Imaging myocellular lipid droplet dynamics in relation to insulin sensitivity

Impact paragraph

What is the aim of the thesis and what are the main results and conclusions?

Diabetes effects the quality of life of more than 1 million people in the Netherlands and is amongst the top 3 diseases with the highest disease burden. Ninety percent of the people with diabetes suffers from type 2 diabetes, who have high blood glucose (sugar) levels and a decreased functioning of the hormone insulin. Insulin regulates the uptake of blood sugar into the tissues after a meal, to make sure that the blood glucose levels will not be too high. When tissues do not respond properly to insulin, which is observed in type 2 diabetes, the tissues in the body take up less glucose and it remains in the blood. This can lead to high blood sugar levels. The muscle is an important tissue in this process as it takes up about 80% of all the blood sugar after a meal. When the muscle becomes less responsive to insulin, known as insulin resistance, this is one of the first signs of developing type 2 diabetes.

How the muscle becomes less responsive to insulin (insulin resistance) is not yet known. Fat accumulation in the muscle can play a role and interfere with the signals of insulin to the muscle. Fat, or often in science called lipid, is mostly stored in fat tissue. However, it can also be stored on other places such as in the liver, muscle or heart. People that are obese or have type 2 diabetes store large amounts of fat in the muscle and this may lead to insulin resistance. Interestingly, people who run marathons or cycle long distances also store large amounts of fat in their muscle, but they remain very responsive to insulin (also called insulin sensitivity). Perhaps these active people use their muscle fat more efficiently or store it in a different way than the muscle fat in patients with type 2 diabetes. Muscle fat is normally stored in the form of lipid droplets. If we look more in detail, we see indeed that active people store their muscle fat in smaller and more lipid droplets, especially in the red muscle fibres and a protein called PLIN5 is more present. Perhaps this could explain why these active people have more muscle fat but still remain responsive to insulin, and people with type 2 diabetes become insulin resistant.

In **chapter 2** we looked at scientific literature to see how diet and exercising can affect muscle fat. We have seen that muscle fat is dynamic and adapts to the type of diet and exercise of a person. When someone eats more than he or she uses, muscle fat increases and when a person reduces his calories, the muscle fat decreases again. During a single bout of exercise muscle fat can be used as fuel to provide energy. When someone trains for a longer time, the total muscle fat increases. The muscle adapts to the new lifestyle and an increase of muscle fat is part of this. This suggests that if the muscle fat is used and stays dynamic this may not lead to unhealthy consequences. However, a lot of questions still remain unanswered. How does muscle fat stay dynamic? When is muscle fat healthy? Could we modulate the muscle fat of patients with type 2 diabetes to become more dynamic and therefore make them healthier?

To make patients with type 2 diabetes healthier, many different risk factors have been studied and treatments have been proposed. A new possible risk factor is the changes in day-night rhythm. People who work as shift workers (so work during the night and sleep during the day for a short period of time and then change back to their 'normal' rhythm) show that the muscle becomes insulin resistant and these people are more at risk of becoming obese or develop type 2 diabetes. In **chapter 4** we wanted to see if the muscle fat was also altered. When shift work was mimicked in the lab, we found a decrease in insulin sensitivity, but no changes in the muscle fat. Perhaps the experiment was too short and maybe muscle fat will change on the longer term. The participants were also young and healthy and they had relative low levels of muscle fat. With specialized techniques we looked further at different kind of lipids and we discovered that some lipid species were changed during shift work. These species might be interesting to look at in the future and if they have influence on insulin sensitivity.

In the other chapters we examined certain treatment options to improve the responsiveness of insulin (insulin sensitivity) and see if there were also changes in muscle fat, which could give more information about the relation between muscle fat and insulin sensitivity. In **chapter 3**, humans were exposed to short-term cold

exposure and this led to increased energy expenditure and fat oxidation. To our surprise, the lipid levels in the blood did not drop, which was earlier seen in animal studies. Perhaps it works different in humans and the body keeps the blood fat levels high to provide energy to stay warm. If the cold exposure would have been bit longer or if we would have activated shivering (which was not the case here, despite the cold environment), we could have observed changes in lipid levels in the blood. Shivering activates the muscle and this may lead to more uptake of lipids by the muscle and therefore lowers blood lipid levels.

In **chapter 5**, we studied the effect of the nutritional supplement resveratrol. Resveratrol is naturally present in grapes and peanuts and boosts energy expenditure. Some studies showed that resveratrol also increased muscle fat without causing insulin resistance. We found that resveratrol modulated the muscle fat similar to what we have seen in active people: smaller lipid droplets, stored especially in red muscle fibres with the presence of the PLIN5 protein. Resveratrol can be a possible candidate to modulate the muscle fat to a healthier way of storage. More studies are needed to explore this, especially on the longer term.

In **chapter 6**, we looked more in detail what happens to the muscle fat when you exercise. Thus, we examined muscle fat before exercise, just after exercise and during recovery and investigated how it changes. In addition, does it matter whether you stay fasted during exercises or consume sugars? To study this, one group was only allowed to drink water during this study (the fasted group), while the other drank a sugar drink. We found that in the group with the sugar drink, not much happened with the muscle fat. But in the group that drank water, the muscle fat was used during exercise and during recovery it increased again. During recovery, the muscle fat in the water group was much higher than in the sugar group. The muscle fat was especially stored in the red muscle fibres and in the presence of PLIN5. This shows that drinking calories (sugar) can affect the use of your muscle fat during exercise.

If we combine the findings from this thesis with other scientific literature, we can consider that fat storage in the muscle is not necessarily bad. As long as you store it

in a healthy way and use it. It seems that it is better to store your muscle fat in many small lipid droplets (not a few big ones), especially in muscle fibres that oxidize these lipids (red muscle fibres) and preferably with the protein PLIN5 on these lipid droplets. It also matters what kind of lipid you store in your muscle cells. If your day-night rhythm is disrupted some specific lipid species change, but more research is needed how these lipid species matter and if they can have an effect on insulin sensitivity.

What is the potential contribution of these results to science and society?

This thesis contributes to the knowledge of the metabolism of muscle fat and how it changes in certain circumstances. This broadens our understanding of the behaviour of lipid droplets inside the muscle that form the total fat storage in the muscle. We looked at many details of the muscle fat, like the number and size of lipid droplets, the involvement of the PLIN5 protein and where the muscle fat is located. All these different features can react differently upon diet or exercise and are therefore important to study. Our results show that it is important to not only look at total muscle fat, but to look also at more details to better understand muscle fat metabolism and its health consequences. The amount of muscle fat cannot fully explain whether a muscle is metabolically healthy (here: insulin-sensitive) or not.

As it is still not known how tissues become insulin resistant, so more studies are needed to understand the development of type 2 diabetes. When it is more evident how insulin resistance evolves, this knowledge can be used to prevent the development of type 2 diabetes. Fat storage is associated with obesity, which is an important risk factor for type 2 diabetes. Unhealthy fat storage in the muscle can facilitate the development of insulin resistance. If this relation is better understood, the development of insulin resistance can be prevented and therefore prevent new cases of type 2 diabetes.

The knowledge in this thesis can also be interesting for new studies to modulate muscle fat. For example, we observed that resveratrol can modulate muscle fat into a healthier way of storage. If we could develop strategies to help patients with type 2 diabetes to make their muscle fat more dynamic (and healthier) this might reduce their symptoms and improve the quality of life. Alternatively, specific markers could be developed in the future to identify which person has a healthy muscle fat storage.

In this thesis we used microscopy to study muscle fat. For this, small muscle biopsies were taken from human volunteers and we looked at lipid droplets within the muscle pieces. Microscopy is a powerful tool to obtain information on fat storage within a single muscle cell. Maastricht University has a specialized facility where different kinds of microscopes can be used. With the microscope we are able to look at more details such as number and size of lipid droplets, location of these lipid droplets and examine the PLIN5 protein. By using both human studies and microscopy techniques, we combine the knowledge about what happens in the muscle with the knowledge what happens in the human body. This makes it easier to translate findings to future clinical studies with patients. Furthermore, the microscopy facility enables us to develop more techniques to study the dynamics of muscle fat as we proposed in **chapter 7**. This gives us more knowledge on the cell level, to better understand how fat is stored in the muscle.

To whom would these results be interesting?

The results of this thesis are mainly interesting for researchers. At first for researchers that also study muscle fat metabolism and/or the development of type 2 diabetes. This thesis can provide another piece of the puzzle for the field of muscle fat and insulin sensitivity. Furthermore, fat is not only stored in the muscle but also in other tissues and is associated with many other diseases such as heart diseases, Alzheimer's and cancer. Researchers in this field can also benefit from our findings. Lipid droplets are not only studied in human tissues but also in totally different disciplines. For example, lipid droplets are also important for foetal development, for viruses to infect people or to study the use of lipid droplets in biofuel. Studying lipid droplets itself

can be beneficial for researchers in many different fields.

Microscopy is a growing interest for many researchers and more groups are starting to use the techniques. Researchers that are interested in microscopy would be interested in this thesis to use for example the same techniques or for those who want to cooperate with our facilities.

Most studies performed in this thesis are done to gain more knowledge. However, this knowledge can be relevant for research that is directly involving patients. If we understood how muscle fat works and can be modulated, this can be relevant for patients to improve their quality of life.

The results of this thesis are published in scientific papers, or will be in the future, which are available to read for anyone who is interested. These results are published in journals, online and via social media. Many results have been presented and discussed in national and international conferences with other researchers, patient organizations or representatives from compagnies. In the end, the knowledge can also be used for educational purposes for students or other audiences.