Lipidomic analysis of intracellular *Leishmania (Leishmania) infantum* amastigotes

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Visceral Leishmaniasis, caused by *Leishmania infantum* in Brazil, is caused by a digenetic parasite presenting as promastigote (in the vector insect) and amastigote (intracellular). The amastigotes reside in the cell's phagolysosome. While the promastigote develops inside the phlebotomines gut and relies more on glycolysis, the intracellular amastigote uses β-oxidation as energy sources. Thus, lipids constitute one of the sources of energy for the intracellular parasite. In addition, the amastigote can incorporate lipids from the host into its lipid composition, playing a significant role in the intracellular growth of *Leishmania*. The main aim of the present study is to characterize the lipidome of *L. infantum* amastigotes isolated from infected THP-1 macrophages. Human macrophages derived from THP-1 monocytes were infected with stationary phase *L. infantum* promastigotes , at 10:1 parasite:cell ratio. The intracellular parasites were recovered and isolated through centrifugation in a Ficoll400 column at time zero (after 6 hours of parasite:macrophage co-culture) and 24 hours after infection. A comprehensive analysis of the lipid composition of the amastigotes was conducted through lipidomics at both time points. We analyzed the lipidome of *L. infantum* amastigotes in comparison with the lipid composition of the infected macrophage and identified 265 differentially expressed lipid molecular species, demonstrating significant differences between the lipidome of infected human macrophages and intracellular *L. infantum*. In contrast to the macrophages that exhibited a greater enrichment in phospholipids associated with polyunsaturated fatty acids, sphingomyelins, and triglycerides with long and unsaturated chains, *L. infantum* intracellular amastigotes showed a higher concentration of cholesterol esters, triglycerides containing short and saturated fatty acids, and ceramides. Characterizing the lipids in *L. infantum* amastigotes can contribute to a better understanding of the parasite's lipid metabolism. These data could also reveal new targets for the development of therapeutic strategies and allow the identification of specific lipid biomarkers for the prognosis and monitoring of the progression of Visceral Leishmaniasis.

Keywords
Visceral Leishmaniasis; *Leishmania (L.) infantum*; Lipidomics; Amastigotes; Lipids

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