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Review of PhD thesis

Thesis title: Genetic and environmental interactions in the acquisition of the brown adipocyte phenotype during postnatal development.

General description of the dissertation

As the prevalence of overweight and obesity continues to increase, efforts abound to attract people's attention to the importance of environmental factors, including nutrition, in the determination of body weight. However, these different strategies have failed to stop the epidemic of obesity. The most effective approach seems to be prevention; for this reason, nutritional education with the aim of creating healthy habits, especially in children and adolescents, is of great importance. Once obesity develops, it is very difficult to efficiently and permanently reduce body weight. The idea of affecting the development of brown adipose tissue to enhance its energy dissipation capacity over the whole lifetime thus seems very promising. Moreover, it has been shown that environmental factors that occur during development (prenatal or early postnatal) may have lifelong effects on metabolism, due to phenotypic plasticity and a variety of adaptation mechanisms.

The dissertation submitted for evaluation is written in English and comprises of two parts: the introduction and a description of two articles forming a monothematic cycle of publications. The thesis also contains a list of abbreviations, abstracts (in Polish and in English), and a list of 81 references. The essential part of the dissertation consists of two articles:

1. The critical period for brown adipocyte development: genetic and environmental influences. Chabowska-Kita A., Kozak LP. *Obesity* 2015
2. Low ambient temperature during early post-natal development fails to cause a permanent induction of brown adipocytes. Chabowska-Kita A, Trąbczyńska A, Korytko A, Kaczmarek M, Kozak LP. *FASEB J.* 2015 Aug;29(8):3238–52.

Comments on the introduction:

1. This part of the text shows that the author possesses the ability to present scientific ideas with the use of clear and logic language and without overlong descriptions. This is not a very common skill, especially among young scientists.
2. In my opinion, when the genetic contribution to human obesity is discussed, results of association studies (including genome-wide association studies) should be mentioned.
3. Part of Section 1.4 could be moved to Section 1.5, where stimulation of the brown adipocyte phenotype in WAT is described.

The description of the articles included in the doctoral dissertation is, in my opinion, not really needed.

Comments on the articles included in doctoral dissertation:

1. Review article entitled "The critical period for brown adipocyte development: genetic and environmental influences" published in *Obesity* in 2015 and coauthored by Agnieszka Chabowska-Kita and Leslie P. Kozak.

This review summarizes the recent advances in the origin of brown adipocytes in rodents and humans and presents a model for brown adipocyte development. The article is interesting and well-organized, but in some parts not very easy to follow. A few review articles on brown adipose tissue development have been published recently, but the article by Chabowska-Kita and Kozak do not overlap with them and presents different aspects of this topic.

Abstract: the abstract not exactly reflects the article. The phrase "genetic variability in the expression of brown adipocytes" is unclear.

Conclusions: The existence of enormous interindividual variation in the amount and activity of BAT in humans does not indicate per se that this trait is determined genetically. Large variations may be caused by environmental factors or gene–environment interactions. Also, the phrase "determined genetically" may be misleading to some readers, who may understand it as meaning the trait is monogenic (fully genetically determined). It seems to me that the author meant that this trait also has a genetic component.

p. 2. There is no reference for the sentence beginning with: "In addition, approximately 10% of adipocytes are renewed...".

p. 1. The phrase "food absorption" should read "nutrient absorption".

2. Original article entitled "Low ambient temperature during early post-natal development fails to cause a permanent induction of brown adipocytes" published in *FASEB Journal* in 2015, coauthored by Agnieszka Chabowska-Kita, Anna Trąbczyńska, Agnieszka Korytko, Monika Kaczmarek, and Leslie P. Kozak.

The article, which is written with high scientific accuracy, describes the results of an experiment aimed at manipulating the brown adipocyte phenotype in white adipose tissue using different ambient temperatures during the early postnatal development of mice. The reaction to high-fat diet feeding in mice upon treatment with different ambient temperatures was also analyzed. It was hypothesized that a decreased ambient temperature during early postnatal development would determine the brown adipocyte function and thereby reduce susceptibility to diet-induced obesity. Finally, the reactions of mice to treatments with β-adrenergic receptor agonist or thyroid hormone were analyzed.

The whole experiment is well-designed, very complex, and took much work; it addresses several scientific questions that constitute a comprehensive approach to the problem being studied. Several methods were used in the study, including measurement of food intake, body composition, and total energy expenditure. The effects of the treatments were studied on the molecular level, using global gene expression analysis (and confirmed with real-time PCR) and Western blot analysis. Concentrations of plasma leptin and insulin were measured with the ELISA method. A histological

examination was also performed. All the methods used were appropriate. The conclusions drawn were supported by the obtained results. For all those reasons, but also because of their novelty, I find the obtained results valuable and important.

The declared input of Leslie P. Kozak to this manuscript seems quite low.

Abstract: The experiment's high level of complexity and the great number of results no doubt contributed to the difficulty of writing the abstract. However, I have the impression that the abstract could be improved a little so as to better reflect the content of the manuscript and to more clearly present its ideas.

Methods: It is not quite obvious to me why only 112D mice were mentioned in the description of the tissue collection procedure.

Figures 1A and 5A could be moved to the methods section. Information on all treatments could be added, which would help in understanding the whole experimental procedure at the very beginning of the manuscript.

From how many litters were the mice used for the different phenotypic assays? Since there were 844 mice in the experiment, I expect the number is high enough. However, this information could be added.

The titles of Tables 1 and 2 seem to be misleading.

Conclusions: The question I would consider is how much the activity of BAT contributes to energy expenditure, especially in humans.

Final conclusions

In my opinion, the articles included in the doctoral dissertation have significantly increased our knowledge on the development of brown adipose tissue. Moreover, Agnieszka Chabowska-Kita has demonstrated her broad knowledge of the topic at hand. She has also demonstrated her ability to conduct complex experiments under supervision in order to test scientific hypotheses, and to write well-structured articles. I conclude that the dissertation presented for evaluation meets all the requirements described in the Academic Titles and Degrees Act. I therefore recommend the application of Anna Chabowska-Kita to the Faculty Council of the Medical University of Białystok for further processing of the PhD procedure.

Stwierdzam, że przedstawiona do oceny rozprawa doktorska spełnia wszelkie wymogi stawiane przez USTAWĘ z dnia 14 marca 2003 r. O STOPNIACH NAUKOWYCH I TYTULE NAUKOWYM ORAZ O STOPNIACH I TYTULE NAUKOWYM W ZAKRESIE SZTUKI i wnoszę do Rady Wydziału Lekarskiego o dopuszczenie mgr Anny Chabowskiej-Kity do dalszych etapów przewodu doktorskiego.