

Report on the Thesis for the degree of doctor of philosophy

Specialization: Molecular and cell biology, genetics and virology

Innovation pharmacologique/Ecole Doctorale Biologic-Santé-Biotechnologies

„Regulation of human adipose tissue gene expression in relation to obesity and insulin resistance

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Supervisors: Docent Vladimír Štich

Professor Dominique Langin

The thesis ensued from work within the French-Czech laboratory for Clinical Research on Obesity which was established by doc. Vladimír Štich, 3rd Medical School, Charles University, Prague, and prof. Dominique Langin, INSERM, Toulouse University.

Comments to the manuscript

The objective of the dissertation is to contribute to clarification of the processes involved in changes of insulin sensitivity in obesity with emphasis on the role of adipokines.

Introduction

In the first chapter Eva Klimčáková shows in range of 80 pages an extensive overview of recent knowledge on obesity and adipose tissue physiology and review of obesity management strategies. The first part is focused on the role of adipose tissue in development of insulin resistance. Author gives a detailed current view on molecular basis of the insulin signaling and insulin resistance, role of low grade inflammation and adipokines in insulin resistance development. The review is very well documented. The second part of the first chapter shows overview of current strategies in obesity and insulin resistance management starting from behavioural intervention with change of the diet and physical activity to pharmacotherapy and bariatric surgery.

The thesis is divided into clinical and experimental part.

The clinical part contains 3 studies showing effect of different long-term nutritional and physical activity and fitness interventions in obese subjects on expression of genes involved in regulation of insulin sensitivity and low grade inflammation. The aim of the clinical part was to investigate associations between the diet/ or physical activity-induced modifications of insulin sensitivity and modifications of adipose tissue endocrine characteristics.

In the experimental part the author examined in vitro the effects of pharmacological approach using PPAR agonists and investigated their effect on proteome of human subcutaneous adipose tissue.

Methods:

In clinical part studied groups of patients included 36, 12 and 25 subjects, followed for 6-7 months, 12 weeks and 3 months and treated by VLCD/LCD and weight maintenance period, by aerobic training and by dynamic strength training, respectively. Insulin sensitivity was examined by euglycemic-hyperinsulinemic clamp technique, adipokine gene expression subcutaneous adipose tissue by quantitative PCR technique.

In experimental part human subcutaneous adipose tissue was obtained from 13 obese and overweight women undergoing plastic surgery. The effect of the three PPAR agonists (PPAR alpha, PPAR gamma and PPAR beta/delta) on protein production was followed.

Main results:

In clinical part in the first study author shows effect of 3 months of intensive aerobic training on plasma and mRNA levels of several adipokines in obese women. Increased aerobic fitness and insulin sensitivity and decreased plasma leptin levels were found while the other followed adipokines did not change.

In the second study the impact of 3 months dynamic training on plasma and mRNA levels of adipokines from subcutaneous fat was examined. In obese men examined by euglycemic hyperinsulinemic clamp no weight change was found; significant increase in whole body glucose disposal and insulin sensitivity and decrease of systolic and diastolic blood pressure were shown. Except for decrease of leptin plasma and mRNA levels of adipokine did not change.

In the third study effect of a 3 months dietary regimen starting with 4 weeks of very low calorie diet followed by 2 month low calorie diet and 3-4 months of weight maintenance was examined in a group of obese women. Glucose disposal rate increased during VLCD phase

and remained enhanced. Plasma retinol-binding protein (RBP4) and adipose tissue mRNA decreased after VLCD; after LCD and weight maintenance only plasma RBP4 levels remained significantly decreased. RBP4 is expressed almost exclusively in adipocytes.

In experimental pharmacologic study therapeutic actions of PPAR agonists on production of cytokines in human subcutaneous adipose tissue was followed. PPAR agonists influence adipokine secretion from adipocytes but also from stromal vascular cells of adipose tissue.

The list of the candidate's own publications contains 4 papers where Eva Klimčáková is the first author in 3 of them and second author in one of them and 11 papers where she is co-author. The four publications are in high level journals, 2 publications in *Journal of Clinical Endocrinology*, 1 publication in *Metabolism* and 1 in *Biochem Biophys Res Commun* and also most of the other 11 annexed publications are in journals with high IF. The thesis contains 444 references.

The thesis is very carefully formulated and precisely documented by figures and tables.

Questions

1. In the 2 studies following effects of physical activity in obese subjects the women underwent aerobic activity and the men dynamic endurance training. Could you speculate if there exist sex-related differences in the response to the given type of training?
2. Was there monitoring of energy intake and nutrient composition during the studies based on increased physical activity?
3. Where there any effects of dynamic training on markers of body fat distribution (e.g. waist and hip circumference)?
4. In the study with dynamic training in men, the weight loss or fat loss was not found as a major determinant for the decrease in insulin resistance. Could you speculate on comparison of factors influencing decrease of insulin resistance during different long-term weight management conditions? As shown in conclusions the decrease in leptin levels could be mediator of increase in insulin sensitivity after different types of intervention. Could you speculate on mechanisms?

The thesis of Eva Klimčáková presents valuable and original results published in high level scientific journals. The thesis is carefully elaborated and documented.

I recommend the Thesis to be accepted as a high quality thesis.

Marie Kunešová
Marie Kunešová

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Prague, October 31, 2007

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