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Régulations métaboliques, nutrition et diabètes
Hubert VIDAL, directeur

Report on the Thesis submitted by Eva KLIMCAKOVA to obtain the degree of Doctor of Philosophy (PhD) in *Molecular and Cell Biology of Charles University of Prague* and *Pharmacological Innovation of Paul Sabatier-Toulouse III University.*

Eva Klimcakova presents a manuscript entitled "Regulation of human adipose tissue gene expression in relation to obesity and insulin resistance" in which are documented the results of her experimental works carried out under the joint supervision of Prof. Dominique Langin in INSERM U-586 at Toulouse University, and of Docent Vladimir Stich in the Department of Sport Medicine at Charles University Prague. This Thesis was performed under the rules of the Co-Tutelle program according to a convention between the two Universities and in the frame of the Franco-Czech laboratory for Clinical Research on Obesity, co-headed by D. Langin and V. Stich.

The general objective of Eva Klimcakova's Thesis was to define the potential implication of the adipokines produced by subcutaneous adipose tissue in insulin resistance and in the low-grade inflammation state that characterized obese individuals and that are important determinants in the complications of obesity (such as type 2 diabetes and cardiovascular diseases). Her studies were conducted in Human, combining clinical investigations based on lifestyle interventions to reduce weight and/or improve insulin resistance in obese subjects, molecular analysis in adipose tissue biopsies and in vitro experiments using pharmacological agents with promising actions on adipose tissue metabolism.

The main results obtained by Eva Klimcakova led to the publication of four research articles in international peer-reviewed journals, including three as first author. Two of them are in J. Clin. Endocrinol. Metab. (Impact Factor = 6.02), published in 206 and 2007, and one is in Biochem. Biophys. Res. Commun. (IF = 3.0), published in 2007. The fourth, signed as second author, was published in Metabolism (IF = 2.3) in 2006.

A- Comments on the submitted manuscript

The manuscript is organised in four chapters:

Introduction/Review of the literature:
Eva Klimcakova summarised in about 50 pages most of the relevant literature and current hypotheses regarding the role of adipose tissue, and more specifically of the adipokines, in

insulin resistance. Specific chapters present the current knowledge regarding the main adipokines individually (leptin, adiponectin, TNFa, IL6, IL1ß and RBP4). A second part of the introduction is a rapid overview of the main strategies classically used to tackle obesity and insulin resistance (about 30 pages). This second part is less documented and less precise than the first part, but all the main approaches and methods currently in use for the treatment of obesity are presented and rapidly discussed.
Globally, this review of the literature is very well written and of high quality.

Aims:
This short chapter presents the two major research strategies carried out by Eva Klimcakova during her experimental work, clearly indicating the rational and the objectives of the studies.

Results:
The main results are clearly presented and correctly discussed in view of the current literature. These results are published in international journals.
In the submitted version of the Thesis, the copy of these articles was not included in the text. It will be easier for the readers to have reprints of these papers added in the final version of the Thesis.

Conclusions and Perspectives:
The discussion of the Thesis is excellent, with the presentation of an original hypothesis regarding the potential role of leptin as a central actor of insulin resistance in obesity. This hypothesis is mainly based on the data obtained during the Thesis, but can also conciliate some data from the literature. It is an attractive hypothesis that will certainly be interesting to test more specifically in future experiments.

Globally, the manuscript presented Eva Klimcakova is very well written and correctly illustrated with an iconography of excellent quality. Her working hypotheses are clearly presented and explained. The discussion of the results is extremely interesting, leading to the proposal of a novel hypothesis on the role of leptin in the aetiology of insulin resistance in human obesity.
The few points that needed to be corrected in the manuscript were directly communicated to the candidate who will do the corrections before the public presentation. These minor comments do not have any impact on the quality of the document and of the presented work.

B- Comments on the work performed during the Thesis

During her Thesis, Eva Klimcakova tried to answer important questions regarding the role of adipose tissue, and more specifically the products of adipose tissue, in the complications of obesity. The mechanisms linking obesity and insulin resistance (a major risk factor for type 2 diabetes and cardiovascular diseases) are not well defined. A number of evidence support a role of a low-grade inflammation, eventually produced by adipose tissue. Adipokines are secreted by adipose tissue and, for the majority of them, have pro- (or anti-) inflammatory properties. It is therefore extremely important to better understand the role of adipokines in human obesity.
In three non-pharmacological clinical studies designed to reduce body weight or to improve insulin sensitivity, Eva Klimcakova investigated in details the regulation of the main adipokines and tried to define the relationships between the changes in their plasma levels and gene expression in fat biopsies and the changes in insulin sensitivity in different groups of

obese subjects, submitted to either 3 months of intensive aerobic training, 3 months of dynamic strength training or to low calorie diet programs. Insulin sensitivity was assessed by the mean of the gold standard method (hyperinsulinemic clamp) and gene expression of the main adipokines was determined using quantitative PCR in subcutaneous abdominal adipose tissue biopsies taken before and at the end of the clinical interventions. Globally, she found that while insulin sensitivity was improved in all three studies, none of the tested adipokines, except plasma leptin levels, showed significant variation in their expression levels, either in the plasma or at the gene expression level in adipose tissue. These important observations suggested therefore that the measured adipokines (including adiponectin, TNFa, IL6, IL1ß and RBP4) are not crucial factors in the regulation of insulin responsiveness in obese individuals. The observed changes in leptin levels led to an original hypothesis suggesting that this hormone could play a more important role in insulin action than what was previously supposed.

In a second part of her work, Eva Klimcakova studied the effects of specific agonists of the nuclear receptors PPARs on the production of adipokines by human adipose tissue in vitro. The identification of pharmacological agents able to modulate the expression and the production of adipokines by adipose tissue is a very important objective in obesity and related metabolic diseases. Explants of subcutaneous adipose tissue were incubated with various pharmacological ligands of the three PPAR isoforms and a profiling of the secreted proteins was carried out using a cytokine antibody array allowing analysis of 120 different cytokines in the incubation medium. The main result of this secretome analysis was the identification of HGF, angiogenin and TIMP-1 as new proteins secreted by adipose tissue in response to the PPARs.

Altogether, these studies represent a very impressive sum of work, combining different types of clinical studies with obese patients, molecular analysis of gene expression in fat biopsies and a more focused investigations of adipokine production in vitro, using new methodology based on protein array. It appears therefore that Eva Klimcakova has utilized a very large variety of methods during her thesis. She participated in studies involving various aspects of the modern research in integrative physiology, and she developed fruitful interactions with different collaborators, including clinicians, nutritionists, physiologists and molecular biologists. She clearly succeeded in the realisation of her assigned tasks.
In addition to the four research articles based on the results obtained during the Thesis, it is important to point out that Eva Klimcakova is also co-author of 10 additional papers in international journals published between 2005 and 2007. This is therefore the perfect example of what should be a very good thesis performed in a fruitful "co-tutelle" program.

In conclusion, Eva Klimcakova has performed a very important work during her Thesis. Using a large variety of methodologies, combining clinical investigation and molecular studies, she obtained original results that were published in high quality international journals. The presented manuscript is very well written and does not require modifications.

My recommendation is that the Thesis is highly commended.

Hubert VIDAL
PhD, Director of Research

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