

Causes and consequences of lipid overload in skeletal muscle, liver and heart

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CHAPTER 9

Appendices

VALORIZATION ADDENDUM

SOCIAL RELEVANCE

Obesity is today's most obvious visible health problem. Obesity is escalating and has become a global epidemic. The prevalence of obesity has more than doubled between 1980 and 2015, and without proper interventions, the prevalence of obesity-related conditions is likely to increase as obesity continues to rise in the next few decades. At present, at least 2.8 million people die prematurely each year as a result of being overweight or obese (WHO report March 2013). The health consequences of obesity are related to metabolic diseases, such as type 2 diabetes, cardiovascular disease, hypertension, stroke and certain types of cancer. Next to the metabolic complications, obesity also has social and psychological dimensions affecting essentially all ages and socioeconomic groups. Taken together, obesity and its complications can dramatically impair quality of life and have a big impact on health care costs. Therefore, it is important with immediate action to slow down or stop the global obesity epidemic and its complications. In this context a better understanding of the underlying cause of obesity related complications is needed and could result in the development of more specific therapeutic or preventive strategies for people at risk.

TARGET GROUPS

The obesity epidemic is a result of people consuming high caloric diets and being physically inactive. This results in an imbalance between calories consumed and calories expended, and hence a positive energy balance. This positive energy imbalance leads to an excessive accumulation of fat not only in adipose tissue, but also in non-adipose tissues (ectopic fat accumulation), such as skeletal muscle, liver and heart. In contrast to the fat stored in adipose tissues, the fat stored in the non-adipose tissue is associated with the development of insulin resistance and type 2 diabetes. Therefore, to slow down the development of obesity related complications, such as type 2 diabetes, it is essential to gain a better understanding of why lipids store in these tissues and what the consequences of ectopic fat storage are.

Because obesity affects millions of people worldwide and leads to exploding healthcare costs, the search for novel strategies to counteract obesity and its complications is important for the general public, and especially for those at risk

of developing overweight- and obesity related complications. In this context, discovery of novel therapies and treatments might lead to an improvement of quality of life and long-term health, but also to saving health care costs by e.g. reduced hospitalization. Therefore it is important for obesity and diabetes management that the communication between researchers and health institutions, health care professionals and the general population, is good, so that knowledge can be shared.

ACTIVITIES AND PRODUCTS

The studies described in this thesis have been conducted at the department of Human Biology of Maastricht University Medical Center+. In order to investigate the causes and consequences of ectopic fat accumulation, some studies were done in collaboration with the department of Radiology of the University Hospital of Maastricht. This collaboration was essentially, as the current technique to measure ectopic fat in skeletal muscle, liver and heart non-invasively with Magnetic Resonance Spectroscopy (MRS) was only available in the hospital. All studies outlined in this thesis were conducted in human volunteers and different interventions were applied to manipulate e.g. plasma free fatty acid levels. Both *in vivo* and *ex vivo* state-of-the-art techniques, such as MRS and high-resolution respirometry, were applied to unravel why fat is stored in non-adipose tissues and what the consequences are.

The results described in this thesis have been implemented in original research articles that have been published or submitted to scientific journals in the field of obesity and diabetes. These articles are to be found online and are accessible to other scientists. The present results have also been presented on international conferences and can be used for future studies.

INNOVATION

In obesity there is a failure in the capacity of appropriate adipose tissue expansion, which leads to an increased flux of free fatty acids away from rather than into adipose tissue resulting in an increased storage of fat in non-adipose tissues. This increased influx of free fatty acids is believed to be harmful to the tissues, contributing to insulin resistance and type 2 diabetes, a process referred to as lipotoxicity. Alternatively, impaired oxidative capacity of the mitochondria could also be an underlying factor for ectopic fat accumulation, interestingly, a low mitochondrial function has also been linked to insulin resistance. In this thesis, we mainly focused on the role of FFA in this

ectopic fat accumulation and how this is linked to lipotoxicity, but also investigate mitochondrial function as a factor in lipotoxicity. We recruited human volunteers who underwent a combination of intervention studies, including manipulations of plasma FFA by acute exercise or administration of the antilipolytic-drug acipimox and manipulations of mitochondrial function and oxidative capacity by inactivity. Earlier, the heart and the liver were both inaccessible for human studies. However, due to a development of the non-invasive MRS technique and the implementation on clinical MRI scanners in recent years, we were able to intensively study the ectopic fat accumulation (with proton (¹H) MRS) in skeletal muscle, liver and heart in both healthy volunteers and in people with type 2 diabetes. Additionally, applying phosphorous (³¹P) MRS enabled us to investigate the energy status of the heart and the liver and the *in vivo* mitochondrial function of skeletal muscle. Another strength of this thesis therefore is that we were able to measure ectopic fat accumulation in more tissues enabling a comparison between tissues. The excellent facilities of our lab also enabled us to investigate subjects' substrate metabolism and insulin sensitivity *in vivo* by means of a ventilated hood and a 2-step hyperinsulinemic euglycemic clamp, respectively. Next to performing *in vivo* measurements, muscle biopsies were also withdrawn for *ex vivo* measurements of oxidative capacity, fat- and glucose metabolism and insulin signalling analysis. Oxidative capacity was measured with high-resolution respirometry (Oroboros oxygraph), fat- and glucose metabolism by ¹⁴C oxidation assays and insulin signaling by western blotting. The results provided us with valuable information on the role of FFA on ectopic fat accumulation and organ function. With the mentioned techniques we were able to show that plasma FFA levels are an important determinant for ectopic fat accumulation and that non-adipose tissues seem to take up plasma FFA from the circulation when the availability is high, independent of oxidative needs. However, we could not show that ectopic fat accumulation always leads to organ dysfunction and more research is necessary to answer this. The results of this thesis can be used for future studies focusing on the relevance of fat accumulation and lipotoxicity for the etiology of type 2 diabetes in humans.

PLANNING AND REALIZATION

In this thesis, human intervention studies have been used to study the role of free fatty acids in ectopic lipid accumulation. We demonstrated that plasma concentration of free fatty acids indeed is an important determinant for ectopic fat accumulation, but that an acute increase in ectopic fat depots did not necessarily lead to harmful

effects disturbing organ function. Thus, more research is warranted to unravel the relationship between elevated ectopic fat accumulation and tissue dysfunction. In “The Diabetes and Metabolism Research Group” of Prof. dr. P. Schrauwen and Prof. dr. M. Hesselink, this relationship will further be explored by performing translational research. To this end high-end molecular and cellular biology tools and non-invasive and microscopically imaging and spectroscopy will be combined with state-of-the-art metabolic phenotyping in a predominantly experimental human research setting.

LIST OF PUBLICATIONS

L.Bilet, E. Phielix, E. Moonen-Kornips, V.B. Schrauwen-Hinderling, M.K.C. Hesselink, P. Schrauwen. Differential effects of exercise on IMCL content in the fasted and fed state. In preparation.

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L. Bilet, V.B. Schrauwen-Hinderling, N. A. van Herpen, R.C. Meex, E. Phielix, P. Schrauwen, M.K.C. Hesselink. The novel biomarker for insulin resistance alpha-hydroksybutyrate is not responsive to lifestyle changes affecting liver fat or insulin sensitivity. Submitted.

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