The Fe-nomenal Flexibility of *Toxoplasma* Metabolism

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Iron is essential for almost all life due to its role as a cofactor in vital cellular processes. For the obligate intracellular parasite *Toxoplasma gondii,* iron must be obtained from their host cells. Given *Toxoplasma's* unprecedented host cell range and the role of nutritional immunity in regulating iron levels, *Toxoplasma* must contend with diverse and dynamic levels of accessible iron. The metabolic adaptations *Toxoplasma* makes to survive and propagate in these changing iron environments have not been investigated. While observing mitochondrial morphology, we identified iron dependent changes that were rescued 6 hours after the reintroduction of iron replete media. We also observed changes to mitochondrial membrane potential and using extracellular flux analysis, confirmed that iron deprived parasites displayed significantly reduced mitochondrial oxygen consumption while glycolytic output was sustained.

These changes to energy metabolism prompted us to perform untargeted metabolomics. We observed an accumulation of both citrate and fumarate in iron depleted parasites, suggesting dysregulation of the TCA cycle. We then performed stable isotope labelling using ^{13}C glucose or glutamine which revealed increased incorporation of glucose into lactate and reduced incorporation of glucose into the TCA cycle. We also observed increased incorporation of glutamine into the TCA cycle, suggesting a disconnect between glycolysis and TCA. To understand which energy sources are key under iron deprivation we limited the major carbon sources utilised by *Toxoplasma*. We found that glucose was critical for survival while glutamine was dispensable under low iron conditions. These findings provide the first evidence of Warburg-like adaptations to *Toxoplasma* energy metabolism and underscore the remarkable metabolic flexibility of these parasites in response to nutrient stress.