Molecular analysis of human adipocytes during glucose restriction and (re)feeding

Appendix I:

Summary

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Obesity has become a worldwide critical health issue because it is frequently accompanied by the development of health complications such as type II diabetes, cardiovascular diseases, respiratory problems and certain types of cancer. Weight loss is an optimal method for overweight or obese individuals to decrease the risk of health complications. Approaches towards achieving weight loss include dietary intervention, increased physical activity, pharmacological treatment and surgical treatment. Among these, calorie restriction is a common practice for weight loss. However, up to 80% of the people who lost weight on a low energy diet, regain weight and are unsuccessful in maintaining their weight loss. Consequently, prevention of weight regain after weight loss is the core problem of body weight management. It has become clear that the white adipose tissue (WAT) plays an important role in the increased risk for weight regain after weight loss. A model that the compensatory reflex begins with changes to the shape of adipocytes proposed by the group of Professor Edwin Mariman, draws increasing attention. As adipocytes release fat and shrink, their membranes pull away against the points of focal adhesion to the nearby extracellular matrix (ECM), creating mechanical stress. This in turn sets a multitude of adaptations in motion, although the strength of these responses will differ across individuals. Moreover, changes of ECM- and stress-related gene expression during weight loss by calorie restriction were proved to be linked to the risk for weight regain. However, the detailed information on proteins involved in the pull model mechanism is still lacking, and a clear view on metabolic changes induced by calorie restriction and refeeding is not well-established yet. Therefore, studies in this thesis investigated the molecular changes of human *in vitro* adipocytes during glucose restriction and (re)feeding by using a proteomics approach aiming to better understand the weight cycling effect, more specifically the weight regain. In addition, the relation between several parameters of the glucose/lipid metabolism, including adipocyte volume, with weight regain was studied *in vivo*.

In **Chapter 1**, a general introduction to this thesis is presented. The background of obesity and especially the major player in this, the adipose tissue, is generally introduced in relation to weight regain. Subsequently, the methods are briefly introduced in the context of the advantages and disadvantages of the applied proteomics techniques and the applied human Simpson Golabi Behmel Syndrome (SGBS) cell model.

Chapter 2 presents an established *in vitro* model for the development of hypertrophic adipocytes, of which time-dependent adipocyte morphological changes as well as the cellular proteome and secretome changes were monitored under high glucose and high insulin conditions, simulating a condition of overfeeding. In total, 393 cellular proteins and 246 secreted proteins were identified for further analysis. Pathway analysis, functional clustering analysis, metabolic proteome profiling and morphologic characterization of the adipocytes allowed us to determine time-dependent changes. During the first 4 days of high glucose and high insulin the adipocytes seemed to prefer pyruvate as energy source, whereas beta-oxidation was down-regulated supporting lipid-loading. In addition, glycolysis was being limited which was accompanied by the reduction of protein translation. Over time, lipid droplet fusion instead of lipid uptake became relatively more important for growth of lipid droplets during the last 4 days. Moreover, ECM production shifted towards ECM turnover by the upregulation of proteases over eight days probably to protect the lipid-loaded adipocytes against mechanical rupture. This *in vitro* model provides insight into the molecular and metabolic changes of mature adipocytes under conditions of high glucose and insulin, which may help to understand the process of *in vivo* development of adipocyte hypertrophy in the context of obesity. Notably, this on itself cannot be studied *in vivo*, because adipose tissue is not only composed of adipocytes but of several other cell types.

In **Chapter 3**, an *in vitro* model system based on glucose restriction (GR) and refeeding (RF) was established to uncover cellular proteome differences between GR plus RF versus normal feeding, aiming to find mechanistic leads

at the genomics/proteomics level for weight regain by comparing the data from the *in vitro* model with *in vivo* data. The *in vitro* model revealed 44 proteins differentially expressed after GR with RF versus normal feeding. While most of these proteins reversed their expression during RF, four proteins that were up- or down-regulated during GR, persisted in their change of expression during RF: liver carboxylesterase (CES1), mitochondrial superoxide dismutase [Mn] (SOD2), alpha-crystallin B-chain (CRYAB), alpha-enolase (ENO1). Accordingly, *in vivo* weight loss-induced RNA expression changes linked CES1, CRYAB and ENO1 to weight regain. Moreover, of the 44 proteins CES1 and glucosidase II alpha subunit (GANAB) were correlated with weight regain during follow up. Correlation clustering of *in vivo* protein expression data indicated an interaction of these proteins with structural components of the focal adhesions and cytoplasmic filaments in the adipocytes.

In **Chapter 4**, the same *in vitro* model system (Chapter 3) was used to study changes of the human adipocyte secretome upon refeeding after glucose restriction. Data were compared with data from normal feeding experiments, again to find leads for *in vivo* processes related to weight regain. We identified 338 secreted proteins, of which 49 were described for the first time as being secreted by human adipocytes. In addition, comparison between normal feeding and GR plus RF showed 39 differentially secreted proteins. Functional classification revealed GR plus RF induced changes of enzymes for ECM modification, complement system factors, cathepsins, and several proteins related to Alzheimer's disease. These observations can be used as clues to investigate metabolic consequences of weight regain, weight cycling or intermittent fasting.

In **Chapter 5,** an *in vivo* study is described in which factors that were previously reported as being associated with (the risk of) weight regain, were analyzed during the follow up phase of a weight loss/maintenance intervention (the Yoyo-study) to investigate their potential relation to weight regain. In this study, 61 overweight/obese participants were enrolled in a

randomized, controlled dietary intervention study, of which all participants went through a dietary intervention period (T1-T3) and then a 9-month follow-up free-living period (T3-T4). Here we report for the first time that the changes of triglycerides (TG) and interleukine-6 (IL6) independently associated with weight regain after weight loss in overweight/obese participants. The change of TG robustly correlates with the change in Homeostatic Model Assessment of Insulin Resistance (HOMA-IR). Furthermore, the absolute values of HOMA-IR at T3 and T4 correlated with adipocyte volume. These results indicate that TG and IL6 are directly linked to weight regain via separate mechanisms, and that HOMA-IR and adipocyte volume are indirectly linked to weight regain through the change of TG. Our findings suggest that (partial) insulin resistance leading to increased levels of TG, glucose and insulin, may promote storage of energy and weight regain.

Finally, in **Chapter 6**, a general discussion in the context of advantages and disadvantages of the *in vitro* hypertrophic adipocyte model as well as the GR plus RF model is provided. The results of our *in vitro* and *in vivo* research, including the ECM remodeling, TG as well as IR are discussed in relation to their influence on weight regain.

In summary, the major accomplishments of this thesis are focused on five aspects:

(i) For the first time we have shown the molecular and morphologic dynamics of adipocytes under conditions of high insulin and high glucose *in vitro* to mimic *in vivo* adipocyte hypertrophy.

(ii) We have generated a human adipocyte *in vitro* model of glucose restriction and refeeding as the first surrogate model for weight loss and weight regain.

(iii) We have identified several proteins that *in vitro* persist in their change of expression under adipocyte glucose restriction and refeeding. It suggests that calorie restriction of adipocytes may reset the expression of certain proteins, perhaps by epigenetics.

These proteins are associated with the ECM ─ focal adhesion ─ cytoskeleton interaction.

(iv) We have identified functional classes of adipocyte-secreted proteins that are modified in their expression profile during glucose restriction and refeeding *in vitro*. Their role can now be further studied.

(v) Our *in vivo* observations suggest that (partial) insulin resistance promotes adipocyte growth and weight regain by the creation of increased plasma levels of TG, glucose and insulin. In addition, inflammation seems to act as an independent promoter of weight regain.

In conclusion, we regard this thesis as a valuable step towards more knowledge about weight regulation on the molecular level. Furthermore, results from this thesis can now be used as a lead for follow-up studies to promote the development of successful treatment strategies for obesity and its related complications.