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

Appendix II:

Valorization

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Appendix II

This thesis is focused on gaining more knowledge underlying the dynamic process of the seemingly inevitable weight regain after weight loss in overweight and obese humans.

Social and economic relevance

The prevalence of obesity is increasing globally and to date not a single country has successfully reversed its epidemic [1, 2]. In the past three decades, global overweight (body mass index (BMI) $\geqslant$25 kg/m^2) increased from 28.8% to 36.9% in men and from 29.8% to 38.0% in women [2], while age-standardized obesity (BMI $\geqslant$30 kg/m^2) increased from 3.2% to 10.8% in men and from 6.4% to 14.9% in women [3]. In 2016, the World Health Organization estimated that more than 1.9 billion people aged 18 years and older were overweight, of which over 650 million adults were obese, with at least 2.8 million people dying each year as a result of being overweight or obese [4]. Moreover, obesity also represents a major health challenge because it substantially increases the risk of diseases such as type 2 diabetes [5], fatty liver disease [6], cardiovascular diseases [7], obstructive sleep apnea [8] and several types of cancer [9], thereby contributing to a decline in both quality of life and life expectancy [10]. In addition, obesity not only brings in disasters in the public health management system, but also increases the social economic burden to the whole world due to the direct and indirect medical cost. According to a systematic review, the direct medical costs are 6%-45% higher in obese patients compared with healthy-weight peers [11] and medical spending associated with adult obesity approaches $210 billion a year only in America [12]. Therefore, the obesity epidemic has far-reaching consequences for individuals, society and the economy, and prevention and treatment strategies — both at the individual and population level — are urgently needed.

For overweight and obese people, weight loss is an indicated remedy that can reduce the risk for health complications. It has been demonstrated that losing 5% of body weight already results in significant improvement of health

parameters like a lower blood pressure and plasma glucose and insulin levels [13]. Decrease of calorie intake by diet is a common practice to try and lose weight. However, successful weight loss in the long term has not been achieved yet due to the individual's complex and persistent hormonal, metabolic and neurochemical adaptations defending against weight loss and promoting weight regain. As such, a better understanding of the remarkable dynamic process of weight cycling is crucially important in providing guidance on the most promising intervention strategies. Therefore, this thesis provides an extended view on the molecular and metabolic changes of human adipocytes during glucose restriction and (re)feeding, aiming to form a theoretical basis and to generate leads for weight regain prevention.

Scientific gain of this thesis

This thesis describes a proteomics approach towards understanding weight regain by profiling molecular changes of human adipocytes during glucose restriction and (re)feeding, aiming to generate responsible targets for the weight cycling process, more specifically for weight regain. In addition, the relation between several parameters of the glucose/lipid metabolism, including adipocyte size, with weight regain was studied *in vivo*. In general, the major accomplishments of this thesis are summarized as follows:

- For the first time we have shown the molecular and morphological dynamics of adipocytes under conditions of high insulin and high glucose *in vitro* to mimic *in vivo* adipocyte hypertrophy. 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.
- 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. Although such a model system does not

reflect the *in vivo* situation directly, valuable clues to biological processes could be obtained.

- We have identified several cellular and secretome 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. Furthermore, these proteins are associated with the extracellular matrix ─ focal adhesion ─ cytoskeleton interaction.
- Our *in vivo* observations suggest that partial insulin resistance promotes adipocyte growth and weight regain probably 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. This provides more insight into the mechanisms related to the biological process for weight regain.

Innovation

The present thesis presents various novel findings and insight. Firstly, we were able to establish the *in vitro* model for the development of hypertrophic adipocytes, of which the changes over time of the cellular proteome and of the secretome of human SGBS adipocytes under conditions of high glucose and high insulin were recorded. Based on this, we were able to extend the human *in vitro* model system to glucose restriction and refeeding aiming to shed light on the morphological adaptations as well as the molecular alterations of adipocytes in the context of calorie/glucose restriction and weight regain. Specifically, we have uncovered differentially expressed proteins (CES1, CRYAB, ENO1, GANAB), which are tightly related to the intracellular reorganization of focal adhesions and cytoskeletal filaments and are significantly correlated with weight regain. These proteins may be potential targets for further research on the regulation of weight regain. In parallel, glucose restriction plus refeeding leads to changes in the secretome

of adipocytes which are related to extracellular matrix modification, factors of the complement system and several proteins relevant for Alzheimer's disease. These observations can be used as clues to investigate metabolic consequences of weight regain, weight cycling or intermittent fasting.

Moreover, in this thesis we report for the first time that the changes of triglycerides (TG) and interleukin-6 (IL6) were associated with weight regain in overweight/obese participants, whereas association between weight regain and the homeostasis model assessment of insulin resistance (HOMA-IR) or the angiotensin converting enzyme (ACE) were not supported by the multiple regression analysis. These results suggest that changes in TG and IL6 are linked to weight regain via separate mechanisms, and further *in vivo* results suggest that (partial) insulin resistance leading to increased levels of TG, glucose and insulin, may promote storage of energy and weight regain. Altogether, this thesis provides vital insight for better understanding the dynamic process of weight gain, weight loss and weight regain. This 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.

Target groups

The results from fundamental research described in this thesis are foremost available to the scientific community through publications in international peer-reviewed journals. We have generated several proteins which are uncovered for the first time in the context of calorie/glucose restriction and weight regain, providing an important indication about their role. This can now be used as a lead for follow-up studies and hopefully our results will inspire scientists, including our own research team, to undertake the next step resulting in human studies. Therefore, the scientific gain of this thesis will help to promote the development of successful treatment strategies for obesity and its related complications on the long term.

Meanwhile, the target group of applied research (in Chapter 5) in this thesis reaches beyond the scientific community, because the obese populations can

benefit from it directly. For instance, individuals after weight loss with a higher level of fasting TG and IL6 should receive a more stringent and frequent guidance to keep these parameters to the healthy ranges.

Altogether, these results will enhance the efficiency of weight loss treatment and weight regain prevention, which will directly or indirectly contribute to the reduction of the health and economic burdens for society. Still, to achieve this, high quality and personalized guidelines are needed, which calls for collaborations among research groups, translational medicine companies, dieticians and specialists in hospital to finally make it.

Planning and implementation

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. However, our findings are not the end point of this topic, further research is needed. For instance, the *in vitro* model does not reflect the *in vivo* situation directly which makes it inapplicable in clinical settings immediately. Thus, further identification and translational research should be conducted with more specific and personalized *in vivo* models. It may take years to improve and identify current knowledge, and collaborations from different disciplines such as genomics/proteomics groups, bioinformatics and biostatistics to transform these research data into an applicable model are crucially required. The applicable model then will assist the specialists to provide specific, personalized guidance to people to create optimal conditions for weight maintenance after weight loss.

References

1. Roberto CA, Swinburn B, Hawkes C, Huang TT, Costa SA, Ashe M, Zwicker L, Cawley JH, Brownell KD: **Patchy progress on obesity prevention: emerging examples, entrenched barriers, and new thinking.** *Lancet* 2015, **385:**2400-2409.
2. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, Mullany EC, Biryukov S, Abbafati C, Abera SF, et al: **Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013.** *Lancet* 2014, **384:**766-781.
3. Collaboration NCDRF: **Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19.2 million participants.** *Lancet* 2016, **387:**1377-1396.
4. **World Health Organization: Obesity and overweight** [www.who.int/news-room/fact-sheets/detail/obesity-and-overweight]
5. Verma S, Hussain ME: **Obesity and diabetes: An update.** *Diabetes & Metabolic Syndrome-Clinical Research & Reviews* 2017, **11:**73-79.
6. Polyzos SA, Kountouras J, Mantzoros CS: **Adipose tissue, obesity and non-alcoholic fatty liver disease.** *Minerva Endocrinol* 2017, **42:**92-108.
7. Mandviwala T, Khalid U, Deswal A: **Obesity and Cardiovascular Disease: a Risk Factor or a Risk Marker?** *Curr Atheroscler Rep* 2016, **18:**21.
8. Carneiro G, Zanella MT: **Obesity metabolic and hormonal disorders associated with obstructive sleep apnea and their impact on the risk of cardiovascular events.** *Metabolism-Clinical and Experimental* 2018, **84:**76-84.
9. The L: **The link between cancer and obesity.** *Lancet* 2017, **390:**1716.
10. Bluher M: **Obesity: global epidemiology and pathogenesis.** *Nat Rev Endocrinol* 2019, **15:**288-298.
11. Withrow D, Alter DA: **The economic burden of obesity worldwide: a systematic review of the direct costs of obesity.** *Obes Rev* 2011, **12:**131-141.
12. Spieker EA, Pyzocha N: **Economic Impact of Obesity.** *Prim Care* 2016, **43:**83-95, viii-ix.
13. Horton ES: **Effects of lifestyle changes to reduce risks of diabetes and associated cardiovascular risks: results from large scale efficacy trials.** *Obesity (Silver Spring)* 2009, **17 Suppl 3:**S43-48.