VALORIZATION

Social relevance
The obesity epidemic is growing worldwide and has doubled since 1980 according to the World Health Organization (WHO). In 2014 39% of the adults were overweight and 13% were obese. The rise in obesity is not only a problem of well-developed countries, but is on the rise in developing countries as well. In addition, worldwide more people are obese than people who are underweight. Obesity is a major risk factor for cardiovascular diseases and type 2 diabetes. The prevalence of diabetes increased from 4.7% in 1980 to 8.5% in 2014 and when looking to absolute numbers the amount of people with diabetes has a four-fold increase in this period. According to the International Diabetes Federation 90% from all people who have diabetes has type 2 diabetes (T2DM). Due to increasing rates of obesity and T2DM health care costs are on the rise as well. Not only diabetes itself leads to increased cost, but comorbidities contribute as well. Consequences of uncontrolled diabetes that can occur are blindness, cardiovascular diseases, kidney failure and lower extremity amputations.

Early treatment of T2DM and recognition of symptoms at an early stage will prevent or delay the development of comorbidities. In addition, quality of life will improve or be maintained better if blood glucose can be well-controlled. Much can be gained on the early recognition of symptoms of T2DM. According to the WHO 24% to 62% of the people with diabetes is undiagnosed and thus untreated. These people are at a higher risk for developing comorbidities, which will burden the health care costs even more. Nowadays treatment of T2DM consists of glucose lowering medication, diet, increasing physical activity or a combination of these. However, T2DM is a complex metabolic disease whereby underlying mechanisms are yet not completely understood. Understanding these underlying mechanisms might result in new treatment strategies.

Scientific background
Development of obesity and T2DM is a consequence of a sustained positive energy balance. This positive energy balance can result from a high calorie intake or a reduction in physical activity or both. Obesity and T2DM coincides with lipid storage, not only in adipose tissue, but in non-adipose tissues like the liver, heart and skeletal muscle as well. This pattern of ectopic lipid storage in skeletal muscle associates with insulin resistance. Skeletal muscle is an important organ for whole body glucose homeostasis, as it takes up 80% of the glucose upon meal consumption. However, lipid storage in skeletal muscle is not consistently associated with insulin resistance. In contrast to T2DM patients, endurance-trained athletes store similar amounts of lipids in their skeletal muscle as
patients with T2DM whilst very insulin sensitive. These ectopic lipids are stored in lipid droplets which are nowadays recognized as dynamic organelles. Athletes store lipids in their skeletal muscle as a readily available energy source for performance. Release of lipids for oxidation and rates of fat oxidation are matched. In contrast, T2DM patients store lipids in skeletal muscle as a consequence of a misbalance between fat oxidation rate and fatty acid supply to the mitochondria. This misbalance can lead to lipotoxic events resulting in insulin resistance of the skeletal muscle. Understanding the role of the dynamics of lipid droplets in skeletal muscle, i.e. the release and storage of fatty acids, may give insights in the relationship between ectopic lipid storage and insulin resistance. This thesis aimed to study the characteristics of skeletal muscle lipid droplets in relation to insulin sensitivity to gain a better understanding of the negative association of lipid storage in the skeletal muscle with insulin resistance. These lipid droplet characteristics (lipid droplet size, number, location and chemical composition) were studied in this thesis in human subjects possessing a wide phenotypical range as well as in subjects challenged with a high-fat diet or during a prolonged fast.

**Target groups**

In 2010 the prevalence of physical inactivity in adults was 23% according to the WHO. Physical activity is the best strategy to combat obesity and T2DM. In addition, physical inactivity contributes to the development of obesity and T2DM. Changing from a sedentary lifestyle, while being obese, is not easy, especially when it is not under supervision. So, other ways of treating obesity and T2DM are warranted. Understanding the role of muscular fat in the development of skeletal muscle insulin resistance may lead to new target treatments.

This thesis focused on lipid droplet characteristics in human subjects and revealed conditions under which muscular lipid storage is not detrimental for insulin sensitivity, but rather is protective. What the exact mechanisms are behind this is potentially interesting for the pharmaceutical industry. However, more mechanistically studies are needed to find which pathway needs to be targeted to store lipids in a non-detrimental way for insulin sensitivity.

Although (re)introducing physical activity in a sedentary lifestyle is not easy, the results from this thesis underpin the notion that general practitioners and physiotherapists should stimulate T2DM patients and people with overweight/obesity to become more physically active or providing exercise programs for this patient group. However, a more detailed understanding is still needed for the group of T2DM patients who do not improve their insulin sensitivity upon exercise.


**Activities and products**

The research executed in this thesis is performed at the department of Human Biology and Human Movement Sciences at the Maastricht University in the group of Prof. dr. M.K.C. Hesselink and Prof. dr. P. Schrauwen. This research group is worldwide recognized in performing translational studies in the field of T2DM and is mainly focused on elucidating the underlying mechanisms of insulin resistance of mainly skeletal muscle. In this thesis we took the advantage to analyze muscle biopsies taken in human studies in more detail regarding lipid droplet characteristics with confocal fluorescence microscopy. These microscopic studies were performed in cooperation with the department of Genetics & Cell Biology, division Molecular Biology of the Maastricht University. In addition, the measurements for chemical lipid droplet composition were performed in collaboration with the department of Molecular Spectroscopy of the Max Planck Institute in Mainz.

Results presented in this thesis have been implemented in original scientific articles. These articles are published in or submitted to well-recognized peer-reviewed journals. These articles are available online and can be accessed by scientist all around the world. In addition, knowledge obtained from these studies is also communicated to the scientific community via presentations and posters at national and international conferences.

**Innovation**

A classical way to study lipid storage in skeletal muscle is by biochemically extract triglycerides from homogenates. These lipid extractions only give insights in concentration, but not in location (where in the muscle fiber or which fiber type), lipid droplet size and number. The use of microscopy can image these lipid droplet characteristics. Electron microscopy is a widely used technique to study lipid droplet size, number and location. However, this type of microscopy does not give insight in fiber type differences. Fluorescence microscopy can measure fiber type specific and quite some studies have used this type of microscopy to study lipid droplet characteristics per fiber type. In this thesis high-resolution confocal scanning laser microscopy was used to study lipid droplet morphology, decoration and location. The field of high-resolution microscopy is developing quickly. Super-resolution microscopy techniques like STimulated Emission Depletion (STED), a technique awarded the 2014 Nobel Prize in Chemistry, can theoretically reach spatial resolutions down to the 30 nm level. STED microscopy is nowadays accessible for biomedical scientist and is well on its way to become the new golden standard for colocalization/interaction studies. We have set-up STED imaging to study the putative presence of PLIN5 in the mitochondria. The resolution was high enough to distinguish the inner and outer mitochondrial membrane. This gave us the opportunity to
examine if PLIN5 is present on the inner or outer mitochondrial membrane, or is in close proximity of mitochondria. Besides studying lipid droplet morphology and location we applied Coherent Anti-Stokes Raman Spectroscopy (CARS) to examine the chemical lipid droplet composition. In contrast to the classical way to study lipid composition, no lipid extractions are needed for CARS and lipids can be studied without the need of labels in situ. This technique will give the chemical composition (level of saturation and chain length) of lipid droplets only and can make a distinction between type I and type II fibers when combined with fluorescence microscopy.

We combined the data of lipid droplet characteristics at the level of muscle fibers with whole body physiological data obtained in our lab. Lipid droplet characteristics were studied in relation to insulin sensitivity (measured by a hyperinsulinemic euglycemic clamp) and maximal oxidative capacity. Besides cross-sectional studies we studied changes in lipid droplet characteristics in healthy lean subjects upon interventions that challenge the muscle fibers to handle a high flux of fatty acids. Although these type of studies promote our understanding of how fatty-acid flux is handled by the muscle and sheds novel light on the importance of lipid droplet dynamics in maintaining insulin sensitivity these human studies do not provide causality. Nevertheless, our partly associative studies are important key for the translation of pre-clinical research towards the human situation.

**Planning and realization**

In this thesis, we observed that PLIN5 coated lipid droplets can be protective against the development of insulin sensitivity. However, this is not always the case; South Asians still develop insulin resistance upon a high-fat high-calorie diet despite an increase in PLIN5 protein content. In addition, number of PLIN5 coated lipid droplets and PLIN5 protein content are only associated with levels of insulin sensitivity when maximal oxidative capacity is taken into account. More research is warranted to study role of skeletal muscle lipid droplet dynamics in relation to insulin sensitivity. I will continue to perform human studies whereby imaging techniques, like fluorescence microscopy and mass spectrometry imaging, are combined with whole body physiological data to study the role of lipid droplet and mitochondrial dynamics in skeletal muscle insulin resistance.