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Review of doctoral dissertation
entitled: 'Biomarkers of adipose tissue expansion in obese, aging animals. Biomarkery rozrostu tkanki tłuszczowej u otyłych, starzejących się zwierząt',
written by Magdalena Jura

General description of the doctoral dissertation

The doctoral dissertation, submitted for evaluation, has been prepared at the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn under the supervision of Prof. Ph.D. Leslie P. Kozak. The work, written in English, includes 60 pages and consists of eight chapters: *1) Introduction, 2) Objectives, 3) Materials and methods, 4) Description of the publications included in the PhD dissertation, 5) Further research, 6) Summary, 7) Abstract in Polish and 8) References.* In addition, the list of frequently used abbreviations, the list of publications with their copies as well as statements of co-authors concerning the participation in the preparation of publications composing the doctoral thesis have been included.

Magdalena Jura is the first author of two publications: an original paper published in the *Biochemie* (2015, IF=3.1; 30 points of MNiSW) and a review – published in the *Age* (2016, IF=3.4, 30 points of MNiSW). Based on the attached statements of co-authors regarding their involvement in the above publications, there is no doubt that MS Jura is a leading author, with 60% (the original paper) or 75% (the review) share in their formation. However, I would like to point at the lack of precise contribution of the co-authors that should be indicated.

The analysis of the doctoral dissertation

The studies, undertaken by MS Jura, remain in the scientific area of the Supervisor that concerns mechanisms controlling metabolism of adipose tissue depending on various genetic or environmental milieu. In *Introduction,* (13 pages), the Author concisely provides a background related to obesity phenotype. She briefly presents white/brown adipose tissue features and focuses mainly on biomarkers of white adipose tissue expansion (ATE): mesoderm specific transcript (*Mest*), secreted frizzled-related protein-5 (*Sfrp5*) and caveolins (1 and 2). In the next sections of the PhD thesis, the Author demonstrates objectives (1 page) of the study as well as describes experimental animals, research methods and statistical analysis (5 pages). It should be clearly emphasized that the studies, which the Candidate focused on, are important in the light of increasing worldwide problem of obesity and obesity-

related diseases. The identification of new genes and more profound analysis of discovered genes controlling fat mass extension might be a future target for prevention and treatment of obesity. Particular accent should be given to a broad range of research and an application of different experimental methods (body composition determined by NMR, glucose and insulin tolerance test, adipocyte morphological examination, several gene and protein analyses by quantitative PCR and Western blot). Then, the Author provides a short description (6 pages) of two published papers included to the PhD dissertation.

In the section describing the paper entitled *"Mest and Sfrp5 are biomarkers for healthy adipose tissue"*, the Author presents research aim of the study, animal protocol, results (without showing figures or tables) and shortly discusses the obtained data (without drawing clear final conclusions). The studies, performed on two different genetic models for obesity generation (ob/ob and C57BL/6J mice), provide several interesting findings. They demonstrate time-dependent changes in the expression of the ATE markers – *Mest* and *Sfrp5* – in fat tissue of both animal models. The obtained results suggest the involvement of the above factors in establishing the capacity for maximal ATE. High levels of the genes expression (unfortunately not followed by protein levels) might define the ability to accumulate more fat whereas low expressions limit this capacity. Moreover, there is a limit to the gene expression when maximal adiposity is achieved. In my opinion, the findings are important and valuable. They significantly extend our knowledge of the expression of *Mest* and *Sfrp5* in subcutaneous fat, regarding DIO and ob/ob mice, in relation to the origin and age of obesity development. Although the publication has been solidly evaluated by a few Reviewers, I would like to address some questions/remarks to this section:

- did the Author verify the correlation between caveole components (*Cav1*, *Cav2, Cavin*) gene/protein expression with body weight, adiposity index or fat mass in the tested animal models? Is there any relation with the above parameters, based on available literature?
- some comments regarding the data on the expression of *Glut4*, *FAS*, *PCNA*, *Skp2, TNFα or IL-6* genes should be given in the discussion section. How would the Author connect the lower expression of proliferation markers – *PCNA* and *Skp2* – mRNA levels in the fat of 5-month old DIO or ob/ob mice (compared to the control individuals) with other tested parameters (e.g. adiposity, cell size or markers of ATE)? If possible, could the Author give also a short comment on potential mechanism responsible for a lack of changes in the expression of the pro-inflammatory markers (IL-6 and TNFα) in the fat of 5- and 8-month old DIO mice when compared with control animals (non obese) if such difference was noted in the fat of ob/ob mice?
- how would the Author explain a big difference (unequal expression) in beta-actin products/bands intensity, presented on Western blots, between Fig. 7C (B6 2M and DIO5 – very weak signal) and e.g. Fig. 7B or 7A (strong and equal signal). Furthermore, if possible would the Author give a brief comment on two bands presenting MEST protein in the tissue of the ob/ob mice mostly (fig. 7B). Which band/bands have been considered for the analysis? The protein size marker should be presented in the figures,
- why did the Author decide to shorten an experimental procedure for ob/ob mice to 5 months and did not follow the same protocol as for B6 mice (including analyses at 6-8 months)?

In the section describing the publication entitled *"Obesity and related consequences to aging"*, the Author gives a brief summary of a comprehensive and interesting review on similarities between mechanisms related to obesity and aging, which lead to a valuable and important conclusion. It indicates that age-related changes in body fat distribution and metabolism can accelerate the aging process and increase the risk of age-related diseases, such as insulin resistance, heart attack, stroke or cancer. In my opinion, this is a well-written/well-organized publication, which also includes the Author's own results of previously performed experiments in this field. After reading the review, one question comes to my mind: would the Author give a short comment regarding the possible involvement of other factors produced by adipocytes (e.g. resistin, visfatin) or other tissues (e.g. gonads) in obese-related aging acceleration?

Besides the report on two already published papers, MS M. Jura incorporated to the doctoral dissertation the information on further research (24 pages). This section, consisting of two subsections (each contains research aim, animal protocol, results and discussion), provides a large number of results, which could be sufficient for another PhD dissertation. I would like to underline that the findings, presented in this section (unpublished yet), are interesting. They expand our knowledge concerning: body composition, tissue morphology, gene/protein expression (with particular emphasis on *Mest* and *Sfrp5* genes) in subcutaneous, as well as in visceral fat of mice exposed to long- or short-term dietary protocols at different age (1st subsection). The results also provide some valuable information concerning the effect of cold pre-treatment or intermittent exposure of various mouse strains to high fat diet on body composition, glucose metabolism and various genes expression profile in subcutaneous fat (2nd subsection). For sure, the obtained results will be published in a very good journal. However, I would like to express a few remarks concerning this chapter:

- there is a lack of information about the mouse strain in subsection 5.1.2 or captions under figures/tables,
- experimental design concerning the evaluation of the effect of a long-term dietary protocol in 8 M (months) mice but short-term in 20-22 M mice is rather confusing. What was the reason for not applying the same (one or two) dietary protocols in each experimental group of animals (young and old)? There are two variable values: age and dietary protocol which, in my opinion, impair drawing conclusions,
- the bands presenting beta-actin protein in Fig. 8B (p. 31) are questionable. In addition, why is beta-actin detected using different fluorochromes?

Conclusion

In summary, submitted for evaluation PhD thesis, written by MS Magdalena Jura, contains a large number of valuable results, which markedly increase our knowledge concerning possible mechanisms driving obesity development depending on genetic and various environmental factors. Conducting the studies, at a high scientific level, required from the Candidate the use of a variety of experimental procedures, different research techniques, as well as enormous amount of laboratory work. It should also be underlined that MS Jura has confirmed an extensive knowledge of the scientific literature on the subject of research,

reflected predominantly in a very interesting review. I need to highlight that all my remarks, presented during the analysis of the thesis, do not diminish a high scientific significance of the work.

To conclude, the dissertation written by MS **Magdalena Jura** fulfils all requirements for gaining a PhD degree in Medical Sciences. Therefore, I support the application to the Faculty of Medicine with the Division of Dentistry and Division of Medical Education in English at the Medical University of Białystok for further processing the PhD procedure.

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 r. Nr 65, poz. 595 z późniejszymi zmianami) i wnoszę do Rady Wydziału Lekarskiego Uniwersytetu Medycznego w Białymstoku o dopuszczenie mgr Magdaleny Jury do dalszych etapów przewodu doktorskiego.

Olsztyn, May 19th, 2016

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