

The impact of glucose in *Trypanosoma cruzi* viability, cell growth, cell cycle progression, differentiation, and histone post-translational modifications

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The *Trypanosoma cruzi*, the etiological agent of Chagas disease, is a digenetic flagellated parasite that infects vertebrate and invertebrate hosts. Metacyclogenesis involves the differentiation of epimastigote into metacyclic trypomastigote forms and is triggered by nutritional stress, cellular adhesion and by changes in the microenvironments such as temperature and pH (1). This differentiation induces morphological alterations as well as changes in nuclear position and shape, which occurs in parallel with an increase in the heterochromatin and changes in histone post translation modifications (PTMs). Histone acetylation is associated with an open chromatin status and transcription activation. Importantly, the acetyl-CoA, a central metabolite, is the main source of acetylation indicating an important link between metabolism and epigenetics. As the glycolytic pathway is one of the main sources of acetylCoA, we aimed to evaluate the impact of glucose concentration on the parasite growth, viability, cell cycle progression, metacyclogenesis, and global histone acetylation levels. The *T. cruzi* CL Brener parasites were kept in the Liver Infusion Tryptose (LIT) medium, pH 7.4, supplemented with 10% inactivated fetal bovine serum with a glucose gradient from 0.0 to 0.4% (w/v), at 28°C. The epimastigotes growth curve was initiated at a density of 2.5x10⁶ cells/mL, and the phenotypical analysis (growth, viability, and cell cycle progression) were evaluated at three-time points of the exponential phase, namely: early (at 24 h), middle (at 72 h), and late (at 120 h). As expected, parasite density was higher at glucose concentration of 0.2% and 0.4% compared to 0.0% and 0.1%. Furthermore, at late timepoint, the cell viability (MTT assay) was dropped by around 30% in parasites cultivated with 0-0.1% of glucose. . Flow cytometry analyses indicated that glucose concentration does not interfere in the cell cycle progression. Interestingly, the differentiation rate of epimastigotes to metacyclic trypomastigotes was higher in the parasites kept at 0.0 to 0.2% of glucose compared with those cultivated in higher glucose concentrations. Using an optimized workflow of MS-based data-independent acquisition analysis preliminary data showed no differences in the global histone acetylation levels in parasites cultivated with 0.0 and 0.4%

of glucose. Ongoing research is being performed to associate glucose concentration and the generation of acetyl-CoA and global histone acetylation. The results proposed in this study will be pioneering to uncover the underestimated potential of glucose metabolism in epigenetic changes in parasite protozoa.