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INSTITUT KLINICKÉ A EXPERIMENTÁLNÍ MEDICÍNY
Klinika diabetologie CD
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DISSERTATION THESIS OPPONENT'S REPORT

Candidate:	Ing. Lucia Mráziková
University:	Charles University in Prague, 1st Medical Faculty
Affiliation:	Institute for Organic Chemistry and Biochemistry, Czech Academy of Sciences
Thesis:	Effects of stable analogs of anorexigenic neuropeptides in models of metabolic syndrome
Tutor:	RNDr. Lenka Maletínská, CSc.
Opponent:	doc. MUDr. Jan Kříž, Ph.D.
Affiliation:	Diabetes Centre, Institute for Clinical and Experimental Medicine

Relevance of the topic, formal aspects of the doctoral thesis:

The thesis was prepared as a final part of the postgraduate studies in biomedicine at the 1st Medical Faculty of Charles University, in the branch of Biochemistry and Patho-biochemistry (YBICH). The candidate worked under the supervision of Dr. Lenka Maletinska at her laboratory in the Institute for Organic Chemistry and Biochemistry, Czech Academy of Sciences. All data are experimental and were obtained using rodent models.

The thesis is written in English, follows the required formal structure, contains an abstract, introduction, formulation of objectives and hypotheses, description of methodology, results and discussion. It concludes with a summary and a numerous list of literature used. The appendix lists four papers with IF related to the topic of the thesis, the candidate is the first author of two of them, and three other papers focused elsewhere.

Overweight and obesity is an important multifactorial disease, it is of great importance for the development of many diseases and no reliable pharmacological treatment is available yet. Therefore, many groups are involved in detailed research of the pathogenetic mechanisms of obesity and the resulting potential for the development of new therapeutics. The topic is therefore highly relevant and the results potentially useful for practice.

Commentary to Introduction:

The candidate summarized background of obesity and its relationship to a Type-2 diabetes mellitus and metabolic syndrome, possible approaches to therapy of impaired food intake control. In general, the introduction is adequate and explanatory enough. There are some issues and comments:

1) In part 1.4. the candidate has explained the mechanism of blood glucose regulation by insulin interaction with insulin receptor. The explanation of GLUT-4 transporters translocation to cellular membrane adequately fits to adipose tissue and muscles, but is not correct enough in case of hepatocytes. It is not important for the Thesis outcomes and message, but make me sure, please, that the candidate understands these differences enough – **Question 1**.
2) Formal arrangement of the text is a little bit confusing, i.e the last four lines on page 25. This text probably related to the figure, but is written using the same format as the main text on the section. It is difficult to distinguish between figure comments and regular text from the first view.
3) The second paragraph of 1.5. section on page 21, there are listed several hypothalamic nuclei. Almost all of them are named using full name and a short as well. But the ARC was mentioned just as the short – it looks strange, although is not mentioned in the text for the first time and strictly said it is correct.

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4) The last paragraph on page 21: the short DMH probably is a mistake
5) The last two sentences of the second paragraph on page 23: "*GLP-1 inhibits gastric emptying and glucagon secretion and stimulates glucose-dependent insulin secretion and biosynthesis though this molecule is limited by its short half-life. Therefore, long-acting GLP-1 agonists were developed.*" Highlighted words are not directly related to the information of the first part of sentence. Thus, it would be better to rephrase it: "*This molecule is limited by its short half-life, therefore long-acting GLP-1 agonists were developed*".
6) The last paragraph on page 23: *"Liraglutide is successful for T2DM treatment as well as obesity treatment shown by decreased FI, BW reduction and improvements in metabolic parameters in animal models and in human studies"*. I suppose that the candidate wanted to mention clinical trials as a source of data about effect of liraglutide – human studies are not used in medical terminology for this meaning. In addition to that, liraglutide is commonly used in routine clinical practice, so we have much more proofs for its function then from clinical trials.

Commentary to methods and animals used:

In general, used methods and animal models were chosen adequately for testing hypothesis and could provide convincing data. There are some minor comments:

7) Section 3.1. Substances on page 35: palm11-PrRP31, leptin and luraglutide administration was described, but only for leptin the daily dose was mentioned.
8) At the end of experiments, the oGTT was performed in all groups including collection of plasma for testing of insulin levels. The collection of blood from tail veins is a little bit tricky particularly from conscious mouse. Were your animals anesthetized before the blood collection and oGTT? - **Question 2**.
9) Page 37: *In experiment 2, mice were treated for eight weeks from 16 weeks of age and the following groups of 10 animals were used: WT saline, ob/ob saline, ob/ob leptin (10 µg/kg), ob/ob palm11-PrRP31 (5 mg/kg) and ob/ob leptin + palm11-PrRP31 (5 µg/kg + 5 mg/kg).* The leptin doses were different in these groups. Why? Was it relevant for comparability of these groups? – **Question 3**
10) Page 38: *Alzet osmotic minipumps were implanted IP under a short-term ether anesthesia and were replaced after 4 weeks with the new ones.* Based on my information, the ether anesthesia is prohibited in Czech Republic for research on animals. Are you sure, that your experiments were performed using this kind of anesthesia? – **Question 4**
11) Page 40: Liver histology – in this section was described just the liver sample processing, but there were not mentioned any parameters or signs, which were assessed in livers taken from experimental animal. Clear definition of positive or negative results should be explained.

Commentary to results, discussion and conclusions:

Outcomes are convincing enough and qualified authors for pronouncing of conclusions answering the hypothesis. In conclusion, the presented data suggest a good efficacy of palmitoylated PrRP analogs in rodent models of diet-induced obesity together with a good function of leptin signaling. Therefore, palmitoylated PrRP analogs can be considered promising candidates for anti-obesity and glucose-lowering therapy. The discussion is very well written and the results have been considered from different angles and in the context of previous experiments and literature sources.

There are some minor comments:

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12) Page 45 – the last line: *plasma level of TG and FGF21 compared to WKY HF saline (Table 3).* Why FGF-21? It was not mentioned in Introduction nor Method sections. – **Question 5**
13) Page 72 – Table 6, etc.: it is questionable, whether the table should be located in the discussion or results section. I would prefer to put it in Results but specifically in this thesis it is possible to use it as a structured schematic support for discussion.

In total, the formal preparation of the thesis could be more careful, but is still adequate. Experiments were clearly designed, results are convincing enough and conclusions sensible. The thesis confirms the candidate's ability to propose a scientific hypothesis, work with appropriate laboratory methods and to derive meaningful conclusions. **In my opinion, the submitted thesis qualifies the candidate for the award of the Ph.D. degree in her name, and I recommend its award.**

Questions:

1) In part 1.4. the candidate has explained the mechanism of blood glucose regulation by insulin interaction with insulin receptor. The explanation of GLUT-4 transporters translocation to cellular membrane adequately fits to adipose tissue and muscles, but is not correct enough in case of hepatocytes. It does not change anything in the Thesis outcomes and message, but make me sure please, that candidates understand these differences enough.
2) Were your animals anesthetized before the blood collection and oGTT?
3) The leptin doses were different between groups. Why? Was it relevant for comparability of these groups?
4) Based on my information, the ether anesthesia is prohibited in Czech Republic for research on animals. Are you sure, that your experiments were performed using this kind of anesthesia?
5) FGF-21 was reported in Results section but it was not mentioned in Introduction nor Method sections. Why did you measure it?

V Praze 15. 6. 2022

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