Regulation of host metabolic health by parasitic helminths

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Abstract

Obesity is a worldwide pandemic and major risk factor for the development of metabolic syndrome and type-2 diabetes (T2D). T2D requires lifelong medical support to limit complications and is defined by impaired glucose tolerance, insulin resistance, and chronic low-level systemic inflammation initiating from adipose tissue. The current preventative strategies include a healthy diet, controlled physical activity and medication targeting hyperglycemia, with underexplored underlying

inflammation. Studies suggest a protective role for helminth infection in the prevention of T2D. The mechanisms may involve induction of modified type-2 and regulatory immune responses that suppress inflammation and promote insulin sensitivity. In this review, the roles of helminths in counteracting metabolic syndrome, and prospects for harnessing these protective mechanisms for the development of novel anti-diabetes drugs are discussed.

Keywords: Metabolic syndrome, chronic inflammation, helminth, type 2 immune response, type 2 diabetes

Overview of link between metabolic syndrome, inflammation and helminth infection

Obesity (see **Glossary**) is defined as abnormally excessive deposition of fat that augments a risk to health. In 2020, an estimated 38% of people over 5 years old worldwide were overweight or obese with projections to reach 51% by 2035 [1]. Multiple factors including genetics, dietary intake, physical activity patterns and environmental elements are implicated in the etiology of obesity. Obesity is a pandemic and a leading cause of death worldwide [2]. This condition considerably reduces the number of disease-free years [3] and favors the development of **metabolic syndrome** (**MetS**, see **Box 1**), and more than 200 chronic conditions [4], including non-alcoholic fatty liver disease, cardiovascular disease and **type 2 diabetes (T2D)**. The estimated prevalence of T2D was 10.5% in 2021 which is predicted to increase to 12.2% by 2040 [5]. Recently, the use of glucagon-like peptide-1 receptor agonists (GLP-1RAs) have been highly effective at improving glycemic control, inducing weight loss and treating T2D; however, these drugs require constant treatment and the potential detrimental effects of long-term exposure to these drugs are unclear [6]. Hence, there is still a need for the development of alternative treatments for T2D. The detrimental role of chronic inflammation is well established in

the pathogenesis of metabolic diseases [7,8]. Emanating from the expanding **white adipose tissue (WAT),** system-wide inflammation primarily due to polarization towards increased pro-inflammatory macrophages may induce **insulin resistance**, impair insulin secretion, and dysregulate multiple organs involved in metabolic homeostasis [9]. Conversely, anti-inflammatory type 2 immune responses are instrumental in metabolic homeostasis and preventing MetS and T2D. Hence, limiting the inflammatory cascade may be a realistic approach to the prevention and management of some metabolic diseases. Several anti-diabetic drugs successfully use manipulation of M1/M2 macrophage phenotypes to manage obesity-induced inflammation [10]. Helminth parasites and their **excretory-secretory products (ESPs)** have been investigated as potential inducers of type 2 immune responses, primarily through the expansion of M2 macrophages and eosinophils to restore **glucose homeostasis** [11]. Findings from both human and mouse studies indicate that helminth infections downregulate pro-inflammatory T helper type 1 (Th1) and type 17 (Th17) immune responses and skew towards a modified **Type 2 (Th2) immune response** via the release of biologically active ESPs [12-14]. In this review, the possible role of helminths and their ESPs in preventing and reversing metabolic disturbances and the potential mechanisms that underpin these prophylactic and therapeutic bioactivities have been discussed.

Obesity, metabolic disturbances and immune responses

Metabolic diseases are characterized by sustained low-grade systemic inflammation originating from the expanding WAT [15], including neuroinflammation [16]. Common metabolic disturbances include adipocyte hypertrophy, systemic insulin resistance, abnormal blood lipids and elevated blood pressure, and these phenomena are predominantly driven by underlying systemic inflammation [8]. Moreover, alteration of gut microflora alters metabolism [17] and further fuels inflammation.

Insulin resistance and 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. This hormone is synthesized in and secreted by β cells in the pancreatic islets, and in healthy subjects, the quantity released is intricately linked to blood glucose concentrations to precisely meet metabolic demands [18]. In target cells, insulin binds to its receptor, promoting glucose uptake into the cell that is facilitated by **glucose transporters (GLUTs)**. In the brain, the principal transporter protein GLUT1 is constitutively on the surface of neuronal cells and can extract glucose from the blood at very low levels, such as during fasting. This process does not require insulin stimulation, thus ensuring a consistent energy supply for these heavily glucose-reliant cells. In contrast, adipocytes and myocytes contain GLUT4, which is insulin-dependent, allowing the appropriate response to high postprandial glucose levels [18]. Glucose entry into adipocytes stimulates fatty acid and glycerol synthesis and downregulates lipolysis. In contrast, myocytes and hepatocytes store glucose as glycogen to be utilized as an instant energy source. Low blood glucose and insulin levels stimulate glucagon secretion by α cells in the pancreatic islets which promotes lipolysis in adipocytes and glycogenolysis in muscle and liver [18].

While insulin resistance can result from excessive secretion of counter-regulatory hormones such as glucagon, corticosteroids and catecholamines, post-receptor defects in insulin signaling explain most cases of insulin resistance [18]. 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 [19]. However, in the context of obesity, prolonged insulin resistance further promotes chronic subacute meta-inflammation

in all tissues and becomes the driving force in the development of T2D [20]. Failure of one or more mechanisms involved in cellular glucose absorption results in hyperglycemia and dyslipidemia, causing compensatory intensified pancreatic insulin secretion [21]. Eventually, sustained glucose overstimulation leads to the exhaustion and failure of β cells and development of T2D [22].

The concept of obesity-induced inflammation and adipose tissue-associated immune cells as the critical factor in the development of insulin resistance is now well established [15,23] (**Box 2**). Mechanistic studies have determined that TNF inhibits glucose uptake by interfering with the initial events after insulin binds to its receptors (**Figure 1**) [23]. Chronic TNF exposure also decreases the expression of GLUT4, which further reduces glucose absorption [24]. These initial TNF findings kickstarted a plethora of studies to cement the link between obesity, inflammation and insulin resistance.

Macrophages

Long-term inflammation of adipose tissue increases circulating levels of IL-6 released from proinflammatory M1 macrophages. This pro-inflammatory cytokine has been suggested to attenuate fatty acid metabolism in multiple tissues, including skeletal muscle, liver and brain (**Figure 1**) [15]. Although some controversy exists regarding the exact role of IL-6 within adipose tissue [25], it may stimulate lipolysis and inhibit lipoprotein lipase, impairing adipocyte lipid storage [26]. Increased abundance of free fatty acids (FFAs) in tissues such as the liver and skeletal muscle initiates lipotoxicity, which promotes system-wide metabolic disturbances and fuels insulin resistance and hypertension [27]. Moreover, elevated circulating FFA stimulates the generation of reactive oxygen

species (ROS), enhancing NLRP3 inflammasome activation [28]. Inflammasome activation further fuels insulin resistance and proinflammatory IL-1β secretion.

An anti-inflammatory environment dominates lean**,** healthy subcutaneous and visceral WAT (**Figure 2**). Lean WAT-resident immune cells and mediators predominantly include alternatively activated anti-inflammatory M2-like macrophages and eosinophils, and anti-inflammatory cytokines such as IL-10. Pertinent to this review article, these same immune cells and cytokines are also prime features of helminth infections [29]. In physiological conditions, M2 macrophages express arginase 1, IL-1 receptor antagonist (IL-1Ra), IL-10, transforming growth factor-beta (TGF-β), and the transcription factor peroxisome proliferated-activator receptor gamma (PPAR-γ) and upregulate uncoupling protein 1- a key protein involved in energy expenditure and beiging of adipose tissues [14]. These factors inhibit the expression of pro-inflammatory chemokines, enzymes and cytokines [30], promote insulin sensitivity, and contribute to lipid buffering in a lipid-rich environment [31]. Collectively, these cells maintain optimal adipocyte mitochondrial function, promote tissue homeostasis, and discourage inflammation and fat accumulation, thereby protecting against insulin resistance [32]. As part of the polarized Th2 immune response, M2 macrophages largely participate in parasite clearance [33], phagocytosis of debris and apoptotic cells, suppressing inflammation, promotion of tissue repair and remodeling, and vasculogenesis [34,35]. Recent research suggests that M2 macrophages can be further sub-divided into four main categories, including M2a, M2b, M2c and M2d, each of which is induced by different stimuli and functions in a distinct anti-inflammatory manner [31,36]. Of note, studies using single-cell RNA-seq approaches have highlighted macrophage heterogeneity and niche-specificity, describing new subsets that may play a pathophysiological role, for example lipid-associated macrophages [37,38]. The presence of similar adipose tissue eosinophil heterogeneity has

been proposed [38] and recently demonstrated experimentally in mice [39] but is yet to be explored in obese mouse models or in humans.

Other immune cells and pathways: roles of eosinophils, T cells, regulatory T cells, ILCs, neutrophils and adipokines

The complexity of macrophage differentiation has switched the focus of immune responses in diabetes to eosinophils, T cells, mast cells and other immune cells [40-42]. Eosinophils play a critical role in this network of adipose-resident immune cells partly due to their ability to promote and maintain M2-like macrophages via the release of IL-4 [11,32,43]. A landmark study first demonstrated the role of IL-4-secreting eosinophils in preserving insulin sensitivity in mice, providing the basis for the idea that type 2 immunity is linked to adipose tissue metabolic homeostasis [11]. Mice genetically deficient in eosinophils showed increased weight gain and impaired glucose and insulin tolerance when placed on a high fat diet [11] whereas mice with genetically elevated numbers of eosinophils showed enhanced glucose tolerance on a normal chow diet [11]. Adipose tissue eosinophil numbers decrease in mice with high fat **diet-induced obesity (DIO)** and recover to baseline levels following low-calorie diet restriction-induced weight loss [44]. A recent study suggests that adipose tissue eosinophil numbers and IL-4 levels also inversely correlate with **body mass index (BMI)** and insulin resistance in humans [45].

$CD4^+$ T cell transfer to DIO mice which lack endogenous lymphocytes reversed weight gain and insulin resistance, demonstrating an important role for Th cells [41]. This study also reported an overwhelming number of $GATA3^+$ and $FoxP3^+$ T cells in visceral adipose tissues of lean mice, and significantly higher number of IFNγ-producing Th1 cells in visceral adipose tissue of obese mice,

indicating roles for Th2 and regulatory T cells (Treg) in regulating obesity. Similarly, mast cell knockout moderated both body mass and insulin resistance in mice [42], which raised the possibility that the improvement in insulin resistance is indirectly mediated via the reduction of body mass rather than via direct regulation of insulin resistance.

Following on, several studies investigated other adipose tissue-resident immune cells. Those cells traditionally considered pro-inflammatory, including DCs, neutrophils, natural killer cells, ILCs, Th1 and Th17 cells, γδ T cells and B cells were able to promote inflammation and insulin resistance (**Figure 2**) [41,46-51]. On the other hand, immune cells that were traditionally viewed as anti-inflammatory cells, such as eosinophils, ILC2 and regulatory B cells inhibited obesity-induced inflammation and insulin resistance [11,52,53]. 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.

Hundreds of adipocyte-secreted pleiotropic molecules called **adipokines** are also linked to obesity, inflammation and T2D [20]. The two most studied adipokines are leptin and adiponectin, both of which can dramatically affect whole-body metabolism. Leptin acts on the hypothalamus and other target tissues to regulate appetite and metabolism, as well as insulin sensitivity [54], and may promote inflammatory responses [55]. 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 [56]. Obesity and prolonged consumption of a high-fat diet impairs blood-brain-barrier integrity, resulting in reduced transport of leptin to its target areas and diminished activation of the signaling pathways

involved in body mass regulation [56]. Adiponectin attenuates inflammation, improves lipid profiles and moderates glycemic control [57]. In adipose tissue, adiponectin polarizes macrophages to the M2 phenotype and reduces ROS production [58]. In skeletal muscle, adiponectin reduces triglyceride content, improving GLUT4 translocation and glucose uptake and likely ameliorates insulin resistance [59]. However, insulin lowers circulating adiponectin levels, with hyperinsulinemia and its adiponectin-reducing action possibly causes insulin resistance. The exact cause and effect in this relationship remains to be established [60]. Omentin-1 levels are positively correlated with adiponectin and high-density lipoprotein levels, and negatively correlated with BMI and insulin resistance [61,62]. This adipokine boosts insulin signal transduction and promotes insulin-mediated glucose uptake in adipocytes [63].

Protective roles for parasitic helminths in metabolic diseases

A quarter of the global population (1.5 billion people) suffer from anemia, hypoproteinemia, and loss of disability-adjusted life years caused by soil-transmitted helminth infections. Inhabitants of low to middle-economic regions such as sub-Saharan Africa, China, South America and Asia are endemic for helminthiasis due to low access to clean water, sanitation and hygiene. Conversely, global prevalence of metabolic diseases has increased most dramatically in high socio-demographic index countries [64]. Several studies in diverse populations have reported a protective role for helminth infection in maintaining metabolic health [65-69]. One of the first studies in this area showed an inverse relationship between the prevalence of *Ascaris lumbricoides* infection and T2D in Turkey [65]. In 2010, two epidemiological studies in southern India found a significantly lower prevalence of lymphatic filariasis in subjects with both type 1 and type 2 diabetes compared to uninfected normal glucose tolerant individuals [68,69]. Moreover, two Indonesian cross-sectional studies on Flores

Island reported inverse relationships between current worm infection and risk factors for T2D. Individuals with at least one worm infection had lower BMI, **Homeostatic Model Assessment for Insulin Resistance (HOMA-IR)**, waist-to-hip ratio, total cholesterol and low density lipoprotein levels [70,71]. In Australia, Aboriginal adults who were seropositive for *Strongyloides stercoralis* infection had a significantly reduced risk of T2D compared to unexposed individuals [72]. A 2018 study in China found that people with chronic schistosomiasis had lower BMI, fasting blood glucose, and total cholesterol and serum triglycerides compared to non-infected individuals, and supporting data was generated using an animal model [73]. Further, Egyptian [74] and Ethiopian [75] researchers found a significantly lower prevalence of MetS and its components in individuals with previous schistosome infection. Additionally, a recent systematic review analysis found that anthelmintic treatment could lead to an 82% increase of post-treatment MetS and blood glucose levels in human [76]. Intriguingly, migrants from helminth-endemic to non-endemic regions were increasingly diagnosed with MetS possibly due to combined effects of energy-dense food, parasite-free water and de-worming medications [77]. In contrast, other studies reported a positive association between *S. stercoralis* and glycated hemoglobin (HbA1c) or T2D [66,67]. Htun et al. [78], conversely found a positive association between *Taenia spp.* infection and T2D in adults in Lao PDR. However, the authors proposed that the greater number of diabetic individuals could be caused by a reduced ability to control or clear parasites due to the development of T2D, resulting in an excess of diabetic individuals with chronic infection over time. The presence of unidentified disease conditions or factors might have superseded the inverse relation between worm infection and T2D in this scenario. Further, the effects of helminths on muscle insulin sensitivity and long-term effects of helminth infection on the development of diabetes complications are not known.

We recently conducted a randomized, double-blinded, placebo-controlled trial with the human hookworm *Necator americanus. N. americanus* infective larvae (L3) were shown to be safe and well tolerated in MetS patients [79]. Treatment with 20 or 40 L3 persistently reduced fasting blood glucose and body mass for up to 24 months and insulin resistance compared to the placebo group and was mediated by persistent blood eosinophilia and elevated IL-5 levels. Both 20 or 40 L3 recipients showed similar degrees of hookworm infection intensity (hookworm EPGs) and eosinophil responses necessitating further trials to demonstrate efficacy with optimal dose. The use of both treatments with L3 and metformin or GLP-1R agonists in future trials could allow an improved determination of the efficacy of hookworm treatment in comparison to current frontline treatments for obesity and T2D. Although 44% hookworm-treated participants experienced expected gastro-intestinal symptoms, the symptoms resolved quickly following anthelmintic treatment. This was the first clinical trial undertaken to address the role of an experimental helminth infection in metabolic disease and suggested a potential role for hookworms in improving insulin resistance.

Mechanisms of worm-mediated protection

While the human studies described above do suggest that helminth infection may provide some benefits for metabolic health, animal model studies have been most informative for defining the mechanisms by which worms instigate these benefits. The following section describes the range of mechanisms by which helminths may improve metabolic dysfunction, for example by producing ESP that have direct effects on type-2 and/or regulatory immune responses, or indirectly via gut microbiota changes.

Helminths directly modulate type-2 and regulatory immune responses

Accumulating evidence suggests that helminths proficiently modulate immune responses to ensure life-long survival in the host [79-81]. 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. Helminth mediated immune modulation involves downregulation of pro-inflammatory Th1 and Th17 immune responses and a skewing towards an altered Th2 immune response via the release of biologically active ESP [12-14]. A classical Th2 immune response is characterized by $CD4^+$ Th2 cells, the cytokines IL-4, IL-5, IL-9, and IL-13, and immunoglobulin E (**Figure 3**). 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 [82-84]. However, in most people, helminth infections lead to a modified Th2 response with high levels of M2 macrophages, Tregs, and tolerogenic DCs (or DC2), resulting in an anti-inflammatory and tissue-remodeling environment with exceptions for example; *Heligmosomoides polygyrus* Alarmin Release Inhibitor (*Hp*ARI) protein ameliorates allergic reactivity by interfering the IL-33 pathway in murine model [85]. The modified immune response elicited by helminths prevents unrelated inflammation, promotes wound healing, and enables parasite survival, thus establishing a mutually beneficial state for both host and parasite. Significantly, downregulation of inflammation arising from the type 2 immune response to helminth infections is tightly linked to improved glucose homeostasis and reduced fat mass [86].

A deeper understanding of the mechanisms of worm-mediated protection against metabolic dysregulation has been obtained from studies using rodent models. In the landmark 2011 study first demonstrating the role of IL-4 secreting eosinophils in preserving insulin sensitivity, worms were utilized to physiologically increase eosinophil numbers [11]. The authors infected mice fed a high-fat

diet with the rodent hookworm *Nippostrongylus brasiliensis*. They reported improved insulin sensitivity and glucose tolerance, lower fasting glucose and reduced perigonadal fat mass. Notably, these improvements were maintained for up to 45 days after parasite clearance. The mechanistic investigations of a protective role of worm infection in metabolic homeostasis have since been replicated in numerous other publications (**Table 1**).

Notably, a few rodent model 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 [87]. 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 normalized glucose homeostasis and insulin levels in obese mice. Treatment with *Litomosoides sigmodontis* ESP increased adiponectin levels in mice fed a high-fat diet, which may have ameliorated glucose tolerance [88]. 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 mice, which correlated with a marked decrease in leptin gene expression. As leptin is associated with a pro-inflammatory action and its levels are often increased in obesity, leptin downregulation in *H. polygyrus* infection could be a factor in the protective effect of infection [14].

Given the convincing evidence that helminth crude ESP mixtures can drive Th2 and regulatory responses, there is substantial interest in identifying the bioactive molecular entities. *S. mansoni* soluble egg antigen (SEA) treatment ameliorated metabolic dysfunction by inducing high number of

WAT eosinophils, Th2 cells and M2s in mice [89,90]. Similarly, adult *L. sigmodontis* extracts improved glucose tolerance by inducing eosinophilia, M2, ILC2s and adiponectin production, and reducing Th1 and Th17 response in adipose tissues of HFD mice [88,91]. Recently, our group has shown that ESP from the rodent hookworm *N. brasiliensis* significantly reduced glucose tolerance and body weight gain in HFD mice, likely modulated by eosinophilia and IL-5 [92].

While there are numerous examples of crude ESP driving type 2 and regulatory responses that protect against metabolic diseases, there is less data on the individual bioactive ES molecules. *S. mansoni* omega-1 (ω1) is the dominant factor in SEA that drives Th2 responses, and recombinant ω1 protein significantly reduced body weight gain, and fasting blood glucose, and improved insulin sensitivity, glucose tolerance and WAT beiging in a mouse model of DIO [93,94]. The improvement in metabolic outcomes was mediated by adipose tissue derived IL-33, Th2 cells, Tregs, eosinophils, ILC2s and M2s. Similarly, *Acanthocheilonema viteae* ES-62 (*Av*ES-62) reduced HFD-induced hepatic fibrosis in mice by inducing WAT eosinophilia, and increased levels of IL-4 and IL-5 [95]. However, HFD-fed male (but not female) mice treated prophylactically with a synthetic small molecule analogue of *Av*ES-62 improved glucose homeostasis and insulin resistance but obvious immune changes were not detected [96], indicating that the mechanisms by which helminth molecules confer protection against metabolic diseases are likely multi-factorial. The helminth derived glycan lacto-N-fucopentose III (LNFPIII) was also shown to have similar effects in improving insulin sensitivity and glucose resistance by reducing IL-10 in WAT in DIO mice [97].

Helminths indirectly modulate type-2 immune response via microbiome change

The gut microbiota is closely linked to the development and modulation of the host's immune system. Helminth infections change the gut microbiota composition with accompanying alterations in immune and metabolic responses [98]. Animal and human studies have shown that microbial species diversity changes following helminth infections or anthelmintic medications [98-102]. Alteration of microbial community composition might be due to helminth ESP or impaired glucose absorption. Murine studies with the gastrointestinal nematodes *H. polygyrus* and *Trichuris muris* reported attenuated airway inflammation and protection against colitis with a greater abundance of *Clostridia* spp. in the gut [103,104]. Infection of mice with *H. polygyrus* [101,105], *T. muris* [106], *N. brasiliensis* [107], *Strongyloides venezuelensis* [108], cats with *Toxocara cati* [109], and hamsters with *Opisthorchis viverrini* increased the numbers of *Lactobacillaceae* that have protective mucosal barrier function [110] and induce Treg expansion [111]. A recent study showed that helminth infection protects mice against DIO by M2 macrophage-mediated alterations of the gut microbiota [98]. Infection of STAT6-/- mice (deficient in Th2 responses) with *H. polygyrus* failed to attenuate DIO and blood glucose level. However, reduced DIO and blood glucose levels in *H. polygyrus* infected WT mice or mice with adoptive transfer of M2 macrophages from *H. polygyrus* infected mice was mediated by fecal abundance of *Rikenellaceae*, *Eggerthellaceae* and *Lactobacillaceae*. However, the likelihood similar processes occurring in humans is debatable. Clearance of *A. lumbricoides* and *N. americanus* with albendazole treatment in Kenya significantly increased the proportion of *Clostridiales* and reduced *Enterobacteriales* [112]. Moreover, several other studies did not find any significant changes in gut microbial communities with helminth infections and deworming [99,113-116].

Concluding remarks

In summary, results from cross-sectional studies and clinical trials in humans and experimental animal models have established a beneficial role for worm infection in protection against metabolic disorders (**Figure 4**). The protective effect is likely mediated by induction of an altered type 2 immune response and secretion of adipokines, or other unexplored mechanisms. Worm-based therapies, or development of novel biologics or small molecules derived from helminth ESP could be a potential area of research to minimize the harmful effects of metabolic diseases by inducing a safe Th2-biased anti-inflammatory environment or by promoting healthy gut microbiota, and thereby promoting metabolic homeostasis (see **Outstanding questions**). Identification of specific worm molecules which can regulate particular metabolic disease parameters may have therapeutic potential. Ultimately, the complex interplay between metabolic disease-driven inflammation and the human biota needs to be further unraveled if next-generation therapeutics based on helminths and their secreted molecular entities are to be developed. While the microbiota and its role in inflammation has received much attention, in both academic and industry settings, the macrobiota (helminths) has not received its fair share of recognition, and we encourage more researchers to get on board.

Authors contributions

SS conceptualized the paper and prepared the figures. DP, SS, CM and ERS contributed to literature searches. PG, KGRQ and AL contributed significantly through manuscript writing, editing and helpful discussion.

Acknowledgements

This work was supported by a grant from the Cooperative Research Centre for Developing Northern Australia (H.5.2021062) and a National Health and Medical Research Council (NHMRC) Investigator Grant (2008450) to AL. The funders had no influence on the views expressed in this review.

Declaration of interests

The authors declare no competing interests.

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Glossary

Adipokines: peptide hormones produced and secreted by healthy adipocytes.

Body mass index (BMI): is a person's weight in kilograms divided by the square of height in meters and is expressed in units of kg/m^2. A high BMI indicates high body fat deposition.

Diet-induced obesity (DIO): a strategy where rodents are fed with high fat content diet to mimic human obesity. At least 35% of total calories is consumed from fats.

Excretory-secretory products (ESPs): are molecules and vesicles secreted by parasitic helminths into their surrounding host tissue or culture media if maintained *in vitro*. The ESPs are important mediators of host-parasite communication, and play roles in parasite invasion, migration, feeding and immunomodulation.

Glucose homeostasis: the balance of two pancreatic hormones insulin and glucagon to maintain stable glucose level in blood.

Glucose transporter (GLUT): are a family of membrane proteins that facilitate the transport of glucose across the plasma membrane into the cell by means of facilitated diffusion.

Homeostatic Model Assessment for Insulin Resistance (HOMA-IR): an indirect method for quantifying insulin resistance and pancreatic β-cell function. It is calculated by multiplying fasting plasma insulin (FPI) by fasting plasma glucose (FPG), then dividing by the constant 22.5, i.e. HOMA-IR = (FPI×FPG)/22.5.

Insulin resistance: is a condition when cells in muscles, fat and liver are unable to respond well to insulin and cannot easily uptake glucose from the blood stream.

Metabolic syndrome (MetS): a cluster of conditions that increase the risk of heart disease, diabetes and stroke. It includes high blood pressure, high blood sugar, high blood triglycerides, low levels of high-density-lipoproteins, excess body fat deposition around the waist, abnormal cholesterol levels and insulin resistance.

Obesity: a condition where the body deposits abnormal or excessive fat that presents a risk to health. A BMI of over 30 is considered obese.

Type 2 diabetes (T2D): is a condition where cells do not respond to insulin (insulin resistance) and there is resulting high blood sugar. Major causes are high fat high caloric diet and sedentary lifestyle. T2D can lead to heart disease, vision loss and kidney disease.

Type 2 (Th2) immune response: characterised by the production of interleukin-4 (IL-4), IL-5 and IL-13 produced by type 2 helper T cells (Th2) in response to helminth or allergen exposure.

White adipose tissue (WAT): heterogeneous tissue composed primarily of lipid-filled adipocytes and several other nonadipocyte cells, including endothelial cell, white blood cells, uncharacterised stromal cells and adipocyte precursor cells. WAT store energy and fatty acids are released when fuel is required.

Box 1. Metabolic syndrome disease highlights

MetS is defined by the presence of at least three of the symptoms of: hyperglycaemia, obesity, hypertension, low HDL cholesterol and/or high blood triglycerides. Major MetS features are:

Insulin resistance (IR)

During IR, skeletal muscle, liver and adipose tissues are unable to respond to insulin, hence, circulating blood glucose remain elevated, which leads to pathology. IR is a strong predictor of T2D.

Obesity

Excessive deposition of visceral adipose tissue enhances proinflammatory cell infiltration. Persistent mild inflammation in adipose tissue dominated by TNFα and IL-6 cytokines induce IR and vascular dysfunction.

Endothelial dysfunction

Hyperglycaemia, free fatty acids (FFA), inflammatory cytokines and adipokines, IR and adiposity are associate with endothelial dysfunction. These factors diminish nitric oxide synthase (NOS) phosphorylation resulting in peroxynitrite anion formation that has toxic effects on endothelial cells.

Atherogenic dyslipidaemia

Features of atherogenic dyslipidaemia are high blood triglyceride and LDL levels, and low HDL cholesterol levels. Visceral obesity and IR are predisposing factors of atherogenic dyslipidaemia. IR triggers lipolysis and production of FFAs, which serve as a substrate to produce triglycerides and LDL.

Heart disease

Obesity and hyperglycaemia predispose to congestive heart failure, atherosclerotic heart disease, coronary arterial diseases and cardiac infarction, although the direct link has not been established.

Stroke

Hypertension and hyperglycaemia are associated with stroke by impaired endogenous fibrinolytic capacity, endothelial dysfunction and a pro-inflammatory state, which intensifies the ischaemic damage and hamper arterial recanalization.

Kidney disease

Obesity, hyperglycaemia, hypertension and dyslipidaemia are co-factors of chronic kidney disease and co-occur within individuals. Adipose tissue expansion, inflammation and IR can elicit kidney injuries through endothelial dysfunction, activation of the renin-angiotensin-aldosterone system, microvascular remodelling and adipokine imbalance. Hyperglycaemia enhances mitochondrial dysfunction and thereby progression of renal damage.

Hypertension

Hypertension is the elevated blood pressure that can lead to stroke, and is associated with dysfunction of endothelial activity, renin-angiotensin-aldosterone system, obesity, glucose intolerance, hyperglycaemia and hyperlipidaemia.

Hypertriglyceridemia

Hypertriglyceridemia is an independent risk factor for cardiovascular disease. It is directly associated with acute coronary syndrome and coronary arterial disease due to deposition of lipids and formation of atherosclerotic plaque causing narrowing and consequently, partial or total obstruction of coronary artery. Similar mechanisms cause peripheral arterial disease and cerebrovascular disease as a sequela.

Fatty liver disease

Excessive deposition of fat tissues causes hepatic steatosis, inflammation and hepatocellular ballooning and major causes are insulin resistance, exaggerated lipogenesis, persistent inflammation, hyperinsulinemia and hyperglycaemia.

Box 2. Immune cells in MetS-helminth infection milieu

Macrophages

Macrophages are professional antigen presenting cells essential for phagocytosis of pathogen, tumour cells and dead tissue clearance. Classically activated M1 macrophages are induced by insulin resistance and persistent inflammation, however, alternatively activated M2 macrophages are dominant during helminth infection and maintain immune tissue homeostasis.

Eosinophils

Are important cells of the type 2 innate immune system and are essential for helminth clearance. IL-4 cytokine produced by eosinophils contribute to maintain immune homeostasis by relative reduction of IFN-γ and IL-17 responses. Adipose tissue eosinophils regulate obesity-associated inflammation and MetS.

Neutrophils

Neutrophils are inflammatory cells that are abundant in obese adipose tissue due to adipocyte IL-8 secretion which is a potent neutrophil chemoattractant. Adipose tissue neutrophils are involved in the development of obesity-associated adipose tissue inflammation and insulin resistance.

Innate lymphoid cells (ILCs)

ILCs protect epithelial barriers from infections and maintain tissue homeostasis. Obesity drives accumulation of group 1 ILCs (ILC1) that secret IFN-γ, which is sufficient to polarize M1 macrophage to promote obesity-associated insulin resistance. Conversely, helminths induce ILC2-derived IL-4 and IL-13 production that have role in suppressing inflammation, promoting tissue repair and inducing wound healing by acting on epithelial cells and polarizing M2 macrophages.

Dendritic cells (DCs)

Adipose tissue DCs are considered the master regulators of crosstalk between immune cells in adipose tissues. CD11c+ conventional group 1 DCs (cDC1) dominate in adipose tissue inflammation. However, PPAR-γ+ cDC2 regulate adipose tissue homeostasis.

Natural killer (NK) cells

NK cells play role in obesity-associated inflammation and IR and regulate adipose tissue macrophages. However, helminths induce invariant NK (iNK) cells which can sense lipid antigen presented by adipocytes to produce IL-4 and polarize M2 macrophages.

T cells

Important T cell subsets that produce adipose tissue inflammation are Th1, Th17 and CD8+ T cells by producing IFN-γ, IL-17, TNFα and IL-6 cytokines. Helminth infection induces/maintains formation of healthy adipose tissue by recruitment and subsequent proliferation of Th2 cells which produce IL-4, IL-13, IL-5 and Treg cells producing IL-10.

B cells

Type 2 B cells (B2) aggravate IR and T2D by presenting antigens to T cells, secreting inflammatory cytokines IFN-γ, IL-12, TNFα and producing pathogenic antibodies in obesity-associated systemic and local adipose tissue inflammation. Regulatory B cells and B1 cells supress inflammation by producing IL-10, IL-4, and IL-13 however, the role of helminths in B cells is unexplored.

Table 1. Helminths induce type 2 immune responses and improve metabolic dysfunction.

Helminth treatment	Immune responses	Metabolic/disease outcome	References
Nippostrongylus brasiliensis 500 L3 s.c.	Eosinophilia, ↑IL-4, ↑M2 gene expression	↓glucose tolerance, ↓body weight gain	[11,12]

once/4 week until 31wk in HGI and HFD mice	↑M2 dependent transcellular glucose absorption	↓paracellular and ↑transcellular glucose absorption	[117]
	↑*Il4*, ↑*Il5*, ↑*Il13,* ↓*TNFα,* ↓*IL12p40* transcripts in small intestine	↓glucose tolerance, ↓body weight gain, ↓insulin resistance, ↓hepatic steatosis	[118]
Adult or L3 *N. brasiliensis* ESP 1mg/kg BW i.p. twice weekly in HGI diet C57BL/6 male mice	Eosinophilia, ↑IL-5, ↓IL-6 in AT, ↑IL-6 in liver	↓glucose tolerance, ↓body weight gain	[92]
Heligmosomoides polygyrus (*Hp*) 200 L3 oral in HFD mice or M2 adoptive transfer from *Hp* infected mice	↑M2	↓body weight gain, ↓blood glucose	[98]
Hp 200 L3 oral in HFD mice	↑Th2 cells, Tregs, eosinophils, IL-10 and adiponectin and ↓Th1 & Th17 cells in adipose tissue	↑insulin sensitivity, ↓obesity-associated inflammation, ↓fat accumulation in liver	[119]

Hp 200 L3 oral in HFD mice	↓Tbet, ↓Gata3, ↑RoRγt, ↑FoxP3 mRNA in mesenteric lymph node. ↓IFNγ, ↓IL-17A, ↑IL-4, ↑IL-10 in mesenteric lymph node. ↑IgG1, ↑IgE, ↓IgG1a, ↑Th2 Treg, ↑M2	↑beige adipose tissue, ↓gonadal and s.c. fat pad, ↓body weight, ↓blood glucose, ↓serum triglyceride	[14]
	Eosinophilia and ↑IL-4, IL-13, IL-10, Arg-1, Fizz1, YM1 (for M2) gene expression in small intestine	↓glucose, ↓fat accumulation, ↑HOMA-IR	[120]
Adult *Litomosoides sigmodontis* extracts (*Ls*Ag) in HFD mice	↓adipose tissue Th1, Th17	↑adiponectin	[88]

Daily i.p. injection of adult *Ls*Ag in C57BL/6J DIO mice for 2 weeks	Eosinophilia, ↑M2, ↑ILC2s & total cells in EAT. ↑expression of adiponectin-coding gene (*adipoq*) & its receptor (*adipor2*), insulin signaling genes, *Cd3e* gene, *arginase-1* (for M2), *Gata3, Foxp3*. Downregulation of genes of inflammation *Emr1* for F4/80, *Tnfrsf1b*, *Nlrp3*, *Pycard*, *Casp1*.	↓glucose tolerance	[91]
Schistosoma mansoni cercariae s.c. or egg antigen (SEA) i.p. injection in HFD induced obese C57BL/6 mice	↑WAT eosinophils, ↑M2, ↑IL-4 IL-13 and IL-5 expressing Th2 cells	↓body weight gain, ↓fat mass gain, ↓adipocyte size, ↓insulin resistance and ↓glucose tolerance. ↑peripheral glucose uptake and ↑WAT insulin sensitivity, ↑HOMA-IR	[90]

Helminth antigen LNFPIII or SEA administered to mice i.p. twice a week 4-6 injections in DIO mice	↑IL-10 in WAT	↑insulin sensitivity, ↓glucose tolerance, ↓hepatic steatosis	[97]
Strongyloides venezuelensis L3 s.c. injection in male Swiss mice with HFD	↑M2 in AT, ↑IL-10 in serum	↑insulin signaling and sensitivity. ↑oleic acid that is anti-inflammatory. No changes in the body weight, epididymal AT weight and feed intake.	[108]
S. mansoni egg-derived recombinant ω1 protein or SEA i.p. every 3 days for 4 weeks in HFD mice	↑Th2, eosinophils, M2 in WAT	↓body fat mass, ↑insulin sensitivity and ↓glucose tolerance	[94]
S. mansoni egg-derived recombinant ω1 protein at day 0, 2, and 4 in HFD C57BL/6 mice	↑IL-33, ↑eosinophils, ↑M2, ↑ILC2 in epididymal WAT	↑weight loss, ↑glucose homeostasis	[93]

Abbreviations and symbols: M2: alternatively activated macrophage or macrophage type 2, ILC: innate lymphoid cell, L3: larval stage 3, wk: week, i.p.: intraperitoneal, s.c.: subcutaneous, BW: body weight, AT: adipose tissue, WAT: white adipose tissue, EAT: epididymal adipose tissue, HGI: high glycemic index, HFD: high fat diet, DIO: diet-induced-obese, IL: interleukin, IFN: interferon,

745 TNF: tumour necrosis factor, Th: helper T cell, Tbet: T-box transcription factor expressed in T cells,
746 RoR: RAR-related orphan receptor, FoxP: Forkhead box P, Ig: immunoglobulin, mRNA: messenger
747 ribonucleic acid, ↑: increase, ↓: decrease.
748
749 **Figure 1. Chronic inflammation of adipose tissue dysregulates downstream metabolic pathways.**
750 Inflammation in adipose tissues disrupts glucose transporter 4 (GLUT4), triggering lipolysis.
751 Lipolysis releases Danger Associated Molecular Patterns (DAMPs) to facilitate inflammation and
752 insulin resistance. Interleukin (IL)-6 promotes circulatory free fatty acid (FFA) production that causes
753 systemic metabolic disturbances. Moreover, FFA accelerates release of reactive oxygen species
754 (ROS) that activate NLRP3 inflammasome to secrete IL-1β. The inflamed adipose tissue dysregulates
755 adipokine secretion, which further enhances circulatory triglyceride content, glucose tolerance and
756 insulin resistance. TNF: tumour necrosis factor, NLRP3: Nod-like receptor protein family pyrin
757 domain containing 3.
758
759 **Figure 2. Immune imbalance during adipose tissue inflammation.** Healthy adipose tissue
760 possesses an anti-inflammatory immune phenotype with infiltration of M2 macrophages, eosinophils,
761 Th2 cells, mast cells, type 2 innate lymphoid cells (ILC2), type 2 dendritic cells (DC2) and regulatory
762 B cells that secrete anti-inflammatory cytokines. Conversely, inflammation promotes distinct immune
763 cell types dominated by inflammatory M1 macrophages, neutrophils, Th1, Th17, γδ T cells, ILC1,
764 NK cells, DC1 and B cells that promote inflammation. IL: interleukin, TGF: transforming growth
765 factor, Th: helper T cell, TNF: tumour necrosis factor, ILC: innate lymphoid cell, DC: dendritic cell,
766 IFN: interferon, NK: natural killer.
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Figure 3. Helminths induce a modified type 2 immune response that reduces the risk of obesity and some metabolic diseases. Adult and infective stages (L3) of helminths such as hookworms induce alarmin secretion (IL-33, TSLP, IL-25) by exposed epithelial cells of the intestine and skin. Tissue localized ILC2 are activated by IL-22 and release IL-4, IL-13, and IL-5 cytokines. Type 2 dendritic cells internalize the worm proteins and migrate to draining lymph nodes to activate naïve T cells. Naïve T cells differentiate to Th2, Treg and type 2 follicular helper T cells (Tfh2). Activated Th2 cells migrate to the site of infection to release more IL-4, IL-13 and IL-5. Eosinophils are activated by the effects of IL-5 and basophils/mast cells are activated by IL-4, IL-13 and IgE that interacts with low-affinity IgE receptor. Activated eosinophils and basophils further secrete IL-4. IL-4 polarizes M2 macrophages to secrete anti-inflammatory IL-10 and TGFß cytokines. Tregs secrete IL-10 which further reduces inflammation. Tfh2 cells induce low-affinity IgE production by plasma cells. IL: interleukin, TSLP: thymic stromal lymphopoietin, DC: dendritic cell, L3: larval stage 3, ILC: innate lymphoid cell, Tfh: follicular helper T cell, GCB: germinal centre B cell, Ig: immunoglobulin, Treg: regulatory T cell, AAM/M2: alternatively activated macrophage/macrophage type 2, TGF: transforming growth factor, T2D: type 2 diabetes.

Figure 4. Role of helminth infection in obesity and diabetes. Consistent high caloric diet enhances excess energy deposition as excess adipose tissue increases the risk of obesity and T2D. Fatty liver and inflammatory adipose tissues are characteristics of metabolic disturbances mediated by obesity. Sustained inflammation in the adipose tissue creates an imbalance in immune phenotype by promoting pro-inflammatory responses. Helminths maintain immune balance by inducing type-2 immune responses. T2D: type 2 diabetes, Th: helper T cell, IFN: interferon, TNF: tumour necrosis factor, M1: macrophage type 1, IL: interleukin, M2: macrophage type 2.