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MINIREVIEW

Tryp-ing Up Metabolism: Role of Metabolic Adaptations in Kinetoplastid Disease Pathogenesis

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ABSTRACT Today, more than a billion people—one-sixth of the world's population—are suffering from neglected tropical diseases. Human African trypanosomiasis, Chagas disease, and leishmaniasis are neglected tropical diseases caused by protozoan parasites belonging to the genera *Trypanosoma* and *Leishmania*. About half a million people living in tropical and subtropical regions of the world are at risk of contracting one of these three infections. Kinetoplastids have complex life cycles with different morphologies and unique physiological requirements at each life cycle stage. This review covers the latest findings on metabolic pathways impacting disease pathogenesis of kinetoplastids within the mammalian host. Nutrient availability is a key factor shaping *in vivo* parasite metabolism; thus, kinetoplastids display significant metabolic flexibility. Proteomic and transcriptomic profiles show that intracellular trypanosomatids are able to switch to an energy-efficient metabolism within the mammalian host system. Host metabolic changes can also favor parasite persistence, and contribute to symptom development, in a location-specific fashion. Ultimately, targeted and untargeted metabolomics studies have been a valuable approach to elucidate the specific biochemical pathways affected by infection within the host, leading to translational drug development and diagnostic insights.

KEYWORDS metabolism, disease pathogenesis, kinetoplastids, neglected tropical diseases, host-parasite interactions, *Trypanosoma cruzi*, Chagas disease, *Trypanosoma brucei*, sleeping sickness, *Leishmania*, leishmaniasis, *Trypanosoma*

The neglected tropical diseases (NTDs) are a group of infections that are common among the world's poorest populations, affecting more than one billion people worldwide (1). NTDs remain major public health issues in parts of Asia, Africa, and South America, but they also affect populations in North America and Europe, due to population migration, climate change, or previously undetected endemicity (2). Due to lack of awareness and financial incentive for drug development, NTDs have remained largely underresearched. The trifecta of nutrition, health, and disease is complex, especially when factoring in parasites and their own metabolic needs. In addition, NTD-causing parasites have complex life cycles and modes of transmission (3). Understanding this plasticity on a metabolic level will deepen our understanding of parasitic diseases.

Metabolic pathways degrade macromolecules to produce energy (catabolic metabolic reactions), generate the building blocks of the cell (lipids, nucleotides, amino acids, and sugars; anabolic metabolic reactions), excrete waste products, and synthesize the small molecules involved in cell and system function and cell-cell communication, thus driving organismal phenotype (4, 5). Within a host system, parasites adapt their metabolism depending on their surroundings and nutrient needs. Parasites replicate and continue their life cycle, capitalizing on energy obtained from the host. Thus, parasites benefit from host cell metabolism, with (in some cases) reduced reliance on

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their own metabolic pathways. Parasites achieve this by influencing host cell gene expression and thus host metabolic pathways (6). Therefore, studying host-parasite interactions also means understanding the implications on the overall host metabolism (3), particularly with regard to energy-generating metabolic pathways. The major pathways for energy generation are the degradation of fatty acids, amino acids, and polysaccharide-derived monosaccharides, with the preferred monosaccharide usually glucose, when available. Glucose is degraded via glycolysis, leading to the production of pyruvate. Pyruvate can have multiple fates depending on oxygen availability. When oxygen is plentiful, it is converted to acetyl coenzyme A (acetyl-CoA), which proceeds through the citric acid cycle for full oxidation to CO_2. The citric acid cycle is also called the tricarboxylic acid (TCA) cycle or the Krebs cycle. Glycolysis and the TCA cycle directly produce ATP and GTP. In addition, they produce reducing equivalents ($FADH_2$ and NADH). These are regenerated to FAD and NAD^+ through oxidative phosphorylation (mitochondrial respiration), which is also an ATP-producing process. Fatty acid catabolism (β-oxidation) and polysaccharide catabolism intersect at the level of acetyl-CoA. Amino acids are degraded to pyruvate, acetyl-CoA, or TCA cycle intermediates (Fig. 1) (5).

This review aims to compile literature on the metabolism of mammalian infection and discuss its impact on disease pathogenesis for the three different human kinetoplastid infections: Chagas disease (CD), human African trypanosomiasis (HAT), and various forms of leishmaniasis, caused by *Trypanosoma cruzi*, *Trypanosoma brucei*, and *Leishmania*, respectively. Studying the metabolic aspects of disease pathogenesis can uncover new mechanisms of infection and aid in developing drugs against these diseases.

LEISHMANIASES

Leishmaniases are caused by over 20 different species of *Leishmania*, which are transmitted by infected female phlebotomine sandflies. Leishmaniasis affects people in more than 100 countries in tropical and subtropical regions worldwide (7), with 12 to 15 million people infected (8). Leishmaniases have been called a disease complex rather than a single disease; their clinical manifestations are diverse but occur in three main categories—cutaneous, mucocutaneous, and visceral. Cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) are the most common forms of the disease. CL manifests as ulcerative skin lesions, while symptoms of VL include fever, weight loss, and enlargement of the liver and spleen (7). VL can become fatal; risk of severe disease is heightened by coinfection with other pathogens such as HIV (9). Mucocutaneous leishmaniasis is a severely disfiguring form affecting the mucosal regions of the nose, mouth, and pharynx (10). *Leishmania* organisms have a two-stage life cycle in which they alternate between the extracellular promastigote stage in the sandfly vector and the intracellular amastigote stage in mammalian host macrophage phagolysosomes (7).

Pentavalent antimonial drug compounds remain a mainstay of leishmaniasis treatment (11). These drugs are highly toxic, and parasites are increasingly resistant to antimony in several regions of the world. Alternatives such as paromomycin and miltefosine come with the drawbacks of toxicity and increased drug resistance. Paromomycin in combination with sodium stibogluconate improves treatment success, but sodium stibogluconate also comes with the risk of cardiotoxicity (12). Amphotericin B in liposomal formulations is better tolerated than conventional amphotericin B formulations but can still cause adverse effects and requires parenteral administration (11). Miltefosine, though oral, is teratogenic and cannot be given to pregnant women. Treatment failure can be attributed to the host immune system and nutritional status of the host, parasite mechanisms of survival or drug resistance within the host, and extrinsic factors like access to medical treatment (13).

Approaching treatment failure by better understanding molecular-level changes occurring with infection could lead to more effective drug development (see below

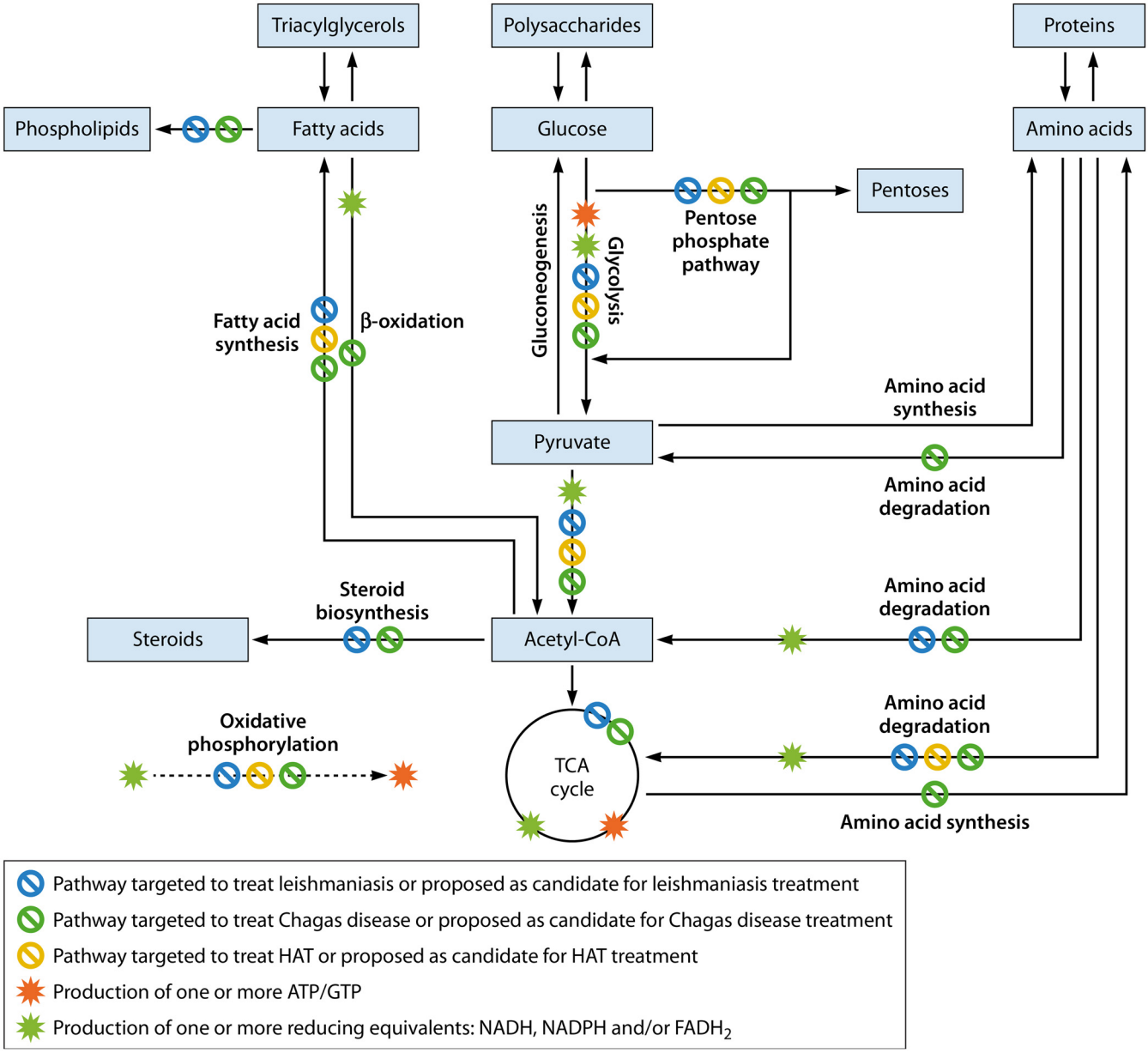

FIG 1 Overall metabolic pathway integration and targets for intervention. Only metabolic pathways and inhibitors discussed in this review are shown. Adaptations unique to specific kinetoplastids and differences between mammalian and parasite metabolism are not displayed. Cofactors, additional reactants, conversion of oxaloacetate to pyruvate and pyruvate to oxaloacetate, energy cost, and urea and CO_2 production are also not drawn. Targeting of parasite versus host metabolic pathways are not differentiated, because this is often hard to distinguish when nonselective inhibitors are used. Metabolites are boxed.

and "Avenues for Intervention"). In the early stages of *Leishmania* infection, catabolism of host-derived amino sugars (*N*-acetylglucosamine and glucosamine) is an essential source of carbon for the intracellular stage of *Leishmania* (14). Although a lack of hexose uptake cannot be compensated by amino acid catabolism *in vivo* (15), gluconeogenesis is also essential for parasite intramacrophage replication and *in vivo* virulence (16). Metabolomic analysis of isolated lysosomes has revealed enrichment of nucleosides, lower levels of specific amino acids (with others showing comparable concentrations), and equivalent levels of glucose and other sugars compared to whole cells (17). However, nutrient availability may be more limited within the *Leishmania*-housing phagolysosome, given that amastigotes engage in a stringent metabolic response, designed for sparing nutrient use. The parasites accomplish this by decreasing usage of glucose and amino acids while increasing catabolism efficiency, with minor increases in fatty acid β-oxidation and reduced

biosynthesis of structural components such as proteins and lipids (18). This stringent response may account for the low growth rate observed for amastigotes *in vivo* (19).

In addition to this stringent metabolic state, *Leishmania* implements several additional mechanisms to compensate for this nutrient shortage, including increased nutrient transporter expression and modulation of host metabolism to favor parasite growth. Such mechanisms are essential given *Leishmania* auxotrophies, which include purines, a critical requirement for exogenous arginine, leucine, lysine, phenylalanine, tryptophan, and valine, with exogenous aspartate, glutamate, glutamine, and serine required to support growth and histidine, isoleucine, methionine, and threonine required to support protein synthesis (20–22). *Leishmania* must also scavenge biopterin, folic acid, riboflavin, and lipoic acid (20). Indeed, an atypical *Leishmania donovani* strain that causes cutaneous lesions instead of VL showed an increase in transporters, possibly representing an adaptation to the nutrient-limiting environment of the skin (23). In the macrophage-residing *Leishmania*, an arginine shortage in the phagolysosome environment is compensated by upregulating an arginine transporter. This upregulation highlights not only the importance of arginine to *Leishmania* survival but also that *Leishmania* monitors levels of arginine in the environment and induces an arginine-deprivation response (24, 25).

Host metabolic hijacking by the parasite has been demonstrated through metabolic alterations in infected macrophages. Macrophage glucose uptake and respiration can be controlled by *Leishmania*: *L. infantum* and *L. major* increase macrophage glycolysis early after infection (26–28). This requires live parasites (28). In contrast, *L. major* organisms downregulate the expression of their own glycolytic genes during the same period (27). At later time points *in vitro*, *L. infantum*, *L. donovani*, and *L. mexicana* promote macrophage oxidative phosphorylation via induction of 5′ AMP-activated protein kinase (AMPK) (26, 29); this has been postulated as a strategy to increase glucose availability for the parasite (30). However, a separate study using mouse bone marrow-derived macrophages infected with *L. major* showed no change in oxidative phosphorylation at 24 h postinfection (28). Analysis of monocyte-derived cells isolated from cutaneous leishmaniasis lesions revealed a decrease in cellular respiration, driven by local nitric oxide levels (31). These parasite-induced metabolic alterations may help reshape the immune response to favor reduced inflammatory cytokine production and thus parasite persistence (31).

L. amazonensis macrophage infection *in vitro* increases alanine, proline, arginine, valine, isoleucine or leucine, ornithine, hypoxanthine, glutamic acid, and histidine, as well as phosphocholine (32). In macrophages infected with *L. donovani*, glutamine consumption and host glutamine metabolism were increased (33). This concurs with findings of decreased glutamine in experimental CL lesions (34). Infected macrophages also upregulate arginine and proline metabolism while downregulating steroid and fatty acid biosynthesis (27), though this contrasts with observations of increased cholesterol uptake, total fatty acids, and lipid droplet formation in other studies (28, 35). Overall, given the parasite energetic needs and amino acid and purine auxotrophies (20, 21), these adaptations likely favor parasite proliferation. In addition, *Leishmania* affects host lipid metabolism, including increasing lysophosphocholine (LPC), total phospholipids, triacylglycerol, diacylglycerol, and monoacylglycerol, as well as specific phosphocholines, phosphoethanolamines, hydroxyoctadecadienoic acid metabolites, and hydroxyeicosatetraenoic acid metabolites, *in vitro* (35–38) and *in vivo* (34). The role of these adaptations in pathogenesis is still unknown but warrants investigation. Given that miltefosine was initially developed to target host phospholipid metabolism for cancer treatment (39), it may restore these infection-induced host phospholipid alterations in addition to directly killing *Leishmania*, although this needs to be demonstrated experimentally.

Strikingly, some of these metabolic alterations are distal to the site of leishmaniasis lesions or even systemic. For example, serum triglyceride levels were significantly higher in VL patients (40), and a study of Indian VL patients showed that an increase in

splenic parasite load came with a decrease in serum cholesterol (41). While systemic metabolic alterations are not unexpected in the case of VL, they have also been reported in CL. Tissue metabolomic analysis showed that specific phosphocholines were increased in the skin beyond the site of the lesion (34). Diffuse cutaneous leishmaniasis was associated with increased plasma cadaverine compared to other CL forms. Ornithine was also lower in plasma of mucocutaneous and localized cutaneous leishmaniasis patients than in healthy controls (42).

Parasite metabolites can also affect immune responses; for example, lipids from *L. braziliensis* amastigotes stimulate nitric oxide production (43). Increased LPC levels worsen dendritic cell infection with *L. major* by decreasing inducible nitric oxide synthase (iNOS) expression (44). Examples of other provirulence *Leishmania* metabolic pathways include mannan metabolism, required for adaptation to variable carbohydrate availability (45), and ether glycerolipid production (46).

Overall, the adaptability of *Leishmania* within its host is a testament to the underlying mechanisms an intracellular parasite uses to survive in different organs of the host. Considerable effort has unveiled metabolic adaptations *in vitro;* exciting advances are beginning to unravel whether these adaptations also occur *in situ* and *in vivo*. New technologies such as single-cell metabolomics and sequencing may enable better characterization of these changes at early or disease resolution time points when parasite burden is low.

CHAGAS DISEASE

CD is caused by the protozoan parasite *T. cruzi*, transmitted by triatomine kissing bugs. It is endemic to 21 Latin American countries, with 6 to 7 million infected individuals worldwide (47). Due to population mobility, its incidence has been increasing in the United States, Canada, and Europe (48). Infection occurs in two stages, acute and chronic. During the acute stage, symptoms include fever, enlarged lymph nodes, and abdominal or chest pain. After 8 to 12 weeks, parasite levels become undetectable by microscopy, and the infected individual enters the chronic stage. Individuals with chronic *T. cruzi* infection who lack disease symptoms have the intermediate form of CD. Symptomatic chronic CD is characterized by cardiac and/or gastrointestinal dysfunction, including cardiac apical aneurysms, megaesophagus, and megacolon (47).

Within the mammalian host, *T. cruzi* alternates between an intracellular amastigote stage in host cell cytoplasm and an extracellular trypomastigote stage, across multiple organs and tissues (47). In mouse chronic CD models, parasite persistence is predominantly in the large intestine, stomach, and gut mesenteric tissue, with transient colonization at multiple other sites (49). This pleiotropism may be facilitated by *T. cruzi* metabolic flexibility, whereby amastigotes can use glucose, amino acids, and fatty acids as their main carbon sources (50–53). Upon interaction with the extracellular matrix, trypomastigotes reduce glycolytic activity (54). This was also observed at the mRNA level early following trypomastigote invasion of fibroblasts, with reemergence of glycolysis-associated transcripts 24 h after invasion (52) and amastigote uptake of glucose from host pools (50). Intracellular amastigotes also upregulate TCA cycle, oxidative phosphorylation, and β-oxidation-associated transcripts and amino acid permeases compared to trypomastigotes (52). This is supported by increased fatty acid oxidation proteins in amastigotes compared to trypomastigotes (51) and adaptations in *T. cruzi* growth rates depending on exogenous nutrient availability (55), further supporting metabolic flexibility.

Deciphering the effect of infection on host metabolism can ultimately lead to a better understanding of how intracellular *T. cruzi* organisms proliferate within the mammalian host. Genes related to mitochondrial function, such as oxidative phosphorylation pathway genes, were downregulated in chronic CD murine models (56). An increased abundance of swollen mitochondria in infected cardiac tissue further indicates that mitochondrial dysfunction may relate to disease severity in chagasic cardiomyopathy (56). Indeed, a general increase in oxidative stress is associated with disease progression in human CD and oxidative stress is linked to damaged mitochondrial

function in chagasic patients (57, 58). Untargeted metabolomics studies of acute experimental infection with *T. cruzi* strain Y parasites also demonstrated oxidative stress in heart tissue and a decline in TCA cycle intermediates such as fumarate, further supporting decreased oxidative metabolism in cardiac tissue (59). This was also observed *in vitro* in two separate studies of macrophage infection and in monocytes isolated from CD patients, was enhanced by gamma interferon (IFN-γ), and was associated with increased production of nitric oxide and reactive oxygen species. These are antiparasitic but also contribute to tissue damage and impair $CD8^+$ T cell function (29, 60, 61). This contrasts with *in vitro* reports of increased mitochondrial respiration in infected fibroblasts (50), suggesting host cell-type-specific effects or differences between *in vitro* and *in vivo* settings.

Increased glucose uptake and glycolysis were observed in infected cardiac tissue (59), *in vitro* in macrophages (60) and fibroblasts and myoblasts (50), and in monocytes isolated from CD patients (61). However, a separate study did not observe increased glycolysis in infected macrophages at 18 h postinfection (29). This discrepancy may be due to the infecting parasite strain, since two of these conflicting studies (29, 60) used the same 18-h postinfection time point, and two studies used human cells (29, 61). Activation of glycolysis promoted parasite clearance in activated macrophages, whereas restriction of glucose availability or replacement of glucose with fructose prevented reactive oxygen and nitrogen species production, with no effects on cytokine production (60). This was only partially confirmed in monocytes isolated from CD patients: similar to *in vitro* murine macrophage infection (60), inhibition of glycolysis reduced nitric oxide production (61). However, glycolysis inhibition also reduced interleukin 1β (IL-1β) secretion (61). Oxidant production was also dependent on the pentose phosphate pathway (60). This observation contrasts with reports for fibroblasts, in which inhibition of glycolysis blocked *T. cruzi* proliferation. The latter reliance on glycolysis was on both host and parasite levels (50, 55).

Differences between these studies suggest a host cell-type-specific relationship between host glycolysis and *T. cruzi* proliferation. This may be related to the presence of other alternative nutrients or higher reliance on alternative pathways for energy metabolism. Indeed, cells that were defective in mitochondrial fatty acid oxidation were also less competent to support the growth of *T. cruzi* amastigotes (62). The amino acids tryptophan, isoleucine, leucine, and valine were increased in infected heart tissue, while purine nucleotides were depleted by infection in heart tissue (59). *T. cruzi*, like *Leishmania*, is auxotrophic for tryptophan, leucine, isoleucine, valine, and purines (21). Parasites also require exogenous glutamine for growth (55). Kynurenine, glycerophosphocholine, and long-chain fatty acids were also increased with infection in heart tissue (59). *T. cruzi* can scavenge and incorporate host lipids (63).

However, *T. cruzi* shows specific tropism even within target organs. In the heart, parasite load was highest at the heart base, which may indicate a preference for a specific cardiac metabolic environment (64). Untargeted metabolomics revealed distinct chemical signatures between the apex and base of the heart. Host nucleosides were elevated at the apex of the heart, specifically, AMP, which regulates AMPK (64). AMPK inhibition *in vitro* promotes intracellular parasite growth (62). In chronic *T. cruzi* strain Sylvio X10/4 infection, significant overall metabolic perturbations in the heart were observed only in the apical region, providing one explanatory mechanism for the localized apical damage observed in human CD. Infection had position-specific impact on specific metabolite families such as acylcarnitines (65). Host metabolism was also affected by *T. cruzi* infection in the gastrointestinal (GI) tract in an experimental mouse model, in a spatially resolved fashion. For example, total and long-chain acylcarnitines were significantly elevated in the small intestine in acute CD. Kynurenine was elevated by infection in the stomach and large intestine in the acute stage. The largest metabolic perturbations were observed in the chronic stage in the esophagus and large intestine, sites of gastrointestinal CD, suggesting a metabolic cause of disease tropism

in this context (66). Thus, *T. cruzi* is able to infect and cause host metabolic alterations in a variety of tissue locations.

Overall, *T. cruzi* depends on nutrient scavenging and on metabolism adaptations for *in vivo* proliferation. Infection also induces adaptive and maladaptive host metabolic adaptations. Thus, there is considerable scope to build on this knowledge to develop new CD treatments (see "Avenues for Intervention" below).

HUMAN AFRICAN TRYPANOSOMIASIS

HAT occurs in sub-Saharan Africa and is transmitted through a tsetse fly vector. The two clinical forms of HAT are the slow-progressing form caused by *Trypanosoma brucei gambiense* in central and western Africa and the fast-progressing form caused by *T. brucei rhodesiense* in parts of eastern and southern Africa (67, 68). Approximately 70 million people live in regions where they are at risk of contracting HAT (69). Unlike *T. cruzi* and *Leishmania*, *T. brucei* is extracellular at all stages (67). Initial symptoms are nonspecific and include fever, headaches, and joint pain (hemolymphatic stage). Eventually, the parasite crosses the blood-brain barrier, leading to psychiatric and behavioral disturbances, ataxia, seizures, coma, and ultimately death (70).

Bloodstream form *T. brucei* lives in glucose-rich environments and thus depends on glucose for its ATP production (71) and anabolic processes (65, 72). Indeed, RNA interference (RNAi) data showed a greater reliance of bloodstream form *T. brucei* on glycolysis, whereas procyclics (insect stage) were more dependent on fatty acid metabolism (73) and proline catabolism, though glucose remains the preferred energy source (74). The observed change in glycolysis products in urine and plasma during experimental *T. brucei brucei* mouse infection (75) could reflect parasite glycolysis or host adaptation to energy needs caused by infection. If reflecting host metabolism, this may lead to competition between host and parasite for glucose. Bloodstream forms of *T. brucei* do not significantly rely on a full TCA cycle (72). Beyond glucose, anabolic processes in bloodstream *T. brucei* use glutamine to produce glutamate, 2-oxoglutarate, and some succinate. Extracellular cysteine is used by the parasite to produce glutathione, trypanothione, and coenzyme A (76). Extracellular acetate feeds into parasite fatty acid biosynthesis (77).

However, there is also metabolic flexibility in *T. brucei*. Bloodstream forms are able to not only survive but also proliferate in a medium containing glycerol instead of glucose (71), with glycerol serving as a source for gluconeogenesis *in vitro* (71, 78). Abundant glycerol could be the reason why parasites associate with adipose tissue, though this has yet to be directly demonstrated. Indirect evidence is provided by elevation of glycolytic/gluconeogenic enzymes in adipose tissue resident parasites (79). A better understanding of glycerol metabolism could open new avenues for developing better drug targets (71). Additional adaptations to adipose tissue tropism compared to bloodstream tropism include increased expression of enzymes involved in the TCA cycle, purine salvage, the pentose phosphate pathway, and sterol and lipid metabolism, including β-oxidation. Parasites isolated from adipose tissue were confirmed *ex vivo* to be capable of metabolizing fatty acids (79). Additional underexplored temporal metabolic flexibility may also occur, with *in vitro* studies showing strong circadian regulation of metabolic genes in cultured bloodstream *T. brucei* forms (80).

Given that *T. brucei* is extracellular (67), the impact on host metabolism will be more indirect than during *Leishmania* or *T. cruzi* infection. Host lipids are highly affected by HAT. A global untargeted metabolic profiling of plasma from *rhodesiense* trypanosomiasis in Uganda showed significant differences in host lipid profiles with infection. HAT patients had lower levels of phosphatidylcholines (PCs) with a high number of saturated bonds compared to the control samples. The concentration of PCs with two or fewer unsaturated bonds was increased with infection (81). Urine and plasma chemical compositions of mice infected with *T. brucei brucei*, a *Trypanosoma brucei* subspecies that causes nonhuman infection, showed enhanced lipid oxidation

with infection (75). The study also showed depleted levels of PC with infection but did not assess degree of saturation (75). There was an increase in the choline concentration in plasma samples, suggesting a breakdown of PCs in the presence of infection (75, 81). Since PCs are among the metabolites most affected by HAT infection in both patient samples and *in vivo* mouse studies, future studies can aim to study the role of PCs in the disease pathogenesis of HAT.

Changes in host amino acid metabolism also occur due to HAT. In the plasma sample study from *rhodesiense* trypanosomiasis in Uganda, higher concentrations of phenylalanine and lower concentrations of other amino acids, like histidine and alanine, occurred with infection (81). Central nervous system (CNS)-stage disease was associated with an increase in 5-hydroxytryptophan and kynurenine and decreased tryptophan in the CNS compared to the case for the hemolymphatic stage (82). A study in voles infected with *T. brucei gambiense* also showed significant perturbations in phenylalanine metabolism, with, however, a decrease in phenylalanine levels in the chronic stage (45- to 49-day infection period) (83). Hypoaminoacidemia was also observed in the plasma of voles infected with *T. brucei gambiense* for 21 days. The aromatic amino acids tryptophan and tyrosine were significantly reduced and may play a role in the neuropsychiatric syndromes associated with HAT (84); these results concur with human CNS studies (82). However, in contrast to the study in patients, the concentration of phenylalanine, also an aromatic amino acid, was unchanged with infection (84). This discrepancy between tyrosine and phenylalanine levels could be attributed to the rate of tyrosine breakdown being considerably greater than the dietary intake of tyrosine as well as the rate at which phenylalanine can be converted to tyrosine (83). Discrepancies between studies could be attributed to the various metabolic differences produced by the two different types of strains used for infection, to the duration of infection, or to the mammalian system studied. *T. brucei* also produces the amino acid metabolites indolepyruvate, hydroxyphenylpyruvate, and phenylpyruvate, which inhibit production of the proinflammatory cytokine IL-1β, via downmodulation of macrophage glycolytic metabolism. This has been postulated to attenuate HAT symptoms and favor host survival long enough to ensure parasite transmission (85, 86). These metabolites also decreased inducible nitric oxide synthase expression and IL-6 induction, likely favoring parasite persistence (86). Additional investigation of immunometabolism in HAT is warranted.

Ultimately, *T. brucei* is able to proliferate and survive within the host system due to its metabolic flexibility. Additionally, infection causes perturbation in overall host lipid and amino acid metabolism.

AVENUES FOR DIAGNOSIS AND PATIENT MONITORING

There is a need for better ways to diagnose leishmaniasis, CD, and sleeping sickness and monitor disease progression and treatment efficacy. Several metabolomics studies have identified metabolites, primarily host derived, that can serve as biomarkers to address these needs. For example, serum samples from an experimental model of CD showed significant perturbations in host amino acid metabolism with infection. The essential amino acids isoleucine, leucine, phenylalanine, tryptophan, and valine were decreased with infection, as was glutamine. Acylcarnitines were also affected by infection (87). Long-chain fatty acids were decreased by infection in plasma, while *p*-cresol sulfate, allantoin, and kynurenine were elevated (59). A machine learning model built on the cardiac metabolome could predict acute experimental *T. cruzi* infection outcome (64); such an approach can serve as a framework to identify disease severity-associated and prognostic circulatory metabolite biomarkers.

The ability to differentiate between the two stages of HAT infection ultimately dictates the course of treatment to be chosen. A metabolomic analysis of cerebrospinal fluid (CSF) and plasma from patients in Angola revealed several biomarkers that were different based on disease stage. Stage 2 HAT could be distinguished from stage 1 by

an increase in neopterin and hydroxytryptophan levels, a slight increase in kynurenine, and a decrease in tryptophan in the CSF samples. In plasma, an increase in aminododecanoic acid and decrease in ornithine are both indicators of stage 2 HAT (82). A second study observed amino acid depletion in the CSF in HAT stage 2 (88). Indolepyruvate produced by the bloodstream form of *T. brucei* could also be an important indicator of parasite presence and disease state (85).

In the context of leishmaniasis, several studies described above reported differences in selected serum or plasma metabolites compared to uninfected controls (40–42). The serum metabolome can also predict CL treatment success, with elevated pretreatment taurine, allantoin, *N*-acetylglutamine, and pyruvate predictive of successful pentavalent antimonial treatment (89).

One cautionary note is that these biomarkers, while useful to monitor disease progression or treatment, may not directly reflect the specific changes occurring at the sites of disease. For example, long-chain fatty acids showed the opposite direction of change in the heart and the plasma during acute *T. cruzi* infection (59), highlighting the importance of studying metabolism right at the site of disease progression to understand pathogenesis. A further limitation is that these metabolic changes are predominantly host derived and often not specific to the kinetoplastid disease of concern. Thus, they may be useful only in combination with other diagnostic methods and of limited utility to determine the presence of an infection. Their strength instead may lie in predicting disease severity (64) or treatment response (89) once infection status has been established.

AVENUES FOR INTERVENTION

Drug development commonly targets the pathogen but can also address pathogen or immune response-induced collateral damage (protolerance mechanism [90]). Though pan-kinetoplastid treatment strategies would be desirable to decrease drug development costs, given the differences in target organs, parasite localization, and timeline of disease progression, treatments of these different diseases will likely require very different pharmacokinetic properties and have different acceptable adverse-effect profiles. Thus, individual optimization of these treatment avenues will likely be necessary for each kinetoplastid disease.

The current HAT treatment, eflornithine, inhibits parasite polyamine metabolism (91). Likewise, miltefosine, in clinical use for leishmaniasis, targets parasite lipid metabolism (92) and allopurinol, used exclusively in veterinary leishmaniasis, inhibits purine salvage (93). Azole family compounds, which failed in clinical trials for CD, targeted parasite ergosterol metabolism (94, 95). By identifying additional metabolic pathways affected by infection, the next generation of drug development can target pathways essential to parasite survival or disease pathogenesis. As described below, there are multiple preclinical drug development studies targeting metabolism in kinetoplastid infection.

For example, *T. cruzi*, *T. brucei*, and *Leishmania* are sensitive to fatty acid biosynthesis inhibitors (19, 96, 97), and inhibitors of glycolysis and respiration kill all three kinetoplastids (98, 99). These need not be nonspecific: compound GNF7686 selectively targets *T. cruzi* mitochondrial respiration with more than a 100-fold selectivity window compared to host cells (100). Some inhibitors of *T. brucei* hexokinase also show low inhibition of host hexokinase (101). Kinetoplastid segregation of initial steps of glycolysis to glycosome organelles (unlike for mammals) also provides an opportunity for the development of selective interventions. For example, interfering with glycosomal enzyme localization impairs ATP production and cures HAT in mouse models. These compounds also showed acceptable efficacy against intracellular *T. cruzi* amastigotes *in vitro*, though *in vivo* activity was not tested (102). Alternatively, research with *T. brucei* suggests that even nonspecific inhibitors (such as those targeting glycolysis, with potential off-target effects on host glycolysis) may be sufficiently safe as long as the inhibitors have a greater impact on flux in the

parasite than in the host (103). Some of these treatments also target host metabolism or the intersection between host and parasite metabolism. For example, chronic-stage CD treatment with resveratrol activates the AMPK pathway, reduces cardiac oxidative stress and parasite load, and improves cardiac function (104). Polyamine production requires metabolism of arginine to ornithine by arginase enzymes. In parallel, arginine is a substrate for host nitric oxide synthase, which produces antiparasitic nitric oxide. The host and parasite both express arginases, with *L. major* arginase expression and *L. infantum* lipids promoting host arginase activity. Arginase inhibition slows cutaneous leishmaniasis lesion progression in BALB/c mice by restricting availability of polyamines without affecting nitric oxide or cytokine production (38, 105, 106), though knocking out arginase in C57BL/6 mice had no impact on cutaneous leishmaniasis lesion size or parasite load (107). In contrast, inhibition of arginase increased nitric oxide production and *T. brucei* killing *in vitro* (108). Arginase may also contribute to CD progression (109), and arginine supplementation reduces *T. cruzi* load and improves disease severity *in vitro* and *in vivo* by increasing nitric oxide production (110).

Indeed, given the key role of metabolism in shaping immune responses (111), several studies have investigated the impact of metabolism modulators on immune responses with the goal of developing new treatments for kinetoplastid infection. For example, 3-hydroxykynurenine is a tryptophan metabolite produced by macrophages and dendritic cells, among others, with immunomodulatory properties. Mice infected with *T. cruzi* and treated with 3-hydroxykynurenine in the acute disease stage showed reduction in chronic-stage electrocardiogram alterations, inflammation and fibrosis in the heart, and reduced parasite load (112). In contrast, inhibiting the enzyme producing these metabolites, indoleamine 2,3 dioxygenase (IDO), significantly reduced cutaneous leishmaniasis lesion size and parasite load (113). Glutaminase inhibition impaired antiparasitic immune responses during *L. donovani* infection, with reduced IFN-γ and increased IL-10 production, supporting the development of strategies that promote glutamine metabolism as new treatments for visceral leishmaniasis (33). Knocking out AMPK in myeloid cells improved *L. infantum* clearance by promoting increased proinflammatory nitric oxide synthase expression and reduced arginase expression (M1 macrophage phenotype) (26). Reducing cholesterol levels via simvastatin treatment improved cutaneous leishmaniasis lesion size via enhanced macrophage antioxidant production (114).

Metabolic modulation can also induce disease tolerance: improved disease severity with no changes in parasite burden. For example, acylcarnitine metabolism was perturbed during acute and chronic CD, and carnitine supplementation improved acute-stage survival *in vivo*, including cardiac function, but without affecting parasite load (66). Metformin, an AMPK modulator that also has AMPK-independent effects (115), improved cardiac function in experimental CD, with no significant effects on parasite load (104).

Drug development can also build on metabolic models, to predict pathways that are essential to the parasite and thus can serve as good targets for antiparasitics. For example, a proteomic network analysis showed reactions involved in carbohydrate metabolism and amino acid metabolism as essential to *T. cruzi* (116). A genome-scale metabolic *T. cruzi* model predicted lethality for drugs targeting pairs of enzymes in the citric acid cycle and gluconeogenesis/glycolysis pathways, in the citric acid cycle and glutamate metabolism, or in glutamate metabolism and oxidative phosphorylation (117). A similar approach in *Leishmania* predicted threonine biosynthesis as a novel target (118). Genes involved in *Leishmania* purine metabolism, methionine metabolism, steroid metabolism, vitamin B_6 metabolism, oxidative phosphorylation, and the pentose phosphate pathway were also predicted to be lethal using a computational, constraint-based *L. donovani* metabolic model (119). Kinetic modeling in *T. brucei* predicts glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and glucose transport as drug targets in this parasite (103). Translation of these *in silico* findings was demonstrated in

T. cruzi, where machine learning based on *T. cruzi* metabolism and prior drug screening hits successfully predicted compounds that then demonstrated *in vitro* and *in vivo* antiparasitic activity (120).

Metabolic modulators do not have to be used alone; instead, they could be combined with existing drugs to facilitate dose reduction, improve safety profiles, or address tissue damage not currently restored by antiparasitics. For example, combining glutamine with miltefosine for the treatment of VL showed improved reduction in parasite load in the spleen (33). Supplementing meglumine antimoniate with allantoin improved *Leishmania* clearance *in vitro* (89). Given that carnitine supplementation improved cardiac function, but without affecting parasite load in acute CD (66), a path forward for clinical implementation will likely require combination with existing antiparasitics. Eflornithine is usually used in combination with nifurtimox for sleeping sickness (121).

Host-parasite metabolomics can also be used to determine drug mechanism of action and whether new drugs proceed via pathways similar to those of previously characterized agents. For example, this approach revealed that the experimental compounds S205, S448, and S1000 have a mechanism of action in CD distinct from that of benznidazole and nifurtimox (122). A similar approach was applied to *Leishmania* (123). This approach can also identify candidate mechanisms of action, for example, by demonstrating that sertraline, a candidate for drug repurposing for leishmaniasis, induces multitarget metabolic dysfunction in the parasite (124). Similar studies used metabolomics to demonstrate that AN5568 acts on *S*-adenosyl-L-methionine metabolism in *T. brucei* and causes redox dysfunction (125) and that miltefosine affects *Leishmania* sphingolipid and sterol metabolism (126). However, one of the barriers that still remains is the high level of metabolite commonalities occurring within the host and parasite and being able to differentiate between these common metabolites for the treated condition (122).

Metabolomics can also help in understanding drug treatment failure or success. For example, metabolomics of miltefosine-resistant *Leishmania* demonstrated resistance-associated changes in phosphatidylcholines (92). Susceptibility to an antiparasitic depends upon the metabolic environment of the parasites and thus the sites of *in vivo* infection. An *in vitro* study showed that in the presence of glutamine, *T. cruzi* amastigotes were more susceptible to the effect of ketoconazole (127). Interestingly, glutamine levels are lower in the large intestine than other gastrointestinal sites (66), which matches sites of reduced parasite clearance during posaconazole treatment (128). Thus, controlling the host metabolic environment may be a possible intervention to increase susceptibility to existing drugs. *T. cruzi* can enter a dormant drug-resistant state (129), although the associated metabolic changes are still unknown. Studying these will require highly sensitive techniques, such as single-cell transcriptomics or metabolomics. Treatment failure can also result from differential tissue damage, leading to altered drug distribution. This has been understudied in the context of infection. Indeed, pharmacokinetic studies are usually performed in uninfected animals (for an example, see reference 130). However, differential metabolism and tissue distribution of the animal anesthetic ketamine were observed in an untargeted metabolomics study of *T. cruzi*-infected hearts (131) and VL lowered liver and spleen amphotericin B levels (132).

Beyond drug treatment, nutrition also impacts disease severity. Host malnutrition is linked to increased death rate in *T. cruzi*-infected mice (133). In the acute stage of CD, a high-fat diet reduced parasitemia, parasite load in the heart, and ultimately mortality (134). In contrast, a murine model of the chronic stage of CD showed that a high-fat diet increased oxidative mitochondrial stress, contributing ultimately to cardiac dysfunction and cardiomyopathy (135). An atherogenic diet is also believed to offer some protection against *L. donovani* infection (136).

CONCLUSIONS AND FUTURE DIRECTIONS

Intracellular pathogens depend on the host to fulfill their carbon as well as other nutrient requirements, and extracellular parasites such as *T. brucei* nevertheless must

scavenge host nutrients. To meet these needs, and in response to infection, kinetoplastids cause several host metabolic alterations. Greater commonality was observed when comparing metabolic adaptations in leishmaniasis and CD than for HAT, CD, and leishmaniasis together. Both *Leishmania* and *T. cruzi* initially downregulate parasite glycolysis upon infection (27, 54), whereas *T. brucei* relies on glycolysis during mammalian bloodstream infection (73). Likewise, both *Leishmania* and *T. cruzi* upregulate parasite β-oxidation during infection, though this increase is small in *Leishmania* (18, 52). *T. brucei* from adipose tissue was also able to consume fatty acids (79). In terms of host metabolic alterations during infection, greater similarities were likewise observed when comparing *Leishmania* and *T. cruzi* infections: for example, both downregulate host glycolysis, at least at early time points (26–28). Other common trends include changes in host oxidative phosphorylation and AMPK modulation as well as changes in phosphocholine and amino acid abundances. However, the direction of changes in these metabolic pathways are often unique to each kinetoplastid infection. For example, *in vitro Leishmania* infection was primarily associated with increased host oxidative phosphorylation (26, 29), while most studies of *T. cruzi* infection indicate decreased host oxidative phosphorylation (29, 60, 61). AMPK activity helped *Leishmania* proliferation (26), while it hindered the growth of *T. cruzi* (62, 104). An increase in phosphocholines was observed in *Leishmania* and *T. cruzi* infection, while a depletion of overall phosphocholines was observed for *T. brucei*, with just specific phosphocholines being increased (27, 43, 61). The bloodstream form of *T. brucei* metabolizes amino acids (76). This impact of infection on amino acid metabolism is common across kinetoplastids, but with different directionality depending on the species: the aromatic amino acids tryptophan and tyrosine decreased with *T. brucei* infection (84). In contrast, the levels of several amino acids were increased by *T. cruzi* infection in cardiac tissue in mouse models (59) and by *Leishmania* infection in macrophages (32), though acute *T. cruzi* infection was associated with depleted tryptophan in the large intestine (66).

A further limitation is the diversity of systems in which these metabolic changes have been evaluated, hampering cross-study comparisons. Although *T. cruzi* colonizes a broad range of tissues *in vivo* (137), few *in vitro* studies consider multiple cell types in parallel, and separate *in vitro* studies have shown conflicting impacts on cellular glycolysis and oxidative phosphorylation (26, 50, 60). Few studies use multiple *Leishmania* species or *Leishmania*, *T. cruzi*, or *T. brucei* strains in parallel. Likewise, while differences in parasite metabolism between vector stages and mammalian stages are unsurprising, there are also large differences between *in vitro* and *in vivo* pathogen metabolism. For example, doubling time for lesional *L. mexicana* is over three times slower than when infecting macrophages *in vitro*, reflected in 5-times-slower fatty acid turnover in the lesion (19). Likewise, *in vitro* settings lack the complex signals derived from multiple intercellular interactions that develop *in vivo*. For example, unlike observations *in vitro* (26), *in vivo L. major* infection was associated with decreased host cell respiration, induced by local immune response-derived nitric oxide (31). Thus, *in vitro* findings should only be extrapolated with caution and should always be validated *in vivo*. Reliance on animal models is unavoidable to enable the invasive tissue collection necessary to assess host and parasite metabolism at sites of disease and to tightly control infection duration, diet, comorbidities, medication usage, etc. Nevertheless, the relevance of each animal model to the disease being represented should always be considered. Concordance between multiple animal models (especially across species, but also across infection durations and diets) strengthens translatability of findings to humans. Nevertheless, sufficient overlap is expected. Indeed, rats and humans share all but eight metabolic enzymes, and only a minority of metabolic subsystems include species-specific reactions (138).

Demonstrating which of these metabolic changes are associated with pathology, which are instead protective and help the host compensate for the stress of infection, and which are merely "passenger" metabolic changes is still in its infancy. Thus, studies of infection metabolism benefit from being coupled with interventional investigations from a fundamental as well as a drug development perspective. This is particularly true for

phospholipid alterations, whose biological significance has been understudied compared to changes in central energy metabolism (except with regard to miltefosine treatment in leishmaniasis). It will also be important to begin differentiating between "keystone" metabolic adaptations and the possibility that infection instead induces a multiplicity of small-scale metabolic alterations that cumulatively affect infection outcome. The latter may be missed by data analysis techniques that rely exclusively on univariate fold change analyses. Furthermore, fold change in metabolomics data is not always a direct representation of the exact fold change in the underlying metabolome, even though directionality of change is usually conserved (139). Similarly, fold change at the transcriptome level is not necessarily the same as fold change at the functional level (140).

Another common trend is parasite metabolic flexibility. *Leishmania* organisms adapt their metabolism to nutrient-restrictive conditions *in vivo* (18), while *T. cruzi* organisms are able to reduce the dependence on glycolysis upon exposure to the extracellular matrix (54). Metabolic flexibility in *T. brucei* allowed the parasites to proliferate in the presence of glycerol rather than glucose *in vitro* (71). However, many of these studies have been performed *in vitro* or have not considered metabolic adaptations to different tissue sites and cellular environments. In addition, there is considerable variability between individual parasite cells (129), although this has not yet been studied at the metabolic level. Future studies should aim to use single-cell approaches like single-cell Raman or single-cell metabolomics, which could provide more insight on the metabolic fingerprint of a single cell (141, 142).

Many studies of the metabolism of infection have only relied on a single or a few time points, and usually a single parasite strain. As costs decrease for omics (especially for liquid chromatography-mass spectrometry-based metabolomics), more time-resolved investigations should be pursued, enabling the determination of disease trajectories on a metabolic level. These advances will enable assessment of metabolic resilience and metabolic disease tolerance, aspects that we are now discovering play key roles in infection outcomes in kinetoplastid diseases and beyond (66). Likewise, usage of multiple parasite strains (and host strains or species in the case of experimental disease models) is essential to enable generalizations on the impact of infection on metabolism and to facilitate translatability to patients. Strain-specific impacts are to be expected, but strain-independent effects should be prioritized for drug and biomarker development, given that the infecting parasite strain is usually unknown in a clinical setting.

The majority of host-kinetoplastid metabolism studies have considered only mammalian and parasite metabolism. However, from a physiological standpoint the human microbiome has often been considered an organ by itself (143). In the GI tract of an *in vivo* CD model, different tissue types had various metabolic and microbiome alterations with infection (66). In CL, skin dysbiosis is not restricted to the site of the skin lesions and a global change in the skin microbiota is observed (144), although microbiome metabolic profiling in this context has not been investigated. Therefore, there is still considerable scope to define how microbiota metabolism affects kinetoplastid disease progression.

Although targeting metabolism is complex, multiple successes demonstrate that this is possible (see "Avenues for Intervention" above). Several of these successes have been with pleiotropic multitarget agents (e.g., carnitine [66], metformin [104], and resveratrol [104] in CD). One intriguing possibility is that these interventions were successful perhaps exactly because of this multiplicity of effects, enabling restoration of the multiple infection-induced metabolic changes. Such a possibility warrants further investigation as we seek to build on these insights into metabolism of infection to defeat kinetoplastid diseases. There is also still significant scope for exploring immunometabolism modulators to treat these diseases.

Overall, there have been major advances in our understanding of metabolism during kinetoplastid infection. Further advances will be facilitated by new data analysis methods and new technologies, including increased spatial analysis *in situ* in infected tissues and single-cell analyses. These insights will lead to new ways to monitor and treat these infections.

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