Review of the doctoral theses entitled „Effects of obesity on the course of Trypanosoma cruzi infection" by Wunnie Brima, MD

Dr Brima has prepared his theses partly at Department of Medical Biochemistry and Laboratory Diagnostics at 1st Faculty of Medicine, Charles University in Prague and at the Feinstein Institute for Medical Research, New York. The latter Department was the place of his practical experimental work documented by his findings and conclusions written in doctoral disertation.

Manuscript of his doctoral disertation is composed of three main parts. The first chapter includes the review on obesity and metabolic syndrome from the view of cytokine and adipokine changes, then on experimental models of metabolic syndrome including dietary, genetic and streptozotocin models and main characteristics of Chagas disease. The second chapter includes experimental characteristics of mice models which have been used in separate experiments. It contains the most important results obtained from his experiments. Finally, the third part includes short discussion and conclusions.

The whole text has 66 pages, 9 tables, 18 figures and 196 references. There are no own manuscripts with the original results prepared for publication in the journals as a part of the theses. The disertation is therefore the main manuscript of his work.

The experimental work was done on dietary modified mice. Two groups on regular diet and on high fat diet were subdivided into control and metformin treated arms. Both arms included mice either on placebo or infected with Trypanozoma cruzi. Altogether eight groups of mice have been created for experimental studies. Oral glucose tolerance tests, body composition analysis, immunoblot analysis of the heart tissue, cytokine, insulin and leptin measurements in serum as well as histopathologic analysis of the heart tissue were examined. In addition, cell culture experiments were included as well.

Mortality rate was decreased in high fat diet mice infected by Trypanosoma as compared with mice on regular diet (20 % vs 55%). Metformin treated mice had significantly reduced mortality in animals on both high fat diet and regular diet (3% vs 20 % and 25% vs 55 %). Obesity had therefore protective effect in infected animals as evaluated by mortality rate and it was further strenghten by metformin treatment. The infection induced weight loss in both high fat diet and regular diet and the decrease was more pronounced in high fat diet mice.

Fasting blood glucose was elevated in high fat diet mice compared to mice on regular diet. Metformin treatment decreased fasting blood glucose in high fat diet mice but not in those on regular diet. Infection induced significant decrease of fasting blood glucose on day 30 and this was more expressed in high fat diet mice. The results in oral glucose tolerace was not different in uninfected group between regular diet and high fat diet mice. However, in infected animals, high fat diet was associated with enhanced glucose tolerance compared with regular diet mice. Suprisingly, mice treated with metformin had higher fasting glucose levels in mice on regular diet. In infected mice the postglucose levels were lower in high fat diet as in regular diet mice and glucose values were again higher in metformin-treated mice compared to those without metformin. ***Has the author any explanation of these results?***

The expression of two enzymes, pAKT and total AKT from the insulin signaling cascade, was different. pAKT was elevated following infection whereas total AKT was not changed. The metformin treated infected mice demonstrated significantly decreased expression of total AKT in both dietary regimens. ***Is there any explanation? What is the difference between pAKT and total AKT?***

The infection was associated with significantly increased interferon-gamma in the heart tissue. Metformin induced a decrease of INF-γ in uninfected mice but not in infected animals. Tumor necrosis factor alfa was significantly elevated in infected animals without any difference due to metformin treatment.

Following seventy days of the experiment, the plasma insulin concentration was lowered in metformin treated uninfected animals on regular diet but not on high fat diet. The infection caused a significant decrease of insulin levels in all groups of mice. ***Is it the primary effect of the infection on beta-cells or a secondary due to changes in insulin sensitivity?*** Leptin levels reflected the amount of body fat. It was higher in high fat diet mice compared to regular diet whereas metformin reduced leptin levels only in regular diet mice. The infection stronly decreased leptin levels as a consequence of lower body weight. ***Is there any correlation between changes in body weight and leptin concentration?***

The evaluation at 70th day postinfection showed that serum levels of IL-6 were increased in high fat diet mice and the infection further significantly increased its levels. This was not found in mice on regular diet and metformin did not have any significant effect in these mice. TNFα levels had opposite effect between regular and high fat diet mice following infection. TNF was increased in regular diet mice but decreased in high fat diet mice after infection in the 70th day. ***Is there any elucidation of this opposite effect?*** TNF reaction was different in the 30th at tissue level and in the 70th day in serum. No difference was found between both groups after metformin treatment. Serum level of IFN-γ was significantly increased in infected mice and the most significant increase was found in high fat infected mice treated with metformin.

Histopathologic examination showed significant inflammation in the heart in postinfection animals irrespective of the type of diet. Parasitemia was increased later and with higher peak in high fed diet mice compared to regular diet mice. The parasite load was found greater in myocardium and omental fat than in the liver. A reduced level (to about 40 %) of parasitemia was found in both regular and high fed diet mice treated with metformin. ***Is there any explanation of this metformin effect?*** No effect of metformin on the growth of parasites was observed in fibroblast culture.

Summary:

Dr. Brima offers in his theses very interesting experimental data concerning the influence of obesity on infection caused by Trypanosoma cruzi in mice. The results have demonstrated the protecting effects of obesity on death as well as the additive effects of metformin treatment against infection. Parameters of glucose metabolism, inflammation and infection at the level of heart, adipose and liver tissue as well as blood have been evaluated in two streams of animals fed by different diets with or without metformin treatment.

Dr. Brima described experimental results in the field which is very actual because obesity as a part of metabolic syndrome is the increasing problem in both developed and developing

countries. It is evaluated as a cluster of risk factors in development of cardiovascular disease. On the other hand, the role of obesity in acute disease like infection is totally different. It was demonstrated by Brima´s work. He used well designed experiments and received original data which are useful for clinical practice although they need to be further confirmed in human medicine.

Doctoral disertation is clearly written but it is not evident which experimental work has been done by the author himself. In addition, there are not mentioned any references with his participation. Are there any manuscripts prepared from the results of his disertation? However, all these remarks do not decrease the high value of his doctoral theses.

In conclusion, Dr. Brima had the opportunity to work at the outstanding department with prominent researchers in New York. He could receive great experience in basic research and make by his hands original results. Dr. Brima gained practical and theoretical experience in in the field of laboratory work. He formulated hypotheses which were followed by hard data obtained in separate experiments. It is evident that his work fulfilled the criteria which are demanded in doctoral students. I therefore recommend to evaluate his disertation as excellent because it demonstrates his own activity in the experimental work and confirms that he may be a recipient of the title „Ph.D.".

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