

Referee's report on the PhD Thesis of Wunnie Brima, MD, entitled
"Effects of obesity on the course of *Trypanosoma cruzi* infection"

Wunnie Brima has submitted a doctoral thesis on the effect of a high fat diet (HFD) in combination with metformin, on *Trypanosoma cruzi* infection in CD-1 mice. The main outcome of the research was the survival of infected/uninfected mice.

Infection with the protozoan, *T. cruzi* causes Chagas disease in humans. It is found mainly in Latin American countries. Parasite persistence has severe cardiac and gastrointestinal complications and it affects the liver, spleen and bone marrow. It is well-established that white/brown adipose tissue is an important reservoir for the parasite. *T. cruzi* infection-induced lipolysis is a hallmark of acute infection. Beleigoli et al. (2012) reported that overweight/obesity is a protective determinant of mortality in patients with Chagas disease. The inverse relation between overweight/obesity and lower mortality risk was firstly described for heart failure and coronary artery disease and termed the "obesity paradox". Wunnie Brima focused her research on showing that a high fat diet/ combination of HFD with metformin may play a protective role in mammals infected by *T. cruzi*.

The thesis consists of 3 chapters, covers 78 pages and includes 9 tables and 18 figures. There are 196 references. The extent of the thesis is consonant with its significance and the amount of new knowledge it presents, especially given that the results and the authors' research work have been already published in *Diabetes/Metabolism Research and Reviews* 2015;31:346-359.

The first chapter (Literature Review, 28 pp) explains the links between obesity and the metabolic syndrome and provides a physiological background to adipose tissue and cytokines/adipokines. There is a review of experimental animal models, diet types and genetic models that are used in metabolic syndrome studies. The last part of this chapter summarizes Chagas disease.

Chapter 2 (Experimental part, 30 pp) describes experiments in mice and on human foreskin fibroblasts.

Chapter 3 (Discussion and conclusion, 7 pp) is relatively brief but the results look extremely promising. The idea that obesity at an early age can be beneficial to the body and have a positive selection function in infectious disease, however, requires further study. From a formal point of view, the thesis is written and organized very carefully. There are no typing

errors within the text that is written in a clear and concise manner. The only item is Blaser et al. cited on page 64 that is missing in the reference list.

The questions for discussion:

1. Two medications, benznidazole and nifurtimox are used to treat Chagas disease if treatment is initiated at an early stage. Possibly treatment with these medications in combination with a high fat diet would be more effective?
2. These medications kill the parasite by production of free radicals which are toxic to *T. cruzi*. On the other hand, metformin is an antidiabetic agent that acts by suppressing gluconeogenesis and increasing peripheral glucose uptake. It has anti-inflammatory/anti-angiogenic effects and inhibits the production of free radicals. A recent study reports that metformin improves the balance of white/brown adipose in a mouse model of obesity by inducing fibroblast growth factor (FGF21). What is the author's opinion that these different mechanisms of action are effective in the case of *T. cruzi* infection?
3. Can metformin be used for additional treatment to slow the progression of Chagas disease?

My view is that this thesis provides an original contribution to the scientific field in question. The thesis fulfills all the requirements of a PhD thesis. It is well-presented and very interesting to read. The aims are achieved and the results can be applied in ongoing clinic research. Wunnie Brima proves to be a skilled researcher. I recommended the thesis for oral defense, and subsequent award of academic degree of Doctor of Philosophy (PhD).

Olomouc, April 18th 2016

Prof. MUDr. Vilim Šimánek, DrSc.