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Review of the doctoral dissertation
"Biomarkers of adipose tissue expansion in obese, aging animals"
by Magdalena Jura
written under the supervision of Prof. Leslie P. Kozak, PhD

In recent years, obesity has emerged as one of the major health problems affecting millions of people worldwide. In both men and women, the prevalence of obesity increases with age and it is now well established that the age-related increase in visceral adipose tissue mass may lead to several metabolic complications, including insulin resistance and type 2 diabetes mellitus. Therefore, the main aim of the dissertation – that is, the investigation of molecular mechanisms underlying adipose tissue remodelling and accumulation associated with aging – is not only interesting but also of great importance since it provides additional information that may be useful in the development of more efficient therapies to treat obesity and diabetes in the elderly.

General description of the dissertation

The dissertation submitted for evaluation is written in English and comprises two articles published in peer-reviewed scientific journals:

1. Jura M, Jarosławska J, Chu DT, Kozak LP. *Mest* and *Sfrp5* are biomarkers for healthy adipose tissue. *Biochimie* 2016; 124: 124-133. IF=3.1
2. Jura M, Kozak LP. Obesity and related consequences to ageing. *Age* 2016; 38: 23. IF=3.4

According to declarations of co-authorship, signed by all co-authors and the PhD candidate, Ms Magdalena Jura has made a substantial contribution (60%) to the paper published in *Biochimie*, and is the leading author of the paper published in *Age* (her contribution to this paper was assessed at 75%).

In addition to the papers mentioned above, the thesis contains the following sections: List of publications, List of abbreviations, Introduction, Objectives, Materials and methods, Description of the publications included in the dissertation, Further research, Summary (both in English and Polish), and the list of 151 references.

In the Introduction (pp. 1-13), the author provides the definition of obesity, describes the metabolic consequences of obesity, and presents animal models used to study the mechanisms of adipose tissue accumulation.

In the Objectives section, four primary aims of the study are formulated. The main purpose of the study was to evaluate *Mest* and *Sfrp5* gene expression profiles in subcutaneous adipose tissue in relation to age, obesity, insulin resistance as well as adipose tissue mass expansion.

In Materials and methods section (pp. 15-19), the author provides detailed information about experimental protocols, standard and high fat diet composition, animal care, and tissue collection. Sequences of probes and primers used in real-time RT-PCR analysis are presented in table 1. Western blot analysis is also described in detail; primary and secondary antibodies used in the study are listed in table 2 and 3, respectively. The description of the methods is clear and complete.

In the section 4: "Description of the publications included into PhD dissertation", the author describes the main aims and results of two published papers. In the article published in *Biochimie*, the results of research on *Mest* and *Sfrp5* gene expression profiles in adipose tissue in different types of obesity (defined by adiposity index) are presented. The authors used C57BL/6J and *ob/ob* mice fed on different diets as animal models of obesity. On the other hand, review paper published in *Age* summarises recent advances in knowledge of age-related changes in body fat distribution, adipose tissue metabolism, adipokine secretion, and adipose tissue expansion.

Two chapters in the Further research section (pp. 26-50) are devoted to the description of research not included in the published articles:

1. Activation and expression of *Mest* and *Sfrp5* genes are age-independent

2. Effects of a cold treatment effect on the progression of diet-induced obesity.

Both chapters are divided into Research aims, Animal protocol, Results, and Discussion. The aims of the study are well defined and the protocols are sufficiently detailed. The results are presented in 20 figures and 6 tables. *Mest* and *Sfrp5* (and more than 10 other genes) expression profiles in adipose tissue were analysed in young and old mice fed on a standard or a high-fat diet. The author concluded that *Mest* and *Sfrp5* expression levels during various dietary interventions are age-independent. In the second set of experiments, *Mest* and *Sfrp5* expression profiles, together with other biomarkers of adipose tissue accumulation, were analysed in adipose tissue of C57BL/6J and AXB8 mice after 12 and 20 weeks of cold exposure.

Minor concerns

- Energy content (kcal/g) of the diets should be provided.
- Peroxisome proliferator-activated receptors should be named as *Ppara* and *Pparg* (genes) or PPARα and PPARγ (proteins).
- In the declarations of co-authorship, Professor Kozak's contribution seems to be underestimated.
- The findings of the research are discussed after each set of experiments (four times). The final joint discussion of the potential role of *Mest* and *Sfrp5* genes in adipose tissue expansion should be included at the end of the thesis.
- A statement indicating the contribution of PhD candidate to all research included in the thesis should be provided.

Conclusions

The dissertation presented by Ms Magdalena Jura provides valuable scientific information about the physiological role of *Mest* and *Sfrp5* genes in adipose tissue expansion. The whole research project is well-designed and addresses important issues. Several methods were used in the study, including body composition monitoring by nuclear magnetic resonance, glucose and insulin tolerance tests, adipose cell size analysis, gene expression profiling using real-time RT-PCR, and Western blot analysis of protein levels. The thesis provides evidence of the candidate's good knowledge of the literature and wide range of research methods as well as the ability to conduct complex research.

I conclude that the dissertation presented for evaluation meets the requirements for the award of the degree and recommend admission of Ms Magdalena Jura to the further stages of the PhD procedure.

Stwierdzam, że przedstawiona do oceny rozprawa spełnia wymogi stawiane rozprawom doktorskim i wnoszę o dopuszczenie mgr Magdaleny Jury do kolejnych etapów przewodu doktorskiego.

dr hab. Zdzisław Kochan