The interplay of type 2 immunity, helminth infection and the microbiota in regulating metabolism

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Abstract
Type 2 immunity has recently emerged as a critical player in metabolic status, with numerous studies investigating the role of type 2 immune cells within adipose tissue. Metabolic dysfunction is often characterised as a low-grade or chronic inflammatory state within tissues, and type 2 immunity may facilitate a return to metabolic homeostasis. A complex network of type 2 resident cells including M2 macrophages, eosinophils and ILC2s has been identified within adipose tissue. Although the effector cells in this equilibrium have not been clearly identified, any alteration of the type 2 microenvironment resulted in an altered metabolic state. Historically, the type 2 immune response has been associated with helminth infection. The type 2 immune response drives host resistance and plays an important role in promoting tissue repair following the migration of helminth larvae through tissues. Although helminths are largely eradicated in developed countries, infection rates remain high in poor communities within the developing world. Interestingly, there is strong evidence that helminth infection is inversely correlated with autoimmune or inflammatory disorders. Recently, an increasing amount of epidemiological and field studies suggest that it could be the same for obesity and metabolic syndrome. In the current review, we summarise the literature linking type 2 immunity to improved adipose tissue function. We then discuss more recent evidence indicating that helminth infection can provide protection against metabolic syndrome. Lastly, we explore the possible contributions of altered nutrient uptake, adipose tissue function and/or the intestinal microbiota with the ability of helminths to alter metabolic status.

Keywords: adipose tissue, metabolism, microbiota, parasitic infection, type 2 immunity

INTRODUCTION
Globally, there has been a significant rise in the incidences of obesity and metabolic syndrome both of which carry further complications, including the increased risk of developing type 2 diabetes and cardiovascular disease. It is now estimated that more than 2 billion people are overweight or obese worldwide.1 Obesity is established through a large number of
environmental, genetic and molecular factors, but is defined by the accumulation of fat within the body which increases the size of white adipose tissue (WAT). The purpose of these fat depots, which are distributed across different anatomical locations in the body, is to store fat to use later as an energy source. The stress induced by the chronic over-accumulation of fat within the WAT during the development of obesity is associated with a low-grade inflammation. Indeed, inflammation is a major risk factor for cardiovascular disease development and predisposition to type 2 diabetes.\(^2\) One of the first associations made between inflammation and obesity was shown in a rodent model of obesity in which tumor necrosis factor alpha (TNF\(\alpha\)) production by adipocytes could be linked to insulin resistance.\(^3\) Neutralisation of TNF\(\alpha\) was able to increase peripheral glucose uptake in response to insulin, hence showing an improvement on insulin resistance associated with obesity.\(^3\) The strong link between obesity, immunity and inflammation has been a key player in driving research in the novel and ever-expanding field of immune-metabolic crosstalk.

More recently, the contribution of type 2 immunity to the regulation of adipose tissue homeostasis has been highlighted as playing a significant role in metabolism. The first evidence came from a phenotypic switch of macrophages that reside within the adipose tissue. Pro-inflammatory classical ‘M1’ macrophages will typically develop following exposure to a number of factors including bacterial stimulation, interferon gamma (IFN\(\gamma\)) and circulating fatty acids, driving inflammation through the release of TNF\(\alpha\), nitric oxide and interleukin-6 (IL-6). Conversely, alternatively activated ‘M2’ macrophages emerge following stimulation by type 2 cytokines IL-4 and IL-13 and play a more anti-inflammatory role by releasing both an IL-1 decoy receptor and an IL-10, and are involved in the maintenance of tissue homeostasis. Obesity alters the balance of macrophages within the adipose tissue, inducing a switch from adipocyte-protective M2 macrophages to pro-inflammatory M1 macrophages that produce TNF\(\alpha\) and drive insulin resistance.\(^4\) The association of type 2 immunity with lean adipose tissue and in protection against obesity came as somewhat of surprise, as this arm of the immune response was long thought to have evolved to primarily to provide protection of mammals against parasitic helminths\(^5\), and in modern societies is typically associated with pathological diseases such as asthma, atopic dermatitis and food allergies.\(^6-8\)

Importantly, studies are now emerging indicating that the ability of helminths to induce type 2 immune responses may also allow these parasites to provide protection against metabolic dysfunction. This raises the possibility that the absence of helminth infection within modern societies may contribute to the increased prevalence of metabolic syndrome and obesity in these regions.

**THE IMPACT OF TYPE 2 IMMUNITY AND ADIPOSE TISSUE**

**Type 2 immune cells limit inflammation within white adipose tissue (WAT)**

The composition and key cellular players within adipose tissue found in metabolically healthy individuals are becoming increasingly appreciated. Type 2 immune cells which reside within adipose tissue, including M2 macrophages, eosinophils, type 2 innate lymphoid cells (ILC2s) and T regulatory cells, promote tissue homeostasis through the release of anti-inflammatory factors, modulating adipocyte function and driving repair. A major role of these cells, which are constitutively active and abundant in lean healthy white adipose tissue (WAT), is to inhibit inflammation, fat accumulation and subsequent insulin resistance, thereby protecting against metabolic dysfunction and obesity (Figure 1).\(^9\)

As previously mentioned, inflammation in adipose tissue is associated with a switch in polarisation from M2 macrophages to a M1 phenotype.\(^4\) Accordingly, bypass surgery (which reduces food intake to help patients lose weight) is correlated with a decrease of inflammatory M1 macrophage populations in adipose tissue.\(^10\) M1 macrophages that are associated with WAT inflammation during obesity are now identified as a specialised population. Called Mme, for metabolic activation, these macrophages can be polarised *in vitro* by treatment with a combination of glucose, insulin and palmitate to mimic the environment found within inflamed adipose tissue.\(^11\) These macrophages were found both in obese mice and in humans and are distinct from classically activated M1 macrophages.\(^11\) Mme cells, like M1 macrophages, produce IL-13 and TNF\(\alpha\), but do not express...
typical M1 surface markers and instead exhibit some markers of M2 macrophages such as CD36 or ABCA1. The key difference lies in the cues that are integrated to drive their polarisation, where M1 macrophages are triggered via toll-like receptor (TLR) signalling and Mme are triggered by metabolic cues such as free fatty acids. The first evidence of M2 macrophages being protective in metabolic syndrome was shown in mice with a specific deletion of peroxisome proliferator-activated receptor gamma (PPARγ) in macrophages. PPARγ-deficient macrophages were unable to polarise into M2 macrophages upon IL-4 stimulation, which was associated with increased insulin resistance and susceptibility to weight gain upon diet-induced obesity (DIO).12

Importantly, treatment with the type 2 cytokine IL-4 during DIO has been demonstrated to reduce insulin resistance via STAT6 activation and attenuate adipose tissue inflammation. A landmark study in the field showed that eosinophils maintain the M2 macrophage pool within adipose tissue through the production of IL-4 (Figure 1).14 Accordingly, the absence of eosinophils in dblGATA1 deficient mice was associated with a significant reduction of M2 macrophages in WAT, and increased susceptibility to DIO through increased body fat and impaired glucose tolerance. Interestingly, infection with the helminth *Nippostrongylus brasiliensis* increased the eosinophil population within WAT and improved glucose tolerance upon DIO. The involvement of M2 macrophages and eosinophils in the healthy ‘lean status’ of WAT has been further endorsed by the discovery of an adipose-resident ILC2 population. These cells are the primary source of IL-5 and IL-13 within the WAT sustaining both eosinophil and M2 macrophage populations, whereby IL-5 deficiency resulted in an increased susceptibility to DIO (Figure 1). Notably, Molofsky and colleagues demonstrated that the alarmin cytokine, IL-33, drives the activation of ILC2s and exogenously promotes a rapid expansion of eosinophils in WAT (Figure 1). Recently, a new population of lipid-associated macrophages (LAMs) have been characterised. These cells arise via the activation of Trem2 during the development of obesity and are...
important to slow the course of metabolic syndrome.\textsuperscript{17} Although LAMs share some phenotypic markers with M2, their true lineage with regards to the M1/M2 paradigm is still to be elucidated.\textsuperscript{17}

Other cell types not classically associated with type 2 immune response have been linked to the maintenance of healthy adipose tissue. For example, invariant natural killer T cells (iNKT) have been described to be resident in WAT and can be activated upon recognition of complex glycolipids that can be presented by CD1d on adipocytes (Figure 1).\textsuperscript{18} iNKT cells have been shown to produce IL-4 and IL-10 to support the maintenance of M2 macrophage populations,\textsuperscript{19,20} as well as producing IL-2 which controls the abundance and activation of T regulatory cells (Tregs) that reside within WAT (Figure 1).\textsuperscript{20} Tregs can also arise through PPAR\textgamma activation in a similar manner to M2 macrophages and adipocytes.\textsuperscript{21} Tregs within WAT are a distinct population which express GATA3 and ST2 (the IL-33 receptor), markers that are classically associated with ILC2s. In humans, not only is IL-33 produced by adipose tissue, but heightened expression of ST2 in omental adipose tissue Tregs suggests a conserved requirement for IL-33 (Figure 1).\textsuperscript{22,23} In two different studies, IL-33 treatment was associated with improved metabolic parameters and an increase in Tregs within the adipose tissue.\textsuperscript{23,24} Further evidence of the crucial role of IL-33 in humans is shown through the negative correlation between IL-33 and circulating levels of body mass index (BMI).\textsuperscript{25}

Additionally, polymorphisms in the IL-33 gene are potentially linked to decreased obesity.\textsuperscript{26}

Recent literature highlights the central role of IL-33, with this alarmin emerging as a key component of immune regulation within the adipose tissue, acting on adipose tissue-resident Tregs and almost all the other immune cell types via the activation of ILC2s (Figure 1).\textsuperscript{23,24,27} The source of IL-33 within adipose tissue has been shown to be restricted to the stromal compartment; however, the exact population of cells producing this alarmin remains unclear.\textsuperscript{28} Spallanzani et al. were able to identify several mesenchymal stromal populations as IL-33 producers and additionally demonstrated a role for IL-33 in maintaining adipose Treg populations.\textsuperscript{29} Secondly, Mahlakoiv et al. showed that IL-33 deficiency in mice resulted in weight gain and increased adiposity which was associated with less active ILC2s and a decreased eosinophil population within WAT.\textsuperscript{30} Adipose-resident ILC2s express higher levels of ST2, which potentiates their activation by IL-33 compared to other ILC2 populations. Mahlakoiv et al. also identified a further source of IL-33 – a population of adipose stem and progenitor cells (ASPCs) which were spread throughout the WAT. Of note, they could identify similar ASPCs within the human WAT.\textsuperscript{30} These findings were later corroborated by Rana et al., who identified a similar stromal population able to support ILC2s via IL-33 production and ICAM1 expression.\textsuperscript{31} Interestingly, the later study further demonstrated that the stromal cells can respond to IL-4/IL-13 and are involved in eosinophil recruitment.\textsuperscript{31}

**Contribution of type 2 immunity to adaptative thermogenesis and beiging of WAT**

Another function of type 2 immunity in adipose tissue is to support adaptative thermogenesis – a process which serves to maintain core body temperature. Mammals contain a specialised fat tissue called brown adipose tissue (BAT), which carries the primary responsibility of heat generation and is particularly prominent in neonates at the time of birth.\textsuperscript{32} Adaptative thermogenesis is driven in response cold exposure, where brown adipocytes subsequently utilise both glucose and fat reserves to dissipate energy as heat in a process which is regulated by norepinephrine (NE) and the sympathetic nerves associated with the tissue. Brown adipocytes contain a large number of mitochondria and express uncoupling protein 1 (UCP1), which enables the uncoupling of the mitochondrial oxidative phosphorylation, thus inducing heat generation. IL-33 also plays an important role in BAT particularly during the neonatal phase. Indeed, in the absence of IL-33 or ST2, BAT develops normally but is unable to properly express UCP1 mRNA.\textsuperscript{33} It suggests that IL-33 enables uncoupling of the respiration within BAT mitochondria and thermoregulation.\textsuperscript{33}

Another means to generate heat is through a process referred as browning of WAT or ‘beiging’. This process involves the upregulation of UCP1 by adipocytes within subcutaneous WAT in response to cold exposure. This phenomenon also involves NE stimulation, and beiging is linked with the improvement of metabolic disease.\textsuperscript{34} In this
context, genetic loss of eosinophils and/or IL-4/13 signalling resulted in an impaired cold-induced beiging of WAT. Cold exposure is not the only trigger to this mechanism. Exercise has also been linked to WAT beiging in an eosinophil/M2 macrophage-dependent manner. Interestingly, two different studies showed that adipocytes secreted proteins (i.e. adiponectin and CXCL14) could induce beiging via M2 macrophages. The role of M2 macrophages was furthermore highlighted by their reliance on the mTORC2 pathway (an important regulator of cellular metabolism). As this pathway was shown to be crucial for M2 macrophage polarisation and their activity in the context of both adaptive thermogenesis and helminth infection. More recently, the involvement of ILC2s in this process has become better defined. Two studies showed that ILC2s are critical to orchestrate the beiging of white adipocytes with the help of eosinophils and M2 macrophages. During cold exposure, this response induces the differentiation and proliferation of adipocyte precursors into beige adipocytes. ILC2s can also directly induce UCP1 expression in white adipocytes and are found in human adipose tissue, as well as during browning of WAT. Additionally, adipose tissue-resident Tregs are also associated with the beiging of adipose tissue. Indeed, transfer of Tregs exposed to cold or to NE stimulation was able to mediate the beiging of WAT.

Surprisingly, another trigger which can induce beiging of WAT is caloric restriction. The peculiarity of caloric restriction compared to cold exposure is the critical role of the microbiota in the beiging process. Taken together, cellular inducers of beiging have in common a negative energy balance which suggests a conserved adaptation of adipose tissue and type 2 immunity. Importantly, beiging has been shown in many instances to improve metabolic syndrome and obesity and thus make this process an interesting target for the design future therapeutic interventions.

Summary – the dual role of type 2 immunity in adipose tissue function

The current paradigm regarding the role of type 2 immune response in adipose tissue homeostasis is the following: a complex network of immune cells including M2 macrophages, eosinophils, ILC2s, Tregs and iNKT cells maintains an anti-inflammatory milieu within the adipose tissue. This status is disrupted during obesity leading to inflammation and insulin resistance (Figure 1). Although the role M2 macrophages in the adipose tissue is currently unclear and has been subjected to controversies, two recent studies showed a crucial role for M2 macrophages in promoting adipose tissue beiging and metabolic function. Both used transgenic macrophages unable to switch from M2 to M1 and demonstrated an improvement of metabolic parameters upon DIO.

Hence, it appears that type 2 immunity is critical to promote adipose tissue homeostasis by preventing inflammation in WAT. In addition, it is also involved in the process of adaptive thermogenesis and helminth infection, where caloric restriction or excessive vulnerability to cold may occur. Thus, helminth-induced type 2 immunity could help promote survival of the infected animals and/or humans, in part through its impact on adipose tissue.

HELMINTH INFECTION AND METABOLIC STATUS

During helminth infection, type 2 immunity is required to limit worm burdens and to promote the timely repair the damage caused by tissue migrating larval stages of the parasite. This requires the participation of multiple immune cells, but importantly, expression of the IL-4Rx receptor which mediates activity of both IL-4 and IL-13. According to the World Health Organization (WHO), more than 1.5 billion people worldwide are infected with soil-transmitted helminths (STHs). In the developing world, the prevalence of helminth infection is significantly higher due to social determinants such as lower income, limited access to health care and poor sanitation. Accordingly, in these areas chronic helminth infection is associated with morbidity resulting from both malnutrition and anaemia. By contrast, these parasites are largely eradicated from modern societies, where paradoxically their absence is associated with the increased prevalence of inflammatory diseases. Here,
we will discuss the potential impact of helminth infection on metabolic status. An improved understanding of the mechanisms by which helminths impact on metabolic status is important. Indeed, several recent reviews of the literature investigating the association between helminths and obesity conclude that helminth infections are associated with hypophagia and weight reduction, as well as improvements in the metabolic syndrome and type 2 diabetes (Table 1).51,52

**A negative association between helminth infection and metabolic syndrome**

As few field studies with helminth infections and metabolic syndrome have been conducted, data related to helminth infection in humans are derived mostly from epidemiological studies (Table 1). One of the first studies statistically associating helminths with protection against diabetes took place in India and concluded that infection with lymphatic filariasis was negatively correlated with diabetes. Moreover, diabetic patients with lymphatic filariasis showed decreased levels of circulating inflammatory cytokines compared to non-infected diabetic patients.53 Additional studies in China reported that a history of Schistosoma infection was negatively correlated with the prevalence of type 2 diabetes and metabolic syndrome, and positively associated with improved glycaemic parameters.54,55 In Indonesia, STH infections were associated with an improvement of insulin sensitivity. Importantly, the effect was still significant when normalising for BMI.56 Another study in Australia, where the impact of *Strongyloides* infection was studied in Aboriginal population, found that *Strongyloides* infection history was negatively correlated with diabetes prevalence.57

| Table 1. The protective role of helminth infection in the context of obesity and diabetes in human and murine hosts |
|---------------------------------------------------------------|
| **Parasite or parasite products** | **Host** | **Affected parameters** | **Putative mechanisms** |
| *Wuchereria bancrofti* or *Brugia malayi* | Human (India) | Diabetes ↓ | circulating pro-inflammatory cytokines ↓ |
| *Schistosoma mansoni* or *Schistosoma japonicum* | Human (China, Uganda) | Diabetes ↓ | Unknown |
| | Human (China) | Triglycerides ↓ | Unknown |
| | LDL-cholesterol ↓ | pro-inflammatory response ↓ |
| soil-transmitted helminths | Human (Indonesia) | Insulin sensitivity ↑ | *S. mansoni*-soluble egg antigens induced: |
| *Strongyloides stercoralis* | Human (Australia, India) | Insulin and glucagon ↓ | - WAT eosinophils ↑ |
| *Nippostrongylus brasiliensis* | Mouse | Fasting glucose ↓ | - WAT M2 macrophages ↑ |
| | | Insulin sensitivity ↑ | - WAT ILC2s, Eosinophils, M2 macrophages ↑ |
| *Schistosoma mansoni* | Mouse | Body weight gain ↓ | S. mansoni-soluble egg antigen binding to CD206 induced: |
| | | Fat mass ↓ | - WAT eosinophils ↑ |
| | | Insulin sensitivity ↑ | - WAT M2 macrophages ↑ |
| *S. mansoni products* | Mouse | Body weight gain ↓ | S. mansoni-soluble egg antigens induced: |
| | | Body weight ↓ | - WAT eosinophils ↑ |
| | | Fat mass ↓ | - WAT M2 macrophages ↑ |
| | | Insulin sensitivity ↑ | *L. sigmodontis* antigen induced: |
| *Heligmosomoides polygyrus* | Mouse | Insulin sensitivity ↑ | - WAT ILC2s, Eosinophils, M2 macrophages ↑ |
| | | Fat mass ↓ | - WAT IL-33 ↑ |
| | | Weight gain ↓ | - WAT ILC2s, Eosinophils, M2 macrophages ↑ |
| *Strongyloides venezuelensis* | Mouse | Insulin sensitivity ↑ | NE production by microbiota ↑ |
| | | Intestinal Lactobacillus spp. ↑ | intestinal tight junction ↑ |
| *Litomosoides sigmodontis* | Mouse | Insulin sensitivity ↑ | - WAT ILC2s, Eosinophils, M2 macrophages ↑ |
Strongyloides infection to assess the impact of anthelmintic therapies on glycaemic parameters. The group demonstrated that Strongyloides infection was associated with improved glycaemic, hormonal and cytokine parameters of type 2 diabetes. More importantly, they could show a significant deterioration of these parameters after anthelmintic therapies.58 Two others studies in Indonesia and Uganda demonstrated similar outcomes after treatment against STHs and S. mansoni, respectively.59,60 Finally, a cross-sectional analyses of several studies linking helminth infection and metabolic syndrome confirmed the trend towards a decreased prevalence of metabolic syndrome in people with past or present helminth infection.61 Although this emerging literature is intriguing, further work is certainly required to show exactly how these effects are mediated.

The potential contribution of nutrient uptake during helminth infection to an altered metabolic status

One of the first documented impacts of experimental helminth infection on energetic metabolism relates to glucose absorption in the gut (Figure 2). In this context, infection with Heligmosomoides polygyrus, an intestinal helminth inducing chronic infection in rodents, reduced glucose absorption by the small intestine. This phenomenon was found to be dependent on STAT6 signalling via either IL-4 or IL-13 stimulation and mast cell activation.62 More recently, the effect of helminth infection on glucose absorption has been further investigated. Using another intestinal helminth, N. brasiliensis, it was demonstrated that M2 macrophages in the gut mediate the downregulation of the insulin-independent glucose transporter SGLT1. This was additionally associated with a M2 macrophage-independent downregulation of GLUT2 and a switch in the glucose absorption pathway leading to the increased expression of GLUT1.63 Considering that GLUT1 is expressed by activated T cells and M1 macrophages, this mechanism of metabolic adaption could function as an immune evasion strategy.64 Evidence exists that it may also be conserved in other helminths, as GLUT2 gene expression was reduced in diabetic mice infected

Type 2 immune cells
(Th2, ILC2s, Eosinophils, M2 macrophages, ...)

Intestinal physiology:
- Leakiness
- Villi size
- Nutrient transporters
- Mucus
- Peristalsis

Helminths or
ES products

Alteration of metabolic functions
- insulin sensitivity
- glucose metabolism
- lipid metabolism
- liver metabolism

Figure 2. Putative mechanisms by which helminth infection could alter the metabolic state of the host. Helminths typically elicit a type 2 immune response which has been associated with modulation of adipose tissue homeostasis, whole-body metabolic changes and changes in intestinal physiology. With the notable exception of S. mansoni, most of the helminth species that have been documented to alter host metabolism include life cycle stages in which the worm resides in the intestinal lumen (as depicted). Helminths can also produce excretory/secretory (ES) products, which modulate the immune response and may also modulate metabolic function. Lastly, helminths have been noted to alter the intestinal microbiota and the microbiota is widely recognised to impact both intestinal physiology and whole-body metabolism. Although research investigating the impact of helminth–microbiota interactions on the host is a rapidly expanding field, to date it is not clear to whether they contribute to the ability of helminths to alter host metabolism.
with *H. polygyrus* infected compared to uninfected diabetic mice (Figure 2).65

**Impact of helminth infection on type 2 immune cell function within adipose tissue**

As discussed earlier, IL-4 and IL-13 are critically associated with the regulation of homeostatic status within adipose tissue, indicating that helminths may impact on metabolic status by altering adipose tissue function (Table 1). *N. brasiliensis* infection in mice has been used to study adipose metabolism because of its ability to induce a strong influx of eosinophils and ILC2s into WAT. Wu *et al.* showed that *N. brasiliensis*-induced eosinophilia within the WAT was associated with an increased insulin sensitivity. Despite the acute nature of *N. brasiliensis* infection, these beneficial effects were observed even 40 to 45 days after the infection14 and were subsequently shown to be mediated via the activation of ILC2s.16 *N. brasiliensis* was further endorsed as exerting beneficial properties regarding obesity and metabolic status by another group who showed that infection was associated with weight loss, improved glycaemic parameters and decreased adipose tissue mass in an IL-13-dependent manner (Figure 2).52 Other parasites have also been linked to improved metabolic parameters in obese animals. For example, *Trichinella* infection in the intestine leads to an improved fasting glucose and insulin sensitivity that was associated with increased M2/M1 macrophage balance within the WAT (Figure 2).66 *Schistosoma mansoni* infection, or systemic injection with *S. mansoni* a soluble egg antigens, increased M2 macrophage and eosinophil populations in WAT, as well as improving insulin sensitivity during DIO in mice. Of note, adult *S. mansoni* worms establish themselves in the veins, where they mature and lay eggs. These eggs tend to accumulate in the liver, which may explain why the authors observed the impact of infection both on adipose tissue and on liver metabolism.67 Another team demonstrated that, upon *S. mansoni* antigen injection, adipocytes released IL-33 which led to metabolic improvement. These benefits relied on the activation of ILC2s and infiltration of M2 macrophages and eosinophils within the WAT.68 Whilst these data focused on the role of helminths in the regulation of adipose tissue during obesity, it is important to remember that the role of type 2 immunity within the adipose tissue also extends to adaptive thermogenesis. Recently, several studies linked the latter mechanism to helminth infection. One group showed that M2 macrophages relied on mTORC2 signalling to induce beigeing of WAT and efficiently clear *N. brasiliensis* infection.39 Finally, two other studies used *H. polygyrus*, a natural murine helminth, to investigate the ability of infection to induce adaptive thermogenesis. The first study by Su and colleagues showed that *H. polygyrus* infection provided protection against DIO by increasing thermogenesis via M2 macrophage recruitment (Figure 2).69 The second study by Shimokawa *et al.* also showed that *H. polygyrus* can provide protection against DOI by increasing thermogenesis, and further demonstrated that this effect was dependent on microbiota and NE production. Indeed, depletion of microbiota leads to the loss of the protection and a decreased level of circulating NE (Figure 2).70 This later study indicates that although helminth infections can directly impact metabolism via type 2 immune responses, they may also alter metabolic status by impacting on the microbiota (Figure 1, Table 1).

**Possible contribution of helminth–microbiota interactions on metabolism**

A role for the intestinal microbiota in regulating energetic metabolism is now fully recognised.71 The first evidence came from germ-free mice which increased their energy harvesting and fat storage, without changing their food intake, upon recolonisation with a normal complex microbiota.72 In the context of humans, a seminal study demonstrated the ability of the microbiota to regulate metabolism using human-to-mouse faecal transfer. In this study, the microbiota was taken from twin patients, whereby one twin was lean and the other obese. These microbial communities were then transferred into germ-free mice, where they replicated the lean or obese phenotype.73 Such work demonstrated a ‘causal’ contribution of the microbiota to metabolism and has since lead to the isolation of bacterial species able to improve the metabolic disease. One such species is *Akkermansia muciniphila*, which is currently being assessed as a treatment for obesity.74

Of note, intestinal helminths, such as *N. brasiliensis*, *H. polygyrus* or *Trichuris muris*, are directly at the intersection of type 2 immunity, microbiota and metabolism. These parasites all reside within the intestine as adults, where they are in close proximity to, and have co-evolved with, the microbiota (Figure 2).75 During helminth
infection, type 2 immunity orchestrates the ‘weep and sweep’ response in the gut which involves increased mucus secretion and intestinal contraction. Although the functional role of this response has long been appreciated to be the expulsion of adult worms from the intestinal lumen, it is now recognised that these same responses can mediate changes in the microbiota (Figure 2).76,77 Another mechanism that helminths could use to alter the microbiota is the secretion of excretory/secretory (ES) products, which have mainly been studied in the context of immunomodulation.78 Recent evidence showed that these products could have a direct inhibitory effect on bacteria growth (Figure 2).79 Whilst most data demonstrating the ability of helminths to alter the intestinal microbiota have been acquired through studies in rodents, evidence in humans also exists indicating this is likely to occur across multiple parasitic and host species.80–82

Helminth-induced alterations to microbial communities have important functional consequences for the host as demonstrated in a recent study from our own group that showed that H. polygyrus infection provides protection against asthma by modulating the intestinal microbiota to increase the availability of short-chain fatty acids (SCFA).80 SCFAs are a group of bacterial metabolites produced during the fermentation of fibre or mucus. The role of the type 2 immune response in the alteration of the microbiota is particularly important when considering mucus production, as mucus provides a niche for specific bacterial populations and be used as food source by others. Importantly, increased SCFA production in helminth infected mice was associated with changes in the microbial communities (Figure 2).78 Clostridiales, a bacterial family that includes potent SCFA producers and is able to induce Tregs, have been noted to increase across multiple infection models77,80,83 Interestingly, feeding of mice with SCFAs, or modulation of the SCFA signalling pathways, has been shown to alter the course of DIO and to change insulin resistance.84,85 In addition, SCFA can promote adaptive thermogenesis.85 These data raise the possibility that helminth infection may also alter metabolic status by impacting on SCFA availability.

Another family of bacteria affected by the presence of helminths are the Lactobacillaceae.86 H. polygyrus, N. brasiliensis and T. muris infections were all shown to induce an increase in Lactobacillaceae, and members of this family are widely recognised as having probiotic potential by virtue of their ability to regulate immune responsiveness.76,87,88 Recently, a study investigating Strongyloides venezuelensis and DIO showed an improvement of insulin sensitivity upon infection, and these benefits were linked to a modification of microbiota, including an increase in several species of Lactobacillus, and a reduction of intestinal permeability (Figure 2).89 As mentioned earlier, one recent study reported that the beneficial effects of H. polygyrus infection on DIO in mice were positively associated with a modification of the microbiota leading to an increased level of circulating NE.70 As also mentioned earlier, adapting to stress, such a helminth infection, requires proper energetic balance and helminths are able to alter glucose uptake within the intestine,62 likely resulting in restricted caloric intake. Interestingly, the microbiota has also been shown to play a role in helminth-induced alterations to nutrient uptake, with one group demonstrating that T. muris infection altered the nutrient processing within the gut in association with a reduced prevalence of Prevotella and Parabacteroides species (Figure 2).90

Taken together, these studies support the hypothesis that helminths can modulate host metabolic status, at least in part, by modulating the intestinal microbiota (Figure 2).

**Helminth infection and metabolic dysfunction – an evolutionary convergence for improved fitness?**

This review highlights the strong link between helminth infection and adipose tissue homeostasis, either via the type 2 immune response, via modification of the microbiota, or both. Through a long process of co-evolution, all mammalian species have adapted to the near ubiquitous presence of helminths to maximise host survival (by reducing worm burdens) and likely also to minimise host morbidity. It is notable that much of the morbidity resulting from helminth infection is asserted through malnutrition, indicating that host mechanisms to minimising or offset the effects of caloric restriction would be strongly selected for. It is perhaps not surprising, therefore, that many of the positive impacts of helminth infection noted for adipose tissue function are similar to those observed following caloric restriction.64 Finally, adaptive thermogenesis in response to helminth
infection may represent a compensatory mechanism to the potential harmful impact of helminth infection on survival during winter months, or in conditions of food scarcity. However, this latter point is based on only a few observations in laboratory models and requires further investigation. This view may provide a rationale behind the observed link between type 2 immunity, microbiota, helminth infection and adipose tissue function.

CONCLUSIONS AND FUTURE OUTLOOK

Considerations on how helminth infection may improve metabolic status presents a unique and exciting opportunity to identify novel approaches to combat the current obesity epidemic. However, to date, there are no published intervention studies assessing the effect of helminths or helminthic products on obese people. Helminth-based therapies have been used for other inflammatory diseases such as IBD; however, their effects have tended to be subtle, and currently, there is little evidence to support live helminth therapy as a treatment against inflammation-driven diseases. Instead, most work is currently focused on screening the molecular secretome of helminths as potential therapeutic candidates, with more recent work additionally fueling interest in investigating the helminth-altered microbiota as a new avenue for identifying therapeutic metabolites. Together with the expansion of multi-omics analysis, the microbiota is now considered a gold mine for the discovery of metabolites and their usage as therapies. Thus, we argue that the interaction between helminths infection and microbiota should be investigated further and could be especially useful in terms of metabolic disease.

It is not yet clear how helminths are capable of modulating adipose tissue homeostasis. It could be via secreted factors, through changes in the organs they colonise (e.g. the intestine) or solely through their ability to induce a type 2 immune response. Most of the current studies focus on adipose tissue, but there is suggestion that helminths may also alter other metabolic organs, such as the liver. It would therefore be critical to determine how helminths impact these other tissues in the context of metabolic adaptation. In conclusion, more research is needed to understand the systemic and tissue-specific metabolic adaptations associated with helminth infection to foster the possible translation of these observations into the clinic.

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CONFICT OF INTEREST

We declare no conflict of interest.

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