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REVIEW OF PhD THESIS

Thesis Title: The effect of ambient temperature and the gut microbiota on the development of diet-induced obesity

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The gut mucosa is the largest immunologically active organ in the body and protects the host from invading microorganisms, which are collectively known as the gut microbiota. Although these microorganisms may affect inflammation and cause infectious diseases, they are also beneficial and have important functions including nutrient absorption, vitamin production and metabolism of xenobiotic compounds (Caesar et al. 2010, J Internal Med). In the presented thesis, PhD candidate Marika Ziętak, describes interesting research project, in which she investigated whether reduction in ambient temperature causes changes in the gut microbiota that affect energy balance and susceptibility to diet-induced obesity (DIO). To achieve this aim two *in vivo* experiments were performed. In Experiment I C57BL6/J male mice were fed a standard chow diet or high-fat diet and were housed at ambient temperatures of 29, 17, or 12°C for 4 weeks to determine the effects of ambient temperature on adiposity and energy metabolism phenotypes together with associated changes in gut microbial metabolism. The idea of the Experiment II was to investigate whether the development of or protection from DIO at reduced ambient temperature could be

transferred to a germ-free host through gut microbiota present in the caecal material of the donors. Both experiments were carefully planned and performed.

The Dissertation is written in English, has typical and appropriate structure on 95 pages with 5 tables and 16 figures. It is starting with Table of Contents. Next there are the following chapters: List of Abbreviations, Introduction, Aim of the Research, Material and Methods, Results, Discussion, Conclusions, Lists of Tables and Figures, Literature, Summary in English and Summary in Polish.

In the Introduction chapter Author describes white and brown adipose tissue biology. Then information about gut microbiota are provided. This part is prepared concisely and easily introduces the readers in the subject of studies. Only one small disadvantage is lack of any schemes to facilitate understanding of some complicated metabolic processes described in this chapter.

The aim of the study is divided into three clearly formulated research tasks which are tightly connected with performed two *in vivo* experiments. Nevertheless, the chapter describing aims of the study should be re-organized, because most of it constitute details about carried experiments which are also presented in the next chapter "Materials and Methods".

In general, provided methods description is sufficient to understand and repeat experiments. However the measurement of "Bile acid analysis" and "Short-chain fatty acid analysis", even if were done as outsourcing, should be described in more details. E.g. information about eluents used for UPLC or a type of the column used for GC should be provided. Principal Coordinate Analysis which results are presented in Figure 9B should be described in Statistics paragraph in Methods chapter.

The results are presented in 16 figures. This is the most interesting part of dissertation. Obtained results show that in reduced ambient temperatures (12 or 17°C) DIO was suppressed in high-fat diet fed mice with increased thermogenic energy expenditure. Moreover, cold exposure improved glucose and lipid metabolism in high-fat diet fed mice. Gut microbiota, which diversity and composition at phylum and family levels were markedly changed due to diet and ambient temperature might constitute potential mechanism linking the reduction of diet induced obesity at reduced ambient temperature. There is no doubt that this work is original and contributes significantly to an understanding of basic mechanism of DIO development.

One of unexpected results presented in the dissertation is that mice fed chow diet housed at 29°C are insulin resistant (Figure 3B). I would like this issue to be elaborated during the defense of the PhD thesis. Please discuss what are the

molecular mechanisms leading to such surprising effect. Are people living in warmer climate more insulin resistant than people from colder regions?

There are some minor remarks regarding results of Western blot analysis presented in Results chapter:

1. Figures 5B, C – there is lack of pictures of Western blot analysis presenting UCP1 protein levels after chow diet feeding although they are presented on the graphs. Why? – please explain.
2. Densitometric analysis in Figures 6B, C, E shows increase in pAMPK, pACC and CPT1 proteins in high-fat diet fed mice housed at 12°C when compared to 29°C. However presented in dissertation pictures of Western blot analysis do not show these changes.
3. Figure 10 is very small and therefore illegible.
4. Figures 12 E, F - there is lack of pictures of Western blot analysis presenting TGR5 protein level after chow diet feeding although they are presented on the graphs. Additionally, TGR5 and beta actin form almost one band – how densitometry analysis was done? Figure 12E – Y axis label is incorrect.
5. Figure 14E - shows quantitative decrease of CPT1 protein in high-fat diet fed mice housed at 29°C when compared to 12°C. However, presented picture of Western blot analysis do not show such difference.
6. Figure 14H – beta actin protein level is increased at 29°C when compared to 12°C. This effect is not noticeable in Figure 14E. Please explain why?

The last chapter of this dissertation was dedicated to the discussion and completed by conclusion. In the Discussion Author comments her results in comparison with the results of other authors supported by the current knowledge in widely cited references. In this discussion Author is careful and manifests some criticism to her own results. The conclusions are presented in six points being an answer to the aim of the study.

Altogether, Ms. Marika Ziętek presented in her PhD thesis interesting and original results. She also proved that she is able to design and carry out experiments as well as correctly interpret results. Marika Ziętek clearly demonstrated her leading role in the research performed and her great scientific possibilities and abilities.

I conclude that the dissertation presented for evaluation meets the requirements of the LAW ABOUT SCIENTIFIC DEGREES AND TITLE OF SCIENTIFIC AND THE GRADE AND TITLE IN THE FIELD OF ART and recommend to the Scientific Council of the Medical University of Białystok admission of Ms. Marika Olga Ziętak to the further stages of Ph.D. proceedings.

Stwierdzam, że przedstawiona do oceny rozprawa doktorska spełnia wymogi stawiane przez **USTAWĘ z dnia 14 marca 2003 r. O STOPNIACH NAUKOWYCH I TYTULE NAUKOWYM ORAZ O STOPNIACH I TYTULE W ZAKRESIE SZTUKI** (Dz. U. z 2003 roku Nr 65, poz. 595 z późniejszymi zmianami) i wnoszę do Rady Wydziału Lekarskiego Uniwersytetu Medycznego w Białymstoku o dopuszczenie mgr Mariki Olgi Ziętak do dalszych etapów przewodu doktorskiego.

Agnieszka Dolnyś