Considering the detrimental role of inflammation on adipose tissue function, the anti-inflammatory effect of helminth infection may protect against disrupted adipose tissue signalling. However, few studies have previously investigated this interaction. In Indonesia, treatment of multiple soil-transmitted helminth infections significantly increased the leptin/adiponectin ratio (Tahapary, de Ruiter, Martin, Brienen, van Lieshout, Djuardi, et al., 2017). As leptin promotes inflammatory immune responses, this increase in leptin/adiponectin ratio may have contributed to the observed increase in insulin resistance in previously infected individuals. In contrast, *S. stercoralis*-infected Indian individuals had significantly lower levels of adiponectin than uninfected that reversed following anthelminthic treatment (Rajamanickam et al., 2018). In the current trial, I explored potential changes in key adipokine (omentin, leptin, and adiponectin) serum levels

during *Na* infection and found no compelling evidence for infection-associated changes in these adipokines.

The physiological role of omentin-1 remains incompletely understood but includes insulin-mediated improvement in glucose uptake, particularly in adipose tissue (de Souza Batista et al., 2007; Yang et al., 2006). Baseline median absolute omentin was near identical in all three groups and in a range found in healthy individuals (> 22.2 pg/mL). In the Placebo and L3-20 groups, median omentin trended downwards but remained stable in the L3-10 group, which may indicate that low-dose infection was protective against omentin decline. However, no significant difference was evident between groups or over time. As with other parameters, individual responses were heterogeneous, but I found no evidence of an association between changes in omentin and HOMA-IR in the worm treatment groups. Surprisingly, omentin change correlated significantly with HOMA-IR change at wk26 in the Placebo group, where a reduction in omentin was associated with HOMA-IR improvement. Previous studies suggested down-regulation of omentin-1 in obesity (Auguet et al., 2011; de Souza Batista et al., 2007; Moreno-Navarrete et al., 2011; Pan et al., 2010; Rothermel et al., 2020; Tan et al., 2008), T2DM (As Habi et al., 2019; Pan et al., 2019), and impaired glucose tolerance (As Habi et al., 2019; Kaushik & et al., 2018). Further, omentin-1 concentrations were inversely related to HOMA-IR in T2DM patients (Kaushik & et al., 2018). Therefore, the correlation in the Placebo group contrasted with these previous observations. It should be noted that most previous studies investigated patients with established T2DM, whereas the current trial's cohort was at risk of developing T2DM. Similar to our findings, previous study results were heterogeneous and associative, prohibiting causal conclusions about this correlation in the Placebo group.

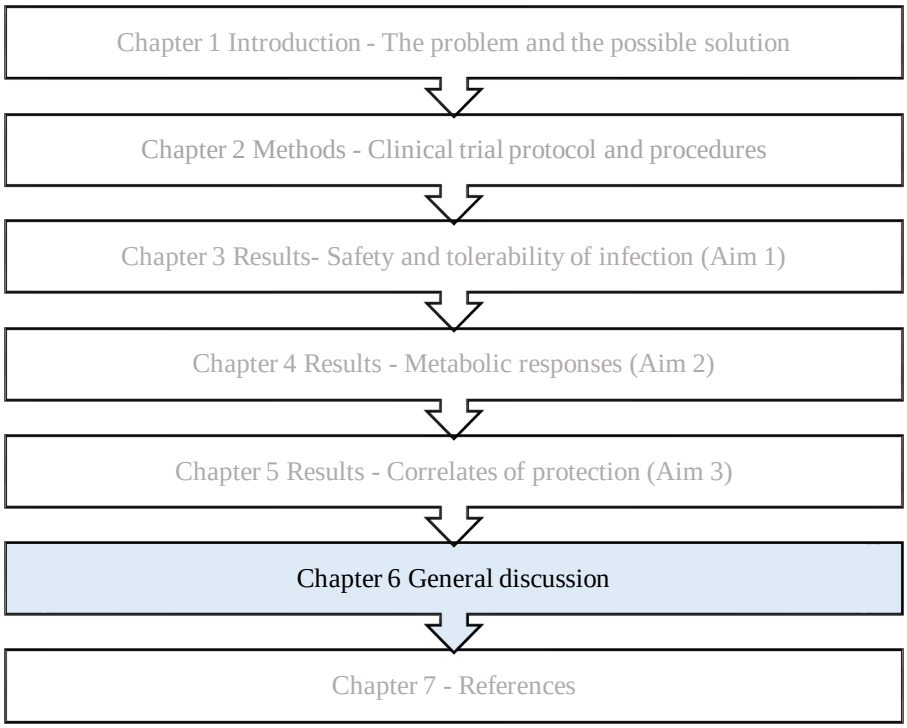
Leptin acts on the hypothalamus and targets tissues to regulate appetite and metabolism (Klok et al., 2007), improves insulin sensitivity (Paz-Filho, Mastronardi, Wong, et al., 2012), and may promote inflammatory responses (La Cava, 2017). Normal leptin concentrations range from 0.5-15.2 ng/mL in females and 0.5-12.5 ng/mL in males (Cleveland Clinic, 2022). Notably, hyperleptinaemia and resistance to normalising body mass are typical characteristics of obesity (Izquierdo et al., 2019; Liu et al., 2020), reflecting a state of leptin resistance. Baseline median leptin concentrations indicated that our cohort in all three treatment groups was leptin resistant with concentrations of up to 28 ng/mL. No differences between groups or over time were evident, suggesting no significant effect of *Na* infection on leptin concentrations. As with omentin, baseline concentrations and changes were heterogeneous, but I found no correlation between leptin changes and HOMA-IR changes.

Plasma adiponectin concentrations have been shown as inversely related to increasing adiposity (Ahl et al., 2015). Some evidence suggests that higher adiponectin concentrations can improve the blood lipid profile, glycaemic control, and inflammation in diabetic individuals (Li et al., 2009; Mantzoros et al., 2005). Adiponectin achieves its insulin-sensitising effect by reducing tissue triglycerides and upregulating insulin signalling (Yadav et al., 2013). In contrast to previous studies

that suggested modulation of adipokines in helminth infection (Surendar et al., 2019; Tahapary, de Ruiter, Martin, Brienen, van Lieshout, Djuardi, et al., 2017), *Na* infection did not induce significant changes in any measured adipokines in the current study. Yet, while adiponectin changes were not significantly correlated with HOMA-IR changes, a trend for reduced HOMA-IR with increased adiponectin existed at wk52, which approached significance ($p = 0.0895$). Individuals with adiponectin increases typically had improved HOMA-IR, while those with decreased adiponectin tended to have worsening HOMA-IR. Despite the lack of significance, the data are compelling and point towards a role of adiponectin in the helminth-mediated improvement of insulin resistance. Future studies with larger cohorts may help to verify this tentative association.

In summary, sex, age, physical activity, basophils, and adipokines were not significantly associated with HOMA-IR changes. In contrast, adherence to a Mediterranean diet, body mass, and eosinophils correlated significantly with HOMA-IR changes in hookworm-treated people. Further, IL-5 increased significantly in the combined worm treatment group and correlated with eosinophil and basophil expansion

6 GENERAL DISCUSSION



6.1 The research field: then and now

The rapidly rising prevalence of obesity-induced metabolic disorders such as T2DM presents a significant global economic and public health crisis. Conservative projections assuming a constant average expenditure per person and constant diabetes prevalence rate estimate a global healthcare expenditure on diabetes of one trillion USD by 2045. Besides the economic burden, T2DM also inflicts great personal strain on the affected individuals and their families. In particular, low- and middle-income countries, where prevalence is rising exponentially, will suffer disproportionately due to limited resources for early diagnosis and the disproportionally higher cost of treatment. Worryingly, an estimated 50% of all adults living with diabetes worldwide remain undiagnosed and are unaware of their condition. Approximately 85% of these live in low-to middle-income countries, where limited resources and low priority of diagnostic screening hinder early diagnosis. Late diagnosis magnifies the likelihood of future diabetic complications and demand for healthcare services and healthcare expenditure, compounding economic and personal burdens for developing nations (International Diabetes Federation, 2017). Once established, T2DM is costly to manage and cannot be cured due to enduring damage to the pancreatic β-islets. However, caloric restrictions and weight loss may promote reversal (remission).

Preventative treatment in individuals at risk of T2DM, such as people with MetS, provides an excellent but often disregarded opportunity to intervene before permanent pancreatic damage has evolved. As a sedentary lifestyle and diet-induced obesity primarily drive MetS, lifestyle interventions, including increased physical activity and dietary modifications, can successfully and cost-effectively reverse obesity and its associated MetS components. Unlike pharmaceutical drugs that primarily target hyperglycaemia, physical activity and a healthy diet can also address the underlying inflammation and have no side effects that need to be managed with secondary treatments. Sadly, these lifestyle interventions remain grossly underused, with health systems favouring pharmaceutical treatment and patients' reluctance to abandon their unhealthy behaviours.

Over the last couple of decades, helminth infection has emerged as a potential candidate for treating so-called Western diseases, including inflammatory disorders such as MetS and obesity. Human cross-sectional and deworming have described improved MetS components, including glucose homeostasis and body mass, in different cohorts infected with a range of helminth species, suggesting that helminth infection may protect against metabolic disease. Experimental animal studies have corroborated these associations and shed light on some of the mechanistic aspects of protection, which included immunomodulatory actions and adjustments in gut microbial composition. Yet, before the present trial, no causal evidence was available to support a protective role of helminth infection against the development of T2DM.

In this world-first proof of principle trial, I aimed to close the gap between observational evidence from human studies and experimental data from animal studies by adding some causal confirmation of a beneficial effect of helminth infection in metabolic disease. Indeed, infection with a low dose of 20 hookworm larvae induced a Type 2 immune response (increased eosinophils, IL-5, basophils) and significantly reduced insulin resistance, which was associated with modest body mass reductions and some improvements in mood and well-being compared to people treated with a placebo. Greater adherence to the Mediterranean diet and increased physical activity could not explain these changes. Adiponectin trended to correlate inversely with insulin resistance, yet this association was not significant. Compositional and functional induced by *Na* infection changes in the gut microflora could have contributed to improved HOMA-IR, and future microbiome analyses in progress will clarify these.

Future studies should verify my findings in larger and more diverse cohorts and finetune an intervention protocol that maximises benefits while maintaining participant safety. These interventions may include live worm infection, administration of worm-derived molecules, or even genetically-modified worms able to produce specific molecules recognised as beneficial in metabolic disease.

6.2 Strengths and limitations

6.2.1.1 Strengths

The study was designed as a randomised, double-blind, placebo-controlled trial, which can provide some of the most robust levels of evidence of clinical efficacy. This trial was the first experimental hookworm infection study in humans with metabolic disease, contributing some much-needed causal support for a protective role of helminth infection against metabolic disease. Besides further establishing the safety, tolerability, and acceptability of experimental hookworm infection, the trial integrated multiple outcomes and determinants that allowed exploring some of the mechanistic aspects previously reported in animal studies. These outcomes included lifestyle modifications, immune responses, and hormonal changes, with microbiome analyses to follow. Unlike previous cross-sectional trials that only provided a snapshot of associations between helminth infection and metabolic health, the current trial's two-year follow-up period allowed a unique insight into the short-and long-term consequences of hookworm infection in humans with metabolic disease.

The present highly informative trial will help to set the parameters for future clinical trials. Despite being a long and demanding trial, participant retention was quite respectable, especially considering that no incentives were offered. This outcome highlights the exceptional level of care provided by the trial team and the volunteers' admirable dedication. The trial structure enabled the collection of precious biological samples to interrogate biological mechanisms of protection against T2DM in a longitudinal manner.

The remarkable degree of HOMA-IR improvement I observed is considerably greater than any differences between infected and non-infected or pre- and post-infection previously reported in the few human studies that assessed insulin resistance as a metabolic outcome. Chen et al. (Chen et al., 2013) noted a 0.53-unit lower HOMA-IR in Chinese individuals with previous schistosome infection, while Tahapary et al. reported increased HOMA-IR from 1.08 to 1.17 units following Albendazole treatment on Flores Island, Indonesia (Tahapary, de Ruiter, Martin, Brienen, van Lieshout, Cobbaert, et al., 2017). In a cross-sectional study also on Flores Island, Wiria et al. (2015) found 0.15 units lower HOMA-IR in infected compared to non-infected individuals. In contrast, in this experimental infection study that added some causal evidence for improved glycaemic control with helminth infection, I found decreases in median HOMA-IR up to 1.48 units, considerably higher than any previously reported differences. Importantly, these improvements were independent of lifestyle changes, such as caloric restrictions and increased physical activity. While these lifestyle changes would still be desirable for other reasons beyond the prevention of T2DM, such significant improvement could have exciting therapeutic potential in individuals at risk of T2DM who fail to make lifestyle changes for various reasons.

6.2.1.2 Limitations

A key limitation of this trial was the small sample size, limiting the power of our findings. We screened over 200 volunteers, indicating that a lack of interest and unacceptability of the intervention were not the limiting factors. While the region's small population may have augmented recruitment challenges, the inclusion/exclusion criteria proved to be the principal reason for screening failures. Despite no shortage of individuals with abdominal obesity, most MetS components were either too far advanced or not advanced enough to qualify the applicant for entry. Several applicants were taking prescription medication for unrelated conditions, such as polycystic ovarian syndrome, that excluded them from participation.

Compounding the recruitment challenges, the trial's long duration proved to be a double-edged sword. On the one side, the long follow-up period benefitted data acquisition; on the other side, a two-year commitment complicated participant retention as everyday life events such as career changes or family obligations interfered, further reducing numbers in each group. While we aimed to retain participants that moved away by offering Zoom consultations or a visit with their local general practitioner, motivation to stay in the trial dropped quickly once participants had moved to their new location. Related to waning participant motivation, many participants joined the trial hoping to see considerable body mass improvements with infection implied by previous mouse study results. When these losses didn't eventuate, several participants opted for gastric sleeve surgery to assist with weight and health management, which excluded them from continued participation.

A further limitation was that blinding participants in hookworm trials is inherently challenging, given the hallmark inoculation features. As reported in previous trials, a transient erythematous rash at the inoculation site and abdominal discomfort interfered somewhat with the blinding process in the worm treatment groups. Also, several infected participants underwent pathology testing at their usual doctor for unrelated purposes, who pointed out a high eosinophil count indicating the presence of parasites. Most participants in the worm groups correctly guessed their status early during the trial. In contrast, the majority of participants given the Placebo remained unaware of their status until the end of the trial, with some genuinely surprised to learn during unblinding that they did not receive the worms. Whether or not infected, participants may have unconsciously modified their lifestyle based on their presumed infection status and thus distorted the results. However, deciphering if and how much this issue may have interfered with trial outcomes is beyond the scope of this trial.

Additionally, eliciting the desired outcomes may be dose-dependent. Naturally infected individuals often harbour large worm burdens, which may indicate that a higher dose is required to induce the protective effect of helminth infection. However, in the present trial that tested two different hookworm doses based on safety reports from previous trials, the lower dose of 20 worms was superior at reducing insulin resistance compared to the higher dose. This phenomenon could result from our participants' helminth-naïve status, where even a small dose might induce beneficial immune responses.

As discussed in Chapter 6, further considerations for the lesser response in the high-dose group may be baseline differences in HOMA-IR and age, organ-specific immune responses at higher worm burden, or an artefact of low participant numbers.

A 2019 systematic review suggested active infection was most beneficial to improving metabolic outcomes (Rennie et al., 2021), so it may be necessary to repeat treatment with a small dose at regular intervals to maintain a constant level of protection without compromising safety. This schedule would more closely mimic natural infection than administering a one-off high dose. Under experimental conditions, careful dose control and health monitoring could prevent potential detrimental changes associated with a high worm burden, such as iron deficiency anaemia. A group in the Netherlands inoculated volunteers with 1 x 50, 2 x 50, and 3 x 50 L3 two weeks apart with no significant difference in the number of adverse events between the doses (Hoogerwerf et al., 2021). While the higher doses did not raise any safety concerns, this trial did not investigate metabolic outcomes. Hence, it is unclear if the higher dose could provide added benefits.

Another worm-related limitation is the potential for variable infection success. All infected individuals developed eosinophil responses of varying extent, indicating infection success, but eosinophil responses fail to reflect the number of worms successfully attached to the intestinal lining. Although great care was taken to ensure the selection of fit and motile larvae for our inocula, consistent worm fitness was not guaranteed. We could neither verify the proportion of initial worm attachment nor their long-term survival, which may have had implications for metabolic and immune responses.

Further, the trial's first evaluation visit was scheduled for 26 weeks after the first inoculation. Given that some immune responses may peak early after inoculation in response to the worms' migration through the host, investigations at this point are likely to miss the early responses or reflect only the dwindling tail end.

Finally, participant selection bias limits the generalisability of my findings. Most participants were of Caucasian descent, and it is unclear if different populations, such as First Nations people or individuals of Asian descent, would experience similar improvements. Additionally, participants did not suffer from severe comorbidities that could modify the response to helminth infection. Although previous studies noted beneficial effects of natural helminth infections in diverse cohorts, this needs to be verified in experimental infection.

In summary, the trial's robust study design and integration of multiple outcomes allowed the first causal insight into the effects of experimental hookworm infection on metabolic outcomes, immune responses, and hormonal changes in humans with metabolic diseases. Future gut microbiome analyses will add further details to our understanding of the events in *Na* infection. However, my findings are limited by low numbers due to recruitment obstacles and dropouts. Blinding participants to hookworm infection is inherently challenging, and participants' perception of their infection status may have

interfered with their normal behaviour, distorting the trial outcomes. While the low dose successfully improved insulin resistance, this effect may be transient and depend on the continuity of infection. Additionally, variable worm quality and selection bias may have impacted the trial outcomes.

6.3 Bigger and better

The current trial's encouraging results indicate that a low dose of hookworm infection may improve metabolic disorders. Yet, the abovementioned limitations demand further studies to confirm efficacy in different cohorts, finetune an intervention schedule, and optimise worm production.

6.3.1 Participants

To substantiate the present findings, a multicentre Phase 2 clinical trial in different regions may offer the best opportunity to recruit suitable participants. Ideally, this larger cohort would include participants of different ethnic backgrounds to inform our understanding of efficacy in diverse cohorts. In particular, Australia's First Nations people should be actively encouraged to participate in a future trial, as this population group is almost four times more likely than non-indigenous Australians to develop T2DM.

The current selection criteria could be modified to include only individuals with increased WC and elevated HOMA-IR rather than accepting other MetS components for inclusion (hypertension and abnormal blood lipid profile or liver function test), narrowing the focus on insulin-resistant participants. While the current trial investigated hookworm infection as a preventative intervention, exploring helminths' therapeutic potential in individuals with established disease could be appealing and has shown promising results in murine studies (Khudhair et al., 2022). However, already diagnosed diabetics are likely to receive pharmaceutical treatment such as metformin, which would prohibit any definite conclusions about the effect of hookworm infection.

6.3.2 Study design

Although the long trial duration in the present trial resulted in a high dropout rate and subsequent loss of power, a similar or even extended duration could be appropriate for future trials. The longer time frame facilitates a more in-depth observation of the chronic response to hookworm infection, which will inform the future therapeutic use of hookworm infection. In a larger trial, power could still be retained even with a dropout rate of 40% observed in the present trial.

Future investigations could modify the trial protocol by adding an early evaluation visit. The first evaluation at wk26 was likely too late in the current schedule to capture acute immune responses. Although the participants came for their second inoculation eight weeks after the first, the protocol for this visit only included a blood draw for peripheral blood mononuclear cell separation but not pathology

testing or serum storage. This time point could provide an optimal opportunity to assess early cellular and molecular immune changes and could be adopted as the first evaluation visit in future trials.

6.3.3 Intervention

6.3.3.1 Investigational product quality

A key consideration in future trials will be the standardisation of the investigational product and procedures at all sites to ensure consistency of the intervention. In particular, a steady supply of uniform, high-quality worms will need to become a priority. Multiple local production sites would likely impact the consistency of larval quality. Therefore, one leading production site supplying larvae to all trial sites would be desirable. One major hurdle in this setup is transporting larvae from the production site to each trial site. Results from a previous randomised placebo-controlled trial using *Na* infection in coeliacs noted infection failure in nearly one-quarter of participants despite strict production supervision. All failures occurred in the overseas cohort, suggesting hookworm larvae do not travel well (Croese et al., 2020). Future studies must optimise the production method, transportation, and storage of the infective larvae to guarantee their integrity. Efforts in a New Zealand group to advance the production of Good Manufacturing Practice (GMP) worms that can be transported frozen are well underway (personal communication). Once optimised, this production method will significantly advance the provision of a standardised investigational product that can be produced at one site for consistency and shipped to other trial sites.

6.3.3.2 Dosage

In the present trial, infection with 2 x 10 L3 achieved superior results compared to 2 x 20 L3, indicating that the lower dose could be the preferred intervention in a follow-up trial. Improvement in HOMA-IR was highest mid-way through the trial but started to abate slightly by the two-year mark. This weakening response may be due to declining worm numbers or slowly waning protective immune responses. It is worth noting that, based on my data, induction of a more robust eosinophil response does not produce greater benefits regarding insulin resistance; therefore, it is not surprising that the lower worm burden caused a more effective response. A progressive intervention rather than a one-off initial dose might compensate for declining worm numbers or diminishing immune response and correct this waning response. For example, ten L3 could be administered every six months over two years. Such a gradual intervention would also more closely resemble natural infection and may be superior in maintaining a protective immune response.

6.3.4 Comparator

Improvement in HOMA-IR was associated with body mass reduction in the present trial. Therefore, it would be intriguing to clarify if a similar body mass reduction without helminth infection can achieve comparable HOMA-IR improvements. To explore this association, future trials may want to compare the efficacy of helminth infection in improving insulin resistance to a known obesity medication. For example, a comparator group with BMI >30 could receive Orlistat known to reduce body mass by inhibiting gut lipase (Australian Prescriber, 2000). Their HOMA-IR results could then be compared to a hookworm intervention group with similar adiposity to ascertain if helminth infection provided additional benefits to conventional body mass reduction.

6.3.5 Outcomes

Insulin resistance assessed by HOMA-IR was the primary metabolic outcome in the current trial. In clinical practice, glycated haemoglobin (HbA1c) measurement is an extensively used and convenient tool in diabetes screening (Mainous et al., 2016), as the patient does not need to attend in a fasting state, making it an attractive alternative to HOMA-IR. However, HbA1c sensitivity is lower than other diabetes screening tests, and by the time its levels rise noticeably, significant insulin resistance and β-cell dysfunction have likely already developed (Kanat et al., 2015). In contrast, insulin resistance occurs before β cells begin to fail (Pories & Dohm, 2012), and fasting insulin levels could identify insulin resistance well in advance of rising blood glucose and HbA1c. A 2018 systematic review concluded that fasting insulin levels were indicative of the early physiologic shifts in MetS and T2DM. Therefore, HOMA-IR, which incorporates fasting insulin, is a valuable early screening tool in identifying prediabetes before β-cell dysfunction. Further, reductions in HOMA-IR can indicate a remission or postponed risk of T2DM (Switzer et al., 2018), highlighting HOMA-IR as a suitable primary metabolic outcome in trials assessing the prevention of T2DM.

Additionally, finetuning the cut-off HOMA-IR value for inclusion in the study should be considered in future trials. The current trial used a cut-off value of 2.12 units regardless of sex and obesity status (BMI) based on the results from a Spanish cross-sectional study. However, other population studies indicate the cut-off values for insulin resistance (HOMA1-IR) and MetS (HOMA2-IR) could be considerably lower in some cohorts and differ between sexes and obesity status. For example, an Iranian study including nearly 5000 adults suggested the following cut-off values for HOMA1-IR and HOMA2-IR in females and males (Table 6-1) (Ghasemi et al., 2015).

An oral glucose tolerance test could be considered an additional glycaemic control measure. This simple and widely used test reflects the body's glucose disposal efficiency after a glucose load or a meal. However, this test requires four blood samples over the course of 120 minutes to determine glucose and insulin, imposing considerable time demands on participants (Muniyappa et al., 2000).

Further, this test assesses glucose tolerance, which is not equivalent to insulin sensitivity, so it should not replace HOMA-IR as the primary metabolic outcome.

Table 6-1 Optimal cut-off values of HOMA1-IR and HOMA2-IR in obese and non-obese females and males

	Cut-off point	
	Females	**Males**
HOMA1-IR		
BMI < 30 kg/m^2	1.58	1.22
BMI ≥ 30 kg/m^2	2.21	2.21
HOMA2-IR		
BMI < 30 kg/m^2	0.88	0.70
BMI ≥ 30 kg/m^2	1.18	1.23

HOMA1-IR, HOMA-IR index to identify insulin resistance; BMI, body mass index;HOMA2-IR, HOMA-IR index to identify metabolis syndrome.

Given these findings, future trials should first consider upon which HOMA-IR category (HOMA1-IR or HOMA2-IR) to base inclusion and then employ discrete HOMA-IR cut-off values depending on sex and obesity status. In contrast, age appears to be only marginally associated with insulin resistance (Stern et al., 2005) and did not increase HOMA-IR in the Iranian study. Further, the inclusion of the homeostatic model of β-cell function (HOMA-B) as an additional outcome should be evaluated. The combined report of HOMA-IR and HOMA-B may improve the prediction of T2DM as both insulin resistance and loss of β-cell function contribute to the pathogenesis of T2DM (Khardori, 2013; Meier & Bonadonna, 2013), warranting the assessment of both measures.

6.3.6 Lessons from the current trial

6.3.6.1 Faecal sample collection

The request for the collection of a faecal sample proved a challenging task for participants. While all participants submitted the baseline sample, compliance worsened with each consecutive evaluation visit despite the best efforts to make the process as convenient as possible. Participants received a sample collection kit at each evaluation that included instructions, gloves, and collection essentials, together with a team member's contact number to call for collection. The team member would either drive by their home or meet with them at a place nominated by the participant. Several reminders were sent by text message and email if samples were not received within a short time after the evaluation visit. However, the task seemed too unpleasant for some despite their continued enthusiasm and

dedication to the trial. Maybe future trials could provide a small incentive to encourage compliance with this request, as these samples are valuable elements in the assessment of helminth infection.

6.3.6.2 Body composition scan

Participants also did not appear to enjoy the yearly body composition measurement by DEXA scan. Like with faecal samples, all participants attended their baseline scan, with two participants exceeding the table's weight limits. At the one-year mark, only 18 attended, shrinking to six at the two-year mark despite numerous reminders. The reluctance to attend may be related to discomfort during the process, the required time commitment, or displeasure with a confronting body image on the report. Considering the relatively modest body mass reduction seen in the present trial and the additional measurement of WC, future trials could consider dismissing this assessment.

6.4 A treasure chest of samples for future exploratory studies

Blood and faecal samples collected and stored throughout the two-year trial afford superb opportunities to explore immune responses, metabolomics, and metagenomics in more detail in further exploratory studies. Further, whole-blood polyunsaturated fatty acid (PUFA) analysis may provide another insight into possible mediators of glycaemic control.

6.4.1 Immune profiling

While I have included some more basic immunological analyses in this thesis, the stored blood samples offer an exciting opportunity to probe deeper. Focusing on the PBMCs I collected will allow investigation of the cellular immune responses in much greater detail.

For example, it would be important to examine how hookworm infection affected the function of immune cell subsets in the blood. Bulk sequencing (RNA-seq) of peripheral blood mononuclear cells (PBMCs) or RNAseq on sorted T cells or specific innate cell subsets could be used as a genomic approach to study cellular responses by detecting and quantitatively analysing protein-encoding mRNA molecules. Analysing the transcriptome would allow the characterisation of differential T cell or macrophage activation status in the three treatment groups at each evaluation visit, adding to our understanding of distinct treatment-induced immune responses. With the future advance and likely associated price drop of single-cell sequencing, cellular heterogeneity in each phenotype could further be investigated, and previously unrecognised disease-associated cell populations or functional states may be identified. Proteomics analysis of PBMCs and serum could then be used to validate transcriptomics findings. These detailed immunological analyses may identify specific immune signatures most associated with improved metabolism, revealing new strategies for immune modulation not requiring live worms. Lastly, unlike previous studies involving people with immune conditions such as coeliac disease, multiple sclerosis or IBD, my study represented a unique trial where we infected "healthy" people with no prior exposure to a hookworm pathogen. Hence, detailed immune profiling in samples taken from my study will prove informative for understanding the general immune response to hookworm infection, which may have implications for the development of anti-helminth vaccines.

6.4.2 Metabolomics

Metabolomics of faeces and serum samples would complement transcriptomics and proteomics as a powerful approach to provide a snapshot of the underlying biochemical activity of cells and the molecular phenotype. Targeted metabolomics allows the study of a broad spectrum of gut microbiota metabolites as biomarkers of health and disease. Changes in specific gut metabolites, including SCFA's, bile acids, tryptophane, branched-chain amino acids, trimethylamine N-oxide, and indole derivatives, implicated in metabolic disorders could be the focus of future studies. Similarly, the serum metabolic

profile is altered with metabolic disorders. For example, the carbon number of triacylglycerols inversely relates to diabetes risk (Rhee et al., 2011). Further, branched-chain amino acids, α-hydroxybutyrate, and oleic acid correlated positively and glycine negatively with insulin resistance (Gall et al., 2010) and should thus be explored.

6.4.3 Metagenomics

Analyses of the microbiome are currently in progress. We are first examining potential changes in richness and diversity, followed by compositional analyses targeting families, genera, and species previously associated with metabolic disease and helminth infection, such as *A. muciniphila*. Finally, functional metagenomics of the gut microbiome data will enable comprehensive insights beyond compositional changes during helminth infection and/or metabolic disorders. This approach may lead to the discovery of novel functional microbial pathways associated with metabolic disorders or the identification of up-and-down-regulated functions such as SCFA synthesis during helminth infection or dysbiosis.

6.4.4 Lipid-associated pathways

Genomic analyses have identified a large contingent of genes and proteins associated with lipid biosynthesis and transportation in parasitic worms (International Helminth Genomes Consortium, 2019). Further, helminth-derived lipids regulate essential signalling pathways during worm development, host invasion, and infection establishment (Wang et al., 2021). Given the integrative role of lipids in helminth infection. Lipidomics of *Na* and its ES products could advance our understanding of lipid dynamics, interactions, and pathways. The potential discovery of lipids with immunomodulatory functions could inform our understanding of helminth-mediated protection in inflammatory diseases and have implications for future therapeutic interventions.

S. venezuelensis infection has been shown to significantly increase blood oleic acid, a monounsaturated fatty acid known to decrease adiposity and increase the *Lactobacillus* genus in the gut microbiota, which was associated with improved insulin resistance (Pace et al., 2018). Preliminary genomic analysis in platyhelminths indicated the presence of enzymes for *de novo* omega-3 polyunsaturated fatty acids (PUFAs) biosynthesis, providing a pathway through which helminths may increase the overall PUFA pool. Omega-3 PUFAs have been shown to prevent pancreatic β-cell destruction and insulin resistance through several mechanisms, including the reduction of inflammation and modulation of adipokine secretion (Baynes et al., 2018). In the present trial, blood spot collection cards were completed at baseline at all evaluation visits for future PUFA analysis, which may identify a further mechanism of helminth-mediated improvement of glycaemic control. Additionally, more subtle changes in serum lipid composition than in total triglyceride and total cholesterol levels may have occurred in response to helminth infection, which may be unveiled by in-depth phenotyping of

circulating lipid species by lipidomic approach, including some species previously reported as being associated with impaired metabolic homeostasis.

6.5 Worms and metabolism – how does it work?

Previously, experimental studies using various animal models have consistently shown a protective role of helminth infection against obesity and metabolic disorders. Similarly, human cross-sectional and deworming studies have reported improved glucose homeostasis and lower adiposity in helminth-infected individuals, although specific effects may vary depending on helminth species (Rennie et al., 2021). Yet, to date, evidence from human studies could not infer causality. The current trial was the first human experimental study providing causal evidence of a beneficial role of helminth infection in metabolic disorders. A low dose of 20 hookworm larvae administered in two doses of ten eight weeks apart significantly reduced insulin resistance in humans with MetS.

Additionally, the present trial explored potential correlates and mechanisms of protection, including sex, age, lifestyle factors, body mass reduction, immune responses, and hormonal shifts. Despite previous reports of diverse immune responses in males and females, as described in Section 5.1, sex did not appear to influence glucose outcomes in the present trial. However, the numbers were low and skewed towards female dominance, which prohibits accurate evaluation of a sex effect. Similarly, age seemed to be unrelated to HOMA-IR changes. Yet individual body mass reductions may have contributed to helminth-induced improvements in insulin resistance. The low-dose hookworm group experienced a median body mass reduction of approximately 10%, but individual responses were heterogeneous, with a positive correlation between body mass losses and HOMA-IR improvement. Body mass reductions may have been mediated solely by infection or resulted from greater adherence to a Mediterranean diet or a combination of both. It is further plausible that participants made subtle lifestyle changes that contributed to the observed body mass changes. Physical activity appeared unrelated to improved glycaemic control, and most participants, including those with significant HOMA-IR reductions, declared lower than baseline physical activity during the trial, ascribed to COVID restrictions and lockdowns. These restrictions may have also facilitated body mass increases in some participants. However, attempts at evaluating COVID restrictions' actual impact on trial outcomes appear futile.

Corresponding with the findings from animal studies, helminth infection induced a strong type 2 immune response correlated with HOMA-IR changes. Specifically, I found significant increases in eosinophils, basophils, and IL-5, where IL-5 was positively associated with eosinophil and basophil fold changes. These findings confirm that helminth-induced processes previously described in mouse studies (D. Wu et al., 2011) are replicated in humans and afford a mechanistic basis to explain the observed improvement in insulin resistance. Assuming similar immune actions of these cellular and molecular markers in humans and mice, the presence of eosinophils, basophils, and IL-5 likely promoted anti-inflammatory immune responses, including polarisation of AAMs and Tregs differentiation, which we aim to confirm in follow-up studies exploiting the stored Biobank samples.

The observed increases in adiponectin concentrations in the worm treatment groups may reflect potential anti-inflammatory type 2 immune responses. Although not significant, adiponectin increases trended to correlate inversely with insulin resistance, suggesting a potential role of adiponectin in improved glucose homeostasis (Surendar et al., 2019). As WAT inflammation dysregulates adipokine release, normalising adipokine release may result in a downstream effect of reduced information.

Changes in gut microbiota composition may indirectly moderate inflammation and insulin resistance. In particular, compromised gut barrier integrity during dysbiosis facilitates the translocation of inflammatory mediators from Gram-negative bacteria, such as lipopolysaccharides, to the systemic circulation, resulting in systemic inflammation and hyperendotoxemia. Helminth infections have been shown to induce beneficial alterations in gut microbial composition associated with improved gut barrier integrity. Specifically, populations of *Lactobacillaceae,* a Gram-positive *Firmicute,* reportedly expanded in the murine gastrointestinal tract following infection with multiple helminth species in different animal models. *Lactobacillaceae* have been shown to protect the mucosal barrier function (Resta-Lenert & Barrett, 2006) and expand regulatory T cell populations in the mesenteric lymph nodes (Kwon et al., 2010), thereby modulating inflammatory diseases. Interestingly, studies have established a mutualistic relationship between helminths and *Lactobacillaceae,* where helminth infections increase populations of *Lactobacillaceae* and these microbes, in turn, promote helminth infections (Dea-Ayuela et al., 2008 14417; Reynolds et al., 2014). Further, gut microbial composition mediates nutrient availability and inflammation via the breakdown of plant-derived carbohydrates and the production of SCFAs. Short-chain fatty acids provide an essential source of nutrients for colonocytes and are known to enter the systemic circulation and contribute to whole-body energy homeostasis via Treg induction and secretion of interleukins.

During the present trial, participants were asked to provide faecal samples to analyse potential changes in gut microflora composition and function. I have sent extracted DNA of participant samples at baseline and wk26 to the Ramaciotti Centre in Sydney for deep sequencing. Unfortunately, the delayed receipt of the sequencing results prohibited timely analysis and inclusion of the microbiome data into this thesis. However, analyses of compositional and functional changes are in process and will be prepared for publication shortly.

In summary, helminth-induced type 2 immune response, potentially supported by reduced adiposity, may have dampened inflammation, translating to improved glycaemic control. Further, increased adiponectin may have been correlated with protection.

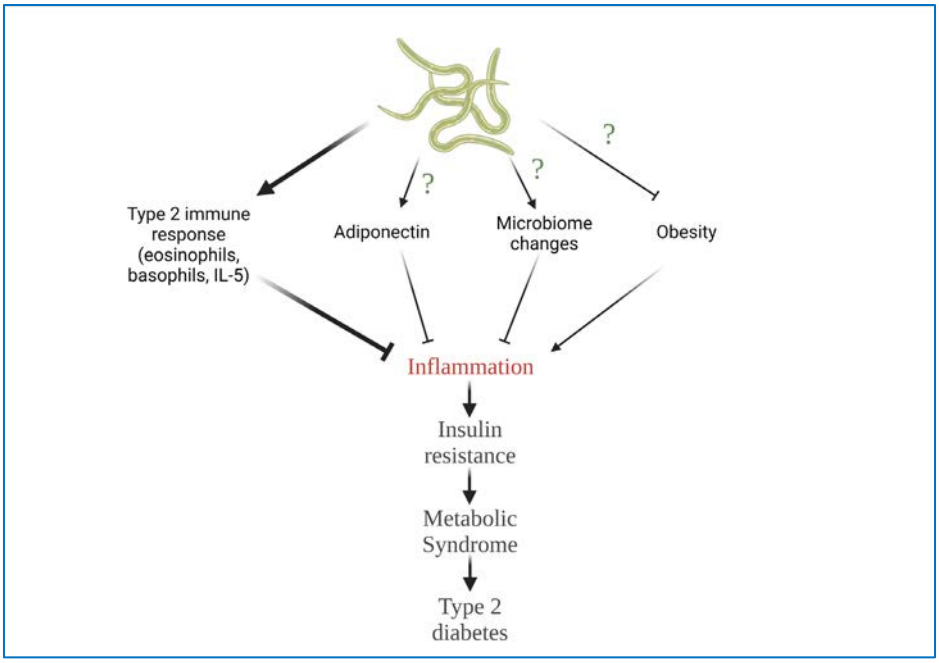


Figure 6-1 Potential mechanisms of helminth-mediated regulation of insulin resistance In the present trial, *Necator americanus* infection induced a type 2 immune response characterised by increased levels of eosinophils, basophils, and IL-5, which possible dampened inflammation. Modest adiponectin increases and body mass reductions in infected individuals may have contributed to reduced inflammation. Further, compositional changes of the gut microflora could have indirectly moderated inflammation and adiposity, which remains to be explored.

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6.6 Validating the mechanisms

The validity of my findings could be clarified in mouse models of obesity. For example, the treatment of obese mice with adult worm extracts of *Na* could be assessed for its potential to induce similar responses to mine, including immune and adipokine responses and body mass changes. In mouse models, manipulating inflammatory and anti-inflammatory mediators and/or selective depletion of cell populations such as macrophages may allow further elucidation of the immune cells and processes involved.

The gut microbiome analyses in progress might highlight characteristic shifts in gut microbiota composition and function following helminth infection. Should this be the case, targeted microbiota transfer or administration of a probiotic cocktail in a mouse model of obesity could provide an avenue to validate their beneficial consequences on metabolic disease. Ridaura et al. have previously used "humanised" animals to demonstrate the reproducibility of taxonomic features via faecal transfer (Ridaura et al., 2013). They colonised germ-free mice with uncultured faecal microbiota from each member of twin pairs discordant for obesity and found significantly greater body mass and adiposity in mice receiving the obese twin's microbiota. The "humanised" mouse model could be adopted to investigate if helminth infection produces a "healthier" microbiome. For example, faecal samples from pre-diabetics pre-and post-hookworm or placebo treatment could be transferred into germ-free mice. I hypothesise that if the hookworm treatment is associated with a "healthier" microbiome, the faecal samples from people post-hookworm treatment may be less likely to make mice diabetic than their baseline or placebo samples.

6.7 Future directions for worm-based treatments

Traditionally, worm-based treatment relied on live worm infection and later expanded to treatment with select worm proteins or protein cocktails. In the future, treatment with genetically-modified helminths might also become an option. With helminth infection, one size certainly does not fit all, and each option attracts arguments for or against its use.

In the context of T2DM prevention, live worm infection addresses both inflammation and insulin resistance. Given the complexity of helminth-induced immune responses to the worm's migration and its ES products, identifying a single molecule to replace this "whole package" with a single molecule or a combination of a few select molecules appears like a herculean effort. On the other hand, the administration of ES products could have comparable therapeutic potential in animal models of metabolic disease (Maizels et al., 2018; Ruyssers et al., 2008).

A key advantage of live infection is the ease of administration and long-lasting effects without frequent treatment. In contrast, the pharmacokinetic properties of helminth-derived therapeutic products

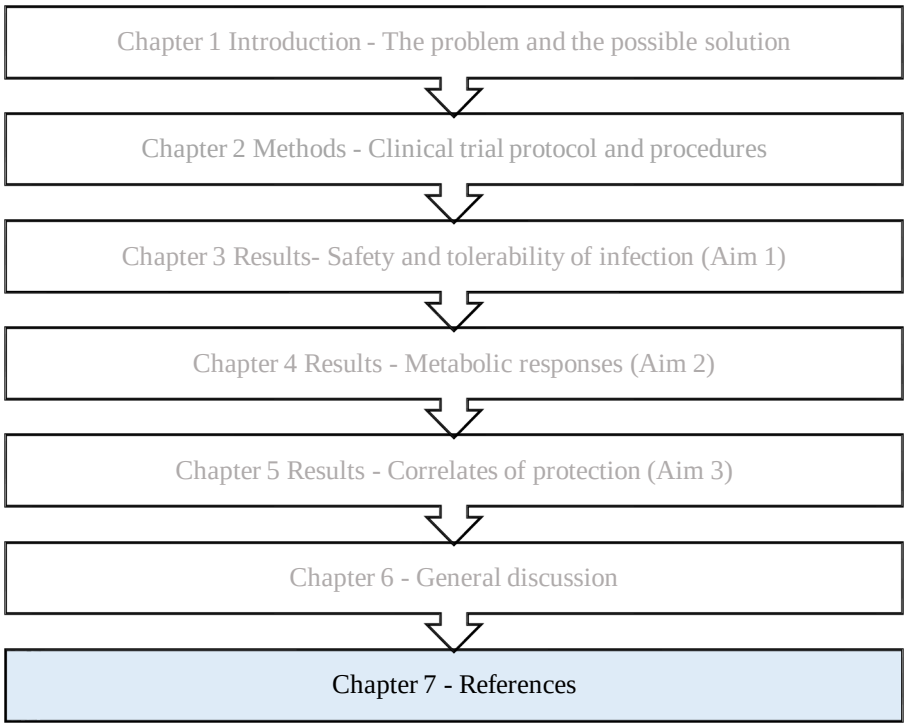
and identifying the optimal delivery route to the target tissue present a significant challenge. Although the symptoms of live worm infection are generally mild to moderate, administration of ES products could bypass this issue altogether. Further, variations in the production of live worms constrain the supply of a standardised product that meets strict quality control guidelines for a product to be distributed widely. Therefore, efforts enabling the future production of infective larvae under GMP guidelines are underway, which would also resolve storage- and transport-related complications of live worm production. In contrast, ES products can successfully and cost-effectively be produced and stored in large quantities under GMP guidelines.

A substantial barrier to large-scale live worm infection may be the hesitance of some individuals to harbour intestinal parasites, which were traditionally regarded as undesirable or even detrimental to health. Additionally, some individuals seem naturally resistant to helminth infection, precluding live worm infection in these individuals. Administration of ES molecules or cocktails may be more acceptable and might overcome the potential resistance to live worm infection.

Recently, live worm infection with genetically modified worms has also been proposed (https://www.scimex.org/newsfeed/parasites-to-fight-chemical-and-biological-weapons). Although initially expected to provide American soldiers with protection from bioterrorism agents such as anthrax (Burt, 2021), this research could have applications beyond the battlefield. While immediate benefits include funding to establish GMP protocols, future developments could see worms engineered to release drugs to combat multiple diseases, including metabolic disease. For example, glucagon-like peptide-1 receptor agonists approved in diabetes management could be conveniently secreted by helminths, replacing daily or weekly injections to improve effectiveness and increase patient comfort. Alternatively, hookworms could be engineered to secrete cytokines/adipokines that promote metabolic health (adiponectin, omentin, MCP-1, IL-13) or gut barrier integrity (secretory IgA, IL-22). While this prospect is enticing, several challenges must be addressed before such treatment can be rolled out. A primary concern would be the difficulty of controlling the amount of drug produced and secreted. Too little would render the treatment ineffective, while too much may have detrimental consequences. Further, the treatment would likely fail in helminth-resistant patients, and last, some individuals may oppose the idea of hosting genetically modified organisms

In summary, this study has added some much-needed causal support for a beneficial role of helminth infection in humans with metabolic disease. My findings should be confirmed in future trials that can also address the current trial's limitations regarding participant numbers, generalisability, and consistency of worm quality. The stored Biobank samples will allow further exploration of immune responses and gut microbiota composition and functional changes that may have moderated HOMA-IR improvements. While live worm therapy might not appeal to everyone, worm-derived molecules could provide an acceptable alternative in the future.

7 REFERENCES



References

Abner, S. R., Parthasarathy, G., Hill, D. E., & Mansfield, L. S. (2001). Trichuris suis: Detection of antibacterial activity in excretory-secretory products from adults. *Experimental Parasitology*, *99*(1), 26-36. https://doi.org/10.1006/expr.2001.4643

Afzal, S., Tybjærg-Hansen, A., Jensen, G. B., & Nordestgaard, B. G. (2016). Change in Body Mass Index associated with lowest mortality in Denmark, 1976-2013. *JAMA*, *315*(18), 1989-1996. https://doi.org/10.1001/jama.2016.4666

Agarwal, A., & Ioannidis, J. P. A. (2019). PREDIMED trial of Mediterranean diet: Retracted, republished, still trusted? *BMJ*, *364*, l341. https://doi.org/10.1136/bmj.l341

Ahl, S., Guenther, M., Zhao, S., James, R., Marks, J., Szabo, A., & Kidambi, S. (2015). Adiponectin levels differentiate metabolically healthy vs unhealthy among obese and nonobese white individuals. *Journal of Clinical Endocrinology and Metabolism*, *100*(11), 4172-4180. https://doi.org/10.1210/jc.2015-2765

Ainsworth, B. E., Haskell, W. L., Hermann, S. D., Meckes, N., Bassett Jr, D. R., Tudor-Locke, C., Gereer, J. L., Vezina, J., C., W.-G. M., & Leon, A. S. (2011). *The Compendium of Physical Activities Tracking Guide*. Retrieved January 30 2022 from https://sites.google.com/site/compendiumofphysicalactivities/

Ajendra, J. (2021). Lessons in type 2 immunity: Neutrophils in helminth infections. *Seminars in Immunology*, *53*, 101531. https://doi.org/10.1016/j.smim.2021.101531

Ajendra, J., Chenery, A. L., Parkinson, J. E., Chan, B. H. K., Pearson, S., Colombo, S. A. P., Boon, L., Grencis, R. K., Sutherland, T. E., & Allen, J. E. (2020). IL-17A both initiates, via IFNgamma suppression, and limits the pulmonary type-2 immune response to nematode infection. *Mucosal Immunology*, *13*(6), 958-968. https://doi.org/10.1038/s41385-020-0318-2

Alberti, K. G., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., Fruchart, J. C., James, W. P., Loria, C. M., Smith, S. C., Jr., International Diabetes Federation Task Force on, E., Prevention, National Heart, L., Blood, I., American Heart, A., World Heart, F., International Atherosclerosis, S., & International Association for the Study of, O. (2009). Harmonizing the metabolic syndrome: A joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*, *120*(16), 1640-1645. https://doi.org/10.1161/CIRCULATIONAHA.109.192644

Alberti, K. G., & Zimmet, P. Z. (1998). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabetic Medicine*, *15*(7), 539-553. https://doi.org/10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S

Allen, J. E., & Maizels, R. M. (2011). Diversity and dialogue in immunity to helminths. *Nature reviews. Immunology*, *11*(6), 375-388. https://doi.org/10.1038/nri2992

Allen, J. E., & Sutherland, T. E. (2014). Host protective roles of type 2 immunity: Parasite killing and tissue repair, flip sides of the same coin. *Seminars in Immunology*, *26*(4), 329-340. https://doi.org/10.1016/j.smim.2014.06.003

Allen, J. E., Sutherland, T. E., & Ruckerl, D. (2015). IL-17 and neutrophils: unexpected players in the type 2 immune response. *Current Opinion in Immunology*, *34*, 99-106. https://doi.org/10.1016/j.coi.2015.03.001

Amadori, A., Zamarchi, R., De Silvestro, G., Forza, G., Cavatton, G., Danieli, G. A., Clementi, M., & Chieco-Bianchi, L. (1995). Genetic control of the CD4/CD8 T-cell ratio in humans. *Nature Medicine*, *1*(12), 1279-1283. https://doi.org/10.1038/nm1295-1279

Anthony, R. M., Urban, J. F., Jr., Alem, F., Hamed, H. A., Rozo, C. T., Boucher, J. L., Van Rooijen, N., & Gause, W. C. (2006). Memory T(H)2 cells induce alternatively activated macrophages to mediate protection against nematode parasites. *Nature Medicine*, *12*(8), 955-960. https://doi.org/10.1038/nm1451

Aravindhan, V., Mohan, V., Surendar, J., Muralidhara Rao, M., Pavankumar, N., Deepa, M., Rajagopalan, R., Kumaraswami, V., Nutman, T. B., & Babu, S. (2010). Decreased prevalence

of lymphatic filariasis among diabetic subjects associated with a diminished pro-inflammatory cytokine response (CURES 83). *PLoS Neglected Tropical Diseases*, *4*(6), e707. https://doi.org/10.1371/journal.pntd.0000707

Aravindhan, V., Mohan, V., Surendar, J., Rao, M. M., Ranjani, H., Kumaraswami, V., Nutman, T. B., & Babu, S. (2010). Decreased prevalence of lymphatic filariasis among subjects with type-1 diabetes. *American Journal of Tropical Medicine and Hygiene*, *83*(6), 1336-1339. https://doi.org/10.4269/ajtmh.2010.10-0410

Armougom, F., Henry, M., Vialettes, B., Raccah, D., & Raoult, D. (2009). Monitoring bacterial community of human gut microbiota reveals an increase in Lactobacillus in obese patients and Methanogens in anorexic patients. *PloS One*, *4*(9), e7125. https://doi.org/10.1371/journal.pone.0007125

As Habi, A., Sadeghi, M., Arab, A., & Hajianfar, H. (2019). The association between omentin and diabetes: A systematic review and meta-analysis of observational studies. *Diabetes, Metabolic Syndrome and Obesity*, *12*, 1277-1286. https://doi.org/10.2147/DMSO.S206981

Auguet, T., Quintero, Y., Riesco, D., Morancho, B., Terra, X., Crescenti, A., Broch, M., Aguilar, C., Olona, M., Porras, J. A., Hernandez, M., Sabench, F., del Castillo, D., & Richart, C. (2011). New adipokines vaspin and omentin. Circulating levels and gene expression in adipose tissue from morbidly obese women. *BMC Medical Genetics*, *12*, 60. https://doi.org/10.1186/1471-2350-12-60

Australian Prescriber. (2000). *Orlistat*. Retrieved July 16 2022 from https://www.nps.org.au/australian-prescriber/articles/orlistat

Avogaro, P., Crepaldi, G., Enzi, G., & Tiengo, A. (1965). [Metabolic aspects of essential obesity]. *Epatologia*, *11*(3), 226-238. https://www.ncbi.nlm.nih.gov/pubmed/5871768 (Aspetti metabolici dell'obesita essenziale.)

Babayan, S. A., Sinclair, A., Duprez, J. S., & Selman, C. (2018). Chronic helminth infection burden differentially affects haematopoietic cell development while ageing selectively impairs adaptive responses to infection. *Scientific Reports*, *8*(1), 3802. https://doi.org/10.1038/s41598-018-22083-5

Babu, S., Bhat, S. Q., Pavan Kumar, N., Lipira, A. B., Kumar, S., Karthik, C., Kumaraswami, V., & Nutman, T. B. (2009). Filarial lymphedema is characterized by antigen-specific Th1 and th17 proinflammatory responses and a lack of regulatory T cells. *PLoS Neglected Tropical Diseases*, *3*(4), e420. https://doi.org/10.1371/journal.pntd.0000420

Bäckhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., Semenkovich, C. F., & Gordon, J. I. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(44), 15718-15723. https://doi.org/10.1073/pnas.0407076101

Bancroft, A. J., Artis, D., Donaldson, D. D., Sypek, J. P., & Grencis, R. K. (2000). Gastrointestinal nematode expulsion in IL-4 knockout mice is IL-13 dependent. *European Journal of Immunology*, *30*(7), 2083-2091. https://doi.org/10.1002/1521-4141(200007)30:7<2083::AID-IMMU2083>3.0.CO;2-3

Banks, W. A., Coon, A. B., Robinson, S. M., Moinuddin, A., Shultz, J. M., Nakaoke, R., & Morley, J. E. (2004). Triglycerides induce leptin resistance at the blood-brain barrier. *Diabetes*, *53*(5), 1253-1260. https://doi.org/10.2337/diabetes.53.5.1253

Banks, W. A., DiPalma, C. R., & Farrell, C. L. (1999). Impaired transport of leptin across the blood-brain barrier in obesity. *Peptides*, *20*(11), 1341-1345. https://doi.org/10.1016/s0196-9781(99)00139-4

Barczynska, R., Litwin, M., Slizewska, K., Szalecki, M., Berdowska, A., Bandurska, K., Libudzisz, Z., & Kapusniak, J. (2018). Bacterial microbiota and fatty acids in the faeces of overweight and obese children. *Polish Journal of Microbiology*, *67*(3), 339-345. https://doi.org/10.21307/pjm-2018-041

Barona, J., Jones, J. J., Kopec, R. E., Comperatore, M., Andersen, C., Schwartz, S. J., Lerman, R. H., & Fernandez, M. L. (2012). A Mediterranean-style low-glycemic-load diet increases plasma carotenoids and decreases LDL oxidation in women with metabolic syndrome. *The Journal of Nutritional Biochemistry*, *23*(6), 609-615. https://doi.org/10.1016/j.jnutbio.2011.02.016

Bashi, T., Bizzaro, G., Ben-Ami Shor, D., Blank, M., & Shoenfeld, Y. (2015). The mechanisms behind helminth's immunomodulation in autoimmunity. *Autoimmunity Reviews*, *14*(2), 98-104. https://doi.org/10.1016/j.autrev.2014.10.004

Baynes, H. W., Mideksa, S., & Ambachew, S. (2018). The role of polyunsaturated fatty acids (n-3 PUFAs) on the pancreatic beta-cells and insulin action. *Adipocyte*, *7*(2), 81-87. https://doi.org/10.1080/21623945.2018.1443662

Belzer, C., & de Vos, W. M. (2012). Microbes inside--from diversity to function: the case of Akkermansia. *ISME J*, *6*(8), 1449-1458. https://doi.org/10.1038/ismej.2012.6

Berbudi, A., Ajendra, J., Wardani, A. P., Hoerauf, A., & Hubner, M. P. (2016). Parasitic helminths and their beneficial impact on type 1 and type 2 diabetes. *Diabetes/Metabolism Research and Reviews*, *32*(3), 238-250. https://doi.org/10.1002/dmrr.2673

Berbudi, A., Surendar, J., Ajendra, J., Gondorf, F., Schmidt, D., Neumann, A. L., Wardani, A. P., Layland, L. E., Hoffmann, L. S., Pfeifer, A., Hoerauf, A., & Hubner, M. P. (2016). Filarial infection or antigen administration improves glucose tolerance in diet-induced obese mice. *Journal of Innate Immunity*, *8*(6), 601-616. https://doi.org/10.1159/000448401

Berg, A. H., & Scherer, P. E. (2005). Adipose tissue, inflammation, and cardiovascular disease. *Circulation Research*, *96*(9), 939-949. https://doi.org/10.1161/01.RES.0000163635.62927.34

Bernink, J. H., Germar, K., & Spits, H. (2014). The role of ILC2 in pathology of type 2 inflammatory diseases. *Current Opinion in Immunology*, *31*, 115-120. https://doi.org/10.1016/j.coi.2014.10.007

Bervoets, L., Van Hoorenbeeck, K., Kortleven, I., Van Noten, C., Hens, N., Vael, C., Goossens, H., Desager, K. N., & Vankerckhoven, V. (2013). Differences in gut microbiota composition between obese and lean children: a cross-sectional study. *Gut Pathogens*, *5*(1), 10. https://doi.org/10.1186/1757-4749-5-10

Bhargava, P., Li, C., Stanya, K. J., Jacobi, D., Dai, L., Liu, S., Gangl, M. R., Harn, D. A., & Lee, C. H. (2012). Immunomodulatory glycan LNFPIII alleviates hepatosteatosis and insulin resistance through direct and indirect control of metabolic pathways [Article]. *Nature Medicine*, *18*(11), 1665-1672. https://doi.org/10.1038/nm.2962

Bjorbaek, C., Elmquist, J. K., Frantz, J. D., Shoelson, S. E., & Flier, J. S. (1998). Identification of SOCS-3 as a potential mediator of central leptin resistance. *Molecular Cell*, *1*(4), 619-625. https://doi.org/10.1016/s1097-2765(00)80062-3

Boden, G. (2008). Obesity and free fatty acids. *Endocrinology and Metabolism Clinics of North America*, *37*(3), 635-646, viii-ix. https://doi.org/10.1016/j.ecl.2008.06.007

Boden, G., Cheung, P., Stein, T. P., Kresge, K., & Mozzoli, M. (2002). FFA cause hepatic insulin resistance by inhibiting insulin suppression of glycogenolysis. *American Journal of Physiology: Endocrinology and Metabolism*, *283*(1), E12-19. https://doi.org/10.1152/ajpendo.00429.2001

Boissier, J., Chlichlia, K., Digon, Y., Ruppel, A., & Mone, H. (2003). Preliminary study on sex-related inflammatory reactions in mice infected with Schistosoma mansoni. *Parasitology Research*, *91*(2), 144-150. https://doi.org/10.1007/s00436-003-0943-1

Bommer, C., Heesemann, E., Sagalova, V., Manne-Goehler, J., Atun, R., Barnighausen, T., & Vollmer, S. (2017). The global economic burden of diabetes in adults aged 20-79 years: A cost-of-illness study. *Lancet Diabetes & Endocrinology*, *5*(6), 423-430. https://doi.org/10.1016/S2213-8587(17)30097-9

Bonne-Annee, S., Kerepesi, L. A., Hess, J. A., O'Connell, A. E., Lok, J. B., Nolan, T. J., & Abraham, D. (2013). Human and mouse macrophages collaborate with neutrophils to kill larval Strongyloides stercoralis. *Infection and Immunity*, *81*(9), 3346-3355. https://doi.org/10.1128/IAI.00625-13

Bosello, O., Donataccio, M. P., & Cuzzolaro, M. (2016). Obesity or obesities? Controversies on the association between body mass index and premature mortality. *Eating and Weight Disorders*, *21*(2), 165-174. https://doi.org/10.1007/s40519-016-0278-4

Bosurgi, L., Cao, Y. G., Cabeza-Cabrerizo, M., Tucci, A., Hughes, L. D., Kong, Y., Weinstein, J. S., Licona-Limon, P., Schmid, E. T., Pelorosso, F., Gagliani, N., Craft, J. E., Flavell, R. A., Ghosh, S., & Rothlin, C. V. (2017). Macrophage function in tissue repair and remodeling requires IL-

4 or IL-13 with apoptotic cells. *Science*, *356*(6342), 1072-1076. https://doi.org/10.1126/science.aai8132

Bouchery, T., Kyle, R., Camberis, M., Shepherd, A., Filbey, K., Smith, A., Harvie, M., Painter, G., Johnston, K., Ferguson, P., Jain, R., Roediger, B., Delahunt, B., Weninger, W., Forbes-Blom, E., & Le Gros, G. (2015). ILC2s and T cells cooperate to ensure maintenance of M2 macrophages for lung immunity against hookworms. *Nature Communications*, *6*, 6970. https://doi.org/10.1038/ncomms7970

Bouchery, T., Le Gros, G., & Harris, N. (2019). ILC2s-Trailblazers in the host response against intestinal helminths. *Frontiers in Immunology*, *10*, 623. https://doi.org/10.3389/fimmu.2019.00623

Bouchery, T., Moyat, M., Sotillo, J., Silverstein, S., Volpe, B., Coakley, G., Tsourouktsoglou, T. D., Becker, L., Shah, K., Kulagin, M., Guiet, R., Camberis, M., Schmidt, A., Seitz, A., Giacomin, P., Le Gros, G., Papayannopoulos, V., Loukas, A., & Harris, N. L. (2020). Hookworms evade host immunity by secreting a deoxyribonuclease to degrade neutrophil extracellular traps. *Cell Host & Microbe*, *27*(2), 277-289 e276. https://doi.org/10.1016/j.chom.2020.01.011

Bouchery, T., Volpe, B., Shah, K., Lebon, L., Filbey, K., LeGros, G., & Harris, N. (2017). The study of host immune responses elicited by the model murine hookworms Nippostrongylus brasiliensis and Heligmosomoides polygyrus. *Current Protocols in Mouse Biology*, *7*(4), 236-286. https://doi.org/10.1002/cpmo.34

Boudina, S., & Graham, T. E. (2014). Mitochondrial function/dysfunction in white adipose tissue. *Experimental Physiology*, *99*(9), 1168-1178. https://doi.org/10.1113/expphysiol.2014.081414

Boyd, A., Ribeiro, J. M., & Nutman, T. B. (2014). Human CD117 (cKit)+ innate lymphoid cells have a discrete transcriptional profile at homeostasis and are expanded during filarial infection. *PloS One*, *9*(9), e108649. https://doi.org/10.1371/journal.pone.0108649

Bremer, A. A., Devaraj, S., Afify, A., & Jialal, I. (2011). Adipose tissue dysregulation in patients with metabolic syndrome. *Journal of Clinical Endocrinology and Metabolism*, *96*(11), E1782-1788. https://doi.org/10.1210/jc.2011-1577

Brigger, D., Riether, C., van Brummelen, R., Mosher, K. I., Shiu, A., Ding, Z., Zbaren, N., Gasser, P., Guntern, P., Yousef, H., Castellano, J. M., Storni, F., Graff-Radford, N., Britschgi, M., Grandgirard, D., Hinterbrandner, M., Siegrist, M., Moullan, N., Hofstetter, W., Leib, S. L., Villiger, P. M., Auwerx, J., Villeda, S. A., Wyss-Coray, T., Noti, M., & Eggel, A. (2020). Eosinophils regulate adipose tissue inflammation and sustain physical and immunological fitness in old age. *Nature Metabolism*, *2*(8), 688-702. https://doi.org/10.1038/s42255-020-0228-3

Brignardello, J., Morales, P., Diaz, E., Romero, J., Brunser, O., & Gotteland, M. (2010). Pilot study: alterations of intestinal microbiota in obese humans are not associated with colonic inflammation or disturbances of barrier function. *Alimentary Pharmacology and Therapeutics*, *32*(11-12), 1307-1314. https://doi.org/10.1111/j.1365-2036.2010.04475.x

Brooker, S., Bethony, J., & Hotez, P. J. (2004). Human hookworm infection in the 21st century. *Advances in Parasitology*, *58*, 197-288. https://doi.org/10.1016/S0065-308X(04)58004-1

Brooker, S., Hotez, P. J., & Bundy, D. A. (2008). Hookworm-related anaemia among pregnant women: A systematic review. *PLoS Neglected Tropical Diseases*, *2*(9), e291. https://doi.org/10.1371/journal.pntd.0000291

Brosschot, T. P., & Reynolds, L. A. (2018). The impact of a helminth-modified microbiome on host immunity. *Mucosal Immunology*, *11*(4), 1039-1046. https://doi.org/10.1038/s41385-018-0008-5

Brumpt, L. C., & Gujar, B. J. (1948). The treatment of polycythemia by artificial infection with Ancylostoma duodenale. *Indian Medical Gazette*, *83*(4), 166-169. https://www.ncbi.nlm.nih.gov/pubmed/29014749

Burt, J. (2021). *James Cook University to genetically modify intestinal worms to protect US military personnel from bioweapons*. Retrieved July 21 2022 from https://www.abc.net.au/news/2021-07-29/genetically-modify-worms-for-us-miltary-james-cook-uni/100331756

Camberis, M., Le Gros, G., & Urban, J., Jr. (2003). Animal model of Nippostrongylus brasiliensis and Heligmosomoides polygyrus. *Current Protocols in Immunology*, *Chapter 19*, Unit 19.12. https://doi.org/https://dx.doi.org/10.1002/0471142735.im1912s55

Camell, C. D., Sander, J., Spadaro, O., Lee, A., Nguyen, K. Y., Wing, A., Goldberg, E. L., Youm, Y. H., Brown, C. W., Elsworth, J., Rodeheffer, M. S., Schultze, J. L., & Dixit, V. D. (2017). Inflammasome-driven catecholamine catabolism in macrophages blunts lipolysis during ageing. *Nature*, *550*(7674), 119-123. https://doi.org/10.1038/nature24022

Campbell, S. C., Wisniewski, P. J., Noji, M., McGuinness, L. R., Haggblom, M. M., Lightfoot, S. A., Joseph, L. B., & Kerkhof, L. J. (2016). The effect of diet and exercise on intestinal integrity and microbial diversity in mice. *PloS One*, *11*(3), e0150502. https://doi.org/10.1371/journal.pone.0150502

Cani, P. D. (2018). Human gut microbiome: hopes, threats and promises. *Gut*, *67*(9), 1716-1725. https://doi.org/10.1136/gutjnl-2018-316723

Cani, P. D., Amar, J., Iglesias, M. A., Poggi, M., Knauf, C., Bastelica, D., Neyrinck, A. M., Fava, F., Tuohy, K. M., Chabo, C., Waget, A., Delmee, E., Cousin, B., Sulpice, T., Chamontin, B., Ferrieres, J., Tanti, J. F., Gibson, G. R., Casteilla, L., Delzenne, N. M., Alessi, M. C., & Burcelin, R. (2007). Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes*, *56*(7), 1761-1772. https://doi.org/10.2337/db06-1491

Cani, P. D., Bibiloni, R., Knauf, C., Waget, A., Neyrinck, A. M., Delzenne, N. M., & Burcelin, R. (2008). Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes*, *57*(6), 1470-1481. https://doi.org/10.2337/db07-1403

Cani, P. D., & de Vos, W. M. (2017). Next-generation beneficial microbes: The sase of Akkermansia muciniphila. *Frontiers in Microbiology*, *8*, 1765. https://doi.org/10.3389/fmicb.2017.01765

Cantacessi, C., Giacomin, P., Croese, J., Zakrzewski, M., Sotillo, J., McCann, L., Nolan, M. J., Mitreva, M., Krause, L., & Loukas, A. (2014). Impact of experimental hookworm infection on the human gut microbiota. *The Journal of Infectious Diseases*, *210*, 1431-1434. https://doi.org/10.1093/infdis/jiu256

Cao, H., Gerhold, K., Mayers, J. R., Wiest, M. M., Watkins, S. M., & Hotamisligil, G. S. (2008). Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. *Cell*, *134*(6), 933-944. https://doi.org/10.1016/j.cell.2008.07.048

Cardoso, V., Chesné, J., Ribeiro, H., García-Cassani, B., Carvalho, T., Bouchery, T., Shah, K., Barbosa-Morais, N. L., Harris, N., & Veiga-Fernandes, H. (2017). Neuronal regulation of type 2 innate lymphoid cells via neuromedin U. *Nature*, *549*, 277. https://doi.org/10.1038/nature23469

Centre for Disease Control and Prevention. (2019). *Hookworm*. Retrieved July 19 2022 from https://www.cdc.gov/parasites/hookworm/biology.html

Cereijo, R., Gavalda-Navarro, A., Cairo, M., Quesada-Lopez, T., Villarroya, J., Moron-Ros, S., Sanchez-Infantes, D., Peyrou, M., Iglesias, R., Mampel, T., Turatsinze, J. V., Eizirik, D. L., Giralt, M., & Villarroya, F. (2018). CXCL14, a brown adipokine that mediates brown-fat-to-macrophage communication in thermogenic adaptation. *Cell Metabolism*, *28*(5), 750-763 e756. https://doi.org/10.1016/j.cmet.2018.07.015

Chapman, P. R., Giacomin, P., Loukas, A., & McCarthy, J. S. (2021). Experimental human hookworm infection: A narrative historical review. *PLoS Neglected Tropical Diseases*, *15*(12), e0009908. https://doi.org/10.1371/journal.pntd.0009908

Chapman, P. R., Webster, R., Giacomin, P., Llewellyn, S., Becker, L., Pearson, M. S., De Labastida Rivera, F., O'Rourke, P., Engwerda, C. R., Loukas, A., & McCarthy, J. S. (2021). Vaccination of human participants with attenuated Necator americanus hookworm larvae and human challenge in Australia: a dose-finding study and randomised, placebo-controlled, phase 1 trial. *Lancet Infectious Diseases*. https://doi.org/10.1016/S1473-3099(21)00153-5

Chatterjee, S., Khunti, K., & Davies, M. J. (2017). Type 2 diabetes. *Lancet*, *389*(10085), 2239-2251. https://doi.org/10.1016/S0140-6736(17)30058-2

Chau, J., Chey, T., Burks-Young, S., Engelen, L., & Bauman, A. (2017). Trends in prevalence of leisure time physical activity and inactivity: Results from Australian National Health Surveys 1989 to 2011. *Australian and New Zealand Journal of Public Health*, *41*(6), 617-624. https://doi.org/10.1111/1753-6405.12699

Chen, F., Liu, Z., Wu, W., Rozo, C., Bowdridge, S., Millman, A., Van Rooijen, N., Urban, J. F., Jr., Wynn, T. A., & Gause, W. C. (2012). An essential role for TH2-type responses in limiting acute tissue damage during experimental helminth infection. *Nature Medicine, 18*(2), 260-266. https://doi.org/10.1038/nm.2628

Chen, F., Wu, W., Millman, A., Craft, J. F., Chen, E., Patel, N., Boucher, J. L., Urban, J. F., Jr., Kim, C. C., & Gause, W. C. (2014). Neutrophils prime a long-lived effector macrophage phenotype that mediates accelerated helminth expulsion. *Nature Immunology, 15*(10), 938-946. https://doi.org/10.1038/ni.2984

Chen, G. Y., & Nunez, G. (2010). Sterile inflammation: Sensing and reacting to damage. *Nature Reviews: Immunology, 10*(12), 826-837. https://doi.org/10.1038/nri2873

Chen, K., Li, F. H., Li, J., Cai, H. B., Strom, S., Bisello, A., Kelley, D. E., Friedman-Einat, M., Skibinski, G. A., McCrory, M. A., Szalai, A. J., & Zhao, A. Z. (2006). Induction of leptin resistance through direct interaction of C-reactive protein with leptin. *Nature Medicine, 12*(4), 425-432. https://doi.org/10.1038/nm1372

Chen, T., Huang, J. B., Dai, J., Zhou, Q., Raj, J. U., & Zhou, G. (2018). PAI-1 is a novel component of the miR-17~92 signaling that regulates pulmonary artery smooth muscle cell phenotypes [Article]. *American Journal of Physiology - Lung Cellular and Molecular Physiology, 315*(2), L149-L161. https://doi.org/10.1152/ajplung.00137.2017

Chen, Y., Lu, J., Huang, Y., Wang, T., Xu, Y., Xu, M., Li, M., Wang, W., Li, D., Bi, Y., & Ning, G. (2013). Association of previous schistosome infection with diabetes and metabolic syndrome: A cross-sectional study in rural China. *Journal of Clinical Endocrinology and Metabolism, 98*(2), E283-E287. https://doi.org/10.1210/jc.2012-2517

Choo, H. J., Kim, J. H., Kwon, O. B., Lee, C. S., Mun, J. Y., Han, S. S., Yoon, Y. S., Yoon, G., Choi, K. M., & Ko, Y. G. (2006). Mitochondria are impaired in the adipocytes of type 2 diabetic mice. *Diabetologia, 49*(4), 784-791. https://doi.org/10.1007/s00125-006-0170-2

Christian, P., Khatry, S. K., & West, K. P., Jr. (2004). Antenatal anthelmintic treatment, birthweight, and infant survival in rural Nepal. *Lancet, 364*(9438), 981-983. https://doi.org/10.1016/S0140-6736(04)17023-2

Chu, Y., Yang, J., Shi, J., Zhang, P., & Wang, X. (2020). Obesity is associated with increased severity of disease in COVID-19 pneumonia: A systematic review and meta-analysis. *European Journal of Medical Research, 25*(1), 64. https://doi.org/10.1186/s40001-020-00464-9

Church, T. (2011). Exercise in obesity, metabolic syndrome, and diabetes. *Progress in Cardiovascular Diseases, 53*(6), 412-418. https://doi.org/10.1016/j.pcad.2011.03.013

Cinti, S., Mitchell, G., Barbatelli, G., Murano, I., Ceresi, E., Faloia, E., Wang, S., Fortier, M., Greenberg, A. S., & Obin, M. S. (2005). Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *Journal of Lipid Research, 46*(11), 2347-2355. https://doi.org/10.1194/jlr.M500294-JLR200

Clements, A. C. A., & Addis Alene, K. (2022). Global distribution of human hookworm species and differences in their morbidity effects: A systematic review. *The Lancet Microbe, 3*(1), e72-e79. https://doi.org/10.1016/S2666-5247(21)00181-6

Cleveland Clinic. (2022, 23/02/2022). *Leptin and leptin resistance*. Retrieved July 08 2022 from https://my.clevelandclinic.org/health/articles/22446-leptin

Coakley, G., McCaskill, J. L., Borger, J. G., Simbari, F., Robertson, E., Millar, M., Harcus, Y., McSorley, H. J., Maizels, R. M., & Buck, A. H. (2017). Extracellular vesicles from a helminth parasite suppress macrophage activation and constitute an effective vaccine for protective immunity. *Cell Reports, 19*(8), 1545-1557. https://doi.org/10.1016/j.celrep.2017.05.001

Coakley, G., Volpe, B., Bouchery, T., Shah, K., Butler, A., Geldhof, P., Hatherill, M., Horsnell, W. G. C., Esser-von Bieren, J., & Harris, N. L. (2020). Immune serum-activated human macrophages coordinate with eosinophils to immobilize Ascaris suum larvae. *Parasite Immunology, 42*(7), e12728. https://doi.org/10.1111/pim.12728

Cooper, P., Walker, A. W., Reyes, J., Chico, M., Salter, S. J., Vaca, M., & Parkhill, J. (2013). Patent human infections with the whipworm, Trichuris trichiura, are not associated with alterations in the faecal microbiota. *PloS One, 8*(10), e76573. https://doi.org/10.1371/journal.pone.0076573

Cooper, P. J., Chico, M. E., Rodrigues, L. C., Ordonez, M., Strachan, D., Griffin, G. E., & Nutman, T. B. (2003). Reduced risk of atopy among school-age children infected with geohelminth

parasites in a rural area of the tropics. *Journal of Allergy and Clinical Immunology*, *111*(5), 995-1000. https://doi.org/10.1067/mai.2003.1348

Cordeiro, F., Epstein, D. A., Thomaz, E., Bales, E., Jagannathan, A. K., Abowd, G. D., & Fogarty, J. (2015). Barriers and negative nudges: Exploring challenges in food journaling. *Proceedings of the 33rd Annual ACM Conference on Human Factors in Computing Systems*, *2015*, 1159-1162. https://doi.org/10.1145/2702123.2702155

Cornick, S., Tawiah, A., & Chadee, K. (2015). Roles and regulation of the mucus barrier in the gut. *Tissue Barriers*, *3*(1-2), e982426. https://doi.org/10.4161/21688370.2014.982426

Cornier, M. A., Dabelea, D., Hernandez, T. L., Lindstrom, R. C., Steig, A. J., Stob, N. R., Van Pelt, R. E., Wang, H., & Eckel, R. H. (2008). The metabolic syndrome. *Endocrine Reviews*, *29*(7), 777-822. https://doi.org/10.1210/er.2008-0024

Cortes-Selva, D., & Fairfax, K. (2021). Schistosome and intestinal helminth modulation of macrophage immunometabolism. *Immunology*, *162*(2), 123-134. https://doi.org/10.1111/imm.13231

Cosovanu, C., & Neumann, C. (2020). The many functions of Foxp3(+) regulatory T Cells in the intestine. *Frontiers in Immunology*, *11*, 600973. https://doi.org/10.3389/fimmu.2020.600973

Costantini, L., Pasquarella, C., Odone, A., Colucci, M. E., Costanza, A., Serafini, G., Aguglia, A., Belvederi Murri, M., Brakoulias, V., Amore, M., Ghaemi, S. N., & Amerio, A. (2021). Screening for depression in primary care with Patient Health Questionnaire-9 (PHQ-9): A systematic review. *Journal of Affective Disorders*, *279*, 473-483. https://doi.org/10.1016/j.jad.2020.09.131

Crewe, C., An, Y. A., & Scherer, P. E. (2017). The ominous triad of adipose tissue dysfunction: inflammation, fibrosis, and impaired angiogenesis. *Journal of Clinical Investigation*, *127*(1), 74-82. https://doi.org/10.1172/JCI88883

Croese, J., Giacomin, P., Navarro, S., Clouston, A., McCann, L., Dougall, A., Ferreira, I., Susianto, A., O'Rourke, P., Howlett, M., McCarthy, J., Engwerda, C., Jones, D., & Loukas, A. (2015). Experimental hookworm infection and gluten microchallenge promote tolerance in celiac disease. *Journal of Allergy and Clinical Immunology*, *135*(2), 508-516. https://doi.org/10.1016/j.jaci.2014.07.022

Croese, J., Miller, G. C., Marquart, L., Llewellyn, S., Gupta, R., Becker, L., Clouston, A. D., Welch, C., Sidorenko, J., Wallace, L., Visscher, P. M., Remedios, M. L., McCarthy, J. S., O'Rourke, P., Radford-Smith, G., Loukas, A., Norrie, M., Masson, J. W., Gearry, R. B., Rahman, T., & Giacomin, P. R. (2020). Randomized, placebo controlled trial of experimental hookworm infection for improving gluten tolerance in celiac disease. *Clinical and Translational Gastroenterology*, *11*(12), e00274. https://doi.org/10.14309/ctg.0000000000000274

Croese, J., O'Neil, J., Masson, J., Cooke, S., Melrose, W., Pritchard, D., & Speare, R. (2006). A proof of concept study establishing Necator americanus in Crohn's patients and reservoir donors. *Gut*, *55*(1), 136. https://doi.org/10.1136/gut.2005.079129

Crowe, J., Lumb, F. E., Doonan, J., Broussard, M., Tarafdar, A., Pineda, M. A., Landabaso, C., Mulvey, L., Hoskisson, P. A., Babayan, S. A., Selman, C., Harnett, W., & Harnett, M. M. (2020). The parasitic worm product ES-62 promotes health- and life-span in a high calorie diet-accelerated mouse model of ageing. *PLoS Pathogens*, *16*(3), e1008391. https://doi.org/10.1371/journal.ppat.1008391

Danilowicz-Luebert, E., O'Regan, N. L., Steinfelder, S., & Hartmann, S. (2011). Modulation of specific and allergy-related immune responses by helminths. *Journal of Biomedicine and Biotechnology*, *2011*, 821578. https://doi.org/10.1155/2011/821578

Dao, M. C., Everard, A., Aron-Wisnewsky, J., Sokolovska, N., Prifti, E., Verger, E. O., Kayser, B. D., Levenez, F., Chilloux, J., Hoyles, L., Dumas, M.-E., Rizkalla, S. W., Doré, J., Cani, P. D., Clément, K., Le Mouhaër, S., Cotillard, A., Kennedy, S. P., Pons, N., Le Chatelier, E., Almeida, M., Quinquis, B., Galleron, N., Batto, J.-M., Renault, P., Zucker, J.-D., Ehrlich, S. D., Blottière, H., Leclerc, M., Juste, C., De Wouters, T., Lepage, P., & Consortium, M. I.-O. (2016). Akkermansia muciniphila and improved metabolic health during a dietary intervention in obesity: Relationship with gut microbiome richness and ecology. *Gut*, *65*(3), 426-436. https://doi.org/10.1136/gutjnl-2014-308778

Daveson, A., Jones, D., Gaze, S., McSorley, H., Clouston, A., Pascoe, A., Cooke, S., Speare, R., Macdonald, G., Anderson, R., McCarthy, J., Loukas, A., & Croese, J. (2011). Effect of

hookworm infection on wheat challenge in celiac disease: A randomised double-blinded placebo controlled trial. *PloS One*, *6*(3), e17366. https://doi.org/10.1371/journal.pone.0017366

De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J. B., Massart, S., Collini, S., Pieraccini, G., & Lionetti, P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(33), 14691-14696. https://doi.org/10.1073/pnas.1005963107

de la Cuesta-Zuluaga, J., Mueller, N. T., Corrales-Agudelo, V., Velasquez-Mejia, E. P., Carmona, J. A., Abad, J. M., & Escobar, J. S. (2017). Metformin is associated aith higher relative abundance of mucin-degrading Akkermansia muciniphila and several short-chain fatty acid-producing microbiota in the gut. *Diabetes Care*, *40*(1), 54-62. https://doi.org/10.2337/dc16-1324

de Los Reyes Jimenez, M., Lechner, A., Alessandrini, F., Bohnacker, S., Schindela, S., Trompette, A., Haimerl, P., Thomas, D., Henkel, F., Mourao, A., Geerlof, A., da Costa, C. P., Chaker, A. M., Brune, B., Nusing, R., Jakobsson, P. J., Nockher, W. A., Feige, M. J., Haslbeck, M., Ohnmacht, C., Marsland, B. J., Voehringer, D., Harris, N. L., Schmidt-Weber, C. B., & Esser-von Bieren, J. (2020). An anti-inflammatory eicosanoid switch mediates the suppression of type-2 inflammation by helminth larval products. *Science Translational Medicine*, *12*(540). https://doi.org/10.1126/scitranslmed.aay0605

De Pauw, A., Tejerina, S., Raes, M., Keijer, J., & Arnould, T. (2009). Mitochondrial (dys)function in adipocyte (de)differentiation and systemic metabolic alterations. *American Journal of Pathology*, *175*(3), 927-939. https://doi.org/10.2353/ajpath.2009.081155

de Ruiter, K., Jochems, S. P., Tahapary, D. L., Stam, K. A., König, M., van Unen, V., Laban, S., Höllt, T., Mbow, M., Lelieveldt, B. P. F., Koning, F., Sartono, E., Smit, J. W. A., Supali, T., & Yazdanbakhsh, M. (2020). Helminth infections drive heterogeneity in human type 2 and regulatory cells [Article]. *Science Translational Medicine*, *12*(524), Article eaaw3703. https://doi.org/10.1126/scitranslmed.aaw3703

de Ruiter, K., Tahapary, D. L., Sartono, E., Soewondo, P., Supali, T., Smit, J. W. A., & Yazdanbakhsh, M. (2017). Helminths, hygiene hypothesis and type 2 diabetes. *Parasite Immunology*, *39*(5), e12404, Article e12404. https://doi.org/doi:10.1111/pim.12404

de Souza Batista, C. M., Yang, R. Z., Lee, M. J., Glynn, N. M., Yu, D. Z., Pray, J., Ndubuizu, K., Patil, S., Schwartz, A., Kligman, M., Fried, S. K., Gong, D. W., Shuldiner, A. R., Pollin, T. I., & McLenithan, J. C. (2007). Omentin plasma levels and gene expression are decreased in obesity. *Diabetes*, *56*(6), 1655-1661. https://doi.org/10.2337/db06-1506

Dea-Ayuela, M. A., Rama-Iniguez, S., & Bolas-Fernandez, F. (2008). Enhanced susceptibility to Trichuris muris infection of B10Br mice treated with the probiotic Lactobacillus casei. *International Immunopharmacology*, *8*(1), 28-35. https://doi.org/10.1016/j.intimp.2007.10.003

Delaleu, N., & Bickel, M. (2004). Interleukin-1 beta and interleukin-18: Regulation and activity in local inflammation. *Periodontology 2000*, *35*, 42-52. https://doi.org/10.1111/j.0906-6713.2004.003569.x

den Besten, G., van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D., & Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research*, *54*(9), 2325-2340. https://doi.org/10.1194/jlr.R036012

Depommier, C., Everard, A., Druart, C., Plovier, H., Van Hul, M., Vieira-Silva, S., Falony, G., Raes, J., Maiter, D., Delzenne, N. M., de Barsy, M., Loumaye, A., Hermans, M. P., Thissen, J.-P., de Vos, W. M., & Cani, P. D. (2019). Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: A proof-of-concept exploratory study. *Nature Medicine*. https://doi.org/10.1038/s41591-019-0495-2

Derrien, M., Belzer, C., & de Vos, W. M. (2017). Akkermansia muciniphila and its role in regulating host functions. *Microbial Pathogenesis*, *106*, 171-181. https://doi.org/10.1016/j.micpath.2016.02.005

Derrien, M., Vaughan, E. E., Plugge, C. M., & de Vos, W. M. (2004). Akkermansia muciniphila gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. *International Journal of Systematic and Evolutionary Microbiology*, *54*(Pt 5), 1469-1476. https://doi.org/10.1099/ijs.0.02873-0

Despres, J. P., Lemieux, I., Bergeron, J., Pibarot, P., Mathieu, P., Larose, E., Rodes-Cabau, J., Bertrand, O. F., & Poirier, P. (2008). Abdominal obesity and the metabolic syndrome: Contribution to global cardiometabolic risk. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *28*(6), 1039-1049. https://doi.org/10.1161/ATVBAHA.107.159228

Dinarello, C. A. (1998). Interleukin-1 beta, interleukin-18, and the interleukin-1 beta converting enzyme. *Annals of the New York Academy of Sciences*, *856*, 1-11. https://doi.org/10.1111/j.1749-6632.1998.tb08307.x

Doehner, W., Clark, A., & Anker, S. D. (2010). The obesity paradox: Weighing the benefit. *European Heart Journal*, *31*(2), 146-148. https://doi.org/10.1093/eurheartj/ehp339

Donath, M. Y. (2016). Multiple benefits of targeting inflammation in the treatment of type 2 diabetes. *Diabetologia*, *59*(4), 679-682. https://doi.org/10.1007/s00125-016-3873-z

Donath, M. Y., Dinarello, C. A., & Mandrup-Poulsen, T. (2019). Targeting innate immune mediators in type 1 and type 2 diabetes. *Nature Reviews Immunology*. https://doi.org/10.1038/s41577-019-0213-9

Doolan, R., & Bouchery, T. (2022). Hookworm infections: Reappraising the evidence for a role of neutrophils in light of NETosis. *Parasite Immunology*, *44*(6), e12911. https://doi.org/10.1111/pim.12911

Duan, Q., Xiong, L., Liao, C., Liu, Z., Xiao, Y., Huang, R., Tan, T., Ouyang, Y., Cai, J., Xiao, M., Xu, H., Chen, Z., Chen, L., Yang, T., & Huang, H. (2018). Population based and animal study on the effects of Schistosoma japonicum infection in the regulation of host glucose homeostasis. *Acta Tropica*, *180*, 33-41. https://doi.org/10.1016/j.actatropica.2018.01.002

Duan, Y., Zeng, L., Zheng, C., Song, B., Li, F., Kong, X., & Xu, K. (2018). Inflammatory links between high fat diets and diseases. *Frontiers in Immunology*, *9*, 2649. https://doi.org/10.3389/fimmu.2018.02649

Duarte, A. M., Jenkins, T. P., Latrofa, M. S., Giannelli, A., Papadopoulos, E., de Carvalho, L. M., Nolan, M. J., Otranto, D., & Cantacessi, C. (2016). Helminth infections and gut microbiota - a feline perspective. *Parasit Vectors*, *9*(1), 625. https://doi.org/10.1186/s13071-016-1908-4

Dubé, J. J., Allison, K. F., Rousson, V., Goodpaster, B. H., & Amati, F. (2012). Exercise dose and insulin sensitivity: Relevance for diabetes prevention. *Medicine and Science in Sports and Exercise*, *44*(5), 793-799. https://doi.org/10.1249/MSS.0b013e31823f679f

Duncan, G. E. (2006). Exercise, fitness, and cardiovascular disease risk in type 2 diabetes and the metabolic syndrome. *Current Diabetes Reports*, *6*(1), 29-35. https://doi.org/10.1007/s11892-006-0048-1

Easton, A. V., Quinones, M., Vujkovic-Cvijin, I., Oliveira, R. G., Kepha, S., Odiere, M. R., Anderson, R. M., Belkaid, Y., & Nutman, T. B. (2019). The impact of anthelmintic treatment on human gut microbiota based on cross-sectional and pre- and post deworming comparisons in Western Kenya. *MBio*, *10*(2). https://doi.org/10.1128/mBio.00519-19

Egholm, C., Heeb, L. E. M., Impellizzieri, D., & Boyman, O. (2019). The Regulatory Effects of Interleukin-4 Receptor Signaling on Neutrophils in Type 2 Immune Responses. *Frontiers in Immunology*, *10*, 2507. https://doi.org/10.3389/fimmu.2019.02507

El-Den, S., Chen, T. F., Gan, Y. L., Wong, E., & O'Reilly, C. L. (2018). The psychometric properties of depression screening tools in primary healthcare settings: A systematic review. *Journal of Affective Disorders*, *225*, 503-522. https://doi.org/10.1016/j.jad.2017.08.060

El-Haschimi, K., Pierroz, D. D., Hileman, S. M., Bjorbaek, C., & Flier, J. S. (2000). Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. *Journal of Clinical Investigation*, *105*(12), 1827-1832. https://doi.org/10.1172/JCI9842

Elliott, D. E., & Weinstock, J. V. (2017). Nematodes and human therapeutic trials for inflammatory disease. *Parasite Immunology*, *39*(5), Article e12407. https://doi.org/10.1111/pim.12407

Elsaid, N. H., Sadik, N. A., Ahmed, N. R., Fayez, S. E., & Mohammed, N. A. E. (2018). Serum omentin-1 levels in type 2 diabetic obese women in relation to glycemic control, insulin resistance and metabolic parameters. *Journal of Clinical & Translational Endocrinology*, *13*, 14-19. https://doi.org/10.1016/j.jcte.2018.05.003

Endemann, D. H., & Schiffrin, E. L. (2004). Nitric oxide, oxidative excess, and vascular complications of diabetes mellitus. *Current Hypertension Reports*, *6*(2), 85-89. https://doi.org/10.1007/s11906-004-0081-x

Engeland, A., Bjørge, T., Søgaard, A. J., & Tverdal, A. (2003). Body Mass Index in adolescence in relation to total mortality: 32-Year follow-up of 227,000 Norwegian boys and girls. *American Journal of Epidemiology*, *157*(6), 517-523. https://doi.org/10.1093/aje/kwf219

Esposito, K., Maiorino, M. I., Bellastella, G., Chiodini, P., Panagiotakos, D., & Giugliano, D. (2015). A journey into a Mediterranean diet and type 2 diabetes: A systematic review with meta-analyses. *BMJ Open*, *5*(8), e008222. https://doi.org/10.1136/bmjopen-2015-008222

Esposito, K., Marfella, R., Ciotola, M., Di Palo, C., Giugliano, F., Giugliano, G., D'Armiento, M., D'Andrea, F., & Giugliano, D. (2004). Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: A randomized trial. *JAMA*, *292*(12), 1440-1446. https://doi.org/10.1001/jama.292.12.1440

Esser-von Bieren, J., Mosconi, I., Guiet, R., Piersgilli, A., Volpe, B., Chen, F., Gause, W. C., Seitz, A., Verbeek, J. S., & Harris, N. L. (2013). Antibodies trap tissue migrating helminth larvae and prevent tissue damage by driving IL-4Ralpha-independent alternative differentiation of macrophages. *PLoS Pathogens*, *9*(11), e1003771. https://doi.org/10.1371/journal.ppat.1003771

Estruch, R., Martinez-Gonzalez, M. A., Corella, D., Salas-Salvado, J., Ruiz-Gutierrez, V., Covas, M. I., Fiol, M., Gomez-Gracia, E., Lopez-Sabater, M. C., Vinyoles, E., Aros, F., Conde, M., Lahoz, C., Lapetra, J., Saez, G., Ros, E., & Investigators, P. S. (2006). Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. *Annals of Internal Medicine*, *145*(1), 1-11. https://doi.org/10.7326/0003-4819-145-1-200607040-00004

Estruch, R., & Ros, E. (2020). The role of the Mediterranean diet on weight loss and obesity-related diseases. *Reviews in Endocrine & Metabolic Disorders*, *21*(3), 315-327. https://doi.org/10.1007/s11154-020-09579-0

Everard, A., Belzer, C., Geurts, L., Ouwerkerk, J. P., Druart, C., Bindels, L. B., Guiot, Y., Derrien, M., Muccioli, G. G., Delzenne, N. M., de Vos, W. M., & Cani, P. D. (2013). Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(22), 9066-9071. https://doi.org/10.1073/pnas.1219451110

Expert Panel on Detection, E., and Treatment of High Blood Cholesterol in Adults. (2001). Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA*, *285*(19), 2486-2497. https://doi.org/10.1001/jama.285.19.2486

Falcone, F. H., Telford, G., Hooi, D., Brown, A. P., Seabra, R., Feary, J., Venn, A., Britton, J., & Pritchard, D. I. (2009). Antigen-driven basophil activation is indicative of early Necator americanus infection in IgE-seronegative patients. *Journal of Allergy and Clinical Immunology*, *124*(6), 1343-1350 e1347. https://doi.org/10.1016/j.jaci.2009.07.039

Fallon, P. G., Ballantyne, S. J., Mangan, N. E., Barlow, J. L., Dasvarma, A., Hewett, D. R., McIlgorm, A., Jolin, H. E., & McKenzie, A. N. (2006). Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. *Journal of Experimental Medicine*, *203*(4), 1105-1116. https://doi.org/10.1084/jem.20051615

Faty, A., Ferre, P., & Commans, S. (2012). The acute phase protein Serum Amyloid A induces lipolysis and inflammation in human adipocytes through distinct pathways. *PloS One*, *7*(4), e34031. https://doi.org/10.1371/journal.pone.0034031

Feary, J., Venn, A., Brown, A., Hooi, D., Falcone, F. H., Mortimer, K., Pritchard, D. I., & Britton, J. (2009). Safety of hookworm infection in individuals with measurable airway responsiveness: A randomized placebo-controlled feasibility study. *Clinical and Experimental Allergy*, *39*(7), 1060-1068. https://doi.org/10.1111/j.1365-2222.2009.03187.x

Feary, J. R., Venn, A. J., Mortimer, K., Brown, A. P., Hooi, D., Falcone, F. H., Pritchard, D. I., & Britton, J. R. (2010). Experimental hookworm infection: A randomized placebo-controlled trial in asthma. *Clinical and Experimental Allergy*, *40*(2), 299-306. https://doi.org/10.1111/j.1365-2222.2009.03433.x

Feng, X., Uchida, Y., Koch, L., Britton, S., Hu, J., Lutrin, D., & Maze, M. (2017). Exercise prevents enhanced postoperative neuroinflammation and cognitive decline and rectifies the gut microbiome in a rat model of metabolic syndrome. *Frontiers in Immunology*, *8*(1768). https://doi.org/10.3389/fimmu.2017.01768

Fernandes, J., Su, W., Rahat-Rozenbloom, S., Wolever, T. M., & Comelli, E. M. (2014). Adiposity, gut microbiota and faecal short chain fatty acids are linked in adult humans. *Nutrition & Diabetes, 4*, e121. https://doi.org/10.1038/nutd.2014.23

Feuerer, M., Herrero, L., Cipolletta, D., Naaz, A., Wong, J., Nayer, A., Lee, J., Goldfine, A. B., Benoist, C., Shoelson, S., & Mathis, D. (2009). Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nature Medicine, 15*(8), 930-939. https://doi.org/10.1038/nm.2002

Fink, S. L., & Cookson, B. T. (2006). Caspase-1-dependent pore formation during pyroptosis leads to osmotic lysis of infected host macrophages. *Cellular Microbiology, 8*(11), 1812-1825. https://doi.org/10.1111/j.1462-5822.2006.00751.x

Fischer, C. P., Berntsen, A., Perstrup, L. B., Eskildsen, P., & Pedersen, B. K. (2007). Plasma levels of interleukin-6 and C-reactive protein are associated with physical inactivity independent of obesity. *Scandinavian Journal of Medicine and Science in Sports, 17*(5), 580-587. https://doi.org/10.1111/j.1600-0838.2006.00602.x

Flegal, K. M., Carroll, M. D., Ogden, C. L., & Curtin, L. R. (2010). Prevalence and trends in obesity among US adults, 1999-2008. *JAMA, 303*(3), 235-241. https://doi.org/10.1001/jama.2009.2014

Flegal, K. M., Kit, B. K., Orpana, H., & Graubard, B. I. (2013). Association of all-cause mortality with overweight and obesity using standard body mass index categories: A systematic review and meta-analysis. *JAMA, 309*(1), 71-82. https://doi.org/10.1001/jama.2012.113905

Flier, J. S., Cook, K. S., Usher, P., & Spiegelman, B. M. (1987). Severely impaired adipsin expression in genetic and acquired obesity. *Science, 237*(4813), 405-408. https://doi.org/10.1126/science.3299706

Flier, J. S., & Maratos-Flier, E. (2017). Leptin's physiologic role: Does the emperor of energy balance have no clothes? *Cell Metabolism, 26*(1), 24-26. https://doi.org/10.1016/j.cmet.2017.05.013

Forslund, K., Hildebrand, F., Nielsen, T., Falony, G., Le Chatelier, E., Sunagawa, S., Prifti, E., Vieira-Silva, S., Gudmundsdottir, V., Pedersen, H. K., Arumugam, M., Kristiansen, K., Voigt, A. Y., Vestergaard, H., Hercog, R., Costea, P. I., Kultima, J. R., Li, J., Jorgensen, T., Levenez, F., Dore, J., Meta, H. I. T. c., Nielsen, H. B., Brunak, S., Raes, J., Hansen, T., Wang, J., Ehrlich, S. D., Bork, P., & Pedersen, O. (2015). Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature, 528*(7581), 262-266. https://doi.org/10.1038/nature15766

Franks, P. W., Hanson, R. L., Knowler, W. C., Sievers, M. L., Bennett, P. H., & Looker, H. C. (2010). Childhood obesity, other cardiovascular risk factors, and premature death. *New England Journal of Medicine, 362*(6), 485-493. https://doi.org/10.1056/NEJMoa0904130

Fricke, W. F., Song, Y., Wang, A. J., Smith, A., Grinchuk, V., Mongodin, E., Pei, C., Ma, B., Lu, N., Urban, J. F., Jr., Shea-Donohue, T., & Zhao, A. (2015). Type 2 immunity-dependent reduction of segmented filamentous bacteria in mice infected with the helminthic parasite Nippostrongylus brasiliensis. *Microbiome, 3*, 40. https://doi.org/10.1186/s40168-015-0103-8

Fujiwara, R. T., Geiger, S. M., Bethony, J., & Mendez, S. (2006). Comparative immunology of human and animal models of hookworm infection [Review]. *Parasite Immunology, 28*(7), 285-293. https://doi.org/10.1111/j.1365-3024.2006.00821.x

G. B. D. Risk Factor Collaborators. (2018). Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990-2017: A systematic analysis for the Global Burden of Disease Study 2017. *Lancet, 392*(10159), 1923-1994. https://doi.org/10.1016/S0140-6736(18)32225-6

G.B.D. Obesity Collaborators. (2017). Health effects of overweight and obesity in 195 countries over 25 years. *New England Journal of Medicine, 377*(1), 13-27.

Galioto, A. M., Hess, J. A., Nolan, T. J., Schad, G. A., Lee, J. J., & Abraham, D. (2006). Role of eosinophils and neutrophils in innate and adaptive protective immunity to larval Strongyloides stercoralis in mice. *Infection and Immunity, 74*(10), 5730-5738. https://doi.org/10.1128/IAI.01958-05

Gall, W. E., Beebe, K., Lawton, K. A., Adam, K. P., Mitchell, M. W., Nakhle, P. J., Ryals, J. A., Milburn, M. V., Nannipieri, M., Camastra, S., Natali, A., Ferrannini, E., & Group, R. S. (2010).

alpha-hydroxybutyrate is an early biomarker of insulin resistance and glucose intolerance in a nondiabetic population. *PloS One*, *5*(5), e10883. https://doi.org/10.1371/journal.pone.0010883

Garcia-Martinez, I., Shaker, M. E., & Mehal, W. Z. (2015). Therapeutic opportunities in damage-associated molecular pattern-driven metabolic diseases. *Antioxidanta & Redox Signaling*, *23*(17), 1305-1315. https://doi.org/10.1089/ars.2015.6383

Gause, W. C., Wynn, T. A., & Allen, J. E. (2013). Type 2 immunity and wound healing: Evolutionary refinement of adaptive immunity by helminths. *Nature Reviews: Immunology*, *13*(8), 607-614. https://doi.org/10.1038/nri3476

Gayoso-Diz, P., Otero-González, A., Rodriguez-Alvarez, M. X., Gude, F., García, F., De Francisco, A., & Quintela, A. G. (2013). Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a general adult population: Effect of gender and age: EPIRCE cross-sectional study. *BMC Endocrine Disorders*, *13*(1), 47. https://doi.org/10.1186/1472-6823-13-47

Gaze, S., McSorley, H. J., Daveson, J., Jones, D., Bethony, J. M., Oliveira, L. M., Speare, R., McCarthy, J. S., Engwerda, C. R., Croese, J., & Loukas, A. (2012). Characterising the mucosal and systemic immune responses to experimental human hookworm infection. *PLoS Pathogens*, *8*(2), e1002520. https://doi.org/10.1371/journal.ppat.1002520

Gebreselassie, N. G., Moorhead, A. R., Fabre, V., Gagliardo, L. F., Lee, N. A., Lee, J. J., & Appleton, J. A. (2012). Eosinophils preserve parasitic nematode larvae by regulating local immunity. *Journal of Immunology*, *188*(1), 417-425. https://doi.org/10.4049/jimmunol.1101980

Geiger, S. M., Fujiwara, R. T., Santiago, H., Correa-Oliveira, R., & Bethony, J. M. (2008). Early stage-specific immune responses in primary experimental human hookworm infection. *Microbes and Infection*, *10*(14-15), 1524-1535. https://doi.org/10.1016/j.micinf.2008.09.003

Gerbe, F., Sidot, E., Smyth, D. J., Ohmoto, M., Matsumoto, I., Dardalhon, V., Cesses, P., Garnier, L., Pouzolles, M., Brulin, B., Bruschi, M., Harcus, Y., Zimmermann, V. S., Taylor, N., Maizels, R. M., & Jay, P. (2016). Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. *Nature*, *529*(7585), 226-230. https://doi.org/10.1038/nature16527

Ghasemi, A., Tohidi, M., Derakhshan, A., Hasheminia, M., Azizi, F., & Hadaegh, F. (2015). Cut-off points of homeostasis model assessment of insulin resistance, beta-cell function, and fasting serum insulin to identify future type 2 diabetes: Tehran Lipid and Glucose Study. *Acta Diabetologica*, *52*(5), 905-915. https://doi.org/10.1007/s00592-015-0730-3

Giacomin, P., Agha, Z., & Loukas, A. (2016). Helminths and intestinal flora team up to improve gut health. *Trends in Parasitology*, *32*(9), 664-666. https://doi.org/10.1016/j.pt.2016.05.006

Giacomin, P., Zakrzewski, M., Croese, J., Su, X., Sotillo, J., McCann, L., Navarro, S., Mitreva, M., Krause, L., Loukas, A., & Cantacessi, C. (2015). Experimental hookworm infection and escalating gluten challenges are associated with increased microbial richness in celiac subjects. *Scientific Reports*, *5*, 13797. https://doi.org/10.1038/srep13797

Giacomin, P., Zakrzewski, M., Jenkins, T. P., Su, X. P., Al-Hallaf, R., Croese, J., de Vries, S., Grant, A., Mitreva, M., Loukas, A., Krause, L., & Cantacessi, C. (2016). Changes in duodenal tissue-associated microbiota following hookworm infection and consecutive gluten challenges in humans with coeliac disease. *Scientific Reports*, *6*, 36797. https://doi.org/10.1038/srep36797

Giacomin, P. R., Siracusa, M. C., Walsh, K. P., Grencis, R. K., Kubo, M., Comeau, M. R., & Artis, D. (2012). Thymic stromal lymphopoietin-dependent basophils promote Th2 cytokine responses following intestinal helminth infection. *Journal of Immunology*, *189*(9), 4371-4378. https://doi.org/10.4049/jimmunol.1200691

Gieseck, R. L., 3rd, Ramalingam, T. R., Hart, K. M., Vannella, K. M., Cantu, D. A., Lu, W. Y., Ferreira-González, S., Forbes, S. J., Vallier, L., & Wynn, T. A. (2016). Interleukin-13 activates distinct cellular pathways leading to ductular reaction, steatosis, and fibrosis. *Immunity*, *45*(1), 145-158. https://doi.org/10.1016/j.immuni.2016.06.009

Gillies, C. L., Abrams, K. R., Lambert, P. C., Cooper, N. J., Sutton, A. J., Hsu, R. T., & Khunti, K. (2007). Pharmacological and lifestyle interventions to prevent or delay type 2 diabetes in people with impaired glucose tolerance: systematic review and meta-analysis. *BMJ*, *334*(7588), 299. https://doi.org/10.1136/bmj.39063.689375.55

Giordano, A., Murano, I., Mondini, E., Perugini, J., Smorlesi, A., Severi, I., Barazzoni, R., Scherer, P. E., & Cinti, S. (2013). Obese adipocytes show ultrastructural features of stressed cells and die

of pyroptosis. *Journal of Lipid Research*, *54*(9), 2423-2436. https://doi.org/10.1194/jlr.M038638

Gleeson, M., Bishop, N. C., Stensel, D. J., Lindley, M. R., Mastana, S. S., & Nimmo, M. A. (2011). The anti-inflammatory effects of exercise: Mechanisms and implications for the prevention and treatment of disease. *Nature Reviews Immunology*, *11*(9), 607-615. https://doi.org/10.1038/nri3041

Golbidi, S., & Laher, I. (2014). Exercise-induced adipokine changes and the metabolic syndrome. *Journal of Diabetes Research*, *2014*, 726861. https://doi.org/10.1155/2014/726861

Goldfine, A. B., Fonseca, V., Jablonski, K. A., Chen, Y. D., Tipton, L., Staten, M. A., Shoelson, S. E., & Targeting Inflammation Using Salsalate in Type 2 Diabetes Study, T. (2013). Salicylate (salsalate) in patients with type 2 diabetes: A randomized trial. *Annals of Internal Medicine*, *159*(1), 1-12. https://doi.org/10.7326/0003-4819-159-1-201307020-00003

Goldfine, A. B., Fonseca, V., Jablonski, K. A., Pyle, L., Staten, M. A., Shoelson, S. E., & Team, T.-T. D. S. (2010). The effects of salsalate on glycemic control in patients with type 2 diabetes: A randomized trial. *Annals of Internal Medicine*, *152*(6), 346-357. https://doi.org/10.7326/0003-4819-152-6-201003160-00004

Goldfine, A. B., Silver, R., Aldhahi, W., Cai, D., Tatro, E., Lee, J., & Shoelson, S. E. (2008). Use of salsalate to target inflammation in the treatment of insulin resistance and type 2 diabetes. *Clinical and Translational Science*, *1*(1), 36-43. https://doi.org/10.1111/j.1752-8062.2008.00026.x

Golia, E., Limongelli, G., Natale, F., Fimiani, F., Maddaloni, V., Pariggiano, I., Bianchi, R., Crisci, M., D'Acierno, L., Giordano, R., Di Palma, G., Conte, M., Golino, P., Russo, M. G., Calabro, R., & Calabro, P. (2014). Inflammation and cardiovascular disease: From pathogenesis to therapeutic target. *Current Atherosclerosis Reports*, *16*(9), 435. https://doi.org/10.1007/s11883-014-0435-z

Gong, T., Liu, L., Jiang, W., & Zhou, R. (2020). DAMP-sensing receptors in sterile inflammation and inflammatory diseases. *Nature Reviews Immunology*, *20*(2), 95-112. https://doi.org/10.1038/s41577-019-0215-7

Goossens, G. H., Bizzarri, A., Venteclef, N., Essers, Y., Cleutjens, J. P., Konings, E., Jocken, J. W., Cajlakovic, M., Ribitsch, V., Clement, K., & Blaak, E. E. (2011). Increased adipose tissue oxygen tension in obese compared with lean men is accompanied by insulin resistance, impaired adipose tissue capillarization, and inflammation. *Circulation*, *124*(1), 67-76. https://doi.org/10.1161/CIRCULATIONAHA.111.027813

Gray, N., Picone, G., Sloan, F., & Yashkin, A. (2015). The Relationship between BMI and onset of diabetes mellitus and its complications. *Southern Medical Journal*, *108*(1), 29-36. https://doi.org/10.14423/smj.0000000000000214

Greenwood, B. M. (1968). Autoimmune disease and parasitic infections in Nigerians. *Lancet*, *2*(7564), 380-382. https://doi.org/10.1016/s0140-6736(68)90595-3

Grover, S. A., Kaouache, M., Rempel, P., Joseph, L., Dawes, M., Lau, D. C., & Lowensteyn, I. (2015). Years of life lost and healthy life-years lost from diabetes and cardiovascular disease in overweight and obese people: a modelling study. *Lancet Diabetes & Endocrinology*, *3*(2), 114-122. https://doi.org/10.1016/S2213-8587(14)70229-3

Grundy, S. M. (2015). Adipose tissue and metabolic syndrome: Too much, too little or neither. *European Journal of Clinical Investigation*, *45*(11), 1209-1217. https://doi.org/10.1111/eci.12519

Guerrero, R., Vega, G. L., Grundy, S. M., & Browning, J. D. (2009). Ethnic differences in hepatic steatosis: An insulin resistance paradox? *Hepatology*, *49*(3), 791-801. https://doi.org/10.1002/hep.22726

Guigas, B., & Molofsky, A. B. (2015). A worm of one's own: How helminths modulate host adipose tissue function and metabolism. *Trends in Parasitology*, *31*(9), 435-441. https://doi.org/10.1016/j.pt.2015.04.008

Guilherme, A., Virbasius, J. V., Puri, V., & Czech, M. P. (2008). Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nature Reviews Molecular Cell Biology*, *9*, 367. https://doi.org/10.1038/nrm2391

Guo, H., Callaway, J. B., & Ting, J. P. (2015). Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nature Medicine*, *21*(7), 677-687. https://doi.org/10.1038/nm.3893

Guo, Z., Hensrud, D. D., Johnson, C. M., & Jensen, M. D. (1999). Regional postprandial fatty acid metabolism in different obesity phenotypes. *Diabetes*, *48*(8), 1586-1592. https://doi.org/10.2337/diabetes.48.8.1586

Hakansson, A., & Molin, G. (2011). Gut microbiota and inflammation. *Nutrients*, *3*(6), 637-682. https://doi.org/10.3390/nu3060637

Halim, T. Y., Steer, C. A., Matha, L., Gold, M. J., Martinez-Gonzalez, I., McNagny, K. M., McKenzie, A. N., & Takei, F. (2014). Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. *Immunity*, *40*(3), 425-435. https://doi.org/10.1016/j.immuni.2014.01.011

Hallal, P. C., Andersen, L. B., Bull, F. C., Guthold, R., Haskell, W., Ekelund, U., & Lancet Physical Activity Series Working, G. (2012). Global physical activity levels: Surveillance progress, pitfalls, and prospects. *Lancet*, *380*(9838), 247-257. https://doi.org/10.1016/S0140-6736(12)60646-1

Hallberg, S. J., Gershuni, V. M., Hazbun, T. L., & Athinarayanan, S. J. (2019). Reversing type 2 diabetes: A narrative review of the evidence. *Nutrients*, *11*(4). https://doi.org/10.3390/nu11040766

Haller, H., & Hanefeld, M. (1975). Synoptische Betrachtung Metabolischer Risikofaktoren. In H. Haller, M. Hanefeld, & W. Jaross (Eds.), *Lipidstoffwechselstörungen* (pp. 254-264). Gustav Fischer Verlag.

Halleux, C. M., Takahashi, M., Delporte, M. L., Detry, R., Funahashi, T., Matsuzawa, Y., & Brichard, S. M. (2001). Secretion of adiponectin and regulation of apM1 gene expression in human visceral adipose tissue. *Biochemical and Biophysical Research Communications*, *288*(5), 1102-1107. https://doi.org/10.1006/bbrc.2001.5904

Hamburg, N. M., McMackin, C. J., Huang, A. L., Shenouda, S. M., Widlansky, M. E., Schulz, E., Gokce, N., Ruderman, N. B., Keaney, J. F., Jr., & Vita, J. A. (2007). Physical inactivity rapidly induces insulin resistance and microvascular dysfunction in healthy volunteers. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *27*(12), 2650-2656. https://doi.org/10.1161/ATVBAHA.107.153288

Hams, E., Bermingham, R., Wurlod, F. A., Hogan, A. E., O'Shea, D., Preston, R. J., Rodewald, H. R., McKenzie, A. N., & Fallon, P. G. (2016). The helminth T2 RNase omega1 promotes metabolic homeostasis in an IL-33- and group 2 innate lymphoid cell-dependent mechanism. *FASEB Journal*, *30*(2), 824-835. https://doi.org/10.1096/fj.15-277822

Hancock, C. R., Han, D. H., Chen, M., Terada, S., Yasuda, T., Wright, D. C., & Holloszy, J. O. (2008). High-fat diets cause insulin resistance despite an increase in muscle mitochondria. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(22), 7815-7820. https://doi.org/10.1073/pnas.0802057105

Hanefeld, M., Schaper, F., & Ceriello, A. (2007). Geschichte und Definition(en) des metabolischen Syndroms. *Der Internist*, *48*(2), 117-125. https://doi.org/10.1007/s00108-006-1786-5

Harman-Boehm, I., Bluher, M., Redel, H., Sion-Vardy, N., Ovadia, S., Avinoach, E., Shai, I., Kloting, N., Stumvoll, M., Bashan, N., & Rudich, A. (2007). Macrophage infiltration into omental versus subcutaneous fat across different populations: Effect of regional adiposity and the comorbidities of obesity. *Journal of Clinical Endocrinology and Metabolism*, *92*(6), 2240-2247. https://doi.org/10.1210/jc.2006-1811

Hays, R., Esterman, A., Giacomin, P., Loukas, A., & McDermott, R. (2015). Does Strongyloides stercoralis infection protect against type 2 diabetes in humans? Evidence from Australian Aboriginal adults. *Diabetes Research and Clinical Practice*, *107*(3), 355-361. https://doi.org/10.1016/j.diabres.2015.01.012

Hays, R., Giacomin, P., Olma, L., Esterman, A., & McDermott, R. (2017). The relationship between treatment for Strongyloides stercoralis infection and type 2 diabetes mellitus in an Australian Aboriginal population: A three-year cohort study. *Diabetes Research and Clinical Practice*, *134*, 8-16. https://doi.org/10.1016/j.diabres.2017.09.012

Heilbronn, L. K., & Campbell, L. V. (2008). Adipose tissue macrophages, low grade inflammation and insulin resistance in human obesity. *Current Pharmaceutical Design*, *14*(12), 1225-1230. https://doi.org/10.2174/138161208784246153

Heinonen, S., Jokinen, R., Rissanen, A., & Pietilainen, K. H. (2019). White adipose tissue mitochondrial metabolism in health and in obesity. *Obesity Reviews*. https://doi.org/10.1111/obr.12958

Heiston, E. M., Liu, Z., Ballantyne, A., Kranz, S., & Malin, S. K. (2021). A single bout of exercise improves vascular insulin sensitivity in adults with obesity. *Obesity*, *29*(9), 1487-1496. https://doi.org/https://doi.org/10.1002/oby.23229

Hepworth, M. R., Hardman, M. J., & Grencis, R. K. (2010). The role of sex hormones in the development of Th2 immunity in a gender-biased model of Trichuris muris infection. *European Journal of Immunology*, *40*(2), 406-416. https://doi.org/10.1002/eji.200939589

Heymsfield, S. B., & Wadden, T. A. (2017). Mechanisms, pathophysiology, and management of obesity. *New England Journal of Medicine*, *376*(3), 254-266. https://doi.org/10.1056/NEJMra1514009

Hida, S., Tadachi, M., Saito, T., & Taki, S. (2005). Negative control of basophil expansion by IRF-2 critical for the regulation of Th1/Th2 balance. *Blood*, *106*(6), 2011-2017. https://doi.org/10.1182/blood-2005-04-1344

Hii, S. F., Senevirathna, D., Llewellyn, S., Inpankaew, T., Odermatt, P., Khieu, V., Muth, S., McCarthy, J., & Traub, R. J. (2018). Development and evaluation of a multiplex quantitative real-time polymerase chain reaction for hookworm species in human stool. *American Journal of Tropical Medicine and Hygiene*, *99*(5), 1186-1193. https://doi.org/10.4269/ajtmh.18-0276

Himmerich, H., Berthold-Losleben, M., & Pollmacher, T. (2009). [The relevance of the TNF-alpha system in psychiatric disorders]. *Fortschritte der Neurologie-Psychiatrie*, *77*(6), 334-345. https://doi.org/10.1055/s-0028-1109420 (Die Bedeutung des TNF-alpha-Systems fur Erkrankungen des Gehirns.)

Hindmarch, I. (2002). Beyond the monoamine hypothesis: Mechanisms, molecules and methods. *European Psychiatry*, *17*(S3), 294s-299s. https://doi.org/10.1016/S0924-9338(02)00653-3

Hoagland, K. E., & Schad, G. A. (1978). Necator americanus and Ancylostoma duodenale: Life history parameters and epidemiological implications of two sympatric hookworms of humans. *Experimental Parasitology*, *44*(1), 36-49. https://doi.org/10.1016/0014-4894(78)90078-4

Hoddy, K. K., Axelrod, C. L., Mey, J. T., Hari, A., Beyl, R. A., Blair, J. B., Dantas, W. S., & Kirwan, J. P. (2021). Insulin resistance persists despite a metabolically healthy obesity phenotype. *Obesity*, *n/a*(n/a). https://doi.org/https://doi.org/10.1002/oby.23312

Holm, J. B., Sorobetea, D., Kiilerich, P., Ramayo-Caldas, Y., Estelle, J., Ma, T., Madsen, L., Kristiansen, K., & Svensson-Frej, M. (2015). Chronic Trichuris muris infection decreases diversity of the intestinal microbiota and concomitantly increases the abundance of Lactobacilli. *PloS One*, *10*(5), e0125495. https://doi.org/10.1371/journal.pone.0125495

Hoogerwerf, M. A., Coffeng, L. E., Brienen, E. A. T., Janse, J. J., Langenberg, M. C. C., Kruize, Y. C. M., Gootjes, C., Manurung, M. D., Dekker, M., Becker, L., Erkens, M. A. A., van der Beek, M. T., Ganesh, M. S., Feijt, C., Winkel, B. M. F., Westra, I. M., Meij, P., Loukas, A., Visser, L. G., de Vlas, S. J., Yazdanbakhsh, M., van Lieshout, L., & Roestenberg, M. (2019). New insights into the kinetics and variability of egg excretion in controlled human hookworm infections. *Journal of Infectious Diseases*. https://doi.org/10.1093/infdis/jiz218

Hoogerwerf, M. A., Koopman, J. P. R., Janse, J. J., Langenberg, M. C. C., van Schuijlenburg, R., Kruize, Y. C. M., Brienen, E. A. T., Manurung, M. D., Verbeek-Menken, P., van der Beek, M. T., Westra, I. M., Meij, P., Visser, L. G., van Lieshout, L., de Vlas, S. J., Yazdanbakhsh, M., Coffeng, L. E., & Roestenberg, M. (2021). A randomized controlled trial to investigate safety and variability of egg excretion after repeated controlled human hookworm infection. *Journal of Infectious Diseases*, *223*(5), 905-913. https://doi.org/10.1093/infdis/jiaa414

Hooper, L. V., Littman, D. R., & Macpherson, A. J. (2012). Interactions between the microbiota and the immune system [10.1126/science.1223490]. *Science*, *336*(6086), 1268-1273. https://doi.org/10.1126/science.1223490

Hotamisligil, G. S. (2006). Inflammation and metabolic disorders. *Nature*, *444*(7121), 860. http://www.nature.com/articles/nature05485.pdf

Hotamisligil, G. S., Shargill, N. S., & Spiegelman, B. M. (1993). Adipose expression of tumor necrosis factor-alpha: Direct role in obesity-linked insulin resistance. *Science*, *259*(5091), 87-91. https://doi.org/10.1126/science.7678183

Hotamisligil, G. S., Wiesbrock, S. M., Marino, M. W., & Uysal, K. T. (1997). Protection from obesity-induced insulin resistance in mice lacking TNF-α function. *Nature*, *389*(6651), 610-614. https://doi.org/10.1038/39335

Hotez, P., & Whitham, M. (2014). Helminth infections: A new global women's health agenda. *Obstetrics and Gynecology*, *123*(1), 155-160. https://doi.org/10.1097/AOG.0000000000000025

Hotez, P. J., Brooker, S., Bethony, J. M., Bottazzi, M. E., Loukas, A., & Xiao, S. (2004). Hookworm infection. *New England Journal of Medicine*, *351*(8), 799-807. https://doi.org/10.1056/NEJMra032492

Hotta, K., Funahashi, T., Arita, Y., Takahashi, M., Matsuda, M., Okamoto, Y., Iwahashi, H., Kuriyama, H., Ouchi, N., Maeda, K., Nishida, M., Kihara, S., Sakai, N., Nakajima, T., Hasegawa, K., Muraguchi, M., Ohmoto, Y., Nakamura, T., Yamashita, S., Hanafusa, T., & Matsuzawa, Y. (2000). Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *20*(6), 1595-1599. https://doi.org/10.1161/01.atv.20.6.1595

Houlden, A., Hayes, K. S., Bancroft, A. J., Worthington, J. J., Wang, P., Grencis, R. K., & Roberts, I. S. (2015). Chronic Trichuris muris infection in C57BL/6 mice causes significant changes in host microbiota and metabolome: Effects reversed by pathogen clearance. *PloS One*, *10*(5), e0125945. https://doi.org/10.1371/journal.pone.0125945

Howitt, M. R., Lavoie, S., Michaud, M., Blum, A. M., Tran, S. V., Weinstock, J. V., Gallini, C. A., Redding, K., Margolskee, R. F., Osborne, L. C., Artis, D., & Garrett, W. S. (2016). Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. *Science*, *351*(6279), 1329-1333. https://doi.org/10.1126/science.aaf1648

Htun, N. S. N., Odermatt, P., Muller, I., Yap, P., Steinmann, P., Schindler, C., Gerber, M., Du Randt, R., Walter, C., Puhse, U., Utzinger, J., & Probst-Hensch, N. (2018). Association between gastrointestinal tract infections and glycated hemoglobin in school children of poor neighborhoods in Port Elizabeth, South Africa. *PLoS Neglected Tropical Diseases*, *12*(3), e0006332. https://doi.org/10.1371/journal.pntd.0006332

Htun, N. S. N., Odermatt, P., Paboriboune, P., Sayasone, S., Vongsakid, M., Phimolsarn-Nusith, V., Tran, X. D., Ounnavong, P. S., Andriama-Hefasoa, N., Senvanpan, N. D., Homsana, A., Lianosay, B., Xayavong, D., Robinson, D. R., Bounsavath, P., Prasayasith, P. P., Syphan, S. D., Lu, Y. X., Thilakoun, K., Xaiyaphet, X. S., Vongngakesone, P. T., Eze, I. C., Imboden, M., Sripa, B., Reinharz, D., & Probst-Hensch, N. (2018). Association between helminth infections and diabetes mellitus in adults from the Lao People's Democratic Republic: a cross-sectional study. *Infectious Diseases of Poverty*, *7*(1), 105. https://doi.org/10.1186/s40249-018-0488-2

Hu, F. B. (2011). Globalization of diabetes: The role of diet, lifestyle, and genes. *Diabetes Care*, *34*(6), 1249-1257. https://doi.org/10.2337/dc11-0442

Huang, Y., Guo, L., Qiu, J., Chen, X., Hu-Li, J., Siebenlist, U., Williamson, P. R., Urban, J. F., Jr., & Paul, W. E. (2015). IL-25-responsive, lineage-negative KLRG1(hi) cells are multipotential 'inflammatory' type 2 innate lymphoid cells. *Nature Immunology*, *16*(2), 161-169. https://doi.org/10.1038/ni.3078

Hung, L. Y., Lewkowich, I. P., Dawson, L. A., Downey, J., Yang, Y., Smith, D. E., & Herbert, D. R. (2013). IL-33 drives biphasic IL-13 production for noncanonical Type 2 immunity against hookworms. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(1), 282-287. https://doi.org/10.1073/pnas.1206587110

Hurst, S. D., Muchamuel, T., Gorman, D. M., Gilbert, J. M., Clifford, T., Kwan, S., Menon, S., Seymour, B., Jackson, C., Kung, T. T., Brieland, J. K., Zurawski, S. M., Chapman, R. W., Zurawski, G., & Coffman, R. L. (2002). New IL-17 family members promote Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25. *Journal of Immunology*, *169*(1), 443-453. https://doi.org/10.4049/jimmunol.169.1.443

Hussaarts, L., García-Tardón, N., Van Beek, L., Heemskerk, M. M., Haeberlein, S., Van Der Zon, G. C., Ozir-Fazalalikhan, A., Berbée, J. F. P., Van Dijk, K. W., Van Harmelen, V., Yazdanbakhsh,

M., & Guigas, B. (2015). Chronic helminth infection and helminth-derived egg antigens promote adipose tissue M2 macrophages and improve insulin sensitivity in obese mice. *FASEB Journal*, *29*(7), 3027-3039. https://doi.org/10.1096/fj.14-266239

Hussaarts, L., van der Vlugt, L. E., Yazdanbakhsh, M., & Smits, H. H. (2011). Regulatory B-cell induction by helminths: Implications for allergic disease. *Journal of Allergy and Clinical Immunology*, *128*(4), 733-739. https://doi.org/10.1016/j.jaci.2011.05.012

International Diabetes Federation. (2006). *The IDF consensus worldwide definition of the metabolic syndrome*. Retrieved December 10 2019 from https://www.idf.org/our-activities/advocacy-awareness/resources-and-tools/60:idfconsensus-worldwide-definitionof-the-metabolic-syndrome.html

International Diabetes Federation. (2017). *IDF Diabetes Atlas 8th edition.* Retrieved June 20 2019 from https://diabetesatlas.org/resources/2017-atlas.html

International Diabetes Federation. (2022). *IDF Diabetes Atlas 10th Edition 2021*. Retrieved June 11 2022 from https://diabetesatlas.org/

International Helminth Genomes Consortium. (2019). Comparative genomics of the major parasitic worms. *Nature Genetics*, *51*(1), 163-174.

Izquierdo, A. G., Crujeiras, A. B., Casanueva, F. F., & Carreira, M. C. (2019). Leptin, obesity, and leptin resistance: Where are we 25 years later? *Nutrients*, *11*(11). https://doi.org/10.3390/nu11112704

Jacobsen, E. A., Helmers, R. A., Lee, J. J., & Lee, N. A. (2012). The expanding role(s) of eosinophils in health and disease. *Blood*, *120*(19), 3882-3890. https://doi.org/10.1182/blood-2012-06-330845

Jais, A., & Bruning, J. C. (2017). Hypothalamic inflammation in obesity and metabolic disease. *Journal of Clinical Investigation*, *127*(1), 24-32. https://doi.org/10.1172/JCI88878

Jaitin, D. A., Adlung, L., Thaiss, C. A., Weiner, A., Li, B., Descamps, H., Lundgren, P., Bleriot, C., Liu, Z., Deczkowska, A., Keren-Shaul, H., David, E., Zmora, N., Eldar, S. M., Lubezky, N., Shibolet, O., Hill, D. A., Lazar, M. A., Colonna, M., Ginhoux, F., Shapiro, H., Elinav, E., & Amit, I. (2019). Lipid-associated macrophages control metabolic homeostasis in a Trem2-dependent manner. *Cell*(in press). https://doi.org/10.1016/j.cell.2019.05.054

Jakobsen, J. C., Gluud, C., Wetterslev, J., & Winkel, P. (2017). When and how should multiple imputation be used for handling missing data in randomised clinical trials - a practical guide with flowcharts. *BMC Medical Research Methodology*, *17*(1), 162. https://doi.org/10.1186/s12874-017-0442-1

Jastreboff, A. M., Kotz, C. M., Kahan, S., Kelly, A. S., & Heymsfield, S. B. (2019). Obesity as a disease: The Obesity Society 2018 Position Statement. *Obesity*, *27*(1), 7-9. https://doi.org/10.1002/oby.22378

Jenkins, T. P., Rathnayaka, Y., Perera, P. K., Peachey, L. E., Nolan, M. J., Krause, L., Rajakaruna, R. S., & Cantacessi, C. (2017). Infections by human gastrointestinal helminths are associated with changes in faecal microbiota diversity and composition. *PloS One*, *12*(9), e0184719. https://doi.org/10.1371/journal.pone.0184719

Jensen, M., Ryan, D., Apovian, C., Ard, J., Comuzzie, A., & Donato, K. (2014). 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. *Circulation*, *129*(25 Suppl 2), S139-S140.

Jensen, M. D., Haymond, M. W., Rizza, R. A., Cryer, P. E., & Miles, J. M. (1989). Influence of body fat distribution on free fatty acid metabolism in obesity. *Journal of Clinical Investigation*, *83*(4), 1168-1173. https://doi.org/10.1172/JCI113997

Jian, X., Sen, L., Hui-Qin, Q., Hai-Nan, R., Tie-Hua, L., Hai-Chou, X., Hotez, P. J., & Shu-Hua, X. (2003). Necator americanus: Maintenance through one hundred generations in golden hamsters (Mesocricetus auratus). I. host sex-associated differences in hookworm burden and fecundity. *Experimental Parasitology*, *104*(1), 62-66. https://doi.org/10.1016/S0014-4894(03)00094-8

Jie, Z., Xia, H., Zhong, S. L., Feng, Q., Li, S., Liang, S., Zhong, H., Liu, Z., Gao, Y., Zhao, H., Zhang, D., Su, Z., Fang, Z., Lan, Z., Li, J., Xiao, L., Li, J., Li, R., Li, X., Li, F., Ren, H., Huang, Y., Peng, Y., Li, G., Wen, B., Dong, B., Chen, J. Y., Geng, Q. S., Zhang, Z. W., Yang, H., Wang, J., Wang, J., Zhang, X., Madsen, L., Brix, S., Ning, G., Xu, X., Liu, X., Hou, Y., Jia, H., He,

K., & Kristiansen, K. (2017). The gut microbiome in atherosclerotic cardiovascular disease. *Nat Commun, 8*(1), 845. https://doi.org/10.1038/s41467-017-00900-1

Jong, E. C., Chi, E. Y., & Klebanoff, S. J. (1984). Human neutrophil-mediated killing of schistosomula of Schistosoma mansoni: augmentation by schistosomal binding of eosinophil peroxidase. *American Journal of Tropical Medicine and Hygiene, 33*(1), 104-115. https://doi.org/10.4269/ajtmh.1984.33.104

Jourdan, P. M., Lamberton, P. H. L., Fenwick, A., & Addiss, D. G. (2018). Soil-transmitted helminth infections. *Lancet, 391*(10117), 252-265. https://doi.org/10.1016/S0140-6736(17)31930-X

Kabra, D. G., Pfuhlmann, K., Garcia-Caceres, C., Schriever, S. C., Casquero Garcia, V., Kebede, A. F., Fuente-Martin, E., Trivedi, C., Heppner, K., Uhlenhaut, N. H., Legutko, B., Kabra, U. D., Gao, Y., Yi, C. X., Quarta, C., Clemmensen, C., Finan, B., Muller, T. D., Meyer, C. W., Paez-Pereda, M., Stemmer, K., Woods, S. C., Perez-Tilve, D., Schneider, R., Olson, E. N., Tschop, M. H., & Pfluger, P. T. (2016). Hypothalamic leptin action is mediated by histone deacetylase 5. *Nature Communications, 7*, 10782. https://doi.org/10.1038/ncomms10782

Kadowaki, T., Yamauchi, T., Kubota, N., Hara, K., Ueki, K., & Tobe, K. (2006). Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *Journal of Clinical Investigation*, *116*(7), 1784-1792. https://doi.org/10.1172/JCI29126

Kahn, S. E., Hull, R. L., & Utzschneider, K. M. (2006). Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature, 444*(7121), 840-846. https://doi.org/10.1038/nature05482

Kanat, M., DeFronzo, R. A., & Abdul-Ghani, M. A. (2015). Treatment of prediabetes. *World Journal of Diabetes, 6*(12), 1207-1222. https://doi.org/10.4239/wjd.v6.i12.1207

Karlsson, F. H., Tremaroli, V., Nookaew, I., Bergstrom, G., Behre, C. J., Fagerberg, B., Nielsen, J., & Backhed, F. (2013). Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature, 498*(7452), 99-103. https://doi.org/10.1038/nature12198

Kaur, J. (2014). A comprehensive review on metabolic syndrome. *Cardiology Research and Practice, 2014*, 943162. https://doi.org/10.1155/2014/943162

Kaushik, R. K., Piyush Dixit, Mool Kumar Tyagi, JK Gambhir, SV Madhu, ., & et al. (2018). Plasma omentin-1 level and its relationship with insulin resistance in obese prediabetics. *Journal of Clinical and Diagnostic Research, 12*(4), BC10-BC14. https://doi.org/10.7860/jcdr/2018/31845.11432

Kautzky-Willer, A., Harreiter, J., & Pacini, G. (2016). Sex and gender differences in risk, pathophysiology and complications of type 2 diabetes mellitus. *Endocrine Reviews, 37*(3), 278-316. https://doi.org/10.1210/er.2015-1137

Kay, G. L., Millard, A., Sergeant, M. J., Midzi, N., Gwisai, R., Mduluza, T., Ivens, A., Nausch, N., Mutapi, F., & Pallen, M. (2015). Differences in the faecal microbiome in Schistosoma haematobium infected children vs. uninfected children. *PLoS Neglected Tropical Diseases, 9*(6), e0003861. https://doi.org/10.1371/journal.pntd.0003861

Keys, A., Fidanza, F., Karvonen, M. J., Kimura, N., & Taylor, H. L. (1972). Indices of relative weight and obesity. *Journal of Chronic Diseases, 25*(6), 329-343. https://www.ncbi.nlm.nih.gov/pubmed/4650929

Khardori, R. (2013). Changing paradigms in type 2 diabetes mellitus. *Indian Journal of Endocrinology and Metabolism, 17*(Suppl 1), S68-71. https://doi.org/10.4103/2230-8210.119509

Khudhair, Z., Alhallaf, R., Eichenberger, R. M., Field, M., Krause, L., Sotillo, J., & Loukas, A. (2022). Administration of hookworm excretory/secretory proteins improves glucose tolerance in a mouse model of Type 2 Diabetes. *Biomolecules, 12*(5). https://doi.org/10.3390/biom12050637

Khudhair, Z., Alhallaf, R., Eichenberger, R. M., Whan, J., Kupz, A., Field, M., Krause, L., Wilson, D. T., Daly, N. L., Giacomin, P., Sotillo, J., & Loukas, A. (2021). Gastrointestinal helminth infection improves insulin sensitivity, decreases systemic inflammation, and alters the composition of gut microbiota in distinct mouse models of type 2 diabetes [Article]. *Frontiers in Endocrinology, 11*, Article 606530. https://doi.org/10.3389/fendo.2020.606530

Kiliaan, A. J., Arnoldussen, I. A., & Gustafson, D. R. (2014). Adipokines: A link between obesity and dementia? *Lancet Neurology, 13*(9), 913-923. https://doi.org/10.1016/S1474-4422(14)70085-7

Kim, J., & Lee, J. (2021). Role of obesity-induced inflammation in the development of insulin resistance and type 2 diabetes: History of the research and remaining questions. *Annals of Pediatric Endocrinology & Metabolism, 26*(1), 1-13. https://doi.org/10.6065/apem.2040188.094

Kim, J. K., Kim, Y. J., Fillmore, J. J., Chen, Y., Moore, I., Lee, J., Yuan, M., Li, Z. W., Karin, M., Perret, P., Shoelson, S. E., & Shulman, G. I. (2001). Prevention of fat-induced insulin resistance by salicylate. *Journal of Clinical Investigation, 108*(3), 437-446. https://doi.org/10.1172/JCI11559

Kim, K. N., Yao, Y., & Ju, S. Y. (2019). Short-chain fatty acids and fecal microbiota abundance in humans with obesity: A systematic review and meta-analysis. *Nutrients, 11*(10). https://doi.org/10.3390/nu11102512

Kim, M. S., Yamamoto, Y., Kim, K., Kamei, N., Shimada, T., Liu, L., Moore, K., Woo, J. R., Shoelson, S. E., & Lee, J. (2013). Regulation of diet-induced adipose tissue and systemic inflammation by salicylates and pioglitazone. *PloS One, 8*(12), e82847. https://doi.org/10.1371/journal.pone.0082847

Kirwan, A. M., Lenighan, Y. M., O'Reilly, M. E., McGillicuddy, F. C., & Roche, H. M. (2017). Nutritional modulation of metabolic inflammation. *Biochemical Society Transactions, 45*(4), 979-985. https://doi.org/10.1042/bst20160465

Klok, M. D., Jakobsdottir, S., & Drent, M. L. (2007). The role of leptin and ghrelin in the regulation of food intake and body weight in humans: A review. *Obesity Reviews, 8*(1), 21-34. https://doi.org/10.1111/j.1467-789X.2006.00270.x

Klose, C. S., Mahlakõiv, T., Moeller, J. B., Rankin, L. C., Flamar, A.-L., Kabata, H., Monticelli, L. A., Moriyama, S., Putzel, G. G., & Rakhilin, N. (2017). The neuropeptide neuromedin U stimulates innate lymphoid cells and type 2 inflammation. *Nature, 549*(7671), 282. http://www.nature.com/articles/nature23676

Klotz, C., Ziegler, T., Figueiredo, A. S., Rausch, S., Hepworth, M. R., Obsivac, N., Sers, C., Lang, R., Hammerstein, P., Lucius, R., & Hartmann, S. (2011). A helminth immunomodulator exploits host signaling events to regulate cytokine production in macrophages. *PLoS Pathogens, 7*(1), e1001248. https://doi.org/10.1371/journal.ppat.1001248

Kondo, Y., Yoshimoto, T., Yasuda, K., Futatsugi-Yumikura, S., Morimoto, M., Hayashi, N., Hoshino, T., Fujimoto, J., & Nakanishi, K. (2008). Administration of IL-33 induces airway hyperresponsiveness and goblet cell hyperplasia in the lungs in the absence of adaptive immune system. *International Immunology, 20*(6), 791-800. https://doi.org/10.1093/intimm/dxn037

Koninkx, J. F., Mirck, M. H., Hendriks, H. G., Mouwen, J. M., & van Dijk, J. E. (1988). Nippostrongylus brasiliensis: Histochemical changes in the composition of mucins in goblet cells during infection in rats. *Experimental Parasitology, 65*(1), 84-90. https://doi.org/10.1016/0014-4894(88)90109-9

Kreisinger, J., Bastien, G., Hauffe, H. C., Marchesi, J., & Perkins, S. E. (2015). Interactions between multiple helminths and the gut microbiota in wild rodents. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 370*(1675). https://doi.org/10.1098/rstb.2014.0295

Krljanac, B., Schubart, C., Naumann, R., Wirtz, S., Culemann, S., Kronke, G., & Voehringer, D. (2019). RELMalpha-expressing macrophages protect against fatal lung damage and reduce parasite burden during helminth infection. *Science Immunology, 4*(35). https://doi.org/10.1126/sciimmunol.aau3814

Kroenke, K., Spitzer, R. L., & Williams, J. B. (2001). The PHQ-9: Validity of a brief depression severity measure. *Journal of General Internal Medicine, 16*(9), 606-613. https://doi.org/10.1046/j.1525-1497.2001.016009606.x

Kroenke, K., Spitzer, R. L., Williams, J. B. W., & Löwe, B. (2010). The Patient Health Questionnaire somatic, anxiety, and depressive symptom scales: A systematic review. *General Hospital Psychiatry, 32*(4), 345-359. https://doi.org/10.1016/j.genhosppsych.2010.03.006

Kusminski, C. M., Holland, W. L., Sun, K., Park, J., Spurgin, S. B., Lin, Y., Askew, G. R., Simcox, J. A., McClain, D. A., Li, C., & Scherer, P. E. (2012). MitoNEET-driven alterations in adipocyte mitochondrial activity reveal a crucial adaptive process that preserves insulin sensitivity in obesity. *Nature Medicine, 18*(10), 1539-1549. https://doi.org/10.1038/nm.2899

Kusminski, C. M., & Scherer, P. E. (2012). Mitochondrial dysfunction in white adipose tissue. *Trends in Endocrinology and Metabolism, 23*(9), 435-443. https://doi.org/10.1016/j.tem.2012.06.004

Kwon, H. K., Lee, C. G., So, J. S., Chae, C. S., Hwang, J. S., Sahoo, A., Nam, J. H., Rhee, J. H., Hwang, K. C., & Im, S. H. (2010). Generation of regulatory dendritic cells and CD4+Foxp3+ T cells

by probiotics administration suppresses immune disorders. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(5), 2159-2164. https://doi.org/10.1073/pnas.0904055107

Kylin, E. (1923). Studien über das Hypertonie-Hyperglykämie-Hyperurikämiesyndrome. *Zentralblatt für innere Medizin*, *44*, 105-127.

La Cava, A. (2017). Leptin in inflammation and autoimmunity. *Cytokine*, *98*, 51-58. https://doi.org/10.1016/j.cyto.2016.10.011

Lackey, D. E., & Olefsky, J. M. (2016). Regulation of metabolism by the innate immune system. *Nature Reviews: Endocrinology*, *12*(1), 15-28. https://doi.org/10.1038/nrendo.2015.189

Lambeth, S. M., Carson, T., Lowe, J., Ramaraj, T., Leff, J. W., Luo, L., Bell, C. J., & Shah, V. O. (2015). Composition, diversity and abundance of gut microbiome in prediabetes and type 2 diabetes. *Journal of Diabetes and Obesity*, *2*(3), 1-7. https://doi.org/10.15436/2376-0949.15.031

Landecho, M. F., Tuero, C., Valenti, V., Bilbao, I., de la Higuera, M., & Fruhbeck, G. (2019). Relevance of leptin and other adipokines in obesity-associated cardiovascular risk. *Nutrients*, *11*(11). https://doi.org/10.3390/nu11112664

Langenberg, M. C. C., Hoogerwerf, M. A., Koopman, J. P. R., Janse, J. J., Kos-van Oosterhoud, J., Feijt, C., Jochems, S. P., de Dood, C. J., van Schuijlenburg, R., Ozir-Fazalalikhan, A., Manurung, M. D., Sartono, E., van der Beek, M. T., Winkel, B. M. F., Verbeek-Menken, P. H., Stam, K. A., van Leeuwen, F. W. B., Meij, P., van Diepen, A., van Lieshout, L., van Dam, G. J., Corstjens, P., Hokke, C. H., Yazdanbakhsh, M., Visser, L. G., & Roestenberg, M. (2020). A controlled human Schistosoma mansoni infection model to advance novel drugs, vaccines and diagnostics. *Nature Medicine*, *26*(3), 326-332. https://doi.org/10.1038/s41591-020-0759-x

Lantz, C. S., Boesiger, J., Song, C. H., Mach, N., Kobayashi, T., Mulligan, R. C., Nawa, Y., Dranoff, G., & Galli, S. J. (1998). Role for interleukin-3 in mast-cell and basophil development and in immunity to parasites. *Nature*, *392*(6671), 90-93. https://doi.org/10.1038/32190

Lantz, C. S., Min, B., Tsai, M., Chatterjea, D., Dranoff, G., & Galli, S. J. (2008). IL-3 is required for increases in blood basophils in nematode infection in mice and can enhance IgE-dependent IL-4 production by basophils in vitro. *Laboratory Investigation*, *88*(11), 1134-1142. https://doi.org/10.1038/labinvest.2008.88

Larsen, N., Vogensen, F. K., Van Den Berg, F. W. J., Nielsen, D. S., Andreasen, A. S., Pedersen, B. K., Al-Soud, W. A., Sørensen, S. J., Hansen, L. H., & Jakobsen, M. (2010). Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PloS One*, *5*(2), e9085. https://doi.org/10.1371/journal.pone.0009085

Lassenius, M. I., Pietilainen, K. H., Kaartinen, K., Pussinen, P. J., Syrjanen, J., Forsblom, C., Porsti, I., Rissanen, A., Kaprio, J., Mustonen, J., Groop, P. H., Lehto, M., & FinnDiane Study, G. (2011). Bacterial endotoxin activity in human serum is associated with dyslipidemia, insulin resistance, obesity, and chronic inflammation. *Diabetes Care*, *34*(8), 1809-1815. https://doi.org/10.2337/dc10-2197

Lavie, C. J., De Schutter, A., Parto, P., Jahangir, E., Kokkinos, P., Ortega, F. B., Arena, R., & Milani, R. V. (2016). Obesity and prevalence of cardiovascular diseases and prognosis—The Obesity Paradox updated. *Progress in Cardiovascular Diseases*, *58*(5), 537-547. https://doi.org/10.1016/j.pcad.2016.01.008

Lawrence, R. A., Gray, C. A., Osborne, J., & Maizels, R. M. (1996). Nippostrongylus brasiliensis: cytokine responses and nematode expulsion in normal and IL-4-deficient mice. *Experimental Parasitology*, *84*(1), 65-73. https://doi.org/10.1006/expr.1996.0090

Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., Almeida, M., Arumugam, M., Batto, J. M., Kennedy, S., Leonard, P., Li, J., Burgdorf, K., Grarup, N., Jorgensen, T., Brandslund, I., Nielsen, H. B., Juncker, A. S., Bertalan, M., Levenez, F., Pons, N., Rasmussen, S., Sunagawa, S., Tap, J., Tims, S., Zoetendal, E. G., Brunak, S., Clement, K., Dore, J., Kleerebezem, M., Kristiansen, K., Renault, P., Sicheritz-Ponten, T., de Vos, W. M., Zucker, J. D., Raes, J., Hansen, T., Meta, H. I. T. c., Bork, P., Wang, J., Ehrlich, S. D., & Pedersen, O. (2013). Richness of human gut microbiome correlates with metabolic markers. *Nature*, *500*(7464), 541-546. https://doi.org/10.1038/nature12506

Lechea, E., Popescu, M., Dimulescu, D., Godoroja, D., & Copaescu, C. (2019). The impact of bariatric surgery on diabetes and other cardiovascular risk factors. *Chirurgia*, *114*(6), 725-731. https://doi.org/10.21614/chirurgia.114.6.725

Lechner, A., Bohnacker, S., & Esser-von Bieren, J. (2021). Macrophage regulation & function in helminth infection. *Seminars in Immunology*, *53*, 101526. https://doi.org/10.1016/j.smim.2021.101526

Lee, B. C., Kim, M. S., Pae, M., Yamamoto, Y., Eberle, D., Shimada, T., Kamei, N., Park, H. S., Sasorith, S., Woo, J. R., You, J., Mosher, W., Brady, H. J., Shoelson, S. E., & Lee, J. (2016). Adipose natural killer cells regulate adipose tissue macrophages to promote insulin resistance in obesity. *Cell Metabolism*, *23*(4), 685-698. https://doi.org/10.1016/j.cmet.2016.03.002

Lee, B. C., & Lee, J. (2014). Cellular and molecular players in adipose tissue inflammation in the development of obesity-induced insulin resistance. *Biochimica et Biophysica Acta*, *1842*(3), 446-462. https://doi.org/10.1016/j.bbadis.2013.05.017

Lee, E. H., Itan, M., Jang, J., Gu, H. J., Rozenberg, P., Mingler, M. K., Wen, T., Yoon, J., Park, S. Y., Roh, J. Y., Choi, C. S., Park, W. J., Munitz, A., & Jung, Y. (2018). Eosinophils support adipocyte maturation and promote glucose tolerance in obesity. *Scientific Reports*, *8*(1), 9894. https://doi.org/10.1038/s41598-018-28371-4

Lee, S. C., Tang, M. S., Lim, Y. A. L., Choy, S. H., Kurtz, Z. D., Cox, L. M., Gundra, U. M., Cho, I., Bonneau, R., Blaser, M. J., Chua, K. H., & Loke, P. (2014). Helminth colonization is associated with increased diversity of the gut microbiota. *PLoS Neglected Tropical Diseases*, *8*(5), e2880. https://doi.org/10.1371/journal.pntd.0002880

Levis, B., Benedetti, A., Thombs, B. D., & Collaboration, D. E. S. D. (2019). Accuracy of Patient Health Questionnaire-9 (PHQ-9) for screening to detect major depression: individual participant data meta-analysis. *BMJ*, *365*, l1476. https://doi.org/10.1136/bmj.l1476

Levy, M., Kolodziejczyk, A. A., Thaiss, C. A., & Elinav, E. (2017). Dysbiosis and the immune system. *Nature Reviews Immunology*, *17*(4), 219-232. https://doi.org/10.1038/nri.2017.7

Ley, R. E., Bäckhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., & Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences*, *102*(31), 11070-11075. http://www.pnas.org/content/pnas/102/31/11070.full.pdf

Ley, R. E., Turnbaugh, P. J., Klein, S., & Gordon, J. I. (2006). Microbial ecology: Human gut microbes associated with obesity. *Nature*, *444*(7122), 1022-1023. https://doi.org/10.1038/4441022a

Li, J., Lin, S., Vanhoutte, P. M., Woo, C. W., & Xu, A. (2016). Akkermansia muciniphila protects against atherosclerosis by preventing metabolic endotoxemia-induced inflammation in Apoe-/- Mice. *Circulation*, *133*(24), 2434-2446. https://doi.org/10.1161/CIRCULATIONAHA.115.019645

Li, R. W., Wu, S., Li, W., Navarro, K., Couch, R. D., Hill, D., & Urban, J. F., Jr. (2012). Alterations in the porcine colon microbiota induced by the gastrointestinal nematode Trichuris suis. *Infection and Immunity*, *80*(6), 2150-2157. https://doi.org/10.1128/IAI.00141-12

Li, S., Shin, H. J., Ding, E. L., & van Dam, R. M. (2009). Adiponectin levels and risk of type 2 diabetes: A systematic review and meta-analysis. *JAMA*, *302*(2), 179-188. https://doi.org/10.1001/jama.2009.976

Li, X., Watanabe, K., & Kimura, I. (2017). Gut microbiota dysbiosis drives and implies novel therapeutic strategies for diabetes mellitus and related metabolic diseases. *Frontiers in Immunology*, *8*. https://doi.org/10.3389/fimmu.2017.01882

Lindhorst, A., Raulien, N., Wieghofer, P., Eilers, J., Rossi, F. M. V., Bechmann, I., & Gericke, M. (2021). Adipocyte death triggers a pro-inflammatory response and induces metabolic activation of resident macrophages. *Cell Death & Disease*, *12*(6), 579. https://doi.org/10.1038/s41419-021-03872-9

Liou, A. P., Paziuk, M., Luevano, J. M., Jr., Machineni, S., Turnbaugh, P. J., & Kaplan, L. M. (2013). Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. *Science Translational Medicine*, *5*(178), 178ra141. https://doi.org/10.1126/scitranslmed.3005687

Litvak, Y., Byndloss, M. X., & Baumler, A. J. (2018). Colonocyte metabolism shapes the gut microbiota. *Science*, *362*(6418). https://doi.org/10.1126/science.aat9076

Liu, J., Divoux, A., Sun, J., Zhang, J., Clement, K., Glickman, J. N., Sukhova, G. K., Wolters, P. J., Du, J., Gorgun, C. Z., Doria, A., Libby, P., Blumberg, R. S., Kahn, B. B., Hotamisligil, G. S., & Shi, G. P. (2009). Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. *Nature Medicine*, *15*(8), 940-945. https://doi.org/10.1038/nm.1994

Liu, L., Feng, J., Zhang, G., Yuan, X., Li, F., Yang, T., Hao, S., Huang, D., Hsue, C., & Lou, Q. (2018). Visceral adipose tissue is more strongly associated with insulin resistance than subcutaneous adipose tissue in Chinese subjects with pre-diabetes. *Current Medical Research and Opinion*, *34*(1), 123-129. https://doi.org/10.1080/03007995.2017.1364226

Liu, W., Zhou, X., Li, Y., Zhang, S., Cai, X., Zhang, R., Gong, S., Han, X., & Ji, L. (2020). Serum leptin, resistin, and adiponectin levels in obese and non-obese patients with newly diagnosed type 2 diabetes mellitus: A population-based study. *Medicine (Baltimore)*, *99*(6), e19052. https://doi.org/10.1097/MD.0000000000019052

Liu, Z., Liu, Q., Pesce, J., Anthony, R. M., Lamb, E., Whitmire, J., Hamed, H., Morimoto, M., Urban, J. F., Jr., & Gause, W. C. (2004). Requirements for the development of IL-4-producing T cells during intestinal nematode infections: what it takes to make a Th2 cell in vivo. *Immunological Reviews*, *201*, 57-74. https://doi.org/10.1111/j.0105-2896.2004.00186.x

Loke, P., Gallagher, I., Nair, M. G., Zang, X., Brombacher, F., Mohrs, M., Allison, J. P., & Allen, J. E. (2007). Alternative activation is an innate response to injury that requires CD4+ T cells to be sustained during chronic infection. *Journal of Immunology*, *179*(6), 3926-3936. https://doi.org/10.4049/jimmunol.179.6.3926

Loukas, A., Hotez, P. J., Diemert, D., Yazdanbakhsh, M., McCarthy, J. S., Correa-Oliveira, R., Croese, J., & Bethony, J. M. (2016). Hookworm infection. *Nature Reviews Disease Primers*, *2*, 16088. https://doi.org/10.1038/nrdp.2016.88

Luft, V. C., Schmidt, M. I., Pankow, J. S., Couper, D., Ballantyne, C. M., Young, J. H., & Duncan, B. B. (2013). Chronic inflammation role in the obesity-diabetes association: a case-cohort study. *Diabetology & Metabolic Syndrome*, *5*(1), 31. https://doi.org/10.1186/1758-5996-5-31

Lumeng, C. N., Bodzin, J. L., & Saltiel, A. R. (2007). Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *Journal of Clinical Investigation*, *117*(1), 175-184. https://doi.org/10.1172/JCI29881

Lumeng, C. N., Deyoung, S. M., Bodzin, J. L., & Saltiel, A. R. (2007). Increased inflammatory properties of adipose tissue macrophages recruited during diet-induced obesity. *Diabetes*, *56*(1), 16-23. https://doi.org/10.2337/db06-1076

Luppino, F. S., de Wit, L. M., Bouvy, P. F., Stijnen, T., Cuijpers, P., Penninx, B. W., & Zitman, F. G. (2010). Overweight, obesity, and depression: A systematic review and meta-analysis of longitudinal studies. *Archives of General Psychiatry*, *67*(3), 220-229. https://doi.org/10.1001/archgenpsychiatry.2010.2

Magkos, F., Fraterrigo, G., Yoshino, J., Luecking, C., Kirbach, K., Kelly, S. C., de Las Fuentes, L., He, S., Okunade, A. L., Patterson, B. W., & Klein, S. (2016). Effects of moderate and subsequent progressive weight loss on metabolic function and adipose tissue biology in humans with obesity. *Cell Metabolism*, *23*(4), 591-601. https://doi.org/10.1016/j.cmet.2016.02.005

Mainous, A. G., Tanner, R. J., Scuderi, C. B., Porter, M., & Carek, P. J. (2016). Prediabetes screening and treatment in diabetes prevention: The impact of physician attitudes. *The Journal of the American Board of Family Medicine*, *29*(6), 663. https://doi.org/10.3122/jabfm.2016.06.160138

Maizels, R. M., & McSorley, H. J. (2016). Regulation of the host immune system by helminth parasites [Article]. *Journal of Allergy and Clinical Immunology*, *138*(3), 666-675. https://doi.org/10.1016/j.jaci.2016.07.007

Maizels, R. M., Smits, H. H., & McSorley, H. J. (2018). Modulation of host immunity by helminths: The expanding repertoire of parasite effector molecules. *Immunity*, *49*(5), 801-818. https://doi.org/10.1016/j.immuni.2018.10.016

Maloy, K. J., Salaun, L., Cahill, R., Dougan, G., Saunders, N. J., & Powrie, F. (2003). CD4+CD25+ T(R) cells suppress innate immune pathology through cytokine-dependent mechanisms. *Journal of Experimental Medicine*, *197*(1), 111-119. https://doi.org/10.1084/jem.20021345

Mantovani, A., Biswas, S. K., Galdiero, M. R., Sica, A., & Locati, M. (2013). Macrophage plasticity and polarization in tissue repair and remodelling. *Journal of Pathology*, *229*(2), 176-185. https://doi.org/10.1002/path.4133

Mantzoros, C. S., Li, T., Manson, J. E., Meigs, J. B., & Hu, F. B. (2005). Circulating adiponectin levels are associated with better glycemic control, more favorable lipid profile, and reduced inflammation in women with type 2 diabetes. *Journal of Clinical Endocrinology and Metabolism*, *90*(8), 4542-4548. https://doi.org/10.1210/jc.2005-0372

Martínez-González, M. A., García-Arellano, A., Toledo, E., Salas-Salvadó, J., Buil-Cosiales, P., Corella, D., Covas, M. I., Schröder, H., Arós, F., Gómez-Gracia, E., Fiol, M., Ruiz-Gutiérrez, V., Lapetra, J., Lamuela-Raventos, R. M., Serra-Majem, L., Pintó, X., Muñoz, M. A., Wärnberg, J., Ros, E., Estruch, R., & for the, P. S. I. (2012). A 14-Item Mediterranean diet assessment tool and obesity indexes among high-risk subjects: The PREDIMED trial. *PloS One*, *7*(8), e43134. https://doi.org/10.1371/journal.pone.0043134

Martinez, F. O., Helming, L., Milde, R., Varin, A., Melgert, B. N., Draijer, C., Thomas, B., Fabbri, M., Crawshaw, A., Ho, L. P., Ten Hacken, N. H., Cobos Jimenez, V., Kootstra, N. A., Hamann, J., Greaves, D. R., Locati, M., Mantovani, A., & Gordon, S. (2013). Genetic programs expressed in resting and IL-4 alternatively activated mouse and human macrophages: Similarities and differences. *Blood*, *121*(9), e57-69. https://doi.org/10.1182/blood-2012-06-436212

Martinez, F. O., Sica, A., Mantovani, A., & Locati, M. (2008). Macrophage activation and polarization. *Frontiers in Bioscience*, *13*, 453-461. https://doi.org/10.2741/2692

Matera, G., Giancotti, A., Scalise, S., Pulicari, M. C., Maselli, R., Piizzi, C., Pelaia, G., Tancre, V., Muto, V., Doldo, P., Cosco, V., Cosimo, P., Capicotto, R., Quirino, A., Scalzo, R., Liberto, M. C., Parlato, G., & Foca, A. (2008). Ascaris lumbricoides-induced suppression of total and specific IgE responses in atopic subjects is interleukin 10-independent and associated with an increase of CD25(+) cells. *Diagnostic Microbiology and Infectious Disease*, *62*(3), 280-286. https://doi.org/10.1016/j.diagmicrobio.2008.06.015

Mawson, W., & Tien-Ming, H. (2021). HbA1c: More than just a number. *Australian Journal for General Practitioners*, *50*(9), 628-632. https://www1.racgp.org.au/ajgp/2021/september/more-than-just-a-number

Mazmanian, S. K., & Kasper, D. L. (2006). The love-hate relationship between bacterial polysaccharides and the host immune system. *Nature Reviews: Immunology*, *6*(11), 849-858. https://doi.org/10.1038/nri1956

McCormick, M. L., Metwali, A., Railsback, M. A., Weinstock, J. V., & Britigan, B. E. (1996). Eosinophils from schistosome-induced hepatic granulomas produce superoxide and hydroxyl radical. *Journal of Immunology*, *157*(11), 5009-5015. https://www.ncbi.nlm.nih.gov/pubmed/8943408

McCracken, E., Monaghan, M., & Sreenivasan, S. (2018). Pathophysiology of the metabolic syndrome. *Clinics in Dermatology*, *36*(1), 14-20. https://doi.org/10.1016/j.clindermatol.2017.09.004

McGuire, E., Welch, C., & Melzer, M. (2019). Is Strongyloides seropositivity associated with diabetes mellitus? A retrospective case-control study in an East London NHS Trust. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *113*(4), 189-194. https://doi.org/10.1093/trstmh/try132

McKenney, E. A., Williamson, L., Yoder, A. D., Rawls, J. F., Bilbo, S. D., & Parker, W. (2015). Alteration of the rat cecal microbiome during colonization with the helminth Hymenolepis diminuta. *Gut Microbes*, *6*(3), 182-193. https://doi.org/10.1080/19490976.2015.1047128

McLaughlin, T., Ackerman, S. E., Shen, L., & Engleman, E. (2017). Role of innate and adaptive immunity in obesity-associated metabolic disease. *Journal of Clinical Investigation*, *127*(1), 5-13. https://doi.org/10.1172/JCI88876

McSorley, H. J., Blair, N. F., Smith, K. A., McKenzie, A. N., & Maizels, R. M. (2014). Blockade of IL-33 release and suppression of type 2 innate lymphoid cell responses by helminth secreted products in airway allergy. *Mucosal Immunology*, *7*(5), 1068-1078. https://doi.org/10.1038/mi.2013.123

McSorley, H. J., Gaze, S., Daveson, J., Jones, D., Anderson, R. P., Clouston, A., Ruyssers, N. E., Speare, R., McCarthy, J. S., Engwerda, C. R., Croese, J., & Loukas, A. (2011). Suppression of

inflammatory immune responses in celiac disease by experimental hookworm infection. *PloS One*, *6*(9), e24092. https://doi.org/10.1371/journal.pone.0024092

McSorley, H. J., Hewitson, J. P., & Maizels, R. M. (2013). Immunomodulation by helminth parasites: Defining mechanisms and mediators. *International Journal for Parasitology*, *43*(3-4), 301-310. https://doi.org/10.1016/j.ijpara.2012.11.011

MedPage Today. (2013). *AMA house votes against council, calls obesity a disease.* Retrieved May 31 2019 from https://www.medpagetoday.com/MeetingCoverage/AMA/39952

Mehta, P., Nuotio-Antar, A. M., & Smith, C. W. (2015). gammadelta T cells promote inflammation and insulin resistance during high fat diet-induced obesity in mice. *Journal of Leukocyte Biology*, *97*(1), 121-134. https://doi.org/10.1189/jlb.3A0414-211RR

Meier, J. J., & Bonadonna, R. C. (2013). Role of reduced beta-cell mass versus impaired beta-cell function in the pathogenesis of type 2 diabetes. *Diabetes Care*, *36 Suppl 2*, S113-119. https://doi.org/10.2337/dcS13-2008

Melgert, B. N., Oriss, T. B., Qi, Z., Dixon-McCarthy, B., Geerlings, M., Hylkema, M. N., & Ray, A. (2010). Macrophages: Regulators of sex differences in asthma? *American Journal of Respiratory Cell and Molecular Biology*, *42*(5), 595-603. https://doi.org/10.1165/rcmb.2009-0016OC

Mena, M. P., Sacanella, E., Vazquez-Agell, M., Morales, M., Fito, M., Escoda, R., Serrano-Martinez, M., Salas-Salvado, J., Benages, N., Casas, R., Lamuela-Raventos, R. M., Masanes, F., Ros, E., & Estruch, R. (2009). Inhibition of circulating immune cell activation: A molecular antiinflammatory effect of the Mediterranean diet. *American Journal of Clinical Nutrition*, *89*(1), 248-256. https://doi.org/10.3945/ajcn.2008.26094

Mendez, S., Valenzuela, J. G., Wu, W., & Hotez, P. J. (2005). Host cytokine production, lymphoproliferation, and antibody responses during the course of Ancylostoma ceylanicum infection in the Golden Syrian hamster. *Infection and Immunity*, *73*(6), 3402-3407. https://doi.org/10.1128/IAI.73.6.3402-3407.2005

Mendonca, S. C., Goncalves-Pires Mdo, R., Rodrigues, R. M., Ferreira, A., Jr., & Costa-Cruz, J. M. (2006). Is there an association between positive Strongyloides stercoralis serology and diabetes mellitus? *Acta Tropica*, *99*(1), 102-105. https://doi.org/10.1016/j.actatropica.2006.06.006

Metenou, S., Dembele, B., Konate, S., Dolo, H., Coulibaly, S. Y., Coulibaly, Y. I., Diallo, A. A., Soumaoro, L., Coulibaly, M. E., Sanogo, D., Doumbia, S. S., Traore, S. F., Mahanty, S., Klion, A., & Nutman, T. B. (2010). At homeostasis filarial infections have expanded adaptive T regulatory but not classical Th2 cells. *Journal of Immunology*, *184*(9), 5375-5382. https://doi.org/10.4049/jimmunol.0904067

Middleton, D., Garza, J. J., Greiner, S. P., & Bowdridge, S. A. (2020). Neutrophils rapidly produce Th2 cytokines in response to larval but not adult helminth antigen. *Parasite Immunology*, *42*(1), e12679. https://doi.org/10.1111/pim.12679

Min, B., Prout, M., Hu-Li, J., Zhu, J., Jankovic, D., Morgan, E. S., Urban, J. F., Jr., Dvorak, A. M., Finkelman, F. D., LeGros, G., & Paul, W. E. (2004). Basophils produce IL-4 and accumulate in tissues after infection with a Th2-inducing parasite. *Journal of Experimental Medicine*, *200*(4), 507-517. https://doi.org/10.1084/jem.20040590

Minutti, C. M., Jackson-Jones, L. H., Garcia-Fojeda, B., Knipper, J. A., Sutherland, T. E., Logan, N., Ringqvist, E., Guillamat-Prats, R., Ferenbach, D. A., Artigas, A., Stamme, C., Chroneos, Z. C., Zaiss, D. M., Casals, C., & Allen, J. E. (2017). Local amplifiers of IL-4Ralpha-mediated macrophage activation promote repair in lung and liver. *Science*, *356*(6342), 1076-1080. https://doi.org/10.1126/science.aaj2067

Mishra, P. K., Palma, M., Bleich, D., Loke, P., & Gause, W. C. (2014). Systemic impact of intestinal helminth infections. *Mucosal Immunology*, *7*(4), 753-762. https://doi.org/10.1038/mi.2014.23

Miyake, K., Shiozawa, N., Nagao, T., Yoshikawa, S., Yamanishi, Y., & Karasuyama, H. (2017). Trogocytosis of peptide-MHC class II complexes from dendritic cells confers antigen-presenting ability on basophils. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(5), 1111-1116. https://doi.org/10.1073/pnas.1615973114

Mjosberg, J., Bernink, J., Golebski, K., Karrich, J. J., Peters, C. P., Blom, B., te Velde, A. A., Fokkens, W. J., van Drunen, C. M., & Spits, H. (2012). The transcription factor GATA3 is essential for

the function of human type 2 innate lymphoid cells. *Immunity*, *37*(4), 649-659. https://doi.org/10.1016/j.immuni.2012.08.015

Mohamed, S. M. A., Saber, T., Taha, A. A., Roshdy, H. S., & Shahien, N. E. (2017). Relation between Schistosome past infection and metabolic syndrome. *Journal of the Egyptian Society of Parasitology*, *47*(1), 137-143. https://www.ncbi.nlm.nih.gov/pubmed/30157342

Molofsky, A. B., Nussbaum, J. C., Liang, H.-E., Dyken, S. J. V., Cheng, L. E., Mohapatra, A., Chawla, A., & Locksley, R. M. (2013). Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. *Journal of Experimental Medicine*, *210*(3), 535-549. https://doi.org/10.1084/jem.20121964

Montes, M., Sanchez, C., Verdonck, K., Lake, J. E., Gonzalez, E., Lopez, G., Terashima, A., Nolan, T., Lewis, D. E., Gotuzzo, E., & White, A. C., Jr. (2009). Regulatory T cell expansion in HTLV-1 and strongyloidiasis co-infection is associated with reduced IL-5 responses to Strongyloides stercoralis antigen. *PLoS Neglected Tropical Diseases*, *3*(6), e456. https://doi.org/10.1371/journal.pntd.0000456

Moreno-Navarrete, J. M., Ortega, F., Castro, A., Sabater, M., Ricart, W., & Fernandez-Real, J. M. (2011). Circulating omentin as a novel biomarker of endothelial dysfunction. *Obesity (Silver Spring)*, *19*(8), 1552-1559. https://doi.org/10.1038/oby.2010.351

Morigny, P., Houssier, M., Mouisel, E., & Langin, D. (2016). Adipocyte lipolysis and insulin resistance. *Biochimie*, *125*, 259-266. https://doi.org/10.1016/j.biochi.2015.10.024

Morimoto, M., Azuma, N., Kadowaki, H., Abe, T., & Suto, Y. (2017). Regulation of type 2 diabetes by helminth-induced Th2 immune response. *Journal of Veterinary Medical Science*, *78*(12), 1855-1864. https://doi.org/10.1292/jvms.16-0183

Morimoto, M., Morimoto, M., Whitmire, J., Xiao, S., Anthony, R. M., Mirakami, H., Star, R. A., Urban, J. F., Jr., & Gause, W. C. (2004). Peripheral CD4 T cells rapidly accumulate at the host: parasite interface during an inflammatory Th2 memory response. *Journal of Immunology*, *172*(4), 2424-2430. https://doi.org/10.4049/jimmunol.172.4.2424

Moro, K., Yamada, T., Tanabe, M., Takeuchi, T., Ikawa, T., Kawamoto, H., Furusawa, J., Ohtani, M., Fujii, H., & Koyasu, S. (2010). Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells. *Nature*, *463*(7280), 540-544. https://doi.org/10.1038/nature08636

Mortimer, K., Brown, A., Feary, J., Jagger, C., Lewis, S., Antoniak, M., Pritchard, D., & Britton, J. (2006). Dose-ranging study for trials of therapeutic infection with Necator americanus in humans. *American Journal of Tropical Medicine and Hygiene*, *75*(5), 914-920. https://doi.org/10.4269/ajtmh.2006.75.914

Motoshima, H., Wu, X., Sinha, M. K., Hardy, V. E., Rosato, E. L., Barbot, D. J., Rosato, F. E., & Goldstein, B. J. (2002). Differential regulation of adiponectin secretion from cultured human omental and subcutaneous adipocytes: Effects of insulin and rosiglitazone. *Journal of Clinical Endocrinology and Metabolism*, *87*(12), 5662-5667. https://doi.org/10.1210/jc.2002-020635

Mourão Dias Magalhães, L., Silva Araújo Passos, L., Toshio Fujiwara, R., & Lacerda Bueno, L. (2020). Immunopathology and modulation induced by hookworms: From understanding to intervention. *Parasite Immunology*, *n/a*(n/a), e12798. https://doi.org/https://doi.org/10.1111/pim.12798

Moyat, M., Coakley, G., & Harris, N. L. (2019). The interplay of type 2 immunity, helminth infection and the microbiota in regulating metabolism. *Clinical & Translational Immunology*, *8*(11), e01089. https://doi.org/10.1002/cti2.1089

Muniyappa, R., Madan, R., & Varghese, R. T. (2000). Assessing insulin sensitivity and resistance in humans. In K. R. Feingold, B. Anawalt, A. Boyce, G. Chrousos, W. W. de Herder, K. Dhatariya, K. Dungan, J. M. Hershman, J. Hofland, S. Kalra, G. Kaltsas, C. Koch, P. Kopp, M. Korbonits, C. S. Kovacs, W. Kuohung, B. Laferrere, M. Levy, E. A. McGee, R. McLachlan, J. E. Morley, M. New, J. Purnell, R. Sahay, F. Singer, M. A. Sperling, C. A. Stratakis, D. L. Trence, & D. P. Wilson (Eds.), *Endotext*. https://www.ncbi.nlm.nih.gov/pubmed/25905189

Murphy, E. F., Cotter, P. D., Healy, S., Marques, T. M., O'Sullivan, O., Fouhy, F., Clarke, S. F., O'Toole, P. W., Quigley, E. M., Stanton, C., Ross, P. R., O'Doherty, R. M., & Shanahan, F. (2010). Composition and energy harvesting capacity of the gut microbiota: Relationship to diet,

obesity and time in mouse models. *Gut*, *59*(12), 1635-1642. https://doi.org/10.1136/gut.2010.215665

Murphy, T. J., Ni Choileain, N., Zang, Y., Mannick, J. A., & Lederer, J. A. (2005). CD4+CD25+ regulatory T cells control innate immune reactivity after injury. *Journal of Immunology*, *174*(5), 2957-2963. https://doi.org/10.4049/jimmunol.174.5.2957

Must, A., Jacques, P. F., Dallal, G. E., Bajema, C. J., & Dietz, W. H. (1992). Long-term morbidity and mortality of overweight adolescents. A follow-up of the Harvard Growth Study of 1922 to 1935. *New England Journal of Medicine*, *327*(19), 1350-1355. https://doi.org/10.1056/NEJM199211053271904

Muthukumar, R., Suttiprapa, S., Mairiang, E., Kessomboon, P., Laha, T., Smith, J. F., & Sripa, B. (2019). Effects of Opisthorchis viverrini infection on glucose and lipid profiles in human hosts: A cross-sectional and prospective follow-up study from Thailand. *Parasitology International*, 102000. https://doi.org/10.1016/j.parint.2019.102000

Myers, J., Kokkinos, P., & Nyelin, E. (2019). Physical activity, cardiorespiratory fitness, and the metabolic syndrome. *Nutrients*, *11*(7). https://doi.org/10.3390/nu11071652

Nair, M. G., Du, Y., Perrigoue, J. G., Zaph, C., Taylor, J. J., Goldschmidt, M., Swain, G. P., Yancopoulos, G. D., Valenzuela, D. M., Murphy, A., Karow, M., Stevens, S., Pearce, E. J., & Artis, D. (2009). Alternatively activated macrophage-derived RELM-α is a negative regulator of type 2 inflammation in the lung. *Journal of Experimental Medicine*, *206*(4), 937-952. https://doi.org/10.1084/jem.20082048

Nair, M. G., & Herbert, D. R. (2016). Immune polarization by hookworms: Taking cues from T helper type 2, type 2 innate lymphoid cells and alternatively activated macrophages [Article]. *Immunology*, *148*(2), 115-124. https://doi.org/10.1111/imm.12601

Nakanishi, K., Maruyama, M., Shibata, T., & Morishima, N. (2001). Identification of a caspase-9 substrate and detection of its cleavage in programmed cell death during mouse development. *Journal of Biological Chemistry*, *276*(44), 41237-41244. https://doi.org/10.1074/jbc.M105648200

Nathan, C. (2006). Neutrophils and immunity: Challenges and opportunities. *Nature Reviews: Immunology*, *6*(3), 173-182. https://doi.org/10.1038/nri1785

National Organization for Rare Disorders. (2018). *Polycythemia vera*. Retrieved June 10 2022 from https://rarediseases.org/rare-diseases/polycythemia-vera/

Nausch, N., Appleby, L. J., Sparks, A. M., Midzi, N., Mduluza, T., & Mutapi, F. (2015). Group 2 innate lymphoid cell proportions are diminished in young helminth infected children and restored by curative anti-helminthic treatment. *PLoS Neglected Tropical Diseases*, *9*(3), e0003627. https://doi.org/10.1371/journal.pntd.0003627

Nazligul, Y., Sabuncu, T., & Ozbilge, H. (2001). Is there a predisposition to intestinal parasitosis in diabetic patients? *Diabetes Care*, *24*(8), 1503-1504. https://doi.org/10.2337/diacare.24.8.1503-a

Neill, D. R., Wong, S. H., Bellosi, A., Flynn, R. J., Daly, M., Langford, T. K., Bucks, C., Kane, C. M., Fallon, P. G., Pannell, R., Jolin, H. E., & McKenzie, A. N. (2010). Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature*, *464*(7293), 1367-1370. https://doi.org/10.1038/nature08900

Nguyen, L. T., Jacobs, J., Mathis, D., & Benoist, C. (2007). Where FoxP3-dependent regulatory T cells impinge on the development of inflammatory arthritis. *Arthritis and Rheumatism*, *56*(2), 509-520. https://doi.org/10.1002/art.22272

Ni, Y., Ni, L., Zhuge, F., Xu, L., Fu, Z., & Ota, T. (2020). Adipose tissue macrophage phenotypes and characteristics: The key to insulin resistance in obesity and metabolic disorders. *Obesity*, *n/a*(n/a). https://doi.org/10.1002/oby.22674

Nielsen, S., Guo, Z., Johnson, C. M., Hensrud, D. D., & Jensen, M. D. (2004). Splanchnic lipolysis in human obesity. *Journal of Clinical Investigation*, *113*(11), 1582-1588. https://doi.org/10.1172/JCI21047

Nieves, D. J., Cnop, M., Retzlaff, B., Walden, C. E., Brunzell, J. D., Knopp, R. H., & Kahn, S. E. (2003). The atherogenic lipoprotein profile associated with obesity and insulin resistance is largely attributable to intra-abdominal fat. *Diabetes*, *52*(1), 172-179. https://doi.org/10.2337/diabetes.52.1.172

Nishimura, S., Manabe, I., Nagasaki, M., Eto, K., Yamashita, H., Ohsugi, M., Otsu, M., Hara, K., Ueki, K., Sugiura, S., Yoshimura, K., Kadowaki, T., & Nagai, R. (2009). CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nature Medicine*, *15*(8), 914-920. https://doi.org/10.1038/nm.1964

Nishimura, S., Manabe, I., Takaki, S., Nagasaki, M., Otsu, M., Yamashita, H., Sugita, J., Yoshimura, K., Eto, K., Komuro, I., Kadowaki, T., & Nagai, R. (2013). Adipose natural regulatory B cells negatively control adipose tissue inflammation. *Cell Metabolism*, *18*(5), 759-766. https://doi.org/10.1016/j.cmet.2013.09.017

Noel, W., Raes, G., Hassanzadeh Ghassabeh, G., De Baetselier, P., & Beschin, A. (2004). Alternatively activated macrophages during parasite infections. *Trends in Parasitology*, *20*(3), 126-133. https://doi.org/10.1016/j.pt.2004.01.004

Nogueiras, R., Wilson, H., Rohner-Jeanrenaud, F., & Tschop, M. H. (2008). Central nervous system regulation of adipocyte metabolism. *Regulatory Peptides*, *149*(1-3), 26-31. https://doi.org/10.1016/j.regpep.2007.09.034

Nutman, T. B. (2015). Looking beyond the induction of Th2 responses to explain immunomodulation by helminths. *Parasite Immunology*, *37*(6), 304-313. https://doi.org/10.1111/pim.12194

Nuttall, F. Q. (2015). Body Mass Index: Obesity, BMI, and health: A critical review. *Nutrition Today*, *50*(3), 117-128. https://doi.org/10.1097/NT.0000000000000092

Nyberg, S. T., Batty, G. D., Pentti, J., Virtanen, M., Alfredsson, L., Fransson, E. I., Goldberg, M., Heikkila, K., Jokela, M., Knutsson, A., Koskenvuo, M., Lallukka, T., Leineweber, C., Lindbohm, J. V., Madsen, I. E. H., Magnusson Hanson, L. L., Nordin, M., Oksanen, T., Pietilainen, O., Rahkonen, O., Rugulies, R., Shipley, M. J., Stenholm, S., Suominen, S., Theorell, T., Vahtera, J., Westerholm, P. J. M., Westerlund, H., Zins, M., Hamer, M., Singh-Manoux, A., Bell, J. A., Ferrie, J. E., & Kivimaki, M. (2018). Obesity and loss of disease-free years owing to major non-communicable diseases: A multicohort study. *Lancet Public Health*, *3*(10), e490-e497. https://doi.org/10.1016/S2468-2667(18)30139-7

O'Neill, S., & O'Driscoll, L. (2015). Metabolic syndrome: A closer look at the growing epidemic and its associated pathologies. *Obesity Reviews*, *16*(1), 1-12. https://doi.org/10.1111/obr.12229

O'Sullivan, T. E., Rapp, M., Fan, X., Weizman, O. E., Bhardwaj, P., Adams, N. M., Walzer, T., Dannenberg, A. J., & Sun, J. C. (2016). Adipose-resident group 1 innate lymphoid cells promote obesity-associated insulin resistance. *Immunity*, *45*(2), 428-441. https://doi.org/10.1016/j.immuni.2016.06.016

Obata-Ninomiya, K., Domeier, P. P., & Ziegler, S. F. (2020). Basophils and eosinophils in nematode infections [Review]. *Frontiers in Immunology*, *11*. https://doi.org/10.3389/fimmu.2020.583824

Obata-Ninomiya, K., Ishiwata, K., Tsutsui, H., Nei, Y., Yoshikawa, S., Kawano, Y., Minegishi, Y., Ohta, N., Watanabe, N., Kanuka, H., & Karasuyama, H. (2013). The skin is an important bulwark of acquired immunity against intestinal helminths. *Journal of Experimental Medicine*, *210*(12), 2583-2595. https://doi.org/10.1084/jem.20130761

Obradovic, M., Sudar-Milovanovic, E., Soskic, S., Essack, M., Arya, S., Stewart, A. J., Gojobori, T., & Isenovic, E. R. (2021). Leptin and obesity: Role and clinical implication. *Frontiers in Endocrinology*, *12*, 585887. https://doi.org/10.3389/fendo.2021.585887

Odegaard, J. I., Ricardo-Gonzalez, R. R., Goforth, M. H., Morel, C. R., Subramanian, V., Mukundan, L., Red Eagle, A., Vats, D., Brombacher, F., Ferrante, A. W., & Chawla, A. (2007). Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. *Nature*, *447*(7148), 1116-1120. https://doi.org/10.1038/nature05894

Ohashi, K., Ouchi, N., Sato, K., Higuchi, A., Ishikawa, T. O., Herschman, H. R., Kihara, S., & Walsh, K. (2009). Adiponectin promotes revascularization of ischemic muscle through a cyclooxygenase 2-dependent mechanism. *Molecular and Cellular Biology*, *29*(13), 3487-3499. https://doi.org/10.1128/MCB.00126-09

Oliphant, C. J., Hwang, Y. Y., Walker, J. A., Salimi, M., Wong, S. H., Brewer, J. M., Englezakis, A., Barlow, J. L., Hams, E., Scanlon, S. T., Ogg, G. S., Fallon, P. G., & McKenzie, A. N. (2014). MHCII-mediated dialog between group 2 innate lymphoid cells and CD4(+) T cells potentiates type 2 immunity and promotes parasitic helminth expulsion. *Immunity*, *41*(2), 283-295. https://doi.org/10.1016/j.immuni.2014.06.016

Oliveira-Sequeira, T. C., David, E. B., Ribeiro, C., Guimaraes, S., Masseno, A. P., Katagiri, S., & Sequeira, J. L. (2014). Effect of Bifidobacterium animalis on mice infected with Strongyloides venezuelensis. *Revista do Instituto de Medicina Tropical de São Paulo*, *56*(2), 105-109. https://doi.org/10.1590/S0036-46652014000200003

Olshansky, S. J., Passaro, D. J., Hershow, R. C., Layden, J., Carnes, B. A., Brody, J., Hayflick, L., Butler, R. N., Allison, D. B., & Ludwig, D. S. (2005). A potential decline in life expectancy in the United States in the 21st century. *New England Journal of Medicine*, *352*(11), 1138-1145. https://doi.org/10.1056/NEJMsr043743

Orliaguet, L., Dalmas, E., Drareni, K., Venteclef, N., & Alzaid, F. (2020). Mechanisms of macrophage polarization in insulin signaling and sensitivity. *Frontiers in Endocrinology*, *11*, 62. https://doi.org/10.3389/fendo.2020.00062

Orliaguet, L., Ejlalmanesh, T., & Alzaid, F. (2020). Metabolic and molecular mechanisms of macrophage polarisation and adipose tissue insulin resistance. *International Journal of Molecular Sciences*, *21*(16). https://doi.org/10.3390/ijms21165731

Ormazabal, V., Nair, S., Elfeky, O., Aguayo, C., Salomon, C., & Zuniga, F. A. (2018). Association between insulin resistance and the development of cardiovascular disease. *Cardiovascular Diabetology*, *17*(1), 122. https://doi.org/10.1186/s12933-018-0762-4

Otsuka, A., Nakajima, S., Kubo, M., Egawa, G., Honda, T., Kitoh, A., Nomura, T., Hanakawa, S., Sagita Moniaga, C., Kim, B., Matsuoka, S., Watanabe, T., Miyachi, Y., & Kabashima, K. (2013). Basophils are required for the induction of Th2 immunity to haptens and peptide antigens. *Nature Communications*, *4*, 1739. https://doi.org/10.1038/ncomms2740

Ouchi, N., Kihara, S., Funahashi, T., Matsuzawa, Y., & Walsh, K. (2003). Obesity, adiponectin and vascular inflammatory disease. *Current Opinion in Lipidology*, *14*(6), 561-566. https://doi.org/10.1097/00041433-200312000-00003

Ouchi, N., Parker, J. L., Lugus, J. J., & Walsh, K. (2011). Adipokines in inflammation and metabolic disease. *Nature Reviews Immunology*, *11*(2), 85-97. https://doi.org/10.1038/nri2921

Pace, F., Carvalho, B. M., Zanotto, T. M., Santos, A., Guadagnini, D., Silva, K. L. C., Mendes, M. C. S., Rocha, G. Z., Alegretti, S. M., Santos, G. A., Catharino, R. R., Paroni, R., Folli, F., & Saad, M. J. A. (2018). Helminth infection in mice improves insulin sensitivity via modulation of gut microbiota and fatty acid metabolism. *Pharmacological Research*, *132*, 33-46. https://doi.org/10.1016/j.phrs.2018.04.008

Pal, M., Febbraio, M. A., & Whitham, M. (2014). From cytokine to myokine: the emerging role of interleukin-6 in metabolic regulation. *Immunology and Cell Biology*, *92*(4), 331-339. https://doi.org/https://doi.org/10.1038/icb.2014.16

Pan, H. Y., Guo, L., & Li, Q. (2010). Changes of serum omentin-1 levels in normal subjects and in patients with impaired glucose regulation and with newly diagnosed and untreated type 2 diabetes. *Diabetes Research and Clinical Practice*, *88*(1), 29-33. https://doi.org/10.1016/j.diabres.2010.01.013

Pan, X., Kaminga, A. C., Wen, S. W., Acheampong, K., & Liu, A. (2019). Omentin-1 in diabetes mellitus: A systematic review and meta-analysis. *PloS One*, *14*(12), e0226292. https://doi.org/10.1371/journal.pone.0226292

Papadaki, A., Johnson, L., Toumpakari, Z., England, C., Rai, M., Toms, S., Penfold, C., Zazpe, I., Martinez-Gonzalez, M. A., & Feder, G. (2018). Validation of the English version of the 14-Item Mediterranean Diet Adherence Screener of the PREDIMED study in people at high cardiovascular risk in the UK. *Nutrients*, *10*(2), e138. https://doi.org/10.3390/nu10020138

Parija, S. C., Chidambaram, M., & Mandal, J. (2017). Epidemiology and clinical features of soil-transmitted helminths. *Tropical Parasitology*, *7*(2), 81-85. https://doi.org/10.4103/tp.TP_27_17

Parto, P., & Lavie, C. J. (2017). Obesity and cardiovascular diseases. *Current Problems in Cardiology*, *42*(11), 376-394. https://doi.org/10.1016/j.cpcardiol.2017.04.004

Paveley, R. A., Aynsley, S. A., Cook, P. C., Turner, J. D., & Mountford, A. P. (2009). Fluorescent imaging of antigen released by a skin-invading helminth reveals differential uptake and activation profiles by antigen presenting cells. *PLoS Neglected Tropical Diseases*, *3*(10), e528. https://doi.org/10.1371/journal.pntd.0000528

Paz-Filho, G., Mastronardi, C., Franco, C. B., Wang, K. B., Wong, M. L., & Licinio, J. (2012). Leptin: Molecular mechanisms, systemic pro-inflammatory effects, and clinical implications. *Arquivos Brasileiros de Endocrinologia e Metabologia*, *56*(9), 597-607. https://doi.org/10.1590/s0004-27302012000900001

Paz-Filho, G., Mastronardi, C., Wong, M. L., & Licinio, J. (2012). Leptin therapy, insulin sensitivity, and glucose homeostasis. *Indian Journal of Endocrinology and Metabolism*, *16*(Suppl 3), S549-555. https://doi.org/10.4103/2230-8210.105571

Peachey, L. E., Jenkins, T. P., & Cantacessi, C. (2017). This gut ain't big enough for both of us. Or is it? Helminth-microbiota interactions in veterinary species. *Trends in Parasitology*, *33*(8), 619-632. https://doi.org/10.1016/j.pt.2017.04.004

Pekala, P., Kawakami, M., Vine, W., Lane, M. D., & Cerami, A. (1983). Studies of insulin resistance in adipocytes induced by macrophage mediator. *Journal of Experimental Medicine*, *157*(4), 1360-1365. https://doi.org/10.1084/jem.157.4.1360

Pekgor, S., Duran, C., Berberoglu, U., & Eryilmaz, M. A. (2019). The role of visceral adiposity index levels in predicting the presence of metabolic syndrome and insulin resistance in overweight and obese patients. *Metabolic Syndrome and Related Disorders*, *17*(5), 296-302. https://doi.org/10.1089/met.2019.0005

Peng, J., & Siracusa, M. C. (2021). Basophils in antihelminth immunity. *Seminars in Immunology*, *53*, 101529. https://doi.org/10.1016/j.smim.2021.101529

Perrigoue, J. G., Saenz, S. A., Siracusa, M. C., Allenspach, E. J., Taylor, B. C., Giacomin, P. R., Nair, M. G., Du, Y., Zaph, C., van Rooijen, N., Comeau, M. R., Pearce, E. J., Laufer, T. M., & Artis, D. (2009). MHC class II-dependent basophil-CD4+ T cell interactions promote T(H)2 cytokine-dependent immunity. *Nature Immunology*, *10*(7), 697-705. https://doi.org/10.1038/ni.1740

Perry, R. J., Peng, L., Barry, N. A., Cline, G. W., Zhang, D., Cardone, R. L., Petersen, K. F., Kibbey, R. G., Goodman, A. L., & Shulman, G. I. (2016). Acetate mediates a microbiome-brain-beta-cell axis to promote metabolic syndrome. *Nature*, *534*(7606), 213-217. https://doi.org/10.1038/nature18309

Pesce, J. T., Ramalingam, T. R., Mentink-Kane, M. M., Wilson, M. S., El Kasmi, K. C., Smith, A. M., Thompson, R. W., Cheever, A. W., Murray, P. J., & Wynn, T. A. (2009). Arginase-1-expressing macrophages suppress Th2 cytokine-driven inflammation and fibrosis. *PLoS Pathogens*, *5*(4), e1000371. https://doi.org/10.1371/journal.ppat.1000371

Pesce, J. T., Ramalingam, T. R., Wilson, M. S., Mentink-Kane, M. M., Thompson, R. W., Cheever, A. W., Urban, J. F., Jr., & Wynn, T. A. (2009). Retnla (relmalpha/fizz1) suppresses helminth-induced Th2-type immunity. *PLoS Pathogens*, *5*(4), e1000393. https://doi.org/10.1371/journal.ppat.1000393

Peters, B. A., Shapiro, J. A., Church, T. R., Miller, G., Trinh-Shevrin, C., Yuen, E., Friedlander, C., Hayes, R. B., & Ahn, J. (2018). A taxonomic signature of obesity in a large study of American adults. *Scientific Reports*, *8*(1), 9749. https://doi.org/10.1038/s41598-018-28126-1

Phasuk, N., Apiwattanakul, N., & Punsawad, C. (2022). Profiles of CD4+, CD8+, and regulatory T cells and circulating cytokines in hookworm-infected children in southern Thailand. *Medical Microbiology and Immunology*, *211*(1), 19-28. https://doi.org/10.1007/s00430-021-00723-4

Pierce, D., Merone, L., Lewis, C., Rahman, T., Croese, J., Loukas, A., McDonald, M., Giacomin, P., & McDermott, R. (2019). Safety and tolerability of experimental hookworm infection in humans with metabolic disease: study protocol for a phase 1b randomised controlled clinical trial. *BMC Endocrine Disorders*, *19*(1), 136. https://doi.org/10.1186/s12902-019-0461-5

Plieskatt, J. L., Deenonpoe, R., Mulvenna, J. P., Krause, L., Sripa, B., Bethony, J. M., & Brindley, P. J. (2013). Infection with the carcinogenic liver fluke Opisthorchis viverrini modifies intestinal and biliary microbiome. *FASEB Journal*, *27*(11), 4572-4584. https://doi.org/10.1096/fj.13-232751

Png, C. W., Linden, S. K., Gilshenan, K. S., Zoetendal, E. G., McSweeney, C. S., Sly, L. I., McGuckin, M. A., & Florin, T. H. (2010). Mucolytic bacteria with increased prevalence in IBD mucosa augment in vitro utilization of mucin by other bacteria. *American Journal of Gastroenterology*, *105*(11), 2420-2428. https://doi.org/10.1038/ajg.2010.281

Poirier, P., Cornier, M. A., Mazzone, T., Stiles, S., Cummings, S., Klein, S., McCullough, P. A., Ren Fielding, C., Franklin, B. A., American Heart Association Obesity Committee of the Council on Nutrition, P. A., & Metabolism. (2011). Bariatric surgery and cardiovascular risk factors: A scientific statement from the American Heart Association. *Circulation*, *123*(15), 1683-1701. https://doi.org/10.1161/CIR.0b013e3182149099

Pories, W. J., & Dohm, G. L. (2012). Diabetes: Have we got it all wrong? Hyperinsulinism as the culprit: Surgery provides the evidence. *Diabetes Care*, *35*(12), 2438-2442. https://doi.org/10.2337/dc12-0684

Pouliot, M. C., Despres, J. P., Nadeau, A., Moorjani, S., Prud'Homme, D., Lupien, P. J., Tremblay, A., & Bouchard, C. (1992). Visceral obesity in men. Associations with glucose tolerance, plasma insulin, and lipoprotein levels. *Diabetes*, *41*(7), 826-834. https://doi.org/10.2337/diab.41.7.826

Pradhan, A. D., Manson, J. E., Rifai, N., Buring, J. E., & Ridker, P. M. (2001). C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA*, *286*(3), 327-334. https://doi.org/10.1001/jama.286.3.327

Priest, C., & Tontonoz, P. (2019). Inter-organ cross-talk in metabolic syndrome. *Nature Metabolism*, *1*(12), 1177-1188. https://doi.org/10.1038/s42255-019-0145-5

Qatanani, M., & Lazar, M. A. (2007). Mechanisms of obesity-associated insulin resistance: many choices on the menu. *Genes and Development*, *21*(12), 1443-1455. https://doi.org/10.1101/gad.1550907

Qin, J., Li, R., Raes, J., Arumugam, M., Tims, S., Vos, d. W. M., Zoetendal, E. G., Kleerebezem, M., & Meta, H. I. T. C. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, *464*(7285), 59-65. https://doi.org/10.1038/nature08821

Rahat-Rozenbloom, S., Fernandes, J., Gloor, G. B., & Wolever, T. M. (2014). Evidence for greater production of colonic short-chain fatty acids in overweight than lean humans. *International Journal of Obesity (2005)*, *38*(12), 1525-1531. https://doi.org/10.1038/ijo.2014.46

Rajamanickam, A., Munisankar, S., Bhootra, Y., Dolla, C., Thiruvengadam, K., Nutman, T. B., & Babu, S. (2018). Metabolic consequences of concomitant Strongyloides stercoralis infection in Type 2 diabetes mellitus. *Clinical Infectious Diseases*. https://doi.org/10.1093/cid/ciy935

Rajamanickam, A., Munisankar, S., Dolla, C., Menon, P. A., Thiruvengadam, K., Nutman, T. B., & Babu, S. (2020). Helminth infection modulates systemic pro-inflammatory cytokines and chemokines implicated in type 2 diabetes mellitus pathogenesis. *PLoS Neglected Tropical Diseases*, *14*(3), e0008101. https://doi.org/10.1371/journal.pntd.0008101

Rajamanickam, A., Munisankar, S., Menon, P. A., Dolla, C., Nutman, T. B., & Babu, S. (2020). Helminth mediated attenuation of systemic inflammation and microbial translocation in helminth-diabetes comorbidity. *Frontiers in Cellular and Infection Microbiology*, *10*, 431. https://doi.org/10.3389/fcimb.2020.00431

Ramanan, D., Bowcutt, R., Lee, S. C., Tang, M. S., Kurtz, Z. D., Ding, Y., Honda, K., Gause, W. C., Blaser, M. J., Bonneau, R. A., Lim, Y. A., Loke, P., & Cadwell, K. (2016). Helminth infection promotes colonization resistance via type 2 immunity. *Science*, *352*(6285), 608-612. https://doi.org/10.1126/science.aaf3229

Ranasinghe, P., Mathangasinghe, Y., Jayawardena, R., Hills, A. P., & Misra, A. (2017). Prevalence and trends of metabolic syndrome among adults in the Asia-Pacific region: A systematic review. *BMC Public Health*, *17*(1), 101. https://doi.org/10.1186/s12889-017-4041-1

Rastelli, M., Knauf, C., & Cani, P. D. (2018). Gut microbes and health: A focus on the mechanisms linking microbes, obesity, and related disorders. *Obesity*, *26*(5), 792-800. https://doi.org/10.1002/oby.22175

Rausch, M. E., Weisberg, S., Vardhana, P., & Tortoriello, D. V. (2008). Obesity in C57BL/6J mice is characterized by adipose tissue hypoxia and cytotoxic T-cell infiltration. *International Journal of Obesity (2005)*, *32*(3), 451-463. https://doi.org/10.1038/sj.ijo.0803744

Rausch, S., Held, J., Fischer, A., Heimesaat, M. M., Kuhl, A. A., Bereswill, S., & Hartmann, S. (2013). Small intestinal nematode infection of mice is associated with increased enterobacterial loads alongside the intestinal tract. *PloS One*, *8*(9), e74026. https://doi.org/10.1371/journal.pone.0074026

Reaven, G. M. (1988). Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes*, *37*(12), 1595-1607. https://doi.org/10.2337/diab.37.12.1595

Reiman, R. M., Thompson, R. W., Feng, C. G., Hari, D., Knight, R., Cheever, A. W., Rosenberg, H. F., & Wynn, T. A. (2006). Interleukin-5 (IL-5) augments the progression of liver fibrosis by regulating IL-13 activity. *Infection and Immunity*, *74*(3), 1471-1479. https://doi.org/10.1128/IAI.74.3.1471-1479.2006

Reimann, F., Tolhurst, G., & Gribble, F. M. (2012). G-protein-coupled receptors in intestinal chemosensation. *Cell Metabolism*, *15*(4), 421-431. https://doi.org/10.1016/j.cmet.2011.12.019

Rennie, C., Fernandez, R., Donnelly, S., & McGrath, K. C. (2021). The impact of helminth infection on the incidence of metabolic syndrome: A systematic review and meta-analysis. *Frontiers in Endocrinology*, *12*, 728396. https://doi.org/10.3389/fendo.2021.728396

Resta-Lenert, S., & Barrett, K. E. (2006). Probiotics and commensals reverse TNF-alpha- and IFN-gamma-induced dysfunction in human intestinal epithelial cells. *Gastroenterology*, *130*(3), 731-746. https://doi.org/10.1053/j.gastro.2005.12.015

Reynolds, L. A., Filbey, K. J., & Maizels, R. M. (2012). Immunity to the model intestinal helminth parasite Heligmosomoides polygyrus. *Seminars in Immunopathology*, *34*(6), 829-846. https://doi.org/10.1007/s00281-012-0347-3

Reynolds, L. A., Smith, K. A., Filbey, K. J., Harcus, Y., Hewitson, J. P., Redpath, S. A., Valdez, Y., Yebra, M. J., Finlay, B. B., & Maizels, R. M. (2014). Commensal-pathogen interactions in the intestinal tract: Lactobacilli promote infection with, and are promoted by, helminth parasites. *Gut Microbes*, *5*(4), 522-532. https://doi.org/10.4161/gmic.32155

Rhee, E. P., Cheng, S., Larson, M. G., Walford, G. A., Lewis, G. D., McCabe, E., Yang, E., Farrell, L., Fox, C. S., O'Donnell, C. J., Carr, S. A., Vasan, R. S., Florez, J. C., Clish, C. B., Wang, T. J., & Gerszten, R. E. (2011). Lipid profiling identifies a triacylglycerol signature of insulin resistance and improves diabetes prediction in humans. *Journal of Clinical Investigation*, *121*(4), 1402-1411. https://doi.org/10.1172/JCI44442

Ricardo-Gonzalez, R. R., Eagle, A. R., Odegaard, J. I., Jouihan, H., Morel, C. R., Heredia, J. E., Mukundan, L., Wu, D., Locksley, R. M., Chawla, A., & Evans, R. M. (2010). IL-4/STAT6 immune axis regulates peripheral nutrient metabolism and insulin sensitivity. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(52), 22617-22622. https://doi.org/10.1073/pnas.1009152108

Ricci, N. D., Fiuza, J. A., Bueno, L. L., Cancado, G. G., Gazzinelli-Guimaraes, P. H., Martins, V. G., Matoso, L. F., de Miranda, R. R., Geiger, S. M., Correa-Oliveira, R., Gazzinelli, A., Bartholomeu, D. C., & Fujiwara, R. T. (2011). Induction of CD4(+)CD25(+)FOXP3(+) regulatory T cells during human hookworm infection modulates antigen-mediated lymphocyte proliferation. *PLoS Neglected Tropical Diseases*, *5*(11), e1383. https://doi.org/10.1371/journal.pntd.0001383

Ridaura, V. K., Faith, J. J., Rey, F. E., Cheng, J., Duncan, A. E., Kau, A. L., Griffin, N. W., Lombard, V., Henrissat, B., Bain, J. R., Muehlbauer, M. J., Ilkayeva, O., Semenkovich, C. F., Funai, K., Hayashi, D. K., Lyle, B. J., Martini, M. C., Ursell, L. K., Clemente, J. C., Van Treuren, W., Walters, W. A., Knight, R., Newgard, C. B., Heath, A. C., & Gordon, J. I. (2013). Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science*, *341*(6150), 1079-U1049. https://doi.org/10.1126/science.1241214

Ridker, P. M., Cushman, M., Stampfer, M. J., Tracy, R. P., & Hennekens, C. H. (1997). Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *New England Journal of Medicine*, *336*(14), 973-979. https://doi.org/10.1056/NEJM199704033361401

Riva, A., Borgo, F., Lassandro, C., Verduci, E., Morace, G., Borghi, E., & Berry, D. (2017). Pediatric obesity is associated with an altered gut microbiota and discordant shifts in Firmicutes populations. *Environmental Microbiology*, *19*(1), 95-105. https://doi.org/10.1111/1462-2920.13463

Robb, J. L., Morrissey, N. A., Weightman Potter, P. G., Smithers, H. E., Beall, C., & Ellacott, K. L. J. (2019). Immunometabolic changes in glia - a potential role in the pathophysiology of obesity and diabetes. *Neuroscience*. https://doi.org/10.1016/j.neuroscience.2019.10.021

Roche, M., & Layrisse, M. (1966). The nature and causes of "hookworm anemia". *American Journal of Tropical Medicine and Hygiene*, *15*(6), 1029-1102. https://www.ncbi.nlm.nih.gov/pubmed/5334793

Rochlani, Y., Pothineni, N. V., Kovelamudi, S., & Mehta, J. L. (2017). Metabolic syndrome: Pathophysiology, management, and modulation by natural compounds. *Therapeutic Advances in Cardiovascular Disease*, *11*(8), 215-225. https://doi.org/10.1177/1753944717711379

Romero-Corral, A., Somers, V. K., Sierra-Johnson, J., Korenfeld, Y., Boarin, S., Korinek, J., Jensen, M. D., Parati, G., & Lopez-Jimenez, F. (2010). Normal weight obesity: A risk factor for cardiometabolic dysregulation and cardiovascular mortality. *European Heart Journal*, *31*(6), 737-746. https://doi.org/10.1093/eurheartj/ehp487

Rosa, B. A., Supali, T., Gankpala, L., Djuardi, Y., Sartono, E., Zhou, Y., Fischer, K., Martin, J., Tyagi, R., Bolay, F. K., Fischer, P. U., Yazdanbakhsh, M., & Mitreva, M. (2018). Differential human gut microbiome assemblages during soil-transmitted helminth infections in Indonesia and Liberia. *Microbiome*, *6*(1), 33. https://doi.org/10.1186/s40168-018-0416-5

Rosen, E. D., & Spiegelman, B. M. (2014). What we talk about when we talk about fat. *Cell*, *156*(1-2), 20-44. https://doi.org/10.1016/j.cell.2013.12.012

Ross, R., Aru, J., Freeman, J., Hudson, R., & Janssen, I. (2002). Abdominal adiposity and insulin resistance in obese men. *American Journal of Physiology: Endocrinology and Metabolism*, *282*(3), E657-663. https://doi.org/10.1152/ajpendo.00469.2001

Ross, R., Freeman, J., Hudson, R., & Janssen, I. (2002). Abdominal obesity, muscle composition, and insulin resistance in premenopausal women. *Journal of Clinical Endocrinology and Metabolism*, *87*(11), 5044-5051. https://doi.org/10.1210/jc.2002-020570

Rothermel, J., Lass, N., Barth, A., & Reinehr, T. (2020). Link between omentin-1, obesity and insulin resistance in children: Findings from a longitudinal intervention study. *Pediatric Obesity*, *15*(5), e12605. https://doi.org/10.1111/ijpo.12605

Roufosse, F. (2018). Targeting the Interleukin-5 pathway for treatment of eosinophilic conditions other than asthma. *Frontiers in Medicine (Lausanne)*, *5*, 49. https://doi.org/10.3389/fmed.2018.00049

Roust, L. R., & Jensen, M. D. (1993). Postprandial free fatty acid kinetics are abnormal in upper body obesity. *Diabetes*, *42*(11), 1567-1573. https://doi.org/10.2337/diab.42.11.1567

Russo, L., & Lumeng, C. N. (2018). Properties and functions of adipose tissue macrophages in obesity. *Immunology*, *155*(4), 407-417. https://doi.org/10.1111/imm.13002

Rutkowski, J. M., Stern, J. H., & Scherer, P. E. (2015). The cell biology of fat expansion. *Journal of Cell Biology*, *208*(5), 501-512. https://doi.org/10.1083/jcb.201409063

Ruyssers, N. E., De Winter, B. Y., De Man, J. G., Loukas, A., Pearson, M. S., Weinstock, J. V., Van den Bossche, R. M., Martinet, W., Pelckmans, P. A., & Moreels, T. G. (2008). Therapeutic potential of helminth soluble proteins in TNBS-induced colitis in mice. *Inflammatory Bowel Diseases*, *15*(4), 491-500. https://doi.org/10.1002/ibd.20787

Ryan, M., McInerney, D., Owens, D., Collins, P., Johnson, A., & Tomkin, G. H. (2000). Diabetes and the Mediterranean diet: A beneficial effect of oleic acid on insulin sensitivity, adipocyte glucose transport and endothelium-dependent vasoreactivity. *QJM*, *93*(2), 85-91. https://doi.org/10.1093/qjmed/93.2.85

Ryan, S. M., Eichenberger, R. M., Ruscher, R., Giacomin, P. R., & Loukas, A. (2020). Harnessing helminth-driven immunoregulation in the search for novel therapeutic modalities. *PLoS Pathogens*, *16*(5), Article e1008508. https://doi.org/10.1371/journal.ppat.1008508

Sakaguchi, S., Mikami, N., Wing, J. B., Tanaka, A., Ichiyama, K., & Ohkura, N. (2020). Regulatory T cells and human disease. *Annual Review of Immunology*, *38*, 541-566. https://doi.org/10.1146/annurev-immunol-042718-041717

Saklayen, M. G. (2018). The global epidemic of the metabolic syndrome. *Current Hypertension Reports*, *20*(2), 12. https://doi.org/10.1007/s11906-018-0812-z

Saltiel, A. R., & Olefsky, J. M. (2017). Inflammatory mechanisms linking obesity and metabolic disease. *Journal of Clinical Investigation*, *127*(1), 1-4. https://doi.org/10.1172/JCI92035

Sanchez-Moreno, C., Cano, M. P., de Ancos, B., Plaza, L., Olmedilla, B., Granado, F., & Martin, A. (2006). Mediterranean vegetable soup consumption increases plasma vitamin C and decreases F2-isoprostanes, prostaglandin E2 and monocyte chemotactic protein-1 in healthy humans. *The Journal of Nutritional Biochemistry*, *17*(3), 183-189. https://doi.org/10.1016/j.jnutbio.2005.07.001

Santacruz, A., Collado, M. C., Garcia-Valdes, L., Segura, M. T., Martin-Lagos, J. A., Anjos, T., Marti-Romero, M., Lopez, R. M., Florido, J., Campoy, C., & Sanz, Y. (2010). Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. *British Journal of Nutrition, 104*(1), 83-92. https://doi.org/10.1017/S0007114510000176

Sanya, R. E., Nkurunungi, G., Biraro, I. A., Mpairwe, H., & Elliott, A. M. (2017). A life without worms. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 111*(1), 3-11. https://doi.org/10.1093/trstmh/trx010

Sanya, R. E., Webb, E. L., Zziwa, C., Kizindo, R., Sewankambo, M., Tumusiime, J., Nakazibwe, E., Oduru, G., Niwagaba, E., Kabuubi Nakawungu, P., Kabagenyi, J., Nassuuna, J., Walusimbi, B., Biraro, I. A., Elliott, A. M., & La, V. t. t. (2019). The effect of helminth infections and their treatment on metabolic outcomes: Results of a cluster-randomised trial. *Clinical Infectious Diseases*. https://doi.org/10.1093/cid/ciz859

Sarhan, M., Land, W. G., Tonnus, W., Hugo, C. P., & Linkermann, A. (2018). Origin and consequences of necroinflammation. *Physiological Reviews, 98*(2), 727-780. https://doi.org/10.1152/physrev.00041.2016

Sato, J., Kanazawa, A., Ikeda, F., Yoshihara, T., Goto, H., Abe, H., Komiya, K., Kawaguchi, M., Shimizu, T., Ogihara, T., Tamura, Y., Sakurai, Y., Yamamoto, R., Mita, T., Fujitani, Y., Fukuda, H., Nomoto, K., Takahashi, T., Asahara, T., Hirose, T., Nagata, S., Yamashiro, Y., & Watada, H. (2014). Gut dysbiosis and detection of "live gut bacteria" in blood of Japanese patients with type 2 diabetes. *Diabetes Care, 37*(8), 2343-2350. https://doi.org/10.2337/dc13-2817

Savage, D. B., Petersen, K. F., & Shulman, G. I. (2007). Disordered lipid metabolism and the pathogenesis of insulin resistance. *Physiological Reviews, 87*(2), 507-520. https://doi.org/10.1152/physrev.00024.2006

Scaffidi, P., Misteli, T., & Bianchi, M. E. (2002). Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature, 418*(6894), 191-195. https://doi.org/10.1038/nature00858

Schimpl, M., Moore, C., Lederer, C., Neuhaus, A., Sambrook, J., Danesh, J., Ouwehand, W., & Daumer, M. (2011). Association between walking speed and age in healthy, free-living individuals using mobile accelerometry - a cross-sectional study. *PloS One, 6*(8), e23299. https://doi.org/10.1371/journal.pone.0023299

Schmiedel, Y., Mombo-Ngoma, G., Labuda, L. A., Janse, J. J., de Gier, B., Adegnika, A. A., Issifou, S., Kremsner, P. G., Smits, H. H., & Yazdanbakhsh, M. (2015). CD4+CD25hiFOXP3+ Regulatory T cells and cytokine responses in human Schistosomiasis before and after treatment with praziquantel. *PLoS Neglected Tropical Diseases, 9*(8), e0003995. https://doi.org/10.1371/journal.pntd.0003995

Schmitz, O., Rungby, J., Edge, L., & Juhl, C. B. (2008). On high-frequency insulin oscillations. *Ageing Research Reviews, 7*(4), 301-305. https://doi.org/10.1016/j.arr.2008.04.002

Schottl, T., Kappler, L., Fromme, T., & Klingenspor, M. (2015). Limited OXPHOS capacity in white adipocytes is a hallmark of obesity in laboratory mice irrespective of the glucose tolerance status. *Molecular Metabolism, 4*(9), 631-642. https://doi.org/10.1016/j.molmet.2015.07.001

Schottl, T., & Klingenspor, M. (2013). Boosting mitochondrial biogenesis in white adipocytes: A route towards improved insulin sensitivity? *Molecular Metabolism, 2*(3), 128-129. https://doi.org/10.1016/j.molmet.2013.07.002

Schöttl, T., Pachl, F., Giesbertz, P., Daniel, H., Kuster, B., Fromme, T., & Klingenspor, M. (2020). Proteomic and metabolite profiling reveals profound structural and metabolic reorganization of adipocyte mitochondria in obesity. *Obesity, n/a*(n/a). https://doi.org/10.1002/oby.22737

Schroder, H., Fito, M., Estruch, R., Martinez-Gonzalez, M. A., Corella, D., Salas-Salvado, J., Lamuela-Raventos, R., Ros, E., Salaverria, I., Fiol, M., Lapetra, J., Vinyoles, E., Gomez-Gracia, E., Lahoz, C., Serra-Majem, L., Pinto, X., Ruiz-Gutierrez, V., & Covas, M. I. (2011). A short screener is valid for assessing Mediterranean diet adherence among older Spanish men and women. *Journal of Nutrition, 141*(6), 1140-1145. https://doi.org/10.3945/jn.110.135566

Schroeder, B. O., & Backhed, F. (2016). Signals from the gut microbiota to distant organs in physiology and disease. *Nature Medicine, 22*(10), 1079-1089. https://doi.org/10.1038/nm.4185

Schwartz, C., Turqueti-Neves, A., Hartmann, S., Yu, P., Nimmerjahn, F., & Voehringer, D. (2014). Basophil-mediated protection against gastrointestinal helminths requires IgE-induced cytokine secretion. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(48), E5169-5177. https://doi.org/10.1073/pnas.1412663111

Sears, B., & Perry, M. (2015). The role of fatty acids in insulin resistance. *Lipids in Health and Disease*, *14*, 121. https://doi.org/10.1186/s12944-015-0123-1

Seuring, T., Archangelidi, O., & Suhrcke, M. (2015). The economic costs of type 2 diabetes: A global systematic review. *Pharmacoeconomics*, *33*(8), 811-831. https://doi.org/10.1007/s40273-015-0268-9

Shapouri-Moghaddam, A., Mohammadian, S., Vazini, H., Taghadosi, M., Esmaeili, S. A., Mardani, F., Seifi, B., Mohammadi, A., Afshari, J. T., & Sahebkar, A. (2018). Macrophage plasticity, polarization, and function in health and disease. *Journal of Cellular Physiology*, *233*(9), 6425-6440. https://doi.org/10.1002/jcp.26429

Sharma, A. M., & Campbell-Scherer, D. L. (2017). Redefining obesity: Beyond the numbers. *Obesity*, *25*(4), 660-661. https://doi.org/10.1002/oby.21801

Sharpe, C., Thornton, D. J., & Grencis, R. K. (2018). A sticky end for gastrointestinal helminths; the role of the mucus barrier. *Parasite Immunology*, *40*(4), e12517. https://doi.org/10.1111/pim.12517

Shaw, A. C., Goldstein, D. R., & Montgomery, R. R. (2013). Age-dependent dysregulation of innate immunity. *Nature Reviews: Immunology*, *13*(12), 875-887. https://doi.org/10.1038/nri3547

Shea-Donohue, T., Sullivan, C., Finkelman, F. D., Madden, K. B., Morris, S. C., Goldhill, J., Pineiro-Carrero, V., & Urban, J. F., Jr. (2001). The role of IL-4 in Heligmosomoides polygyrus-induced alterations in murine intestinal epithelial cell function. *Journal of Immunology*, *167*(4), 2234-2239. https://www.ncbi.nlm.nih.gov/pubmed/11490010

Shen, S. W., Lu, Y., Li, F., Shen, Z. H., Xu, M., Yao, W. F., Feng, Y. B., Yun, J. T., Wang, Y. P., Ling, W., Qi, H. J., & Tong, D. X. (2015). The potential long-term effect of previous schistosome infection reduces the risk of metabolic syndrome among Chinese men. *Parasite Immunology*, *37*(7), 333-339. https://doi.org/10.1111/pim.12187

Shen, T., Kim, S., Do, J. S., Wang, L., Lantz, C., Urban, J. F., Le Gros, G., & Min, B. (2008). T cell-derived IL-3 plays key role in parasite infection-induced basophil production but is dispensable for in vivo basophil survival. *International Immunology*, *20*(9), 1201-1209. https://doi.org/10.1093/intimm/dxn077

Shi, H., Kokoeva, M. V., Inouye, K., Tzameli, I., Yin, H., & Flier, J. S. (2006). TLR4 links innate immunity and fatty acid-induced insulin resistance. *Journal of Clinical Investigation*, *116*(11), 3015-3025. https://doi.org/10.1172/JCI28898

Shimokawa, C., Obi, S., Shibata, M., Olia, A., Imai, T., Suzue, K., & Hisaeda, H. (2019). Suppression of obesity by an intestinal helminth through interactions with intestinal microbiota. *Infection and Immunity*, *87*(6). https://doi.org/10.1128/IAI.00042-19

Shin, N. R., Lee, J. C., Lee, H. Y., Kim, M. S., Whon, T. W., Lee, M. S., & Bae, J. W. (2014). An increase in the Akkermansia spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. *Gut*, *63*(5), 727-735. https://doi.org/10.1136/gutjnl-2012-303839

Shoelson, S. E., Herrero, L., & Naaz, A. (2007). Obesity, inflammation, and insulin resistance. *Gastroenterology*, *132*(6), 2169-2180. https://doi.org/10.1053/j.gastro.2007.03.059

Shoelson, S. E., Lee, J., & Goldfine, A. B. (2006). Inflammation and insulin resistance. *Journal of Clinical Investigation*, *116*(7), 1793-1801. https://doi.org/10.1172/JCI29069

Shulman, G. I. (2000). Cellular mechanisms of insulin resistance. *Journal of Clinical Investigation*, *106*(2), 171-176. https://doi.org/10.1172/JCI10583

Sierksma, A., Patel, H., Ouchi, N., Kihara, S., Funahashi, T., Heine, R. J., Grobbee, D. E., Kluft, C., & Hendriks, H. F. (2004). Effect of moderate alcohol consumption on adiponectin, tumor necrosis factor-alpha, and insulin sensitivity. *Diabetes Care*, *27*(1), 184-189. https://doi.org/10.2337/diacare.27.1.184

Silva, Y. P., Bernardi, A., & Frozza, R. L. (2020). The role of short-chain fatty acids from gut microbiota in gut-brain communication. *Frontiers in Endocrinology*, *11*. https://doi.org/10.3389/fendo.2020.00025

Sleiman, D., Al-Badri, M. R., & Azar, S. T. (2015). Effect of mediterranean diet in diabetes control and cardiovascular risk modification: A systematic review. *Frontiers in Public Health*, *3*, 69. https://doi.org/10.3389/fpubh.2015.00069

Smallie, T., Ricchetti, G., Horwood, N. J., Feldmann, M., Clark, A. R., & Williams, L. M. (2010). IL-10 inhibits transcription elongation of the human TNF gene in primary macrophages. *Journal of Experimental Medicine*, *207*(10), 2081-2088. https://doi.org/10.1084/jem.20100414

Snowling, N. J., & Hopkins, W. G. (2006). Effects of different modes of exercise training on glucose control and risk factors for complications in type 2 diabetic patients: A meta-analysis. *Diabetes Care*, *29*(11), 2518-2527. https://doi.org/10.2337/dc06-1317

Sokol, C. L., Chu, N. Q., Yu, S., Nish, S. A., Laufer, T. M., & Medzhitov, R. (2009). Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. *Nature Immunology*, *10*(7), 713-720. https://doi.org/10.1038/ni.1738

Soriguer, F., Esteva, I., Rojo-Martinez, G., Ruiz de Adana, M. S., Dobarganes, M. C., Garcia-Almeida, J. M., Tinahones, F., Beltran, M., Gonzalez-Romero, S., Olveira, G., & Gomez-Zumaquero, J. M. (2004). Oleic acid from cooking oils is associated with lower insulin resistance in the general population (Pizarra study). *European Journal of Endocrinology of the European Federation of Endocrine Societies*, *150*(1), 33-39. https://doi.org/10.1530/eje.0.1500033

Speare, R., Melrose, W., Cooke, S., & Croese, J. (2008). Techniques to kill infective larvae of human hookworm Necator Americanus in the laboratory and a new Material Safety Data Sheet. *Australian Journal of Medical Science*, *29*(3), 91-96. https://doi.org/10.3316/informit.353647515503667

Spencer, M., Yao-Borengasser, A., Unal, R., Rasouli, N., Gurley, C. M., Zhu, B., Peterson, C. A., & Kern, P. A. (2010). Adipose tissue macrophages in insulin-resistant subjects are associated with collagen VI and fibrosis and demonstrate alternative activation. *American Journal of Physiology: Endocrinology and Metabolism*, *299*(6), E1016-1027. https://doi.org/10.1152/ajpendo.00329.2010

Stefanovic-Racic, M., Yang, X., Turner, M. S., Mantell, B. S., Stolz, D. B., Sumpter, T. L., Sipula, I. J., Dedousis, N., Scott, D. K., Morel, P. A., Thomson, A. W., & O'Doherty, R. M. (2012). Dendritic cells promote macrophage infiltration and comprise a substantial proportion of obesity-associated increases in CD11c+ cells in adipose tissue and liver. *Diabetes*, *61*(9), 2330-2339. https://doi.org/10.2337/db11-1523

Stenberg, E., & Thorell, A. (2020). Insulin resistance in bariatric surgery. *Current Opinion in Clinical Nutrition and Metabolic Care*, *23*(4). https://journals.lww.com/co-clinicalnutrition/Fulltext/2020/07000/Insulin_resistance_in_bariatric_surgery.7.aspx

Stephens, J. M., Lee, J., & Pilch, P. F. (1997). Tumor necrosis factor-alpha-induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT4 expression without a loss of insulin receptor-mediated signal transduction. *Journal of Biological Chemistry*, *272*(2), 971-976. https://doi.org/10.1074/jbc.272.2.971

Stern, S. E., Williams, K., Ferrannini, E., DeFronzo, R. A., Bogardus, C., & Stern, M. P. (2005). Identification of individuals with insulin resistance using routine clinical measurements. *Diabetes*, *54*(2), 333-339. https://doi.org/10.2337/diabetes.54.2.333

Stienstra, R., van Diepen, J. A., Tack, C. J., Zaki, M. H., van de Veerdonk, F. L., Perera, D., Neale, G. A., Hooiveld, G. J., Hijmans, A., Vroegrijk, I., van den Berg, S., Romijn, J., Rensen, P. C., Joosten, L. A., Netea, M. G., & Kanneganti, T. D. (2011). Inflammasome is a central player in the induction of obesity and insulin resistance. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(37), 15324-15329. https://doi.org/10.1073/pnas.1100255108

Stivers, K. B., Chilton, P. M., Beare, J. E., Dale, J. R., Alard, P., LeBlanc, A. J., & Hoying, J. B. (2020). Adipose-resident myeloid-derived suppressor cells modulate immune cell homeostasis in healthy mice. *Immunology and Cell Biology*. https://doi.org/10.1111/imcb.12360

Stolk, W. A., Kulik, M. C., le Rutte, E. A., Jacobson, J., Richardus, J. H., de Vlas, S. J., & Houweling, T. A. (2016). Between-country inequalities in the neglected tropical disease burden in 1990 and 2010, with projections for 2020. *PLoS Neglected Tropical Diseases*, *10*(5), e0004560. https://doi.org/10.1371/journal.pntd.0004560

Stoltzfus, R. J., Chwaya, H. M., Tielsch, J. M., Schulze, K. J., Albonico, M., & Savioli, L. (1997). Epidemiology of iron deficiency anemia in Zanzibari schoolchildren: The importance of hookworms. *American Journal of Clinical Nutrition*, *65*(1), 153-159. https://doi.org/10.1093/ajcn/65.1.153

Stout, R. D., Jiang, C., Matta, B., Tietzel, I., Watkins, S. K., & Suttles, J. (2005). Macrophages sequentially change their functional phenotype in response to changes in microenvironmental influences. *Journal of Immunology*, *175*(1), 342-349. https://doi.org/10.4049/jimmunol.175.1.342

Strasser, B. (2013). Physical activity in obesity and metabolic syndrome. *Annals of the New York Academy of Sciences*, *1281*, 141-159. https://doi.org/10.1111/j.1749-6632.2012.06785.x

Stumvoll, M., & Haring, H. (2002). Resistin and adiponectin--of mice and men. *Obesity Research*, *10*(11), 1197-1199. https://doi.org/10.1038/oby.2002.162

Su, C., Chen, C. Y., Li, Y., Long, S. R., Massey, W., Kumar, D. V., Walker, W. A., & Shi, H. N. (2018). Helminth infection protects against high fat diet-induced obesity via induction of alternatively activated macrophages. *Scientific Reports*, *8*(1), Article 4607. https://doi.org/10.1038/s41598-018-22920-7

Su, C. W., Chen, C. Y., Jiao, L., Long, S. R., Mao, T., Ji, Q., O'Donnell, S., Stanton, C., Zheng, S., Walker, W. A., Cherayil, B. J., & Shi, H. N. (2020). Helminth-induced and Th2-dependent alterations of the gut microbiota attenuate obesity caused by high fat diet. *Cellular and Molecular Gastroenterology and Hepatology*. https://doi.org/10.1016/j.jcmgh.2020.06.010

Suganami, T., Tanimoto-Koyama, K., Nishida, J., Itoh, M., Yuan, X., Mizuarai, S., Kotani, H., Yamaoka, S., Miyake, K., Aoe, S., Kamei, Y., & Ogawa, Y. (2007). Role of the Toll-like receptor 4/NF-kappaB pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophages. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *27*(1), 84-91. https://doi.org/10.1161/01.ATV.0000251608.09329.9a

Sun, K., Kusminski, C. M., & Scherer, P. E. (2011). Adipose tissue remodeling and obesity. *Journal of Clinical Investigation*, *121*(6), 2094-2101. https://doi.org/10.1172/JCI45887

Surendar, J., Frohberger, S. J., Karunakaran, I., Schmitt, V., Stamminger, W., Neumann, A.-L., Wilhelm, C., Hoerauf, A., & Hübner, M. P. (2019). Adiponectin limits IFN-γ and IL-17 producing CD4 T cells in obesity by restraining cell intrinsic glycolysis. *Frontiers in Immunology*, *10*, 2555. https://www.frontiersin.org/article/10.3389/fimmu.2019.02555

Sutherland, T. E., Logan, N., Ruckerl, D., Humbles, A. A., Allan, S. M., Papayannopoulos, V., Stockinger, B., Maizels, R. M., & Allen, J. E. (2014). Chitinase-like proteins promote IL-17-mediated neutrophilia in a tradeoff between nematode killing and host damage. *Nature Immunology*, *15*(12), 1116-1125. https://doi.org/10.1038/ni.3023

Swidsinski, A., Loening-Baucke, V., & Herber, A. (2009). Mucosal flora in Crohn's disease and ulcerative colitis - an overview. *Journal of Physiology and Pharmacology*, *60 Suppl 6*, 61-71. https://www.ncbi.nlm.nih.gov/pubmed/20224153

Switzer, W. B., Stwewart, F., & Paauwe-Weust, J. (2018). *Fasting Insulin vs Hemoglobin A1c: Are We Getting It Right?* Retrieved July 16 2022 from https://www.clinicaladvisor.com/home/topics/diabetes-information-center/fasting-insulin-vs-hemoglobin-a1c-are-we-getting-it-right/

Tahapary, D. L., de Ruiter, K., Martin, I., Brienen, E. A. T., van Lieshout, L., Cobbaert, C. M., Soewondo, P., Djuardi, Y., Wiria, A. E., Houwing-Duistermaat, J. J., Sartono, E., Smit, J. W. A., Yazdanbakhsh, M., & Supali, T. (2017). Effect of anthelmintic treatment on insulin resistance: A cluster-randomized, placebo-controlled trial in Indonesia. *Clinical Infectious Diseases*, *65*(5), 764-771. https://doi.org/10.1093/cid/cix416

Tahapary, D. L., de Ruiter, K., Martin, I., Brienen, E. A. T., van Lieshout, L., Djuardi, Y., Djimandjaja, C. C., Houwing-Duistermaat, J. J., Soewondo, P., Sartono, E., Supali, T., Smit, J. W. A., & Yazdanbakhsh, M. (2017). Effect of anthelmintic treatment on leptin, adiponectin and leptin to adiponectin ratio:Aa randomized-controlled trial. *Nutrition & Diabetes*, *7*(10), e289. https://doi.org/10.1038/nutd.2017.37

Tahapary, D. L., Ruiter, K. d., Martin, I., Lieshout, L. v., Guigas, B., Soewondo, P., Djuardi, Y., Wiria, A. E., Mayboroda, O. A., Houwing-Duistermaat, J. J., Tasman, H., Sartono, E., Yazdanbakhsh, M., Smit, J. W. A., & Supali, T. (2015). Helminth infections and type 2 diabetes: A cluster-

randomized placebo controlled SUGARSPIN trial in Nangapanda, Flores, Indonesia. *BMC Infectious Diseases*, *15*(1), 133-139. https://doi.org/10.1186/s12879-015-0873-4

Talukdar, S., Oh, D. Y., Bandyopadhyay, G., Li, D., Xu, J., McNelis, J., Lu, M., Li, P., Yan, Q., Zhu, Y., Ofrecio, J., Lin, M., Brenner, M. B., & Olefsky, J. M. (2012). Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. *Nature Medicine*, *18*(9), 1407-1412. https://doi.org/10.1038/nm.2885

Tan, B. K., Adya, R., Farhatullah, S., Lewandowski, K. C., O'Hare, P., Lehnert, H., & Randeva, H. S. (2008). Omentin-1, a novel adipokine, is decreased in overweight insulin-resistant women with polycystic ovary syndrome: ex vivo and in vivo regulation of omentin-1 by insulin and glucose. *Diabetes*, *57*(4), 801-808. https://doi.org/10.2337/db07-0990

Tanaka, M., Ikeda, K., Suganami, T., Komiya, C., Ochi, K., Shirakawa, I., Hamaguchi, M., Nishimura, S., Manabe, I., Matsuda, T., Kimura, K., Inoue, H., Inagaki, Y., Aoe, S., Yamasaki, S., & Ogawa, Y. (2014). Macrophage-inducible C-type lectin underlies obesity-induced adipose tissue fibrosis. *Nature Communications*, *5*, 4982. https://doi.org/10.1038/ncomms5982

Tanasescu, R., Tench, C. R., Constantinescu, C. S., Telford, G., Singh, S., Frakich, N., Onion, D., Auer, D. P., Gran, B., Evangelou, N., Falah, Y., Ranshaw, C., Cantacessi, C., Jenkins, T. P., & Pritchard, D. I. (2020). Hookworm treatment for relapsing multiple sclerosis: A randomized double-blinded placebo-controlled trial. *JAMA Neurol*. https://doi.org/10.1001/jamaneurol.2020.1118

Tang, H., Cao, W., Kasturi, S. P., Ravindran, R., Nakaya, H. I., Kundu, K., Murthy, N., Kepler, T. B., Malissen, B., & Pulendran, B. (2010). The T helper type 2 response to cysteine proteases requires dendritic cell-basophil cooperation via ROS-mediated signaling. *Nature Immunology*, *11*(7), 608-617. https://doi.org/10.1038/ni.1883

Tchkonia, T., Corkey, B. E., & Kirkland, J. L. (2006). Current views of the fat cell as an endocrine cell: lipotoxicity. In *Overweight and the metabolic syndrome* (pp. 105-123). Springer.

The Global B. M. I. Mortality Collaboration. (2016). Body-mass index and all-cause mortality: Individual-participant-data meta-analysis of 239 prospective studies in four continents. *Lancet*, *388*(10046), 776-786. https://doi.org/10.1016/S0140-6736(16)30175-1

The Royal Australian College of General Practitioners and Diabetes Australia. (2020, March 2021). *Management of type 2 diabetes: A handbook for general practice*. Retrieved June 29 2022 from https://www.racgp.org.au/getattachment/41fee8dc-7f97-4f87-9d90-b7af337af778/Management-of-type-2-diabetes-A-handbook-for-general-practice.aspx

Thorburn, A. N., Macia, L., & Mackay, C. R. (2014). Diet, metabolites, and "Western-lifestyle" inflammatory diseases. *Immunity*, *40*(6), 833-842. https://doi.org/10.1016/j.immuni.2014.05.014

Tibirica, E., & De Lorenzo, A. (2020). Increased severity of COVID-19 in people with obesity: Are we overlooking plausible biological mechanisms? *Obesity*. https://doi.org/10.1002/oby.22887

Tilg, H., & Moschen, A. R. (2014). Microbiota and diabetes: An evolving relationship. *Gut*, *63*(9), 1513-1521. https://doi.org/10.1136/gutjnl-2014-306928

Tomas, J., Mulet, C., Saffarian, A., Cavin, J. B., Ducroc, R., Regnault, B., Kun Tan, C., Duszka, K., Burcelin, R., Wahli, W., Sansonetti, P. J., & Pedron, T. (2016). High-fat diet modifies the PPAR-gamma pathway leading to disruption of microbial and physiological ecosystem in murine small intestine. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(40), E5934-E5943. https://doi.org/10.1073/pnas.1612559113

Tonnus, W., Meyer, C., Paliege, A., Belavgeni, A., von Massenhausen, A., Bornstein, S. R., Hugo, C., Becker, J. U., & Linkermann, A. (2019). The pathological features of regulated necrosis. *Journal of Pathology*, *247*(5), 697-707. https://doi.org/10.1002/path.5248

Tontonoz, P., & Spiegelman, B. M. (2008). Fat and beyond: The diverse biology of PPARgamma. *Annual Review of Biochemistry*, *77*, 289-312. https://doi.org/10.1146/annurev.biochem.77.061307.091829

Torrero, M. N., Hubner, M. P., Larson, D., Karasuyama, H., & Mitre, E. (2010). Basophils amplify type 2 immune responses, but do not serve a protective role, during chronic infection of mice with the filarial nematode Litomosoides sigmodontis. *Journal of Immunology*, *185*(12), 7426-7434. https://doi.org/10.4049/jimmunol.0903864

Tracey, E. F., McDermott, R. A., & McDonald, M. I. (2016). Do worms protect against the metabolic syndrome? A systematic review and meta-analysis. *Diabetes Research and Clinical Practice, 120*, 209-220. https://doi.org/10.1016/j.diabres.2016.08.014

Trayhurn, P., & Wood, I. S. (2005). Signalling role of adipose tissue: Adipokines and inflammation in obesity. *Biochemical Society Transactions*, *33*(Pt 5), 1078-1081. https://doi.org/10.1042/bst0331078

Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Duncan, A., Ley, R. E., Sogin, M. L., Jones, W. J., Roe, B. A., Affourtit, J. P., Egholm, M., Henrissat, B., Heath, A. C., Knight, R., & Gordon, J. I. (2009). A core gut microbiome in obese and lean twins. *Nature, 457*(7228), 480-484. https://doi.org/10.1038/nature07540

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature, 444*, 1027-1031. https://doi.org/10.1038/nature05414

Turnbaugh, P. J., Ridaura, V. K., Faith, J. J., Rey, F. E., Knight, R., & Gordon, J. I. (2009). The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Science Translational Medicine, 1*(6), 6ra14. https://doi.org/10.1126/scitranslmed.3000322

Turner, J. R. (2009). Intestinal mucosal barrier function in health and disease. *Nature Reviews: Immunology*, *9*(11), 799-809. https://doi.org/10.1038/nri2653

University of Saskatchewan. (2021, June 18). *Quantitative Faecal Flotation - McMaster Egg Counting Technique*. Retrieved January 04 2022 from https://wcvm.usask.ca/learnaboutparasites/diagnostics/quantitative-faecal-flotation-mcmaster.php

Uysal, K. T., Wiesbrock, S. M., & Hotamisligil, G. S. (1998). Functional analysis of tumor necrosis factor (TNF) receptors in TNF-alpha-mediated insulin resistance in genetic obesity. *Endocrinology*, *139*(12), 4832-4838. https://doi.org/10.1210/endo.139.12.6337

Vague, J. (1956). The degree of masculine differentiation of obesities: A factor determining predisposition to diabetes, atherosclerosis, gout, and uric calculous disease. *American Journal of Clinical Nutrition*, *4*(1), 20-34. https://doi.org/10.1093/ajcn/4.1.20

Valdes, A. M., Walter, J., Segal, E., & Spector, T. D. (2018). Role of the gut microbiota in nutrition and health. *BMJ*, *361*, k2179. https://doi.org/10.1136/bmj.k2179

van den Berg, S. M., van Dam, A. D., Kusters, P. J. H., Beckers, L., Den Toom, M., van der Velden, S., Van den Bossche, J., van Die, I., Boon, M. R., Rensen, P. C. N., Lutgens, E., & de Winther, M. P. J. (2017). Helminth antigens counteract a rapid high-fat diet-induced decrease in adipose tissue eosinophils. *Journal of Molecular Endocrinology*, *59*(3), 245-255. https://doi.org/10.1530/JME-17-0112

van der Zande, H. J. P., Gonzalez, M. A., de Ruiter, K., Wilbers, R. H. P., Garcia-Tardon, N., van Huizen, M., van Noort, K., Pelgrom, L. R., Lambooij, J. M., Zawistowska-Deniziak, A., Otto, F., Ozir-Fazalalikhan, A., van Willigen, D., Welling, M., Poles, J., van Leeuwen, F., Hokke, C. H., Schots, A., Yazdanbakhsh, M., Loke, P., & Guigas, B. (2021). The helminth glycoprotein omega-1 improves metabolic homeostasis in obese mice through type 2 immunity-independent inhibition of food intake. *FASEB Journal*, *35*(2), e21331. https://doi.org/10.1096/fj.202001973R

Vega, G. L., Chandalia, M., Szczepaniak, L. S., & Grundy, S. M. (2007). Metabolic correlates of nonalcoholic fatty liver in women and men. *Hepatology*, *46*(3), 716-722. https://doi.org/10.1002/hep.21727

Vernochet, C., Damilano, F., Mourier, A., Bezy, O., Mori, M. A., Smyth, G., Rosenzweig, A., Larsson, N. G., & Kahn, C. R. (2014). Adipose tissue mitochondrial dysfunction triggers a lipodystrophic syndrome with insulin resistance, hepatosteatosis, and cardiovascular complications. *FASEB Journal*, *28*(10), 4408-4419. https://doi.org/10.1096/fj.14-253971

Vernon, G., Baranova, A., & Younossi, Z. M. (2011). Systematic review: The epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Alimentary Pharmacology and Therapeutics*, *34*(3), 274-285. https://doi.org/10.1111/j.1365-2036.2011.04724.x

Villacres, M. C., Longmate, J., Auge, C., & Diamond, D. J. (2004). Predominant type 1 CMV-specific memory T-helper response in humans: Evidence for gender differences in cytokine secretion. *Human Immunology*, *65*(5), 476-485. https://doi.org/10.1016/j.humimm.2004.02.021

vom Steeg, L. G., & Klein, S. L. (2016). SeXX Matters in infectious disease pathogenesis. *PLoS Pathogens*, *12*(2), e1005374. https://doi.org/10.1371/journal.ppat.1005374

von Moltke, J., Ji, M., Liang, H. E., & Locksley, R. M. (2016). Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. *Nature*, *529*(7585), 221-225. https://doi.org/10.1038/nature16161

Walk, S. T., Blum, A. M., Ewing, S. A., Weinstock, J. V., & Young, V. B. (2010). Alteration of the murine gut microbiota during infection with the parasitic helminth Heligmosomoides polygyrus. *Inflammatory Bowel Diseases*, *16*(11), 1841-1849. https://doi.org/10.1002/ibd.21299

Wammes, L. J., Hamid, F., Wiria, A. E., Wibowo, H., Sartono, E., Maizels, R. M., Smits, H. H., Supali, T., & Yazdanbakhsh, M. (2012). Regulatory T cells in human lymphatic filariasis: stronger functional activity in microfilaremics. *PLoS Neglected Tropical Diseases*, *6*(5), e1655. https://doi.org/10.1371/journal.pntd.0001655

Wang, C. C., Goalstone, M. L., & Draznin, B. (2004). Molecular mechanisms of insulin resistance that impact cardiovascular biology. *Diabetes*, *53*(11), 2735-2740. https://doi.org/10.2337/diabetes.53.11.2735

Wang, S. J., Sharkey, K. A., & McKay, D. M. (2018). Modulation of the immune response by helminths: A role for serotonin? *Bioscience Reports*, *38*(5). https://doi.org/10.1042/BSR20180027

Wang, T., Nie, S., Reid, G. E., & Gasser, R. B. (2021). Helminth lipidomics: Technical aspects and future prospects. *Current Research in Parasitology & Vector-Borne Diseases*, *1*, 100018. https://doi.org/https://doi.org/10.1016/j.crpvbd.2021.100018

Watanabe, K., Mwinzi, P. N., Black, C. L., Muok, E. M., Karanja, D. M., Secor, W. E., & Colley, D. G. (2007). T regulatory cell levels decrease in people infected with Schistosoma mansoni on effective treatment. *American Journal of Tropical Medicine and Hygiene*, *77*(4), 676-682. https://www.ncbi.nlm.nih.gov/pubmed/17978070

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante, A. W., Jr. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *Journal of Clinical Investigation*, *112*(12), 1796-1808. https://doi.org/10.1172/JCI19246

Wellen, K. E., & Hotamisligil, G. S. (2005). Inflammation, stress, and diabetes. *Journal of Clinical Investigation*, *115*(5), 1111-1119. https://doi.org/10.1172/JCI200525102

Weyand, C. M., & Goronzy, J. J. (2016). Aging of the immune system. Mechanisms and therapeutic targets. *Ann Am Thorac Soc*, *13 Suppl 5*, S422-S428. https://doi.org/10.1513/AnnalsATS.201602-095AW

White, M. P. J., McManus, C. M., & Maizels, R. M. (2020). Regulatory T-cells in helminth infection: induction, function and therapeutic potential. *Immunology*, *160*(3), 248-260. https://doi.org/10.1111/imm.13190

Wilcox, G. (2005). Insulin and insulin resistance. *Clinical Biochemistry Reviews*, *26*(2), 19-39. https://www.ncbi.nlm.nih.gov/pubmed/16278749

Wilson-Fritch, L., Nicoloro, S., Chouinard, M., Lazar, M. A., Chui, P. C., Leszyk, J., Straubhaar, J., Czech, M. P., & Corvera, S. (2004). Mitochondrial remodeling in adipose tissue associated with obesity and treatment with rosiglitazone. *Journal of Clinical Investigation*, *114*(9), 1281-1289. https://doi.org/10.1172/JCI21752

Winer, D. A., Winer, S., Shen, L., Wadia, P. P., Yantha, J., Paltser, G., Tsui, H., Wu, P., Davidson, M. G., Alonso, M. N., Leong, H. X., Glassford, A., Caimol, M., Kenkel, J. A., Tedder, T. F., McLaughlin, T., Miklos, D. B., Dosch, H. M., & Engleman, E. G. (2011). B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. *Nature Medicine*, *17*(5), 610-617. https://doi.org/10.1038/nm.2353

Winer, S., Chan, Y., Paltser, G., Truong, D., Tsui, H., Bahrami, J., Dorfman, R., Wang, Y., Zielenski, J., Mastronardi, F., Maezawa, Y., Drucker, D. J., Engleman, E., Winer, D., & Dosch, H. M. (2009). Normalization of obesity-associated insulin resistance through immunotherapy. *Nature Medicine*, *15*(8), 921-929. https://doi.org/10.1038/nm.2001

Winn, N. C., Cottam, M. A., Wasserman, D. H., & Hasty, A. H. (2021). Exercise and adipose tissue immunity: Outrunning inflammation. *Obesity (Silver Spring)*, *29*(5), 790-801. https://doi.org/10.1002/oby.23147

Wiria, A. E., Hamid, F., Wammes, L. J., Prasetyani, M. A., Dekkers, O. M., May, L., Kaisar, M. M., Verweij, J. J., Guigas, B., Partono, F., Sartono, E., Supali, T., Yazdanbakhsh, M., & Smit, J. W. (2015). Infection with soil-transmitted helminths Is associated with increased insulin sensitivity. *PloS One*, *10*(6), e0127746. https://doi.org/10.1371/journal.pone.0127746

Wiria, A. E., Sartono, E., Supali, T., & Yazdanbakhsh, M. (2014). Helminth infections, type-2 immune response, and metabolic syndrome. *PLoS Pathogens*, *10*(7), e1004140. https://doi.org/10.1371/journal.ppat.1004140

Wiria, A. E., Wammes, L. J., Hamid, F., Dekkers, O. M., Prasetyani, M. A., May, L., Kaisar, M. M., Verweij, J. J., Tamsma, J. T., Partono, F., Sartono, E., Supali, T., Yazdanbakhsh, M., & Smit, J. W. (2013). Relationship between carotid intima media thickness and helminth infections on Flores Island, Indonesia. *PloS One*, *8*(1), e54855. https://doi.org/10.1371/journal.pone.0054855

Wittebolle, L., Marzorati, M., Clement, L., Balloi, A., Daffonchio, D., Heylen, K., De Vos, P., Verstraete, W., & Boon, N. (2009). Initial community evenness favours functionality under selective stress. *Nature*, *458*(7238), 623-626. https://doi.org/10.1038/nature07840

Wolde, M., Berhe, N., Medhin, G., Chala, F., van Die, I., & Tsegaye, A. (2019). Inverse associations of Schistosoma mansoni infection and metabolic syndrome in humans: A cross-sectional study in Northeast Ethiopia. *Microbiology Insights*, *12*, 1178636119849934. https://doi.org/10.1177/1178636119849934

Wolf, Y., Boura-Halfon, S., Cortese, N., Haimon, Z., Sar Shalom, H., Kuperman, Y., Kalchenko, V., Brandis, A., David, E., Segal-Hayoun, Y., Chappell-Maor, L., Yaron, A., & Jung, S. (2017). Brown-adipose-tissue macrophages control tissue innervation and homeostatic energy expenditure. *Nature Immunology*, *18*(6), 665-674. https://doi.org/10.1038/ni.3746

World Health Organisation. (2016). *ICD-10*. Retrieved August 15 2019 from https://icdcode.info/icd-10/code-e88.81.html

World Health Organization. (2018). *Obesity and overweight*. Retrieved June 26 2019 from https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

World Health Organization. (2019). *Body mass index-BMI*. Retrieved June 26 2019 from http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi

World Health Organization. (2021). *Obesity and overweight*. Retrieved August 02 2021 from https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

World Health Organization. (2022, 10 January 2022). *Soil-transmitted helminth infections.* Retrieved June 02 2022 from https://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections

Worthington, J. J., Klementowicz, J. E., Rahman, S., Czajkowska, B. I., Smedley, C., Waldmann, H., Sparwasser, T., Grencis, R. K., & Travis, M. A. (2013). Loss of the TGFbeta-activating integrin alphavbeta8 on dendritic cells protects mice from chronic intestinal parasitic infection via control of type 2 immunity. *PLoS Pathogens*, *9*(10), e1003675. https://doi.org/10.1371/journal.ppat.1003675

Wu, D., Molofsky, A. B., Liang, H.-E., Ricardo-Gonzalez, R. R., Jouihan, H. A., Bando, J. K., Chawla, A., & Locksley, R. M. (2011). Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. *Science*, *332*(6026), 243-247. https://doi.org/10.1126/science.1201475

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.-Y., Keilbaugh, S. A., Bewtra, M., Knights, D., Walters, W. A., Knight, R., Sinha, R., Gilroy, E., Gupta, K., Baldassano, R., Nessel, L., Li, H., Bushman, F. D., & Lewis, J. D. (2011). Linking long-term dietary patterns with gut microbial enterotypes [10.1126/science.1208344]. *Science*, *334*(6052), 105-108. https://doi.org/10.1126/science.1208344

Wu, X., Motoshima, H., Mahadev, K., Stalker, T. J., Scalia, R., & Goldstein, B. J. (2003). Involvement of AMP-activated protein kinase in glucose uptake stimulated by the globular domain of

adiponectin in primary rat adipocytes. *Diabetes*, *52*(6), 1355-1363. https://doi.org/10.2337/diabetes.52.6.1355

Wynn, T. A. (2004). Fibrotic disease and the T(H)1/T(H)2 paradigm. *Nature Reviews: Immunology*, *4*(8), 583-594. https://doi.org/10.1038/nri1412

Wynn, T. A., Chawla, A., & Pollard, J. W. (2013). Macrophage biology in development, homeostasis and disease. *Nature*, *496*(7446), 445-455. https://doi.org/10.1038/nature12034

Wynn, T. A., & Ramalingam, T. R. (2012). Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nature Medicine*, *18*(7), 1028-1040. https://doi.org/10.1038/nm.2807

Xia, H. J., Zhang, G. H., Wang, R. R., & Zheng, Y. T. (2009). The influence of age and sex on the cell counts of peripheral blood leukocyte subpopulations in Chinese rhesus macaques. *Cellular & Molecular Immunology*, *6*(6), 433-440. https://doi.org/10.1038/cmi.2009.55

Xu, H., Barnes, G. T., Yang, Q., Tan, G., Yang, D., Chou, C. J., Sole, J., Nichols, A., Ross, J. S., Tartaglia, L. A., & Chen, H. (2003). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *Journal of Clinical Investigation*, *112*(12), 1821-1830. https://doi.org/10.1172/JCI19451

Xu, X., Grijalva, A., Skowronski, A., van Eijk, M., Serlie, M. J., & Ferrante, A. W., Jr. (2013). Obesity activates a program of lysosomal-dependent lipid metabolism in adipose tissue macrophages independently of classic activation. *Cell Metabolism*, *18*(6), 816-830. https://doi.org/10.1016/j.cmet.2013.11.001

Yadav, A., Kataria, M. A., Saini, V., & Yadav, A. (2013). Role of leptin and adiponectin in insulin resistance. *Clinica Chimica Acta*, *417*, 80-84. https://doi.org/10.1016/j.cca.2012.12.007

Yamauchi, J., Kawai, Y., Yamada, M., Uchikawa, R., Tegoshi, T., & Arizono, N. (2006). Altered expression of goblet cell- and mucin glycosylation-related genes in the intestinal epithelium during infection with the nematode Nippostrongylus brasiliensis in rat. *APMIS*, *114*(4), 270-278. https://doi.org/10.1111/j.1600-0463.2006.apm_353.x

Yamauchi, T., Kamon, J., Waki, H., Terauchi, Y., Kubota, N., Hara, K., Mori, Y., Ide, T., Murakami, K., Tsuboyama-Kasaoka, N., Ezaki, O., Akanuma, Y., Gavrilova, O., Vinson, C., Reitman, M. L., Kagechika, H., Shudo, K., Yoda, M., Nakano, Y., Tobe, K., Nagai, R., Kimura, S., Tomita, M., Froguel, P., & Kadowaki, T. (2001). The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nature Medicine*, *7*(8), 941-946. https://doi.org/10.1038/90984

Yang, H., Youm, Y. H., Vandanmagsar, B., Ravussin, A., Gimble, J. M., Greenway, F., Stephens, J. M., Mynatt, R. L., & Dixit, V. D. (2010). Obesity increases the production of proinflammatory mediators from adipose tissue T cells and compromises TCR repertoire diversity: implications for systemic inflammation and insulin resistance. *Journal of Immunology*, *185*(3), 1836-1845. https://doi.org/10.4049/jimmunol.1000021

Yang, R. Z., Lee, M. J., Hu, H., Pray, J., Wu, H. B., Hansen, B. C., Shuldiner, A. R., Fried, S. K., McLenithan, J. C., & Gong, D. W. (2006). Identification of omentin as a novel depot-specific adipokine in human adipose tissue: Possible role in modulating insulin action. *American Journal of Physiology: Endocrinology and Metabolism*, *290*(6), E1253-1261. https://doi.org/10.1152/ajpendo.00572.2004

Yang, Z., Grinchuk, V., Smith, A., Qin, B., Bohl, J. A., Sun, R., Notari, L., Zhang, Z., Sesaki, H., Joseph, F. U., Jr., Shea-Donohue, T., & Zhao, A. (2013). Parasitic nematode-induced modulation of body weight and associated metabolic dysfunction in mouse models of obesity. *Infection and Immunity*, *81*(6), 1905-1914. https://doi.org/10.1128/IAI.00053-13

Yassour, M., Lim, M. Y., Yun, H. S., Tickle, T. L., Sung, J., Song, Y. M., Lee, K., Franzosa, E. A., Morgan, X. C., Gevers, D., Lander, E. S., Xavier, R. J., Birren, B. W., Ko, G., & Huttenhower, C. (2016). Sub-clinical detection of gut microbial biomarkers of obesity and type 2 diabetes. *Genome Medicine*, *8*(1), 17. https://doi.org/10.1186/s13073-016-0271-6

Yasuda, K., Muto, T., Kawagoe, T., Matsumoto, M., Sasaki, Y., Matsushita, K., Taki, Y., Futatsugi-Yumikura, S., Tsutsui, H., Ishii, K. J., Yoshimoto, T., Akira, S., & Nakanishi, K. (2012). Contribution of IL-33-activated type II innate lymphoid cells to pulmonary eosinophilia in intestinal nematode-infected mice. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(9), 3451-3456. https://doi.org/10.1073/pnas.1201042109

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R. N., Anokhin, A. P., Heath, A. C., Warner, B., Reeder, J., Kuczynski, J., Caporaso, J. G., Lozupone, C. A., Lauber, C., Clemente, J. C., Knights, D., Knight, R., & Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. *Nature*, *486*(7402), 222-227. https://doi.org/10.1038/nature11053

Yazdanbakhsh, M., Kremsner, P. G., & van Ree, R. (2002). Allergy, parasites, and the hygiene hypothesis. *Science*, *296*(5567), 490-494. https://doi.org/10.1126/science.296.5567.490

Ye, R., & Scherer, P. E. (2013). Adiponectin, driver or passenger on the road to insulin sensitivity? *Molecular Metabolism*, *2*(3), 133-141. https://doi.org/10.1016/j.molmet.2013.04.001

Yoon, J., Um, H. N., Jang, J., Bae, Y. A., Park, W. J., Kim, H. J., Yoon, M. S., Chung, I. Y., & Jung, Y. (2019). Eosinophil activation by Toll-like receptor 4 ligands regulates macrophage polarization. *Front Cell Dev Biol*, *7*, 329. https://doi.org/10.3389/fcell.2019.00329

Yoshimoto, T., Yasuda, K., Tanaka, H., Nakahira, M., Imai, Y., Fujimori, Y., & Nakanishi, K. (2009). Basophils contribute to T(H)2-IgE responses in vivo via IL-4 production and presentation of peptide-MHC class II complexes to CD4+ T cells. *Nature Immunology*, *10*(7), 706-712. https://doi.org/10.1038/ni.1737

You, T., Disanzo, B. L., & Arsenis, N. C. (2013). Aerobic exercise training attenuates obesity-related hypogonadism in male rats. *Medicine and Science in Sports and Exercise*, *45*(7), 1244-1251. https://doi.org/10.1249/MSS.0b013e318285816c

Zaiss, M. M., Rapin, A., Lebon, L., Dubey, L. K., Mosconi, I., Sarter, K., Piersigilli, A., Menin, L., Walker, A. W., Rougemont, J., Paerewijck, O., Geldhof, P., McCoy, K. D., Macpherson, A. J., Croese, J., Giacomin, P. R., Loukas, A., Junt, T., Marsland, B. J., & Harris, N. L. (2015). The intestinal microbiota contributes to the ability of helminths to modulate allergic inflammation [Review]. *Immunity*, *43*(5), 998-1010. https://doi.org/10.1016/j.immuni.2015.09.012

Zajak, A. (2020). *HOMA-IR Calculator - Insulin Resistance*. Retrieved March 10 2018 from https://www.omnicalculator.com/health/homa-ir

Zaragosi, L. E., Wdziekonski, B., Villageois, P., Keophiphath, M., Maumus, M., Tchkonia, T., Bourlier, V., Mohsen-Kanson, T., Ladoux, A., Elabd, C., Scheideler, M., Trajanoski, Z., Takashima, Y., Amri, E. Z., Lacasa, D., Sengenes, C., Ailhaud, G., Clement, K., Bouloumie, A., Kirkland, J. L., & Dani, C. (2010). Activin a plays a critical role in proliferation and differentiation of human adipose progenitors. *Diabetes*, *59*(10), 2513-2521. https://doi.org/10.2337/db10-0013

Zeng, H., & Chi, H. (2013). The interplay between regulatory T cells and metabolism in immune regulation. *Oncoimmunology*, *2*(11), e26586. https://doi.org/10.4161/onci.26586

Zeng, M. Y., Inohara, N., & Nunez, G. (2017). Mechanisms of inflammation-driven bacterial dysbiosis in the gut. *Mucosal Immunology*, *10*(1), 18-26. https://doi.org/10.1038/mi.2016.75

Zhang, D., Liu, X., Liu, Y., Sun, X., Wang, B., Ren, Y., Zhao, Y., Zhou, J., Han, C., Yin, L., Zhao, J., Shi, Y., Zhang, M., & Hu, D. (2017). Leisure-time physical activity and incident metabolic syndrome: a systematic review and dose-response meta-analysis of cohort studies. *Metabolism: Clinical and Experimental*, *75*, 36-44. https://doi.org/10.1016/j.metabol.2017.08.001

Zhang, K., Xu, X., Pasha, M. A., Siebel, C. W., Costello, A., Haczku, A., MacNamara, K., Liang, T., Zhu, J., Bhandoola, A., Maillard, I., & Yang, Q. (2017). Cutting edge: Notch signaling promotes the plasticity of Group-2 innate lymphoid cells. *Journal of Immunology*, *198*(5), 1798-1803. https://doi.org/10.4049/jimmunol.1601421

Zhang, X., Shen, D., Fang, Z., Jie, Z., Qiu, X., Zhang, C., Chen, Y., & Ji, L. (2013). Human gut microbiota changes reveal the progression of glucose intolerance. *PloS One*, *8*(8), e71108. https://doi.org/10.1371/journal.pone.0071108

Zheng, L., Hu, Y., Wang, Y., Huang, X., Xu, Y., Shen, Y., & Cao, J. (2017). Recruitment of neutrophils mediated by Vgamma2 gammadelta T cells deteriorates liver fibrosis induced by Schistosoma japonicum infection in C57BL/6 mice. *Infection and Immunity*, *85*(8). https://doi.org/10.1128/IAI.01020-16

Zhong, H., Ren, H., Lu, Y., Fang, C., Hou, G., Yang, Z., Chen, B., Yang, F., Zhao, Y., Shi, Z., Zhou, B., Wu, J., Zou, H., Zi, J., Chen, J., Bao, X., Hu, Y., Gao, Y., Zhang, J., Xu, X., Hou, Y., Yang, H., Wang, J., Liu, S., Jia, H., Madsen, L., Brix, S., Kristiansen, K., Liu, F., & Li, J. (2019). Distinct gut metagenomics and metaproteomics signatures in prediabetics and treatment-naive type 2 diabetics. *EBioMedicine*, *47*, 373-383. https://doi.org/10.1016/j.ebiom.2019.08.048

Zhou, H., & Liu, F. (2018). Regulation, Communication, and Functional Roles of Adipose Tissue-Resident CD4(+) T Cells in the Control of Metabolic Homeostasis. *Frontiers in Immunology*, *9*, 1961. https://doi.org/10.3389/fimmu.2018.01961

Zinsou, J. F., Janse, J. J., Honpkehedji, Y. Y., Dejon-Agobé, J. C., García-Tardón, N., Hoekstra, P. T., Massinga-Loembe, M., Corstjens, P. L. A. M., van Dam, G. J., Giera, M., Kremsner, P. G., Yazdanbakhsh, M., Adegnika, A. A., & Guigas, B. (2020). Schistosoma haematobium infection is associated with lower serum cholesterol levels and improved lipid profile in overweight/obese individuals [Article]. *PLoS Neglected Tropical Diseases*, *14*(7), 1-16, Article e0008464. https://doi.org/10.1371/journal.pntd.0008464

Zouiouich, S., Loftfield, E., Huybrechts, I., Viallon, V., Louca, P., Vogtmann, E., Wells, P. M., Steves, C. J., Herzig, K.-H., Menni, C., Jarvelin, M.-R., Sinha, R., & Gunter, M. J. (2021). Markers of metabolic health and gut microbiome diversity: findings from two population-based cohort studies. *Diabetologia*, *64*(8), 1749-1759. https://doi.org/10.1007/s00125-021-05464-w

Zuniga, L. A., Shen, W. J., Joyce-Shaikh, B., Pyatnova, E. A., Richards, A. G., Thom, C., Andrade, S. M., Cua, D. J., Kraemer, F. B., & Butcher, E. C. (2010). IL-17 regulates adipogenesis, glucose homeostasis, and obesity. *Journal of Immunology*, *185*(11), 6947-6959. https://doi.org/10.4049/jimmunol.1001269