Published in final edited form as:

Nat Microbiol. 2018 February ; 3(2): 130–131. doi:10.1038/s41564-017-0105-3.

Even malaria parasites watch their host's diet

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Abstract

Standfirst. In recent years there has been a growing appreciation of the role metabolism plays in controlling nearly all aspects of cellular function. Three recent articles explore how host metabolic cues influence different aspects of *Plasmodium* biology during infection, including parasite growth and sexual differentiation.

Despite recent progress towards eradication, malaria remains a significant global health concern. Infection starts when *Plasmodium* sporozoites are delivered into a mammalian host via the bite of an infected female Anopheles mosquito. These sporozoites then travel to the liver where they infect hepatocytes, and within these cells the parasites develop into thousands of merozoites that are released into the circulation, where they proceed to infect red blood cells (RBCs). During intraerythrocytic development a subpopulation of parasites produce merozoites that are committed to differentiation into male or female gametocytes that are eventually taken up by mosquitoes, thereby perpetuating the infectious cycle (Figure 1). Given the interplay between parasite and host processes, it is likely that changes in the host metabolic status can influence disease progression. Indeed, three recent papers explored the influence of biochemical mediators of diet and nutrition on *Plasmodium* biology^{1–3}.

The authors studied the effects of diet and nutrition under three very different conditions at different stages of the parasite life cycle in the mammalian host and *in vitro*, namely: the effect of high fat diet (HFD) in mice on parasite killing at the liver stage of infection¹; the effect of caloric restriction (CR) in mice on parasite growth and replication²; and the effect of lysophosphatidylcholine (LysoPC) on parasite sexual differentiation and growth in culture³. What is fascinating about these studies is the insights they provided into the basic mechanisms controlling parasite functions, consistent with the degree to which metabolism is intertwined with cellular function.

Mice placed on high fat diets were discovered by Zuzarte-Luís *et al.* to kill *P. berghei* ANKA (*PbA*) parasites in the liver after invasion. The authors show convincingly that even a few

days on a high fat diet reduced the *PbA* parasite load in C57BL/6 J mice. This was an unexpected finding as hosts are generally considered to be more or less defenseless against the liver stage of the infection. The authors considered that killing was mediated by inflammation or the production of reactive oxygen species (ROS). The authors excluded inflammation as a cause of *PbA* parasite death using mice deficient in a number of inflammatory pathways. Transcription profiling generated the hypothesis that oxidative stress and, more specifically, increased generation of ROS caused elimination of *Plasmodium*. The data presented are consistent with the hypothesis, but they are insufficient to provide rigorous support for its causality. Only a single measure of ROS was utilized, namely the oxidation of dihydroethidium. The assay was originally proposed to be specific for superoxide, but as implemented in this study, it is not⁴. The observed increase in fluorescence is modest, being only about 40% on the high fat diet compared to the regular diet. While this increase reached an impressive statistical significance, it might not have biological significance. Determination of other well-validated markers and mediators of oxidative stress/damage would have been welcome. Among these, measurement of isoprostanes⁵, hydroxynonenals⁶, and of carbonylated proteins⁷ might be considered since each is elevated by oxidation of fat. Moreover, the isoprostanes, hydroxynonenals, and carbonylated proteins could be the mechanistic cause of reduced parasite load, rather than a direct effect of ROS. Treatment with N-acetyl cysteine blocked increased ROS and restored control levels of parasite proliferation. However, N-acetyl cysteine could also reduce production of isoprostanes, hydroxynonenals, carbonylated proteins, and other potential causal agents. Nonetheless, it is interesting that high fat diets may be protective in malaria and raising the possibility that diet may contribute to the relative malaria resistance of the Fulani ethnic groups in Africa as compared to other ethnic groups with which they live sympatrically⁸. The Fulani are herders and thus may have more ready access to an animal-based high fat diet, which may protect against malaria.

In contrast to a HFD, Mancio-Silva *et al.* put C57BL/6J mice on a caloric restriction (CR) diet for two to three weeks before infecting them with *PbA*. Whereas usually infection with this parasite in these mice causes experimental cerebral malaria (ECM) and results in rapid death within six to nine days, the CR mice did not develop ECM and did not die until 28 days post-infection, presumably from high parasitemia and severe anemia. *PbA* parasites in the CR mice demonstrated a number of transcriptional changes and further mutational analysis indicated that knock out of one kinase, KIN, reversed the effect of CR, resulting in ECM in CR mice. KIN has limited homology to the kinases that undergo activation during CR for energy homeostasis in yeast (sucrose non-fermenting 1; SNF1) and in mammals (AMP-activated kinases; AMPK). The threonine in the predicted active site of *PbA* KIN was changed to aspartic acid, a negatively charged amino acid, that maintains activity of the kinase without requiring phosphorylation⁹. The *PbA* KIN with the mutation (T616D), but not wild type KIN, could replace SNF1 in yeast deficient in SNF1, providing evidence that KIN in *PbA* is equivalent to SNF1/AMPK in yeast and mammals. In yeast and in mammals these enzymes are activated during energy depletion and can be down regulated by glucose, and the author demonstrated similar activity in *PbA*. As SNF1 and AMPK are trimeric proteins, it is unknown if the other two proteins were lost during *Plasmodium* evolution or rather remain unidentified. Nonetheless, this exciting discovery of a nutrient-sensing kinase

in *Plasmodium* was a serendipitous outcome of a search for a mechanism by which CR protected mice from ECM, and it will be interesting to understand if KIN does indeed play a role in resistance to ECM.

In a search to identify factors that influence sexual differentiation in *P. falciparum*, Brancucci *et al.* found that serum-free media markedly enhanced the production of sexual stage parasites and reduced asexual growth *in vitro*. Through an extensive screen of serum components, the authors identified one lipid class, LysoPC, which alone could reverse the effects of serum free media and effectively suppress sexual differentiation and enhance asexual parasite growth. Non-hydrolyzable LysoPC analogs had no effect, arguing against LysoPC working as a direct signal. Instead, the authors found that the parasite utilizes LysoPC from the media to produce fatty-acid- and choline-derived products such as phosphatidylcholine (PC) and diglycerides that are used for membrane synthesis during asexual growth. Transcription profiling of parasites grown in the absence of LysoPC demonstrated the up-regulation of transcripts for genes reported to be associated with gametocytogenesis, including the *ap2-g* transcription factor (PF3D7_1222600)¹⁰ and gametocyte development 1, *gdv1* (PF3D7_0935400)¹¹, in addition to enzymes involved in phospholipid biosynthesis. *P. falciparum* parasites recently isolated from patients were not tested, but LysoPC had similar effects on three *P. falciparum* lines, derived from strains Pf2004, NF54 and HB3. In contrast, in the *P. berghei* rodent malaria model there was a marked effect from LysoPC on asexual growth, but no change in sexual differentiation, suggesting gametocytogenesis is regulated differently in the two species. The differential effect of LysoPC limitation on sexual differentiation and asexual growth is an important advance. It will be exciting to continue to unravel the underlying molecular mechanisms and to translate this finding to the field. One major question is whether gametocyte-committed merozoites selectively survive LysoPC limitation or if the metabolic changes associated with LysoPC starvation actually induce production of gametocytes. LysoPC levels in human serum normally range between 200–300 μM ^{12,13}, which is above the 1–20 μM IC₅₀ found to suppress sexual differentiation *in vitro*, but LysoPC levels could vary in different microenvironments and have been shown to fall significantly during infections¹³, including those with *P. falciparum*¹⁴. The balance between asexual growth and sexual differentiation is essential to maintain malaria transmission, so a better understanding of the role of LysoPC in this process could contribute to new disease control strategies.

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Figure 1. Influence of diet and serum lysophosphatidylcholine on the *Plasmodium* life cycle. Schematic of the *Plasmodium* life cycle depicting 1) death of liver stage *PbA* parasites following a HFD; 2) KIN-mediated decrease in pathogenicity of the intra-erythrocytic stages of in *PbA* parasites following CR; and 3) LysoPC increasing *P. falciparum* asexual growth and reducing sexual differentiation (gametocytogenesis) in culture. Figure adapted from ref. ¹⁵, Macmillan Publishers Ltd.