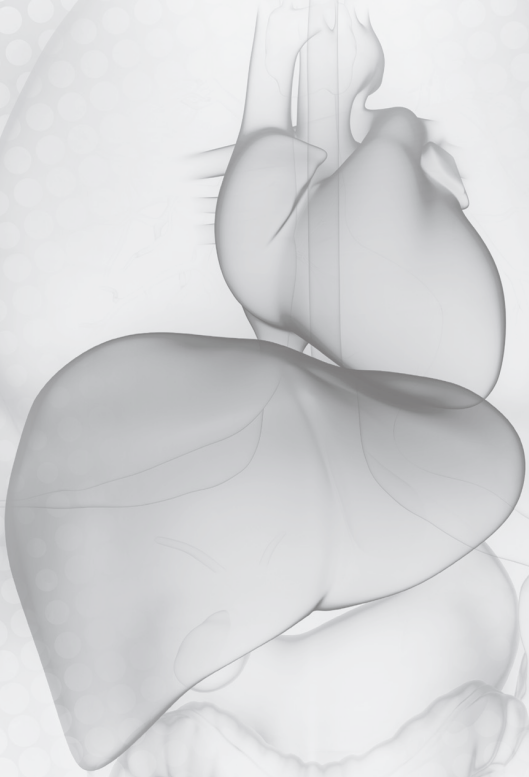
# Chapter 13

## Summary and conclusions



The studies described in this thesis explored the effects of nutritional, exercise and pharmacological interventions on ectopic triglyceride accumulation in obese patients and/or patients with type 1 or type 2 diabetes mellitus. Ectopic fat is defined as triglyceride (TG) storage in tissues, other than adipose tissue, such as the liver, heart and skeletal muscle. The focus of this thesis is on ectopic fat accumulation in two important organs: the liver and the heart. The basis of diabetes treatment is lifestyle intervention with diet and exercise and glucose-lowering medication. Research on the effects of those interventions on ectopic fat compartments in humans has been limited until the introduction of hepatic and, more recently, cardiac proton magnetic resonance spectroscopy ($^{1}$H-MRS). These techniques enable non-invasive assessment of hepatic and myocardial triglyceride accumulation in detail *in vivo.*

**Chapter 2** is a review on the effects of diet and exercise on ectopic fat depositions. This review describes the effects of lifestyle interventions on ectopic fat and the associations between hepatic TG content, intramyocellular lipid accumulation, pericardial fat and myocardial TG content and insulin resistance and cardiac function respectively. The studies described in this review show that the ectopic fat depositions can be reduced by lifestyle interventions and are associated with improved organ function (hepatic and peripheral insulin resistance or myocardial function). Moreover, this review shows that data on lifestyle interventions on myocardial TG content and pericardial fat are limited, especially in diabetes.

**Chapter 3** describes the relation between hepatic triglyceride content and myocardial metabolism and function in type 2 diabetes mellitus (T2DM) male patients without inducible ischemia. This study documented that high liver triglyceride content in T2DM is associated with decreased myocardial perfusion, glucose uptake, and phosphocreatine/adenosine triphosphate ratio in conjunction with decreased insulin sensitivity. Although myocardial metabolism apparently differed between T2DM patients with high and low liver triglyceride content, there were no differences between both groups in myocardial systolic and diastolic function or dimensions.
These data indicate that ectopic triglyceride accumulation in the liver does not occur in isolation in type 2 diabetes mellitus, but is a reflection of disturbed integrative physiology that also involves cardiac metabolism. In addition, these observations indicate that there are profound alterations in tissue metabolism in addition to the altered metabolic parameters in the plasma compartment.

**Chapter 4** studied the effect of a three day very low calorie diet on myocardial TG content and cardiac function in 14 patients with T2DM and cardiac complications. Previous studies by our research group showed an increased myocardial TG content and decreased diastolic cardiac function after a short-term very low calorie diet (VLCD) in healthy subjects and patients

with T2DM without cardiovascular complications (1,2). We hypothesized that this flexibility of the myocardial lipid metabolism would be decreased in patients with T2DM who already have developed cardiac complications. However, we found similar effects of a 3-day VLCD in this study group, with increased plasma levels of non-esterified fatty acids (NEFA) and myocardial TG content and a decrease in diastolic cardiac function. This observation indicates that myocardial triglyceride content is not fixed in patients with T2DM with cardiac complications, but is still amendable to nutritional interventions, just like in healthy subjects and subjects with uncomplicated T2DM. We should comment that the observed changes were subclinical, but nonetheless not in a positive, but rather nonbeneficial direction. However, previous studies by our group have documented that prolongation of a VLCD to 16 weeks is associated with decreased TG accumulation in the heart and improved cardiac function. This raises the concept of biphasic effects of VLCD on the heart in T2DM patients. In the early phase of the VLCD diet, studied in this experiment, the increase in fatty acid levels was associated with increased myocardial TG content, whereas during prolongation of VLCD plasma NEFA levels decrease compared to baseline values, associated with a decreasing fat mass, and associated with decreased myocardial TG accumulation and improved cardiac function.

**Chapter 5** reports on a 14 months follow-up study after a 16-week VLCD intervention in obese T2DM patients on changes in (ectopic) fat compartments. A 16-week VLCD significantly reduces bodyweight, pericardial fat, hepatic TG content, visceral and subcutaneous abdominal fat volumes. Eighteen months after the start of the intervention, weight and (ectopic) fat compartments were still reduced compared to baseline; however all tended to return to baseline values, except for pericardial fat. Chapter 5 and chapter 11 show that pericardial fat is amendable to pharmacological and nutritional interventions. In addition, this study indicates that the dynamics of the studied fat compartments differ, because in contrast to other fat compartments there was no increase in pericardial fat volume upon regain of weight after stopping the VLCD intervention.
The observed differential effect of weight regain on visceral abdominal and pericardial fat is in accordance with chapter 11, where an increase in pericardial fat due to pioglitazone treatment was not accompanied by changes in visceral abdominal fat. Former studies have suggested that pericardial fat thickness is a good proxy for visceral abdominal fat volume, as both compartments are visceral fat depots (3). We assume that pericardial and visceral fat differ in their biological roles and more studies are needed to evaluate the biochemical properties of pericardial fat.

**Chapter 6** describes the long-term effects of a 16-week very low calorie diet on myocardial triglyceride content and cardiac dimensions and function. Patients with T2DM have an increased risk on heart failure, even in absence of coronary artery disease. Myocardial TG accumulation negatively correlates with diastolic function in T2DM. However, myocardial

TG content is not fixed, but amendable to nutritional interventions, even in patients with T2DM (chapter 4). A 16-week VLCD showed a decrease in ectopic fat including myocardial TG content and improved left ventricular diastolic function. However, upon introduction of the regular diet these beneficial effects of VLCD gradually disappear (chapter 5).
Nonetheless, even after 14 additional months of follow up, some beneficial effects of the previous period of VLCD are still present. This sequence of effects also holds true for the cardiac effects of the VLCD. In this chapter we show that although the myocardial TG content returned to baseline values, there are sustained improvements in diastolic function and diastolic left ventricular distensibility. Although most likely stable weight reduction is most desirable, an alternative option would be intermittent periods of VLCD for weight reduction. The present study indicates that the periods of weight regain will not negatively affect the beneficial effects of VLCD on cardiac function. Future research should focus on different approaches to maintain weight loss. Continuous dieting does not seem to be a reasonable option. However, intermittent periods with individual coaching or diet could be options to maintain the positive effects for a longer time.

In **Chapter 7** the effects of a 6-month exercise intervention on ectopic fat compartments in patients with T2DM are studied. The training program consisted of a 6-month individualized training and ended with climbing the Toubkal Mountain. Before training and again after climbing the mountain, patients were studied. We conclude that a 6-month exercise intervention decreases hepatic TG content, visceral abdominal fat and paracardial fat volume in patients with type 2 diabetes mellitus. However, the program did not alter epicardial fat, myocardial or myocellular lipid content. Therefore, the exercise intervention in patients with type 2 diabetes mellitus induces tissue-specific changes in ectopic fat distribution.
Studies in rodents have shown that exercise may improve mitochondrial fatty acid oxidation and suppress expression of proteins involved in hepatic lipogenesis, thereby indicating that the liver is not an innocent bystander, passively adapting to the changes in fatty acid metabolism associated with muscle fitness (4). As we found that moderate exercise could decrease hepatic TG content in T2DM patients, it would be interesting to additionally study the effect on myocardial metabolism, in line with chapter 3.
However, paracardial fat, but not epicardial fat decreased after exercise. Most studies have focused on the role of epicardial fat as possible risk factor for cardiovascular disease. However, epi- and paracardial fat have a different embryonic origin and blood supply. Some studies found a cross-sectional correlation between epicardial and visceral abdominal fat volume. Interestingly, we found a decrease in visceral and paracardial fat, but not in epicardial fat. Therefore, more prospective intervention studies are needed to elucidate the biochemical properties of these fat compartments.

**Chapter 8** evaluates a recently validated magnetic resonance imaging protocol which enables regional assessment of aortic pulse wave velocity (PWV). T2DM patients have increased arterial stiffening as compared to healthy controls. Moreover, aortic stiffness is an independent cardiovascular risk factor for coronary artery disease. Former studies using tonometry and echo Doppler could not accurately measure regional aortic PWV. Current study showed that a standardized oral glucose load induced a decrease of proximal, but not of descending aortic PWV, showing region specific changes in arterial stiffness.
Future studies on the effects of long-term hyperglycemia, may give further insight in possible regional differences of the effects of hyperglycemia on the aortic wall. Moreover, current MRI protocol combined with measurement of cardiac function, could be used to study the association between region specific arterial stiffness and cardiac function.

**Chapter 9** describes nutritional intervention studies designed to increase plasma non-esterified fatty acid (NEFA) levels in both healthy volunteers and T2DM patients. Angiopoietin-like protein 4 (Angptl4) has been identified as an inhibitor of lipoprotein lipase. Little data on determinants of plasma Angptl4 in human are known. However, preliminary data suggest that plasma NEFAs raise plasma Angptl4 levels in humans. We showed that both caloric restriction and caloric excess increased both NEFA levels and plasma Angptl4 levels. It is tempting to speculate that this provides proof of regulation of Angptl4 levels by plasma NEFA levels.
However, nutritional interventions induce tissue-specific and complex endocrine, metabolic and neurogenic changes that cannot be simply dissected into a simple causal relationship between plasma Angptl4 levels and NEFA levels. To further dissect this relationship additional experiments are necessary targeting lipolysis in the same nutritional conditions, e.g. by acipimox which decreases lipolysis and as a consequence plasma NEFA levels. Nonetheless, the experiments described in this study clearly indicate that plasma Angptl4 levels depend on nutritional conditions, just like plasma NEFA levels.

In **Chapter 10** the effect of hyperglycemia caused by reduction in insulin dose on myocardial and hepatic TG content and myocardial function in patients with T1DM is assessed. Ten patients with type 1 diabetes were studied on two occasions: during optimal glucoregulation and after 24 hours of partial insulin deprivation (i.e. reduction of the optimal insulin dose by 50%). Short-term hyperglycemic dysregulation did not alter myocardial or hepatic TG content or myocardial function, despite considerable metabolic derangements, reflected in hyperglycemia and high NEFA levels.
In previous studies from our group, it was shown that in healthy subjects myocardial function and TG content rapidly adapt to changes in metabolic state. Interestingly, this adaptation could not be evoked by short-term hyperglycemia in patients with type 1 diabetes, indicating that the heart is protected from these effects, at least in the conditions of our experiment. Nonetheless, we cannot exclude the possibility that prolongation of the duration of partial

insulin deprivation might have resulted in changes in myocardial function and TG content. This observation is also very interesting with respect to the induction of liver steatosis. Apparently, high plasma NEFA and glucose levels per se are not sufficient to induce liver steatosis in the absence of appropriate insulin levels. This may indicate that hyperinsulinemia is essential for the induction of liver steatosis. This notion may also apply to the relation between plasma NEFA and glucose levels and cardiac triglyceride accumulation.
From a clinical perspective the absence of effects of hypoinsulinemic metabolic derangement on liver and cardiac parameters is fortunate, since this occurs frequently in most patients with type 1 diabetes. The effects of long-term hyperglycemia in T1DM require further investigation.

**Chapter 11** studied the effect of pioglitazone (peroxisome proliferator-activated receptor gamma agonist) versus metformine on pericardial fat in association with other fat compartments and cardiac function in 78 patients with T2DM.
We concluded that pioglitazone increases pericardial fat volume in T2DM patients. This increase in pericardial fat volume did not negatively affect myocardial function. These observations question the notion of a simple inverse causal relationship between pericardial fat volume and myocardial function. Previous studies about the relation between pericardial fat volume and coronary artery disease had a cross-sectional design. In the Framingham Heart Study, pericardial fat was actually inversely associated with measures of left ventricular (LV) function but not independently of other measures of visceral adiposity (5). Correspondingly, there was no association between changes in pericardial fat volume and heart function in the present study, in which we assessed T2DM patients without structural heart disease. In our opinion, there is no proof at present of a direct and causal pathophysiological relation between the size of pericardial fat mass and cardiac function. We postulate that this association reported in other studies is confounded by other factors (e.g. degree of obesity, dyslipidemia, insulin resistance). Otherwise, there might be regional differences in response to interventions of the pericardial fat around the coronary arteries and the myocardium, which may confound observed effects as shown in animal studies (6).

**Chapter 12** demonstrates that 24 weeks of treatment with pioglitazone in T2DM decreases hepatic TG content which is accompanied by a decrease in plasma cholesteryl ester transfer protein (CETP) mass and associated with an increase in HDL cholesterol levels. These results in patients with type 2 diabetes mellitus fully confirm recent findings in mice, showing that a decrease in hepatic lipid content was associated with decreased plasma CETP mass.
Considering the generally observed inverse relationship between plasma triglycerides and HDL cholesterol found in epidemiological studies, a decrease in plasma triglycerides may induce an increase in HDL cholesterol only. However, in the present study the decrease in plasma triglycerides was not different between the groups and plasma HDL cholesterol only

increased in the pioglitazone group, indicating that another mechanism may be responsible for eliciting this difference, i.e., the reduction in CETP.
We found that pioglitazone did not further decrease CETP mass in patients who already used a statin. Experimental studies in APOE*3-Leiden.CETP mice have demonstrated that statins decrease plasma CETP mass by decreasing hepatic mRNA expression, again related to decreased hepatic cholesterol content (7). Apparently, the effect of statins is dominant over the effect of pioglitazone. We hypothesize that statins specifically decrease hepatic cholesterol content and downregulate CETP mRNA expression. Therefore, additional lowering of hepatic triglyceride content will not result in an additional decrease in CETP expression. However, the current study was not designed to assess the effects of statins on these parameters.

Studies in current thesis indeed have shown that lifestyle and pharmacological interventions can change ectopic triglyceride accumulation in (advanced) T2DM and is associated with changes in function. However, the notion emerges that the different fat compartments have differential regulation in relation to exercise, nutrition and pharmacological interventions.
This points to different regulatory mechanisms involved in the accumulation and/or lipolysis of these fat compartments. Alternatively, it is possible that similar mechanisms are involved but with different dose-response relationships. These cannot be derived from the studies described in the current thesis because the aims of these studies were not focussed at these mechanisms underlying the dynamics of the individual fat depots. As patients with obesity and T2DM may have different ectopic fat distribution, this may also affect their (cardiac) risk profile. Manchann et al. (8) already studied the effect of lifestyle intervention versus antidiabetic medication in patients with different fat distribution and found that this could predict intervention failure. More studies in these dynamics will hopefully lead to understanding from which treatments specific patient groups will benefit most.

## CONCLUDING REMARKS

The studies described in the current thesis have yielded the following conclusions:

1. Ectopic triglyceride accumulation in the liver does not occur in isolation in type 2 diabetes mellitus, but is a reflection of disturbed integrative physiology that also involves cardiac metabolism.

2. A three day very low calorie diet paradoxically increases plasma levels of NEFA, and myocardial TG accumulation and decreases diastolic cardiac function in both healthy subjects and T2DM patients with and without cardiovascular complications.

3. Short-term hyperglycemic dysregulation caused by insufficient insulin administration does not modulate myocardial or hepatic TG content or myocardial function, despite considerable metabolic derangements, reflected in hyperglycemia and high NEFA levels.

4. Weight reduction by a 16-week VLCD results in sustained cardiac remodeling and improved diastolic function after 14 months of follow-up, despite weight regain on a regular diet in T2DM patients.

5. Exercise training decreases hepatic triglyceride content, visceral fat and paracardial fat volume in patients with type 2 diabetes mellitus.

6. Pericardial fat volume is amendable to dietary interventions, but with different dynamics than other fat compartments.

7. Pioglitazone increases pericardial fat volume in patients with type 2 diabetes mellitus.

8. An oral glucose load induces region-specific changes in aortic pulse wave velocity.

9. Dietary interventions which induce increased plasma NEFA levels, also increase plasma Angptl4 levels.

10. Pioglitazone decreases hepatic TG content, which is accompanied by a decrease in plasma CETP mass and associated with an increase in HDL cholesterol levels.

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