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1 **Highlights**

- 2 • There is an increasing requirement for the discovery of new anthelmintics with resistance to
- 3 existing drugs on the rise.
- 4 • Current strategies focus on targeting carbohydrate metabolism, with glycolytic enzymes and
- 5 transporters proving to be attractive druggable targets, however these often show a lack of efficacy
- 6 during *in vivo* infections and have a lack of specificity.
- 7 • Recent studies have unearthed the ability of helminths to manipulate short-chain fatty acid and
- 8 bioactive lipid production, as possible alternate immunomodulatory strategies for establishing
- 9 chronic infection.
- 10 • There is an urgent need to establish if these metabolic pathways are critical for parasite survival,
- 11 as these could present as an exciting new avenue for anthelmintic discovery.

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Using our understanding of interactions between helminth metabolism and host immunity to target worm survival

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Abstract

Helminths can adapt to environmental conditions in the host, utilising anaerobic processes like fermentation and malate dismutation to produce energy from carbohydrate. Although targeting carbohydrate metabolism is an established therapeutic strategy to combat helminth infection, questions remain over the metabolic pathways they employ as adults to survive and evade host immunity. Helminths also use amino acid, polyunsaturated fatty acid and cholesterol metabolism, a possible strategy favouring the production immunomodulatory of compounds that may influence survival in the host. Here, we discuss the significance of these differing metabolic pathways and whether targeting of helminth metabolic pathways may allow for the development of novel anthelmintics.

24 **Understanding helminth infections: treatment challenges and emerging strategies**

25 **Helminths** (see **Glossary**) are large multicellular organisms that infect humans and livestock, causing
26 morbidity as well as imposing an economic burden on those affected regions [1,2]. Clinically important
27 parasitic diseases including lymphatic filariasis, soil-transmitted helminthiasis and schistosomiasis are
28 considered “neglected tropical diseases”, exerting a large toll on public health but suffering from a lack
29 of attention from the public health community. These parasites develop in the host following infection,
30 establishing chronic infections in the lymphatic, gastrointestinal tract, and the blood vessels
31 respectively, a process thought to be dependent on their ability to modulate host immunity [3-5].

32 Current treatment strategies in areas endemic with helminth infections focus on mass drug
33 administrations (MDA) of a single class of anthelmintics termed the benzimidazoles [6]. While MDA
34 has demonstrated safety and efficacy in reducing the prevalence of STH infection, sustained MDA was
35 more effective when combined with improved sanitation and economic development [7,8]. Concerns
36 regarding the development of anthelmintic resistance, in particular, have prompted questions about
37 the sustainability of using intensive application of MDA and have called for the development of novel
38 anthelmintics [9]. This demand for new anthelmintics is also paramount in the veterinary sector to
39 address the escalating issues of drug resistance and prevent economic losses associated with
40 decreased productivity and compromised animal welfare [10,11].

41 In an effort to counteract the emerging resistance to existing anthelmintics, and based on the
42 advancing understanding of helminth metabolomes, helminth metabolism has been proposed as a
43 valid target for developing novel anthelmintics [12-14]. In this review we discuss the development of
44 anthelmintics targeting helminth metabolism for both veterinary and human infections, including in
45 pre-clinical rodent models of human helminth infection. We begin by reviewing efforts to target
46 helminth carbohydrate metabolism, including the unique metabolic adaptations helminths make to
47 their host environment. We then explore the potential to target unique features of helminth amino
48 acid and lipid metabolism, which may allow for helminth-specific drug targeting. Based on our
49 increasing knowledge of how metabolites of the amino acid and lipid metabolism pathway influence

50 host immune responses, we propose that targeting helminth regulation of metabolite production
51 presents an exciting strategy for the development of new anthelmintics.

52

53 **Metabolic processes in living organisms: overview and adaptations**

54 Metabolism refers to the sum of chemical reactions a living organism employs to convert amino acids,
55 carbohydrates and lipids from food into life-sustaining molecules. These enzymatic pathways generate
56 the energy required to run processes in the cell, form the building blocks required for life and eliminate
57 metabolic waste [15]. In the presence of oxygen, most differentiated mammalian cells employ aerobic
58 respiration to generate up to 36 molecules of adenosine triphosphate (ATP) from one glucose molecule
59 via 3 steps: glycolysis of glucose to pyruvate, generation of acetyl coenzyme A (acetyl-CoA) via the
60 tricarboxylic acid (TCA) cycle and oxidative phosphorylation (electron transport) (**Figure 1**) [15]. When
61 oxygen is not present, pyruvate undergoes **fermentation** to lactic acid or ethanol, yielding 2 molecules
62 of ATP [15]. Some unicellular organisms prefer fermentation to aerobic respiration, even in the
63 presence of abundant oxygen, for example, yeast species such as *Saccharomyces cerevisiae* produce
64 ethanol as “waste” from fermentation, whereas *Lactobacillus* bacteria produce lactic acid [16].
65 Multicellular organisms such as helminths can utilise aerobic respiration or fermentation, switching
66 metabolic pathways during their life cycle, when oxygen concentration in host tissues is low (**Figure 1**)
67 [17-19]

68

69 **Carbohydrate metabolism: the current target for anthelmintics**

70 As a first step in carbohydrate metabolism, glycolysis is highly conserved among living organisms and
71 used by most, therefore presented as an attractive target for development of anthelmintics
72 [12,20,21]. Genomic data has demonstrated that helminths contain homologues of mammalian
73 glycolytic enzymes, including **hexokinase**, **glucose-6-phosphate isomerase** and **pyruvate kinase**
74 [12,22,23]. The commercially available anthelmintic, Clorsulon, targets the adult liver fluke glycolytic
75 enzymes **phosphoglycerate kinase** and **phosphoglyceromutase**, effectively reducing *Fasciola hepatica*

76 egg **fecundity** in infected cattle [24]. Clorsulon is not metabolised and is secreted in milk therefore,
77 treatment of lactating cows and sheep is forbidden to prevent human consumption [25]. As with many
78 existing anthelmintics, cases of resistance to the drug are reported to be rising [26]. *In vitro*, the
79 antimalarial drug mefloquine reduced activity of *Schistosoma mansoni* **enolase**, which is responsible
80 for the reversible conversion of D-2-phosphoglycerate and phosphoenolpyruvate (PEP) in glycolysis
81 and gluconeogenesis. The drug also reduced juvenile and adult *S.mansoni* worm burden in mice
82 between 80.4-97.8 percent [27]. However, the toxic effects of mefloquine on host cells were concluded
83 to pose a challenge to the intensive use of this drug [28].

84 Derivatives of both glucose and pyruvate have been shown to target hexokinase activity, the enzyme
85 which catalyses the first step in glycolysis [29]. Use of 5-thio-D-Glucose (5-TG) effectively reduced *S.*
86 *mansoni* hexokinase activity but had unwanted side effects, including increased hyperglycaemia and
87 temporary infertility in male mice [30,31]. Use of 3-bromopyruvate (3-BrPA) was reported to exhibit
88 anthelmintic activity against *S. mansoni in vitro* and *Echinococcus multilocularis* metacestodes *in vivo*
89 [32,33]. Although 3-BrPA showed effective anthelmintic activity, treatment *in vivo* resulted in
90 significant adverse effects, such as dose-related hepatotoxicity following intraarterial administration
91 [34]. Furthermore, 3-BrPA was reported to inhibit human hexokinase II, as well as multiple enzymes
92 involved in glycolysis, fermentation and the TCA cycle *in vitro*; therefore the use of 3-BrPA as a potential
93 new anthelmintic has not been pursued [35].

94 Studying the **obligate symbiotic** relationship between *Wolbachia* and the filarial host, genomic analysis
95 revealed that *Brugia malayi* encoded genes for all the enzymes involved in glycolysis, whereas
96 *Wolbachia* did not encode hexokinase, 6-phosphofructokinase, and pyruvate kinase, required for
97 converting glucose into pyruvate [36,37]. Voronin *et al.*, demonstrated that external supplementation
98 with pyruvate increased the *Wolbachia* population in adult male *Brugia pahangi*, whereas inhibition
99 using the pyruvate analogue 3-BrPA significantly reduced the *Wolbachia* population in these worms.
100 3-BrPa treatment also significantly reduced the number of microfilariae secreted by adult female
101 worms and the viability of these worms [38]. Furthermore, *in vitro* addition of an inhibitor specific for

pyruvate kinase reduced the *Wolbachia* population in male worms by 5-fold and the number of microfilariae released from female worms by at least two-fold [38]. A limitation of the use of current anthelmintics for filarial infections, such as Ivermectin, is the triggering of adverse reactions in patients co-infected with the filaria *Onchocerca volvulus* and *Loa loa* [39,40]. Although revealing the metabolic mechanisms of this mutualistic interaction between *Brugia* and *Wolbachia* has allowed the discovery of several novel potential drug targets for adult filaria [38], an important consideration is the *in vivo* effectiveness of these drugs, particularly in the context of filarial co-infection.

It is important to acknowledge a new generation of inhibitors of glycolysis identified in clinical trials for cancer [41,42]. Lonidamine and benserazide are examples of human hexokinase inhibitors known for their high specificity for the enzyme; and demonstrate anti-cancer properties by effectively inhibiting glycolysis within cancer cells [43,44]. The natural product licochalcone A, a component of the plant *Glycyrrhiza uralensis* Fisch, has also been shown to inhibit human hexokinase activity *in vitro* [45,46]. Furthermore, there is evidence *in vitro* of lonidamine and licochalcone A showing anthelmintic properties, highlighting the possibility for repurposing these drugs for *in vivo* investigations [47,48].

Helminth adaption to anaerobic respiration in the host

Targeting glycolysis presents as an attractive target for the development of anthelmintics. However, several helminths utilise anaerobic respiration upon entering their definitive host, highlighting this life-cycle adaptation as a feasible target for novel drugs. For example, while the free-living larvae of *Ascaris suum* utilise oxidative respiration, converting glucose into ATP through the TCA cycle, adult *A. suum* do not express cytochrome c oxidase, the terminal enzyme necessary for aerobic respiration, and instead rely on anaerobic respiration [49,50]. It was proposed that arrival and development of juvenile *F. hepatica* within the anaerobic conditions of the bile duct prompts replacement of the helminth TCA cycle with production of propionate and acetate, formed via the **dismutation** of malate into pyruvate and succinate in the mitochondria of the parasite (**Figure 1**) [51,52]. Helminths including *S. mansoni*

and *B.pahangi* express high levels of pyruvate kinase (PK) activity alongside lactate dehydrogenase (LDH), further metabolising pyruvate to lactic acid during fermentation, whereas *Ascaris lumbricoides*, *Hymenolepis diminuta* and *F. hepatica* express high levels of phosphoenolpyruvate carboxylase (PEP-CK) activity alongside malate dehydrogenase (MDH) to produce malate [36,53,54]. Several benzimidazole anthelmintics were found to inhibit MDH, although it is not their sole and primary target [55]. Interestingly, this study also found clear differences in total MDH activity in distinct sexes and species of helminths, such as *A. suum* and *F. hepatica* [55], which raises implications for the development of drugs targeting MDH as a broad-spectrum anthelmintic.

During mammalian aerobic respiration, complex II, a succinate dehydrogenase, catalyses the oxidation of succinate to fumarate with the reduction of ubiquinone to ubiquinol [56]. The latter step requires electron transfer from complex I, (an NADH-ubiquinone oxidoreductase), the first enzyme of the mitochondrial electron transport chain [56]. Under anaerobic conditions, adult *A.suum* converts Malate into Fumarate via a fumarate hydratase (FH) (**Figure 1**). Here adult *A.suum* complex II functions in the reverse direction, converting Fumarate into Succinate as a fumarate reductase (FDR), a reaction coupled to the electron transfer from complex I by rhodoquinone (RQ) [57] (**Figure 2**).

This is in contrast to the *Ascaris* larval life-stage, which exhibits lower fumarate reductase activity, higher ubiquinone content and succinate dehydrogenase activity [58]. Such variation in the expression of metabolic enzymes across differing life-stages creates both challenges and opportunities for drug targeting. For instance, increasing concentrations of mitochondrial fumarate reductase inhibitors have been demonstrated to significantly decrease the viability of *Echinococcus multilocularis* larvae *in vitro* [59]. The RQ-dependent mechanism of anaerobic metabolism serves as a critical difference between parasites and their mammalian host [17]. Using *Caenorhabditis elegans* as a model organism, Lautens *et al.* identified 9 genes that are required for RQ-dependent metabolism in *C. elegans*, suggesting that their orthologues in helminths play similarly crucial roles [17]. Furthermore, Lautens *et al.* demonstrated that 3 of these genes encode proteins with an active sites that differs between several STH and their mammalian hosts, thus allowing the development of helminth-specific inhibitors [17].

This approach, which enhances our understanding of helminth-specific biology and metabolic machineries, has the potential to identify high-confidence targets for novel anthelmintics.

Alternate approaches for uncovering novel anthelmintics have included *in vitro* screening for inhibitors of NADH-fumarate reductase activity [60]. This approach revealed nafuredin, a compound derived from *Aspergillus niger*, which inhibited NADH-fumarate reductase in adult *A.suum*, as well as NADH-ubiquinone and NADH-rhodoquinone reductase activity in *A.suum* adults and larvae [60]. Notably, this compound did not impact on rat liver complex I or complex II activity [60]. In addition to being a markedly selective inhibitor of helminth complex I, this study reported exerted excellent anthelmintic activity of the drug against *Haemonchus contortus*, significantly reducing egg output in sheep by over 90 percent [60]. However, subsequent screening for inhibitors based on the same strategy led to the discovery of harzianopyridone and its chemically related atpenins. These compounds were found to be potent inhibitors of mammalian complex II, as well as *A.suum* NADH-fumarate reductase, thus limiting their use as anthelmintics [61].

Exploiting substrates other than glucose for metabolism: the hidden secret to helminth survival?

Due to the specific locations inhabited by certain helminths, such as the cecum in the case of whipworms, there is a scarcity of glucose available for carbohydrate metabolism, as glucose absorption primarily occurs in the small intestine [62]. Consequently, these helminths may resort to alternative metabolic pathways for their survival, pathways that are more readily available in their specific environmental conditions.

Genetic analysis of 4 tapeworm species revealed unique features of the metabolism of these organisms, which could be exploited for development of novel anthelmintics. High conservation was found between Cestode and both human and mouse carbohydrate metabolism pathways, whereas lower conservation was found in amino acid and lipid metabolism pathways [63]. Furthermore, these organisms encode fatty acid transporters and lipid elongation enzymes and have tapeworm specific gene families such as fatty acid binding protein (FABP) and the apolipoprotein antigen B, allowing them

to scavenge essential lipids from the host [63]. Several lipid binding proteins are secreted by helminths, were found to be structurally different from those of their host [64] and may serve as potential novel drug targets to disrupt worm survival without affecting host metabolism [63].

In the next two sections of this review, we first discuss our knowledge of helminth amino acid metabolism, then lipid metabolism, highlighting studies that provide evidence supporting the rationale for developing drugs targeting these pathways. We also provide evidence to propose that targeting helminth production of metabolites from both pathways may present a novel strategy to boost host immunity and eradication of helminths.

The importance of amino acid metabolism for helminths

Helminths possess similar pathways of amino acid metabolism to mammals [12,65], nonetheless, disparities do exist in the structure of some metabolic enzymes, notably concerning the metabolism of sulphur amino acids: arginine and proline [66,67]. For example, *S. mansoni* arginase (SmARG) displays similar functional activity to the mammalian enzyme, catalysing the hydrolysis of L-arginine to L-ornithine, but activity relies on unique disulphide bonds [68]. SmARG is expressed in all life-cycle stages of *S. mansoni* and is hypothesized to play a role in immune evasion and reduced killing of *S.mansoni* larvae through the depletion of the L-arginine substrate; typically utilised for nitric oxide (NO) biosynthesis [69]. By contrast, use of arginase-deficient *Leishmania mexicana* demonstrated the importance of enzyme activity in promoting the infection of mice, suggesting this pathway may present a new target for anthelmintic design [70].

Although dietary fibre fermentation is the primary source of short-chain fatty acids (SCFAs), organisms such as the intestinal microbiota can also generate metabolites from protein fermentation, such as short-chain (SC) and branched-chain fatty acids (BCFAs), including acetate (derived from glycine and L-alanine) and isovalerate (derived from leucine) [69]. Both SCFA and BCFA can influence host immune cell function [71,72], thus helminth production of these metabolites may be beneficial for helminth survival by promoting immune-evasion. For example, acetate, propionate and isobutyrate were

detected in the immune-regulatory excretory secretory products of *Trichuris muris* and adult *Ancylostoma caninum* [73,74], with acetate cited as a main end product of energy metabolism for *F. hepatica*, *H. contortus* and *A. suum* [75]. Supplementation of *Heligmosomoides polygyrus* infected mice with the BCFA isovalerate in the drinking water caused a significant increase in egg fecundity [76]. This significant impact on adult worm egg production, coupled with the absence of effects on host intestinal motility and immune cell function suggests helminth production of these amino acid metabolites may play a role in determining worm fitness. This demands further investigation to identify a potential new target for drug development.

Linking helminth modulation of lipid metabolism to the host immune response

Evidence suggests that helminths adapt their lipid profile on contact with the host and during development. Data from Proudfoot *et al.*, identify how the surface of the infective larvae of *Acanthocheilonema viteae*, *Nippostrongylus brasiliensis*, *Trichinella spiralis*, and *Ostertagia ostertagi* becomes more lipophilic on exposure to conditions simulating entry into a mammalian host [77]. By utilising **liquid chromatography/mass spectrometry** (LC/MS), Giera *et al.*, identified lifecycle-specific lipid profiles in *S. mansoni*, including differences in membrane and storage lipids and bioactive lipid mediators, such as arachidonic acid being highly expressed in adult worms but barely detectable in other lifecycle stages [78].

Metabolic products of dietary **polyunsaturated fatty acids** (PUFA), including prostaglandins and thromboxanes have been detected in different lifecycle stages of helminths, as well as within their excretory/secretory products [78,79]. The production of oxylipins from PUFA play an important role in regulation of the host immune response [80], thus helminth production of these metabolites may have implications for helminth survival. Both prostaglandin E₂ (PGE₂) and prostaglandin D₂ (PGD₂) are detectable in *S. mansoni* eggs, with PGE₂ thought to condition dendritic cells to prime type-2 cytokine production by activated T cells [78,81], whereas the presence of PGE₂ and PGD₂ in the lipidome of *S. mansoni* cercariae has been linked to limiting the ability of the host to mount a protective immune

232 response against cercariae [78,82]. PGE₂ was found in both larval forms and adult excretory/secretory
233 products of *Trichuris suis*, where adult PGE₂ modified dendritic cell activation and reduced
234 inflammatory cytokine production by binding PGE₂ receptors [83]. Interestingly, unlike mammalian
235 production of PGE₂, secretion of PGE₂ by *T.suis* was not dependent on the action of a cyclooxygenase
236 (COX) enzyme [83]. By understanding how it is made, we raise the potential to prevent helminth
237 immune modulation by targeting the helminth-specific production of this bioactive lipid. Finally, the *S.*
238 *mansoni* cercariae lifecycle stage contained high levels of lipoxygenase (LOX)-derived oxylipins such as
239 resolvin E2 (RvE2), protectin DX (PDX), and protectin D1 (PD1) [78]. These LOX products are referred
240 to as docosanoids, and are specialised pro-resolving mediators that can suppress inflammatory
241 immune responses and are important for promoting resolution of inflammation [84]. Once cercariae
242 have penetrated the skin they remain for 3-4 days before entering circulation, and it is proposed that
243 production of these pro-resolving mediators during this period increases their chance of survival as
244 these factors can suppress recruitment of immune cells and anti-cercarial immune responses [78,85].
245 Evidence is also accumulating that helminths may manipulate PUFA metabolism in the host via
246 secretion of metabolic enzymes. Following *B. malayi* infection, illumina sequencing demonstrated that
247 macrophage populations expressed high levels of LOX and COX enzymes involved in metabolism of
248 arachidonic acid to produce prostaglandin, thromboxane and hydroxyeicosatetraenoic acid (HETE)
249 [86]. Analysing LC/MS and transcriptomics of **bone marrow-derived macrophages** (bmDMs), Jimenez
250 *et al.*, demonstrated shifts in eicosanoid production and the expression of PUFA metabolic enzymes
251 following *H. polygyrus* larvae (HpbE) exposure [87]. They showed that transfer of HpbE-treated bmDMs
252 deficient in microsomal prostaglandin E synthase (mPGES) failed to reduce the number of eosinophils
253 infiltrating the lung during house dust mite (HDM) airway inflammation, when compared to HpbE-
254 treated mPGES-sufficient bmDMs (**Figure 3**) [87]. Fractionation and proteomics revealed that
255 prostaglandin production by human monocyte-derived macrophages was replicated following
256 treatment with a secreted *H. polygyrus* **glutamate dehydrogenase** (GDH). Moreover, administration of
257 recombinant *H. polygyrus* GDH attenuated HDM-triggered eosinophil infiltration and lung

inflammation [87]. The larvae's capacity to secrete this enzyme implies that the manipulation of these metabolic pathways could play a crucial role in altering immune responses that regulate the establishment of helminth infections, unveiling a potential new candidate for infection prevention.

The final group of lipid metabolites which could contribute to helminth survival by influencing host immune responses are bile acids (BAs), a group of cholesterol-derived molecules deposited into the gut to aid with fat digestion [88,89]. They are thought to promote intestinal homeostasis, limit pro-inflammatory cytokine production and increase the proportion of CD4+Foxp3+ regulatory T cells (Treg) through binding to **nuclear receptors**, including farnesoid X receptor (FXR) (**Figure 4A**) [89]. Lane *et al.* report that infection with *H. polygyrus* disrupted BA homeostasis in infected mice, resulting in reduced concentrations of specific taurine-conjugated primary BAs (T- α -MCA and T-CDCA), reduced expression of BA transporter genes in the proximal small intestine and reduced signalling through the nuclear receptor FXR (**Figure 4B**) [88]. Based on how bile acids influence the immune response (**Figure 4A**), we can propose how *H. polygyrus* modification of FXR signalling may alter immunity (**Figure 4B**). While it was suggested that shifts in the microbiota and a resultant increased activity of specific **bile salt hydrolase** (BSH) enzymes may explain reductions in taurine-conjugated primary BA production, it remains to be established how BSH activity changes following *H. polygyrus* infection [88]. Moreover, it remains unknown whether diminished FXR signalling following *H. polygyrus* infection contributes to the long-term presence of the helminth and whether this pathway presents viable targets for the development of novel anthelmintics.

Experiments by Sukhdeo and Croll demonstrated that bile activated the motility of *H. polygyrus* (previously named *Nematospiriodoides dubius*) and demonstrated that removal or reduction of bile resulted in reduced larval establishment *in vivo* [90]. Both the liver fluke *Opisthorchis viverrini* and the blood fluke *Schistosoma haematobium* excrete oestrogens and oxysterols, steroid compounds known to alter cholesterol levels and promote the production of BAs, respectively [91]. Using radiolabelled cholesterol, male schistosomes were proposed to transfer cholesterol and labelled metabolites to female worms [92], where manipulation of steroid metabolism during *S. mansoni* infection significantly

reduced the number of miracidia excreted in faeces of mice [93]. In addition, pharmacological inhibition of cytochrome p450, required for the enzymatic generation of these molecules, resulted in 100 percent mortality of juvenile *Opisthorchis felinus* liver flukes and adult *S.mansoni* worms *in vitro* [94,95]. Interestingly, cytochrome p450 expression was shown to differ in the lifecycle states of *S.mansoni* and *H.contortus* [95,96], with a decrease in *H.contortus* activity from L3 to adult coinciding with transition from aerobic condition outside the host to anaerobic conditions inside the host [97]. The low sequence identity of *S.mansoni* cytochrome p450 to the human protein [95] makes it an attractive target for anthelmintics, in addition, the unique lack of motifs found in the cytochrome p450 proteins of Trematodes and Cestodes [98] present structurally distinct candidates for drug design. However, cytochrome p450 is involved in other metabolic processes and production of oxysterols can occur through non-enzymatic mechanism, therefore it is important to further characterise the contribution of this metabolic pathway to helminth survival before exploring development of novel anthelmintics that target it.

Concluding remarks

The need for new and novel anthelmintics is a pressing global health issue, with reported resistance to current treatment regimens on the rise. The potential for targeting metabolic enzymes in helminths represents an exciting avenue for therapeutics. Additional insight is needed into the biochemistry and structure of the parasite enzymes to avoid impact on host enzymes and unwanted side-effects. Furthermore, it is critical to define the importance of a range of metabolic pathways in determining regulation of host immunity and subsequent helminth survival, before the design of new anthelmintics can occur (see **Outstanding Questions**).

Existing anthelmintics have limitations, including their promiscuous targeting of multiple metabolic pathways, as well as eliciting unwanted side-effects in the host. The adaptation of helminth carbohydrate metabolism to the host has opened new avenues for drug development. However, these life-stage specific and species-specific differences in enzyme expression create challenges for the

development of broad-spectrum preventative, as well as therapeutic drugs. *In vitro* screening approaches for finding new inhibitors have proven problematic, as exemplified by the discovery of compounds that inhibit both host and parasite NADH-fumarate reductase activity [60,61]. Recent development of genome-scale metabolic models may provide a new approach to accelerate the identification of novel targets through drug repurposing [99].

The possibility remains that there are other metabolic processes helminths manipulate to survive, which may present new avenues for the development of novel anthelmintics. The implications of helminth manipulation of amino acid and lipid metabolism on parasite persistence are discussed in this article.

Ultimately, the discovery of new anthelmintics is reliant on three factors; the need for selective inhibitors, the development of readily available parasitic helminth cell culture systems and robust pre-clinical models in which to understand the influence of helminth metabolism on survival. The effectiveness of compounds and their impact on altering helminth metabolic activity and ATP production can only be assessed through *in vitro* systems. Connecting this to immune modulation and survival, however, necessitates the use of *in vivo* animal models. Recent literature has seen an advancement of the development of *in vitro* helminth models, replicating early interactions between the intestinal epithelium and *T. muris* using two-dimensional caecal organoids [100]. This inexpensive and comparatively simplistic *in vitro* model could allow for the investigation of anthelmintics targeted against several metabolic pathways implicated in helminth establishment (see **Outstanding Questions**).

Declaration of interests

The authors declare no competing interests.

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592

593 Glossary

594 **Bile salt hydrolase:** an enzyme that catalyses the hydrolysis of amide bonds in conjugated bile acids.

595 **Bone-marrow derived macrophages:** macrophage cells that are generated in a research laboratory
596 from mammalian bone marrow cells.

597 **Disability-adjusted life years:** represents the loss of the equivalent of one year of full health.

598 **Dismutation:** a reaction between two molecules in which one is reduced and the other is oxidized.

599 **Enolase:** catalyses the conversion of 2-phosphoglycerate to phosphoenolpyruvate, the ninth and
600 penultimate step of glycolysis.

601 **Fecundity:** a measure of the reproductive capacity of an individual or population.

602 **Fermentation:** an anaerobic process in which energy can be released from glucose even if oxygen is
603 not available.

604 **Fumarate reductase:** the enzyme that converts fumarate to succinate.

605 **Glucose-6-phosphate isomerase:** catalyzes the interconversion of glucose-6 phosphate and fructose-
606 6 phosphate.

607 **Glutamate dehydrogenase:** deaminates the amino acid glutamate into alpha-ketoglutarate.

608 **Helminth:** a parasitic worm from the Nematode or Platyhelminth phyla.

609 **Hexokinase:** the initial enzyme of glycolysis, catalyzing the phosphorylation of glucose by ATP to
610 glucose-6-phosphate.

611 **Liquid chromatography-tandem mass spectrometry:** an analytical chemistry technique that combines
612 the physical separation capabilities of liquid chromatography with the mass analysis capabilities of
613 mass spectrometry.

Nuclear Receptors: a family of transcription factors that play an important role in a number of biological processes including metabolism, reproduction, and inflammation.

Obligate Symbiotic: at least one of the organisms that biologically interact with each other cannot complete its lifecycle on its own.

Phosphoglycerate kinase: catalyses the reversible transfer of a phosphate group from 1,3-bisphosphoglycerate to ADP producing 3-phosphoglycerate.

Phosphoglyceromutase: catalyses the conversion of 3-phosphoglycerate to 2-phosphoglycerate during glycolysis.

Polyunsaturated fatty acids: hydrocarbon chains containing two or more double bonds, and are characterised as either an omega-3 or omega-6 PUFA which refers to the position of the first double bond relative to the methyl end of the fatty acid.

Pyruvate kinase: catalyses the direct transfer of phosphate from phosphoenolpyruvate to ADP to produce ATP and pyruvate.

Rhodoquinone: a modified ubiquinone-like molecule that is an important cofactor used in anaerobic energy metabolism by many organisms.

Figure legends

Figure 1. Helminth metabolic adaptations to the host environment. In the presence of oxygen, immature L2 larvae of *A. suum* produce high levels of ATP through aerobic respiration, comprising 3 processes: glycolysis, the tricarboxylic acid (TCA) cycle, and oxidative phosphorylation (shown in blue). In contrast, L4 *A. suum* adults lack a functional TCA cycle and produce ATP and pyruvate. Helminths like *S. mansoni* and *B. pahangi* express highly active pyruvate kinase (PK) and lactate dehydrogenase (LDH), further metabolizing pyruvate to lactate during fermentation (shown in green), whereas *A. lumbricoides*, *H. diminuta* and *F. hepatica* express high levels of phosphophenyl pyruvate carboxykinase (PEPCK) and cytosolic malate dehydrogenase (cMDH) and produce oxaloacetate and malate during anaerobic respiration (shown in orange). Two examples of drugs which target helminth glycolytic enzymes are shown, with mefloquine targeting enolase and Clorsulon targeting phosphoglycerate kinase (PGK). Original figure using information from Tielens *et al*, 1987 [52]. Created with BioRender.com.

Figure 2. The electron transport chain under aerobic conditions (*A.suum* larvae) and anaerobic conditions (*A.suum* adults). In *A.suum* larvae, electrons from NADH or Succinate are shuttled to

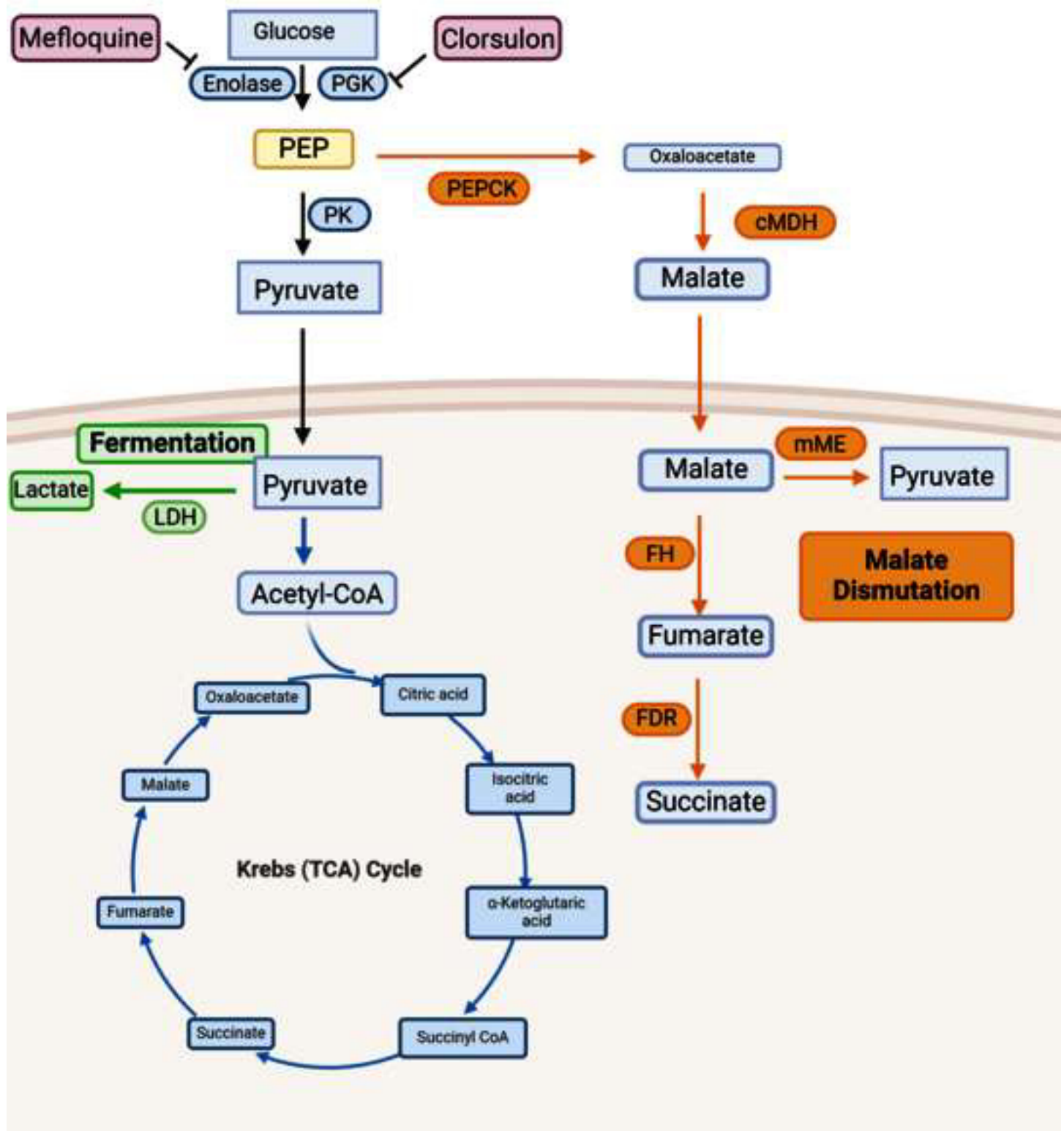
ubiquinone (UQ) via Complex I (I) or Complex II (II) respectively, to generate NAD (NAD⁺), Fumarate and ubiquinol (UQH₂). Electrons from UQH₂ are transferred to complex III (III), cytochrome c and ultimately O₂ via Complex IV. Complex V uses the proton gradient caused by release of protons from complex I, III and IV to synthesize ATP. In *A.suum* adults, electrons from NADH are shuttled to rhodoquinone (RQ) to generate rhodoquinol (RH₂). The RQ pool is regenerated via Complex II (II) functioning as a fumarate reductase to generate Succinate from Fumarate. Created with BioRender.com.

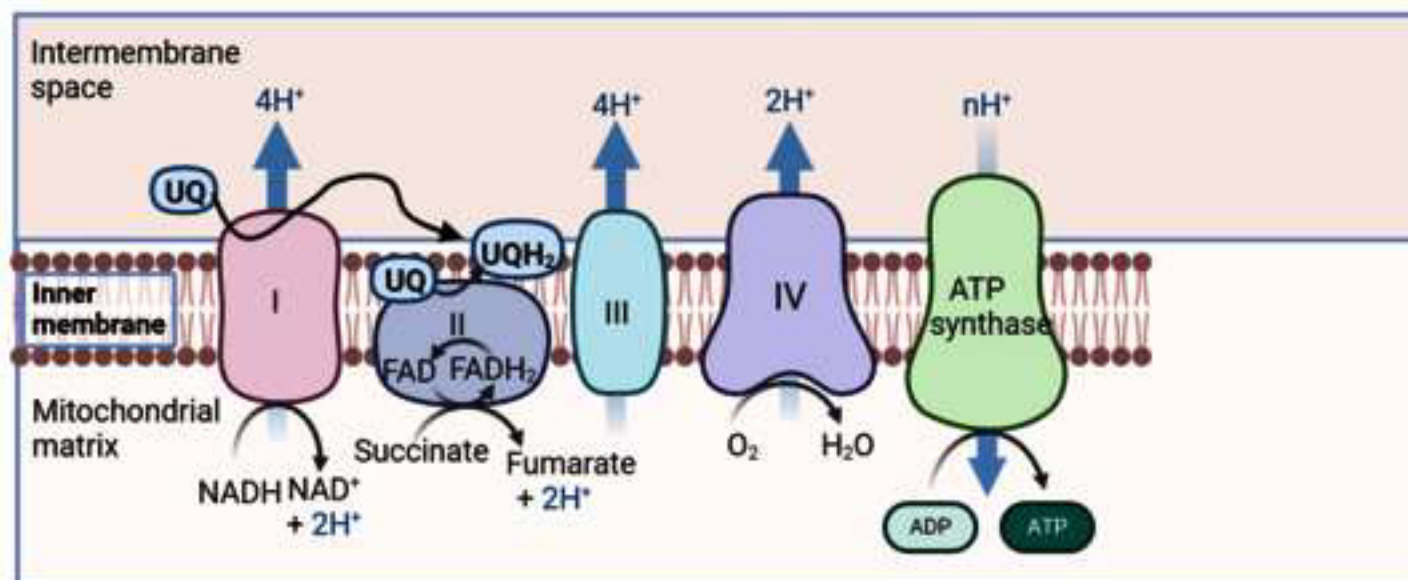
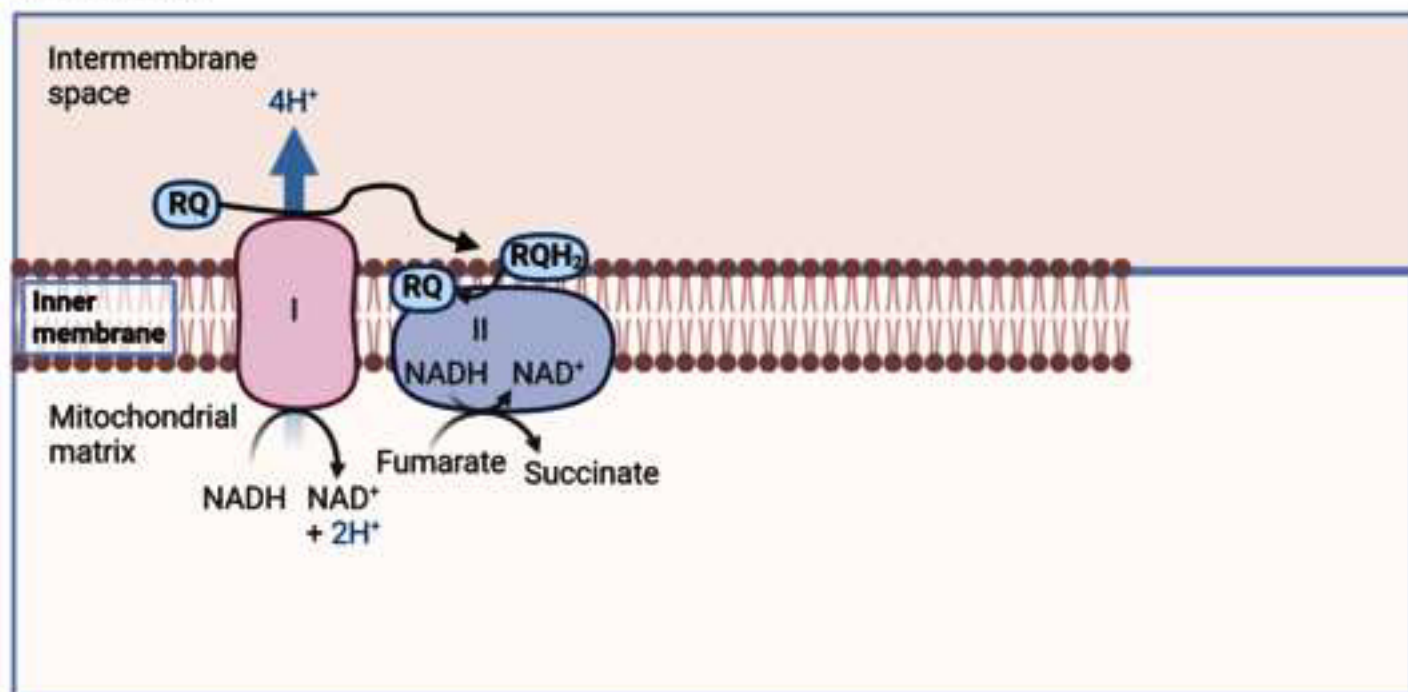
Figure 3. Impact of *H. polygyrus* infection on a human dust mite airway allergy *in vivo* model. The addition of *H. polygyrus* infection causes arachidonic acid to be metabolized by cyclooxygenase (COX) to form prostaglandins, which are further metabolized by microsomal-prostaglandin E synthase (mPGES) to form prostaglandin E₂ (PGE₂). The excretory/secretory products (HES) produced by *H. polygyrus* contain a glutamate dehydrogenase (GDH) enzyme homologue, further increasing the production of PGE₂ from human derived bone marrow macrophages (BMDMs) treated with recombinant GDH. This increase in PGE₂ downregulates eosinophil production, leading to an overall decrease in airway inflammation. In contrast, when COX^{-/-} or mPGES^{-/-} mice were used no change in eosinophil production observed. This indicates that *H. polygyrus* is able to dampen the inflammatory response during HDM-induced airway allergy via the increased production of PGE₂. Original figure created using information from Jimenez *et al*, 2020 [87]. Created with BioRender.com.

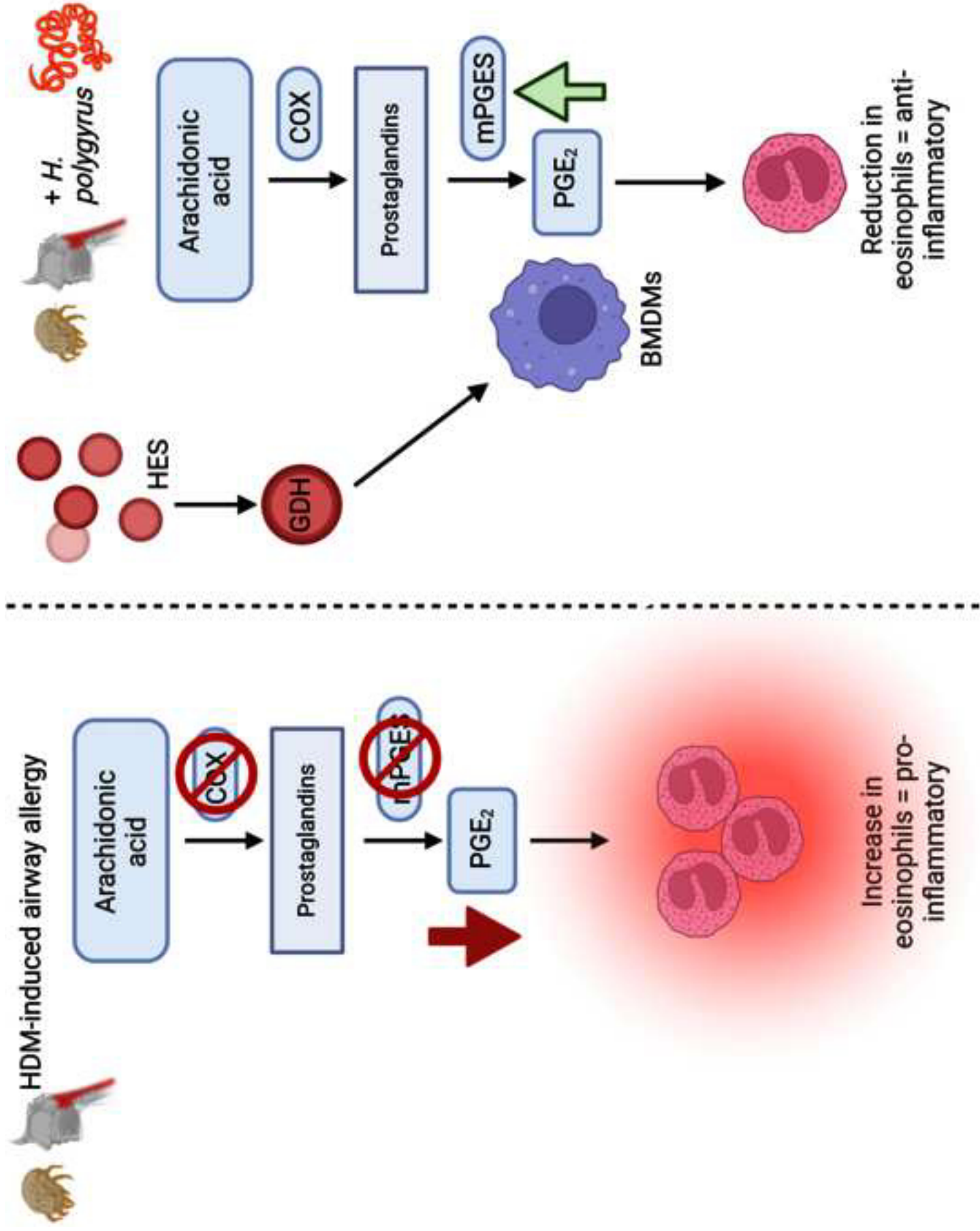
Figure 4. Impact of *H. polygyrus* infection on bile acid homeostasis and signalling. (A) Taurine-conjugated primary bile acids (BAs) such as T- α -MCA and T-CDCA are present in high concentrations in the small intestinal lumen. Bile acid transporters (BAT) present in intestinal epithelial cells are required for the reuptake of BAs from the intestinal lumen. Once transported from the lumen, BAs bind to nuclear receptors present on the cell surface of intestinal immune cells, e.g. the nuclear farnesoid X receptor (FXR). The subsequent receptor signalling cascade results in regulation in intestinal mucosal immunity. **(B)** Following *H. polygyrus* infection, the concentration of T- α -MCA and T-CDCA in the lumen, the expression of genes encoding BATs and FXR signalling was significantly reduced. Reduced FXR signalling may result in an increase in IL-6 and IL-12 production and a decrease in T regulatory cells (Treg). Original figure created using information from Lane *et al.*, 2023 [88] and Chen *et al.*, 2019 [89]. Created with BioRender.com.

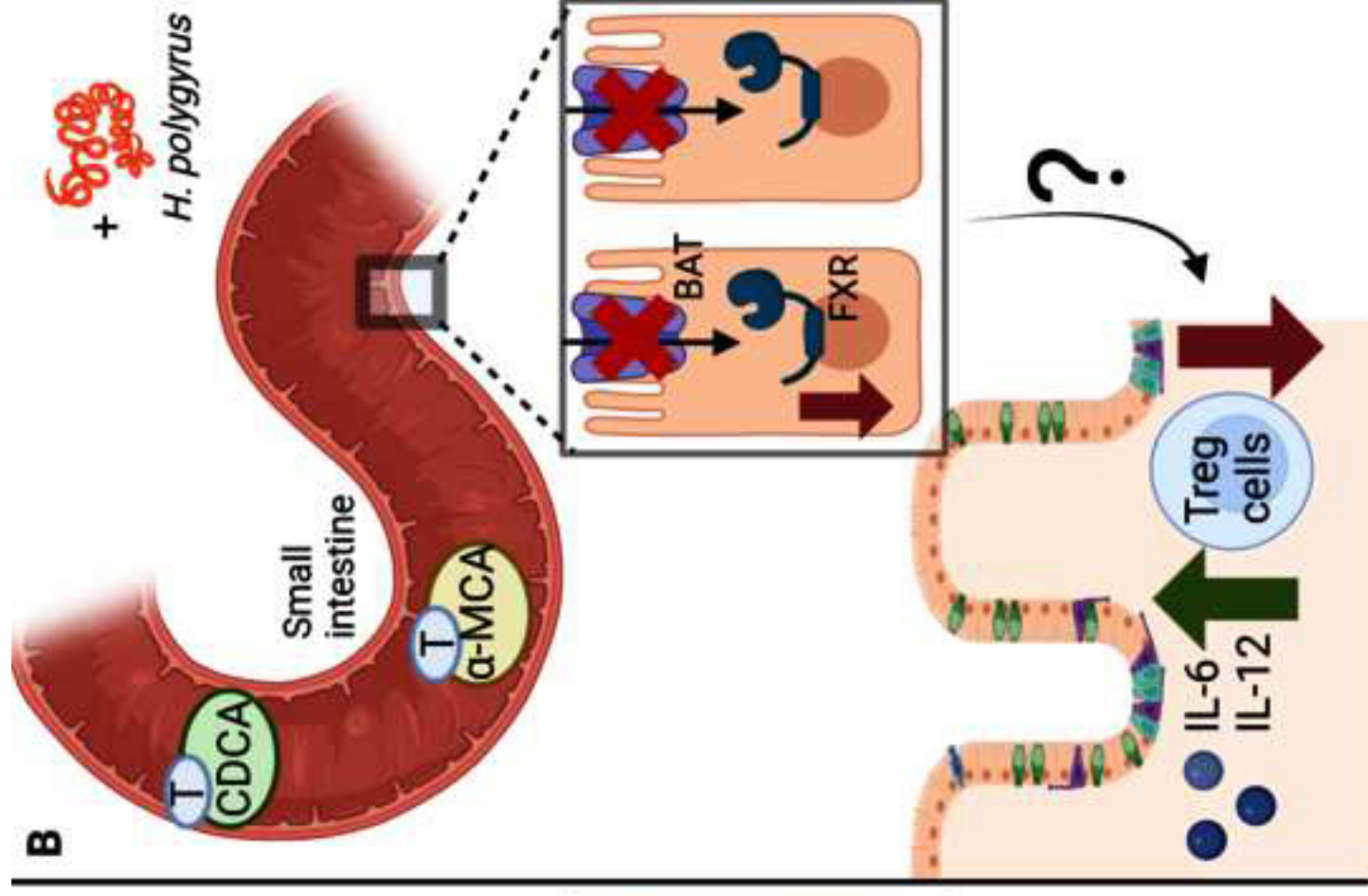
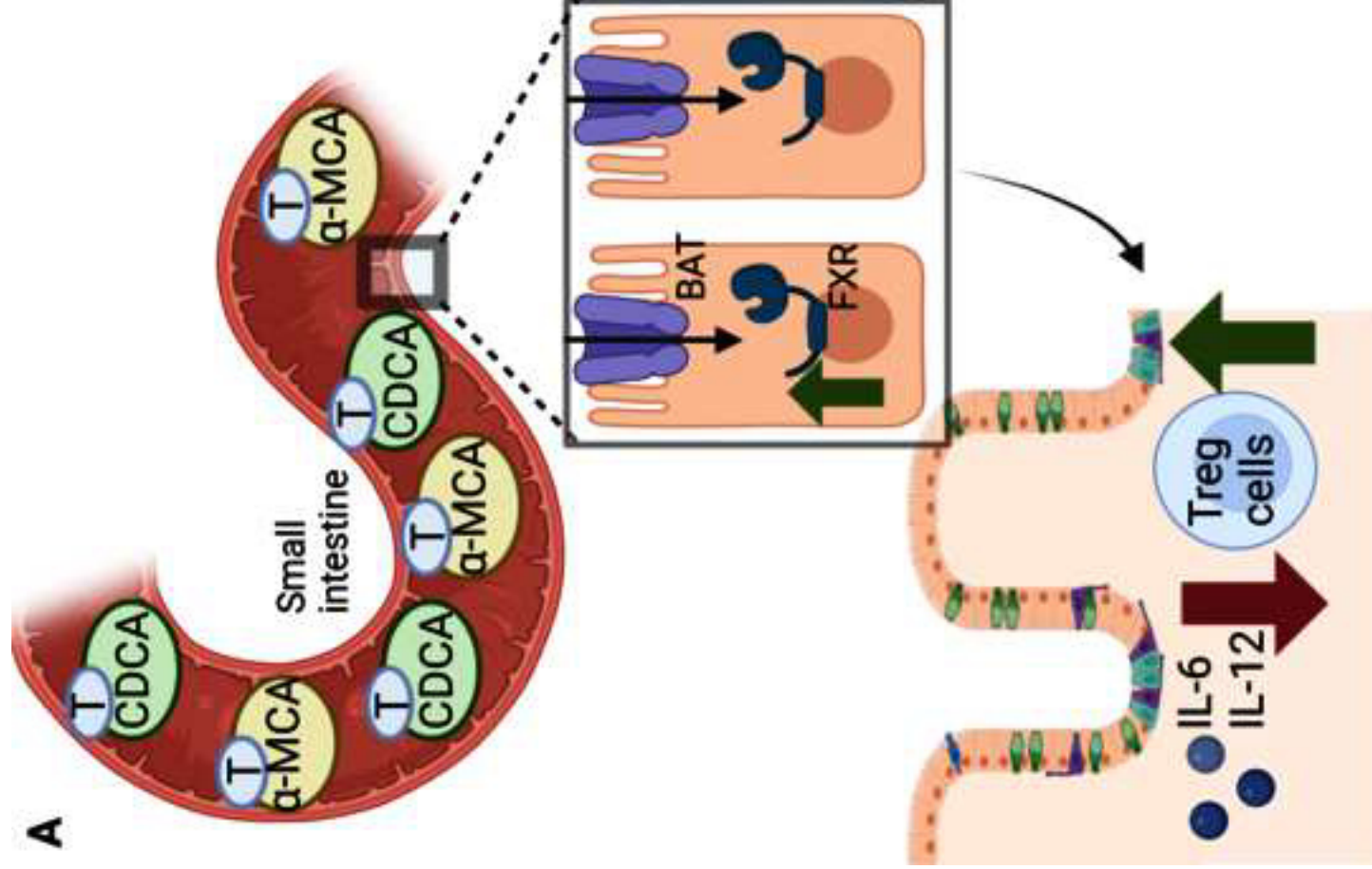
1 **Outstanding questions**

- 2 • Are metabolic pathways the most important target for new anthelmintics in all helminths?
- 3 • How are certain species of helminths able to synthesise their own SCFA, and can we utilise this as
- 4 a drug target?
- 5 • Is the alteration of bile acid metabolism significant to helminth survival, and is this microbiota-
- 6 dependent?
- 7 • If we prevent helminth-induced alteration of eicosanoids, will this be detrimental to parasite
- 8 survival?
- 9 • What are the metabolic mechanisms by which helminths can alter SCFA, PUFA, and bile acid
- 10 metabolism?
- 11 • Is it possible to employ recently developed cell culture techniques to assess the effectiveness of
- 12 novel drugs aimed at metabolism, thus circumventing the necessity for costly in vivo models?



Larval *A.suum***Adult *A.suum***





+
H. polygyrus