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Review

Do worms protect against the metabolic syndrome? A systematic review and meta-analysis

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ABSTRACT

Aims: There is increasing evidence on the role of helminth infections in modifying autoimmune and allergic diseases. These infections may have similar effect in other inflammatory processes, such as insulin resistance. This review aims to examine the literature on the effect of helminthic infections on metabolic outcomes in humans.

Methods: Using the PRISMA protocol, we searched the literature using PubMed, MEDLINE, and a manual review of reference lists. Human studies published in English after 1995 were included. Four papers were included in this review. Data was extracted and a meta-analysis was conducted using a random-effects model. Heterogeneity was assessed using Tau^2 and I^2 tests.

Results: The included studies found that infection was associated with lower glucose levels, less insulin resistance, and/or a lower prevalence of metabolic syndrome (MetS) or type 2 diabetes mellitus (T2DM). Meta-analysis showed that participants with a previous or current helminth infection were 50% less likely to have an endpoint of metabolic dysfunction in comparison to uninfected participants (OR 0.50; 95% CI 0.38–0.66).

Conclusion: This review has shown that helminth infections can be associated with improved metabolic outcomes. Understanding of the mechanisms underlying this relationship could facilitate the development of novel strategies to prevent or delay T2DM.

Contents

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1. Introduction

Helminths have co-evolved with humans over the centuries; evolutionary theory would suggest some mutual benefit for both host and parasite. Publication of the "Hygiene Hypothesis" in 1989 sparked interest in the potential protective effects of helminth infections on human disease [1] and there is now a growing body of literature exploring the role of helminths in prevention and management of certain autoimmune and allergic conditions, including coeliac disease, inflammatory bowel disease, asthma, and type 1 diabetes (T1DM) [2–4]. The apparent anti-inflammatory effect is likely to have facilitated the prolonged survival of the worm in individual hosts and over our evolutionary history. Widespread interruption of this ancient process may even elevate rates of inflammatory, autoimmune, and allergic disease.

For the last half-century, health services around the globe have endeavored to eradicate human helminth infections. In Australia, Aboriginal communities have been the focus of attention, with some researchers advocating for mass drug administration to address widespread *Strongyloides stercoralis* infection [5–8]. However, interpretation of Australian evidence on the prevalence and clinical implications of *S. stercoralis* infection has attracted some controversy [6–8]. This is because it is increasingly apparent that, for the vast majority, *S. stercoralis* causes both a chronic and asymptomatic infection [9]. The most serious and fatal manifestation of the infection, however, is disseminated strongyloidiasis. This has been seen in a small number of cases globally, typically in immunosuppressed patients [6]. Over a 10 year period in the Northern Territory, one of the most endemic areas in Australia for the worm, there were just six known cases of disseminated disease, with one fatality [6].

Coinciding with increased efforts in helminth eradication, T2DM and MetS have reached epidemic proportions across the globe [10]. Metabolic diseases place huge burdens on the health systems of both economically developed and developing societies [3]. The pathogeneses of MetS and T2DM are complex and multifactorial, but it is clear that along with nutritional factors, inflammation plays a critical role. Results from multiple studies have suggested the ability of pro-inflammatory cytokines, classically activated macrophages, and decreased T-regulatory function to drive insulin resistance in hepatic and adipose tissue [11,12]. There is also evidence that demonstrates the ability of anti-inflammatory cytokines, alternatively activated macrophages and T-regulatory cells to protect against insulin resistance in these tissues [11,12].

Recently published data from animal models have shown that helminth infections can reduce insulin resistance through modulation of immune pathways. Such observations have prompted the hypothesis that specific helminth infections may prevent or attenuate the development of insulin resistance in humans. A number of literature reviews have endeavoured to piece together the growing body of epidemiological, experimental, and clinical evidence to support this hypothesis. To date, however, there has been no systematic review or meta-analysis of this work. This paper aims to appraise and synthesise evidence from human studies examining the effect of helminth infection on host metabolic outcomes, including T2DM.

2. Methods

2.1. *Protocol and registration*

The systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines [13]. A review protocol was registered with PROSPERO 2015, registration number CRD42015025486. It is available online.

2.2. Information sources and search strategy

Publications were sourced from database searches of PubMed and MEDLINE via OvidSP; a review of reference lists of included studies; direct contact with authors of retrieved publications; and consultation with experts in the field. One investigator searched PubMed and MEDLINE for publications available until February 8, 2016. The search terms used for both databases was: (helminth OR helminths OR "soil transmitted helminths" OR strongyloides OR strongyloidiasis OR ascaris OR trichuris OR hookworm OR "necator americanus" OR "ancylostoma duodenale" OR schistosomiasis OR schistosoma OR schistosome OR nematode) AND (diabetes OR "type 2 diabetes" OR "insulin resistance" OR "insulin sensitivity" OR "glucose metabolism" OR "glucose tolerance" OR "metabolic syndrome" OR "syndrome x") NOT ("type 1 diabetes"). After removal of duplicates, one investigator screened the titles and abstracts of retrieved citations.

2.3. Eligibility criteria and study selection

This review focused on the influence of chronic helminth infection on human glucose metabolism. Studies about helminth metabolism of glucose, participants with symptomatic helminth infection, those that used non-helminth organisms or extra-intestinal helminths, and studies of T1DM were excluded. Only papers published in English, using human participants and published after 1995 were included. There were no restrictions on study design, sample size, or length of follow-up.

2.4. Data collection process and data items

One reviewer extracted relevant information from each publication using a standardised data collection form. Data items included study design, sample characteristics (sample size, participant characteristics, age and sex distribution of participants), type of helminth(s), method of diagnosis of infection, the metabolic outcome(s) assessed, results and effect estimates, and control for potential confounders. Primary data were presented in standardised units. Two additional reviewers independently verified this process.

2.5. Quality assessment

The first author assessed the quality of the selected studies using the National Institutes of Health's Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies [14]. Criteria six, seven, and 13 were not used as they were not relevant to the cross-sectional methodology of the studies. Two additional reviewers then independently assessed the quality of studies. Questions of publication selection and assessment were discussed amongst the three reviewers and consensus was achieved.

2.6. Summary measures and synthesis of results

The principal summary measure assessed in the literature was the OR for metabolic outcomes based on infection status. ORs for varying metabolic outcomes were available from all included studies. When available, information was also extracted on adjustment for potential confounders, particularly body mass index (BMI).

The senior author of one study was contacted to obtain binary outcome data for the homeostatic model assessment for insulin resistance (HOMA-IR) values for infected and non-infected participants as this was not provided in the original publication [15]. RevMan5.3 was used for meta-analysis of outcomes of metabolic disturbance (hyperglycaemia, T2DM, MetS, and insulin resistance) [16]. ORs were determined with binomial confidence intervals at 95% using the inverse-variance method. Results were obtained using both random- and fixed-effects models. The heterogeneity of the data was interpreted using Tau^2, to estimate the between-study variance, and I^2, to quantify inconsistencies.

3. Results

3.1. Study selection

The study selection process is outlined in Fig. 1. After removal of duplicates, PubMed and MEDLINE database searching identified 413 papers. A search of the reference lists of included papers identified a further 15 papers. After screening of titles and abstracts, 412 papers were excluded. Of the remaining 16 articles, six papers were review articles without original data; three studied the prevalence of helminth infection as an outcome rather than as the exposure; two studied the influence of acute infection on glucose control, and one was a study protocol. Thus, four publications remained.

3.2. Study characteristics

Each of the four peer-reviewed articles that met the inclusion criteria was a cross-sectional study. The characteristics are summarised in Table 1. They came from China ($n = 2$) [17,18], Indonesia ($n = 1$) [15], and Australia ($n = 1$) [19], all published in 2013 or later. The sample sizes ranged from 259 to 3913, with a total of 6415 participants.

The helminths assessed were *Schistosoma* spp. ($n = 2$) [17,18], *S. stercoralis* ($n = 1$) [19], and one study looked at soil-transmitted helminths (STH) as a group, including *Trichuris trichiura*, *Ascaris lumbricoides*, *Necator americanus*, *Ancylostoma duodenale*, and *S. stercoralis* [15]. Helminth infection was diagnosed through a variety of mechanisms. Two studies examined current helminth infections, one using faecal polymerase chain reaction (PCR) [15] and the other using serum enzyme-linked immunosorbent assay (ELISA) [19]. The two Chinese papers examined previous helminth infections. One study used a combination of self-reported infection and medication history, cross-referenced with a local infectious disease registry [17]; and the other used liver ultrasound to detect evidence of previous schistosomal infection (PSI) [18].

There were different outcome measures for each study, but most assessed one or more indicators of glucose metabolism. They included fasting blood glucose (FBG) ($n = 3$) [15,17,18], post-prandial blood glucose (PBG) ($n = 1$) [17],

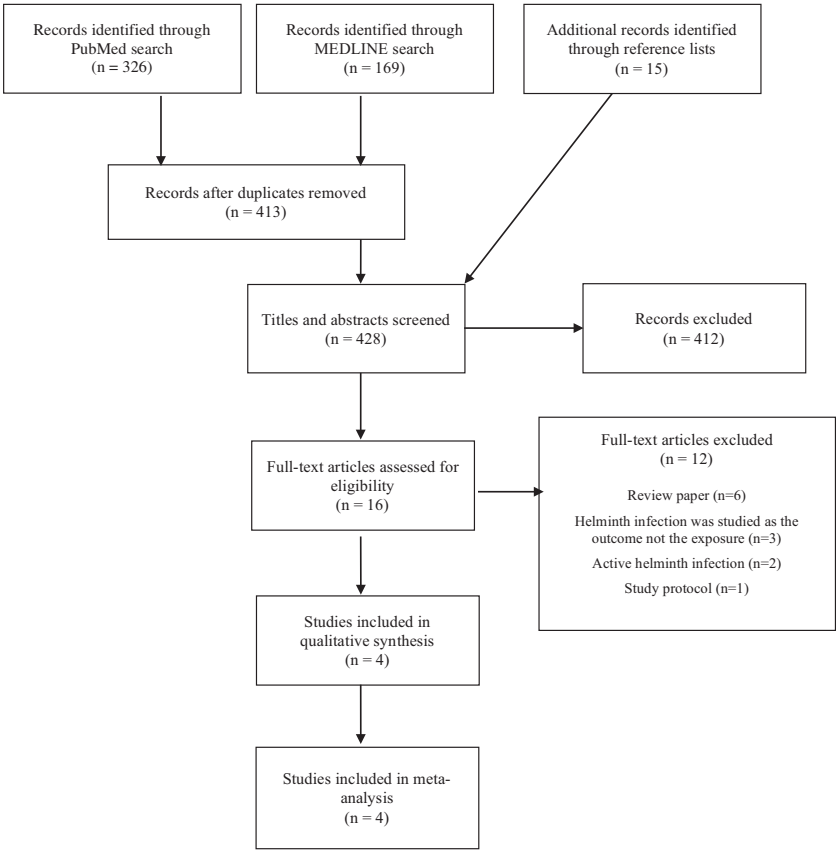

Fig. 1 – PRISMA flow diagram of selection process.

glycated haemoglobin (HbA1c) ($n = 1$) [17], serum insulin ($n = 1$) [15], HOMA-IR insulin resistance index ($n = 2$) [15,17], prevalence of T2DM ($n = 2$) [17,19] and prevalence of MetS ($n = 2$) [17,18]. T2DM was defined by one Chinese study [17] using the World Health Organisation's criteria (fasting glucose $\geqslant$7.0 mmol/L or two-hour glucose $\geqslant$11.1 mmol/L) [20]. The Australian study used similar diagnostic criteria (HbA1c $\geqslant$ 6.5%, random blood glucose > 11.1 mmol/L; or fasting glucose > 7.0 mmol/L) [19]. MetS was defined using two different diagnostic criteria, one study used the National Cholesterol Education Program's criteria (NCEP-ATP III) [21] and the other used the International Diabetes Federation criteria [22].

There was no information available about previous anti-helminth treatment status of the participants in any of the four studies. Two papers treated infected participants with anti-helminth agents after metabolic outcomes had been measured.

3.3. *Quality assessment*

Table 2 summarises the quality assessment of the included human studies. Overall, their quality was acceptable. Two were judged to be of 'fair' quality [17,18] and two 'good' [15,19]. All studies clearly stated their research objectives and defined populations being studied. Exposed and control subjects were recruited from the same population over the same time period in all studies. Inclusion and exclusion criteria were well defined and appropriately applied. The participation rate of eligible subjects was greater than 50% in three of the studies; however, due to a low prevalence of previous schistosomal infection and low prevalence of diabetes in younger populations, one study only analysed data from participants over 60 years of age, potentially introducing selection bias [17]. Sample sizes were clearly stated in all studies; however only one provided a justification for the sample size [17]. The definition of a case of previous helminth infection varied across the studies, with two using objective methods for diagnosis (PCR and ELISA testing) [15,19] and two using subjective measures (self-report and ultrasound findings) [17,18]. One study repeated the ELISA after 287 days for the participants with initially negative results [19]. None of the other studies assessed the exposure more than once. The outcome measures varied between studies, but all used clearly defined, universally accepted methods for assessment of both exposure and control groups. None reported a loss to follow-up. All four conducted multivariate analyses of results to adjust for potential confounding variables, including age and BMI.

3.4. *Results of individual studies*

All the studies found that previous helminth infection was associated with improved glucose homeostasis, reduced insulin resistance, and/or lower prevalence of T2DM or MetS. The results are summarised in Tables 3 and 4.

Table 1 – Characteristics of studies.

Study	Study design		Participant details	Exposure		Outcomes		
	Type	Sample size		Helminth	Method of diagnosis	Primary outcomes	Description of diagnosis	Secondary outcomes
Chen China, 2013 [17]	Cross-sectional	3913	Men and women aged 60 years and older in two communities in Jiading County, China	*Schistosomal* spp.	Self-reported history and medication history, cross-referenced with local government registry data from 1989	MetS prevalence T2DM prevalence	NCEP criteria WHO criteria	HOMA-IR FBG PBG HbA1c
Shen China, 2015 [18]	Cross-sectional	1597	Healthy men, >45 years presenting at a health unit between April and June 2013 in an area in China previously endemic for *S japonicum*. Excluded those with known liver disease or heavy alcohol consumption	*Schistosomal* spp.	Liver U/S showing evidence of chronic infection	MetS prevalence	IDF criteria	FBG
Wiria Indonesia, 2015 [15]	Cross-sectional	646	Community members >18 years who had participated in the ImmunoSPIN project with available stool samples. Nangapanda, an area highly endemic for STH	*T. trichiura* *A. lumbricoides* *N. americanus* *A. duodenale* *S. stercoralis*	Microscopy PCR PCR PCR PCR	HOMA-IR	Fasting serum insulin × fasting glucose/ 22.5	FBG Fasting serum insulin
Hays Australia, 2015 [19]	Cross-sectional	259	Patients attending a clinic in three Aboriginal communities in the Kimberly region, Australia	*S. stercoralis*	ELISA Positive > 0.30	T2DM	Defined by: HbA1c > 6.5% 11.1 mmol/L FBG > 7.0 mmol/L at the time or in the past in patients with known T2DM	

MetS: metabolic syndrome; T2DM: type 2 diabetes mellitus; NCEP: National Cholesterol Education Program Adult Treatment Panel III criteria (three or more of: blood pressure > 130/85; waist circumference (WC) > 102 cm (men) or 88 cm (women); triglycerides (TG) > 1.69 mmol/L, high density lipoprotein cholesterol (HDL-C) < 1.03 mmol/L (men) or < 1.29mml/L (women); fasting blood glucose > 6.1 mmol/L); WHO: World Health Organisation criteria (fasting blood glucose > 7.0 mmol/L; post-prandial blood glucose > 11.1 mmol/L; self-reported diagnosis of diabetes as diagnosed by a physical; or use of antidiabetic medication); HOMA-IR: homeostatic model assessment of insulin resistance; FBG: fasting blood glucose; PBG: post-prandial blood glucose; HbA1c: glycated haemoglobin; U/S: ultrasound; IDF: International Diabetes Federation criteria (central obesity (WC > 90 cm) and any two of TG > 1.7 mmol/L; HDL-C < 1.0 mmol/L or treatment of dyslipidaemia; systolic blood pressure > 130 mmHg or diastolic blood pressure > 85 mmHg; or FBG > 5.6 mmol/L or previous T2DM diagnosis); STH: soil transmitted helminths; PCR: polymerase chain reaction; ELISA: enzyme linked immunosorbent assay; BSL: blood sugar level.

Table 2 – Quality of studies using the NIH's quality assessment of cohort and cross-sectional studies.

	Chen, 2013 [17]	Hays, 2015 [19]	Shen, 2015 [18]	Wiria, 2015 [15]
1. Was the research question or objective in this paper clearly stated?	Yes	Yes	Yes	Yes
2. Was the study population clearly specified and defined?	Yes	Yes	Yes	Yes
3. Was the participation rate of eligible persons at least 50%?	No	NR	Yes	No
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	Yes	Yes	Yes	Yes
5. Was a sample size justification, power description, or variance and effect estimates provided?	Yes	No	No	No
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	N/A	N/A	N/A	N/A
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	N/A	N/A	N/A	N/A
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g. categories of exposure, or exposure measured as continuous variable)?	No	Yes	N/A	Yes
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	No	Yes	No	Yes
10. Was the exposure(s) assessed more than once over time?	No	No	No	No
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes
12. Were the outcome assessors blinded to the exposure status of participants?	Yes	Yes	NR	Yes
13. Was loss to follow-up after baseline 20% or less?	Yes	Yes	Yes	Yes
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	Yes	Yes	Yes	Yes
Overall quality rating (good, fair, or poor)	Fair	Good	Fair	Good

NR: not reported; N/A: not applicable.

3.4.1. *Glucose homeostasis*

Three studies assessed the association between helminth infection and glucose homeostasis through measurement of FBG, PBG, and/or HbA1c [15,17,18]. Three studies that measured FBG found that participants with previous helminth infection had lower mean FBG levels than uninfected participants. This was statistically significant in a cross-sectional study from China [17]. This study also investigated the effect of helminth infection on PBG and demonstrated that participants with previous helminth infection had significantly lower PBG than uninfected groups. Additionally, this study showed that infected groups had a significantly lower HbA1c in comparison to uninfected groups.

3.4.2. *Insulin resistance*

Insulin resistance was assessed in two studies through measurements of HOMA-IR and/or fasting serum insulin concentration [15,17]. HOMA-IR is a validated measure of insulin resistance. It is calculated by multiplying the fasting serum insulin (mU/L) by fasting blood glucose (mmol/L) and then dividing the result by 22.5. Lower HOMA-IR values indicate less insulin resistance [23]. Both studies showed significantly lower HOMA-IR values in infected groups compared to uninfected groups. Also of note, Wiria et al. found that insulin resistance was incrementally reduced with every additional STH species infection [15]. In this study participants with previous STH infections tended to have lower fasting serum insulin levels, though this was not a significant result.

When analysing the ORs for insulin resistance, it should be noted that the two studies used different definitions of an 'abnormal' HOMA-IR result. Chen's study defined insulin resistance as HOMA-IR values of higher than 2.50, whereas in Wiria's study the authors used the 90th percentile to mark an abnormal value, which was given as 1.54.

3.4.3. *Prevalence of metabolic disease: T2DM and MetS*

Table 4 shows the prevalence of T2DM assessed in two studies, both of which showed a significant protective association [17,19]. ORs, when adjusted for BMI, were 0.47 (0.32–0.69) [17]

Table 3 – Primary results.

Study		Measure of glucose or insulin homeostasis					Prevalence	
		FBG (mmol/L)	PBG (mmol/L)	HbA1c in NGSP % (IFCC mmol/mol)	Fasting serum insulin (pmol/L)	HOMA-IR (units)	T2DM (%)	MetS (%)
Chen China, 2013 [17]	Infected ($n = 463$)	5.50**	8.03**	5.8* (40)	–	1.20**	14.90**	14.00**
	Uninfected ($n = 3450$)	5.70**	9.23**	5.9* (41)	–	1.73**	25.40**	35.00**
Hays Australia, 2015 [19]	Infected ($n = 92$)	–	–	–	–	–	34.78**	–
	Uninfected ($n = 167$)	–	–	–	–	–	59.28**	–
Shen China, 2015 [18]	Infected ($n = 465$)	6.21+	–	–	–	–	–	18.28**
	Uninfected ($n = 1132$)	6.38+	–	–	–	–	–	34.01**
Wiria Indonesia, 2015 [15]	Infected ($n = 316$)	5.88+	–	–	45.00+	0.81*	–	–
	Uninfected ($n = 161$)	5.92+	–	–	49.50+	0.97*	–	–

FBG: fasting blood glucose; PBG: post-prandial blood glucose; HbA1c: glycated haemoglobin; NGSP: National Glycohemoglobin Standardization Program; IFCC: International Federation of Clinical Chemistry; HOMA-IR: homeostatic model assessment of insulin resistance; T2DM: type 2 diabetes mellitus; MetS: metabolic syndrome.

+ $p > 0.05$.

* $p < 0.05$.

** $p < 0.01$.

and 0.39 (0.23–0.692) [19]. Two studies found that participants with previous helminth infection were less likely to have MetS, with adjusted ORs of 0.35 (0.25–0.49) [17] and 0.67 (0.48–0.93) [18]. The adjusted OR for the prevalence of MetS in Chen's paper did not include an adjustment for BMI, a potentially confounding variable [17].

3.5. *Synthesis of results*

A meta-analysis was completed using the available data for metabolic end-points, as is shown in Fig. 2. The OR for hyperglycaemia (defined as FBG ⩾ 5.6 mmol/L) in participants with previous helminth infection was 0.72 (0.59, 0.87) (Tau^2: 0.0, I^2: 0%). The OR for the prevalence of T2DM was 0.47 (0.35, 0.63) (Tau^2: 0.01, I^2: 21%). The OR for the prevalence of MetS was 0.36 (0.26, 0.52) (Tau^2: 0.05, I^2: 71%). Of the two studies which reported HOMA-IR as an outcome, only one had results that were able to be used in a meta-analysis [15]. These results defined insulin resistance as a HOMA-IR score above the 90th percentile. The OR for this outcome was 0.60 (0.32, 1.10). When abnormal metabolic outcomes from all studies were combined and assessed using a random-effects model, the OR was 0.50 (0.38, 0.66) (Tau^2: 0.10, I^2: 80%). This result did not significantly differ when using a fixed-effects model (OR 0.49; CI 0.44–0.55). As can be seen in Fig. 2, the subgroups within the meta-analysis are not mutually exclusive. For example, Chen's study includes results for the prevalence of T2DM and MetS. To address this, the analysis was repeated excluding each of the overlapping data sets. The overall OR did not change substantially with these exclusions. The OR range was from 0.47 to 0.56 with confidence intervals between 0.35 and 0.70 in all variations.

4. Discussion

There was a protective association between helminth infection and adverse metabolic outcomes in each of the included studies. Our meta-analysis found that participants with a previous or current helminth infection were 50% less likely to have an outcome of metabolic dysfunction in comparison to participants without evidence of helminth infection. As these were all cross-sectional studies, causality cannot be inferred. However, when reassessed against Bradford-Hill's 'nine viewpoints' the collective results offer some causal insight. According to Bradford-Hill, assessment of causality is facilitated by taking into account the following: the strength of association, consistency of effect, available experimental evidence, a dose-response relationship, timing (the cause must precede the effect), coherence, biologic credibility, specificity, and analogy [24].

All studies included in the review showed at least one significant association between infection and a metabolic outcome, with several showing protective associations for multiple outcomes. The individual measures of effect, as seen in Table 4, demonstrate consistent ORs in favour of a protective association. In the meta-analysis, the combined random-effects OR of 0.50 (0.38, 0.66) demonstrates a strong protective association between helminth infections and metabolic outcomes. The results were consistent across heterogeneous populations, across different countries, and when studying different helminths.

While these human studies are observational, experimental evidence could test the theory in a more robust way to shed light on potential mechanisms. There is a growing body of evidence from animal research supporting the hypothesis

Table 4 – Odds ratios.

Study		Measure of glucose or insulin homeostasis		Prevalence		Comments
		Hyperglycaemia (FBG ⩾ 5.6 mmol/L)	Insulin resistance (Abnormal HOMA-IR)	T2DM	MetS	
Chen China, 2013 [17]	Unadjusted OR (CI, 95%)	–	0.39 (0.30–0.51)**	0.52 (0.39–0.67)**	0.30 (0.23–0.40)**	For insulin resistance and T2DM adjustment was for age, sex, BMI, physical activity, family history of T2DM, and education level. The OR for MetS was adjusted for the above factors excluding BMI. An abnormal HOMA-IR was defined as >2.50
	Adjusted OR (CI, 95%)	–	0.59 (0.40–0.85)**	0.47 (0.32–0.69)**	0.35 (0.25–0.49)**	
Shen China, 2015 [18]	Unadjusted OR (CI, 95%)	0.68 (0.55–0.86)**	–	–	0.43 (0.33–0.57)**	Adjustment was for age and BMI
	Adjusted OR (CI, 95%)	0.87 (0.68–1.10)+	–	–	0.67 (0.48–0.93)*	
Wiria Indonesia, 2015 [15]	Unadjusted OR (CI, 95%)	0.83 (0.57, 1.22)^	0.60 (0.32, 1.10)^	–	–	Abnormal HOMA-IR was a score >90th percentile or >1.54 units.
Hays Australia, 2015 [19]	Unadjusted OR (CI, 95%)	–	–	0.37 (0.22–0.62)**	–	Adjustment was for age, BMI, systolic BP and triglycerides
	Adjusted OR (CI, 95%)	–	–	0.39 (0.23–0.67)**	–	

OR: odds ratio; CI: confidence interval; FBG: fasting blood glucose; HOMA-IR: homeostatic model assessment of insulin resistance; T2DM: type 2 diabetes mellitus; MetS: metabolic syndrome; BMI: body mass index; BP: blood pressure.

^ OR was provided separately by the authors, no *p*-value given.

+ $p > 0.05$.

* $p < 0.05$.

** $p < 0.01$.

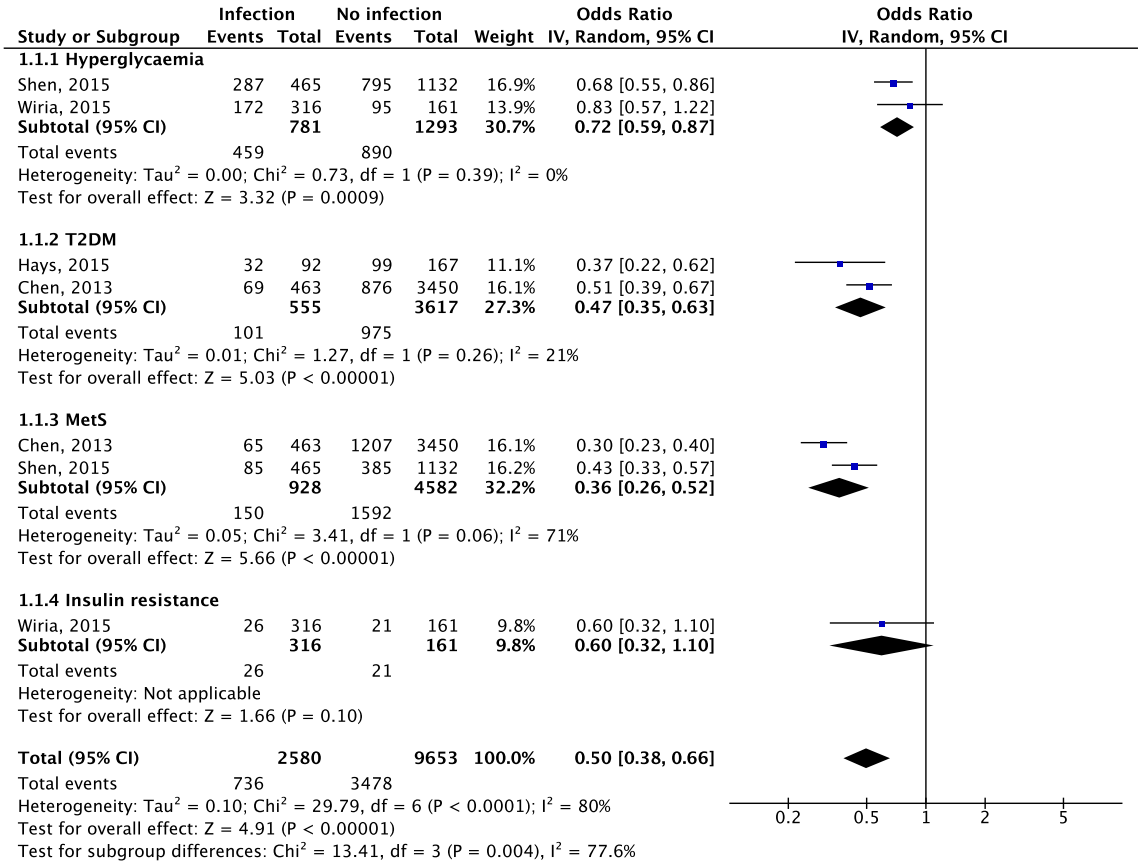

Fig. 2 – A meta-analysis of metabolic end-points.

that specific helminth infections protect against metabolic disease. Mouse studies have shown that experimental infection with helminths can result in reduced fasting glucose concentrations, decreased fasting insulin concentrations, improved glucose and insulin tolerance, and less insulin resistance in comparison to non-infected controls [25–29].

With respect to 'biological gradient', Bradford-Hill nominated dose-response as a key factor in assessing causation [24]. The included study by Wiria et al. found that an increasing number of different helminth infections correlated with incremental reductions in insulin resistance [15]. It is plausible that insulin resistance is reduced in a dose-dependent manner reflected by the infectious load of the worms, but this has not yet been investigated.

Temporality is difficult to establish in cross-sectional studies. Nevertheless, three studies had sufficient chronological data to provide some insight into exposure and outcome. Chen and Shen's studies were located in areas that were highly endemic for *Schistosoma* spp. 30 years ago but are now non-endemic areas [17,18]. Chen et al. defined a PSI case using a self-reported history of infection, cross-referenced with a government registry from 1989 [17]. Shen et al. looked at ultrasound features of chronic schistosomal liver disease [18]. Hays et al. used ELISA for diagnosis of *S. stercoralis* and followed participants with negative results over the course of nine months. They found just three new infections in this period, indicating that it is likely that the majority of participants with positive ELISA results had long-standing, rather than recent, *S. stercoralis* infections [19].

This review has identified a protective association between previous helminth infections and metabolic disease. Three plausible mechanisms to explain this association are discussed below. It is likely that these mechanisms are interactive rather than mutually exclusive.

4.1. *Nutrition*

A nutrition-based hypothesis is that the helminth causes depletion of human energy sources resulting in weight loss, leading to improved metabolic outcomes. Infection was associated with a significantly lower BMI in three studies, but the protective effect of helminth infection on metabolic outcomes persisted in all studies when adjusted for BMI [15,17–19]. Additionally, recent mice experiments have shown improved metabolic outcomes in mice exposed to just proteins of helminths (lacto-N-fucopentaose III and *Schistosoma mansoni* soluble egg antigens) without the parasitic effects of the whole organism [25,26]. Thus it is unlikely that nutritional depletion alone is responsible for improved glucose metabolism.

4.2. *Alterations to the gut microbiome*

Helminths may improve insulin sensitivity in the host through manipulation of the human gut microbiome. Animal

studies of autoimmune disease have shown a correlation between disease activity, helminth infection and parallels in the qualitative and quantitative diversity of the gut microbiome. So far, these studies have focused on idiopathic chronic diarrhoea, ulcerative colitis, and coeliac disease [30]. A number of observational studies and one randomised controlled trial have shown that changes to the gut microbiome can improve insulin resistance [31–33]. The relationship between helminths, the microbiome and metabolic outcomes has yet to be studied.

4.3. *Immunomodulation*

It is postulated that helminth infections may exert an evolutionary advantage by maintaining host immune tolerance through immune-modulation pathways. A recently published paper studied the cytokine profile of asymptomatic infected individuals and found that they had significantly lower circulating levels of pro-inflammatory cytokines and significantly higher levels of anti-inflammatory cytokines [34]. The worms' anti-inflammatory properties may result in reduced insulin resistance. This hypothesis is summarised in Fig. 3. Classically activated macrophages (CAMs) are typically increased transiently in response to bacterial and viral infections. They are also chronically raised with obesity and insulin resistance [35]. As is shown in Fig. 3, high levels of related pro-inflammatory cytokines (TNF-a, IL-1B, and IL-6) reduce the expression of glucose transporters and inhibit the activity of insulin receptors thus impairing insulin sensitivity [36]. Alternatively, helminth infections induce the production of Th2 cells, T regulatory cells, and eosinophils. This skews macrophage polarisation in adipose tissue away from CAMs towards alternatively activated macrophages (AAMs) [35]. A shift towards AAMs is accompanied by the production of anti-inflammatory cytokines, particularly IL-4, IL-5, IL-10, IL-13, and TGF-B [35,36]. These cytokines also inhibit pro-inflammatory responses from CAMs. Thus, through the Th2 pathway, helminths may promote insulin sensitivity to prevent the development of MetS and T2DM.

4.4. *Study limitations*

Only one author of this review conducted the initial literature search and data extraction. Thus, there is a potential source of error in the process. Selection bias is a potential issue in three of the studies. Wiria et al. used participants who had previously provided stool samples as part of the ImmunoSPIN trial [15]. Chen et al. only included an analysis of participants over the age of 60 years due to a low prevalence of PSI and T2DM in younger populations [17]. Shen et al. defined a PSI based on subjective ultrasound findings and would have excluded participants with a history of infection who did

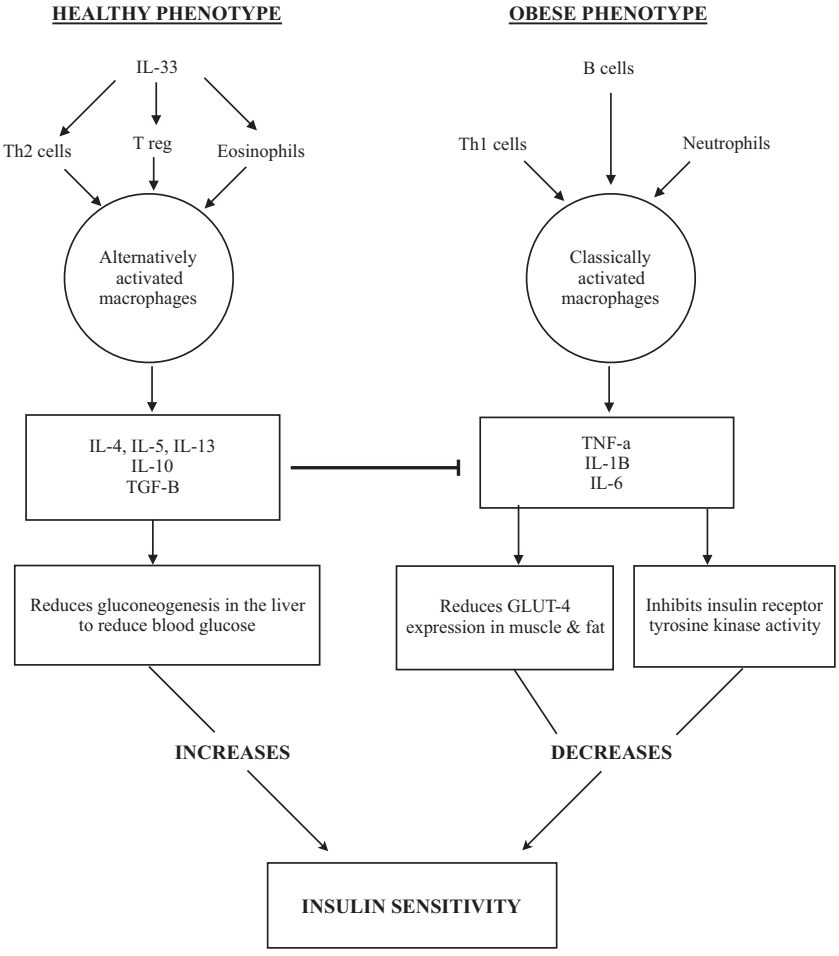

Fig. 3 – A potential immunomodulatory explanation of the effect of helminth infection on insulin resistance.

not have residual features on liver ultrasound [18]. FBG as an outcome of glucose metabolism has shortcomings, with known variation dependent on age, time of day, activity levels, alcohol intake, and duration of fast [37].

There were only a small number of studies available for meta-analysis. Two assessed previous infection whilst two assessed likely current helminth infection. There was no obvious difference in outcome between previous or current infections. With the meta-analysis, a combined outcome was used because, although there were variations in outcomes reported, they all pointed to the metabolic syndrome. Due to the small number of published studies on the subject matter, the evidence for each individual outcome measure was limited. It is likely that the case would be better made with meta-analysis of individual patient data.

It is difficult to interpret the estimates of heterogeneity produced by RevMan5.3. The confidence intervals for heterogeneity estimates are not included in the software's output. It is possible that the high heterogeneity score given by RevMan 5.3 reflects the multiple measures in the combined metabolic syndrome endpoint. Additionally, the chi-squared and I^2 tests have well-recognised shortcomings [38]. In spite of this, the consistency of results across these different study populations and measures suggest a real association.

The random-effects model used should provide more conservative estimates with wider confidence intervals [39]. RevMan5 uses the DerSimonian–Laird method for random-effects analyses. This method has widely recognised faults, including the production of narrow confidence intervals and biased estimates [40]. Though the method has been recognised as one of the optimal methods of analyses when examining less than five studies [39].

It is expected that this study's conclusions will be further validated as more studies are published examining the potential metabolic protective effects of helminth infection.

4.5. *Conclusion*

To our knowledge, this is the first systematic review and meta-analysis on the effect of helminth infections on metabolic disease. It has found a strong protective association between helminth infections and metabolic outcomes. With a greater understanding of the pathways underlying this relationship novel therapeutic strategies can potentially be developed to prevent or delay the onset of T2DM.

Conflicts of interests

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