CHAPTER 2

Ectopic fat and insulin resistance: pathophysiology and effect of diet and lifestyle interventions

Marieke Snel¹, Jacqueline T. Jonker⁎¹, Jan Schoones², Hanno Pijl¹, Johannes W.A. Smit¹, A. Edo Meinders¹, Ingrid M. Jazet¹

⁎Contributed equally to manuscript

Departments of ¹Endocrinology & Metabolism/General Internal Medicine and ²Walaeus Library, Leiden University Medical Center, Leiden, The Netherlands

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ABSTRACT

Obesity predisposes to the development of insulin resistance, type 2 diabetes mellitus (T2DM) and cardiovascular disease. In obese subjects that develop insulin resistance, adipose tissue dysfunction plays a role, causing redirection of triglycerides (TG) towards non-adipose tissues. If in these tissues TG supply exceeds oxidative capacity intracellular lipid accumulation occurs. Ectopic fat is defined as storage of TG in tissues other than adipose tissue, that normally contain only small amounts of fat, such as the liver, skeletal muscle, heart and pancreas. The consequences of ectopic fat accumulation depend on the specific cell type and organ in which TG are deposited. Ectopic fat in the liver and muscle are positively correlated with insulin resistance and T2DM. Myocardial steatosis refers to TG accumulation in the myocardiocytes and is associated with impaired diastolic function. In this review we will discuss the consequences of ectopic fat accumulation in the liver, muscle and heart and, if known, the underlying pathophysiological pathways. In addition, we will discuss the effect of diets with or without exercise on these ectopic fat localizations and the effect of diminishing ectopic fat on insulin resistance in obesity and T2DM.
INTRODUCTION

The amount of people with obesity has increased dramatically over the past decades to an estimated number of 400 million adults worldwide with a projected 700 million in 2015 (http://www.who.int/mediacentre/factsheets/fs311/en/index.html). Obesity predisposes to the development of insulin resistance, type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (1-6). However, about 30% of obese men and women are metabolically healthy (7), that is, do not have hypertension, dyslipidemia or disturbances in glucose metabolism. Vice versa, these metabolic abnormalities occur in 20-30% of normal weight people.

Adipose tissue consists of adipocytes and the so-called stromal-vascular fraction that encompasses blood vessels and stroma with macrophages. Adipose tissue has the unique capacity to store large amounts of energy in the form of triglycerides (TG). Before long, it has been presumed that this was the only function of adipose tissue. However, adipose tissue acts as an endocrine organ by secreting various hormones and cytokines (also referred to as adipokines) with effects on glucose and lipid metabolism and energy homeostasis (8). It now appears that in those obese subjects that develop insulin resistance, adipose tissue dysfunction plays a role as reviewed by Bluher (9). Adipose tissue dysfunction is characterized among others by large adipocytes, secretion of adipokines with a pro-inflammatory profile and ectopic fat deposition. Ectopic fat is defined as storage of TG in tissues other than adipose tissue, that normally contain only small amounts of fat, such as the liver, skeletal muscle, heart and pancreas.

The cause for adipose tissue dysfunction and ectopic fat storage is largely unknown. Bluher recently proposed a model in which genetic and environmental factors lead to adipocyte hypertrophy, hypoxia and endoplasmatic reticulum stress causing inflammation within adipose tissue (via attraction of macrophages) and a different adipokine secretion profile (9). For example, tumor necrosis factor alpha (TNFα) and interleukin 6 (IL6) impair adipocyte differentiation, reduce lipid accumulation and increase lipolysis in adipocytes. Hence, lipids are redirected towards non-adipose tissues. If in these tissues lipid supply exceeds oxidative capacity intracellular lipid accumulation occurs.

The consequences of ectopic fat accumulation depend on the specific cell type and organ in which TG are deposited. Ectopic fat in the liver (10) and muscle (11) are positively correlated with insulin resistance and T2DM. Myocardial steatosis refers to TG accumulation in the myocardiocytes and is associated with impaired diastolic function (12). Recently, increasing interest has focused on fat deposition around the heart: epicardial and pericardial fat. In cross-sectional studies pericardial fat accumulation is associated with coronary artery disease (13) and related to whole body insulin resistance (14). As to the existence and clinical consequences of lipid accumulation in the pancreas controversy exists as elegantly reviewed recently (15). Ectopic fat depositions can be measured with several techniques. Traditionally, biopsies were used to quantify lipid content in liver and skeletal muscle. Nowadays most
studies use non-invasive methods such as computertomografie (CT), ultrasound and proton magnetic resonance spectroscopy (1H-MRS) (Figure 1).

In this review we will discuss the consequences of fat accumulation in the liver, muscle and heart and, if known, the underlying pathophysiological pathways. In addition, we will discuss the effect of diet with or without exercise on these ectopic fat localizations and the effect of diminishing ectopic fat on insulin resistance in obesity and T2DM.

METHODS

The following databases were searched: PubMed (1949 to November 2010), EMBASE (OVID-version, 1980 to November 2010), Web of Science (1945 to November 2010), and Cochrane Library (1990 to November 2010). The search strategy consisted of the AND combination of three main concepts:

1. Type 2 Diabetes Mellitus, Obesity, or Insulin Resistance;
2. Weight Loss, Diet or Exercise;
3. Ectopic Fat.

For these three concepts, all relevant keyword variations were used. References were limited to human studies, adults, written in English or Dutch. In addition, only studies that used techniques that can quantify the amount of lipid accumulation and measured insulin sensitivity were included. Studies using surrogate markers for lipid accumulation (e.g. alanine...
aminotransferase (ALT) or aspartate aminotransferase (AST) as a proxy for hepatic steatosis) were excluded. Hypocaloric diets are defined as containing less calories than required for energy demands and usually contain 1000-1200 kcal/day. Very low calorie diets (VLCD) typically contain less than 800 kcal/day.

**SKELETAL MUSCLE**

**Intramyocellular lipids and peripheral insulin resistance**

Several factors are involved in skeletal muscle insulin resistance: muscle fibre type (less type I, oxidative fibres), impaired capillary recruitment, diminished free fatty acid (FFA) oxidative capacity and an increased plasma FFA concentration (leading to perturbations in insulin signaling). The latter 2 conditions are involved in accumulation of intramyocellular lipid (IMCL). FFAs are taken up by the cell mainly by protein-mediated membrane transport (CD36, fatty acid transport protein (FATP)), along with passive diffusional uptake (16). Inside the cell fatty acid binding protein (FABPc) is the most important cytosolic protein for guiding long-chain fatty acids in the cell to places of oxidation or esterification. Long-chain fatty acyl-CoA is taken up by the mitochondria via carnitine-palmitoyl transferase 1 (CPT1). Inside the mitochondria β-oxidation and further degradation in the tricarboxylic acid cycle takes place. Therefore, IMCL accumulation occurs as a consequence of continuous oversupply of FFAs (caused by enhanced lipolysis, adipocyte dysfunction) together with an impairment in FFA oxidation in the mitochondria.

Accumulation of IMCL is associated with insulin resistance (17-20) and T2DM (11). However, it is not synonymous with the condition given the fact that endurance-trained athletes, who are highly insulin sensitive, also have a high IMCL content (11). Rather, the capacity to oxidize IMCL determines whether they represent a physiological or a pathological role as reviewed by van Loon and Goodpaster (21). In endurance-trained athletes IMCL serve as a readily available energy source. The close proximity of lipid droplets to the mitochondria supports this hypothesis. In these athletes, IMCL are not deleterious because of the increased capacity to oxidize lipids.

T2DM is characterized by a low oxidative capacity which leads to accumulation of lipids and intermediates of fatty acid metabolism such as long chain acyl-CoA (LC-CoA), diacylglycerol (DAG) and ceramides. These fatty acid metabolites induce a sustained activation of serine/threonine kinases such as protein kinase C (PKC) isoforms, IKB-kinase-β and Jun N-terminal kinase, which phosphorylate insulin-receptor substrate (IRS) 1 on serine residues (22). Serine-phosphorylated forms of IRS1 cannot associate with and activate phosphatidylinositol-3-kinase (PI3K), resulting in a decreased glucose transporter 4 (GLUT4) regulated glucose transport over the cell membrane (Figure 2).
The fact that impaired oxidation is involved led to the speculation that mitochondrial dysfunction is the cause of IMCL accumulation and the ensuing insulin resistance. Indeed, a decreased mitochondrial density and/or function has been reported in insulin-resistant offspring of T2DM patients (23-25) and T2DM patients (26-29). However, three of these studies reported decreased mitochondrial function at normal IMCL levels (27-29) suggesting that impaired mitochondrial function is not a prerequisite for IMCL accumulation. Rather, it might be that mitochondrial dysfunction is the consequence of the increased amount of fatty acid metabolites, for example via the formation of lipid peroxides (30). In that case it might be that the lipid-induced mitochondrial dysfunction induces progressive deterioration of oxidative capacity and further accumulation of lipid intermediates in the skeletal muscle cell. Further investigations are warranted to elucidate which one is cause or consequence: IMCL or mitochondrial dysfunction. In this review we focus on IMCL and will not elaborate on mitochondrial function.

In summary, an imbalance between fatty acid supply and oxidation (the contribution of esterification is very low) leads to accumulation of IMCL and lipid intermediates that interfere with insulin signaling and hence reduce insulin-stimulated glucose uptake in the skeletal muscle cell. Therefore, IMCL are associated with insulin resistance with the exception of endurance-trained athletes.

**Effect of diets on IMCL accumulation**

In a 6-month study in normal glucose tolerant (NGT) obese subjects, 25% calorie restriction (amount of calories not mentioned) leading to 10% weight loss (8 kg) had no effect on insulin sensitivity or IMCL. A parallel group that received a low calorie diet (890 kcal/day) but also
had moderate weight loss (14%, 11 kg) showed a significant improvement in insulin sensitivity as measured by the insulin-modified frequently sampled intravenous glucose tolerance test and a tendency to decreased IMCL (measured by 1H-MRS) that was not significant (31). In another 6-month study in morbidly obese NGT subjects, 10% weight loss (14 kg) by hypocaloric diet (1200 kcal/day) also had no significant effect on insulin sensitivity as measured with the hyperinsulinaemic euglycaemic clamp technique nor on IMCL as measured by skeletal muscle biopsies (32). In obese T2DM patients, a similar percentage of weight loss (10%, 8 kg) with a 1200 kcal/day very low fat (3%) diet during an average of 7 weeks, also had no effect on peripheral insulin sensitivity as measured with the hyperinsulinaemic euglycaemic clamp technique nor on IMCL (measured by 1H-MRS) (33).

Moderate weight loss of 10-11% using a VLCD (600-800 kcal/day) for around 8 weeks in obese NGT persons improved insulin sensitivity as measured by homeostasis model assessment insulin resistance index (HOMA-IR) but had no effect on IMCL as measured by skeletal muscle biopsies (34). On the contrary, one study (35) showed a significant decrease in IMCL accumulation but no significant effect on peripheral insulin sensitivity after a 6-days VLCD (700 kcal/day) with a weight loss of 2.5% (in T2DM patients) and 5% (in obese subjects) compared to baseline. It should be noted however IMCL were measured by 1H-MRS in the soleus muscle which has different characteristics than the vastus lateralis muscle. All other studies measured IMCL by muscle biopsy or 1H-MRS of the vastus lateralis muscle. In addition in this study, a very high insulin infusion rate (200 mU/m²/min) was used during the hyperinsulinaemic euglycaemic clamp, leading to supraphysiological insulin concentrations and hence higher glucose uptake rates as compared to other studies.

Moderate weight loss with hypocaloric diets seems to have no effect on IMCL or on insulin sensitivity in either obese or obese T2DM patients (31-33,36-39). More pronounced weight loss using a VLCD leads to a decrease in IMCL. A prolonged VLCD (450 kcal/day, on average 17 weeks duration) leading to 50% reduction of excess weight (mean 22 kg) in obese insulin-treated T2DM patients led to a significant improvement of both peripheral insulin sensitivity as well as IMCL in skeletal muscle biopsies (40) (Table 1).

Effect of diet and exercise on IMCL accumulation

Several studies investigated the effect of the addition of exercise to a hypocaloric or VLCD in obese NGT or T2DM patients. One group investigated the effect of a hypocaloric diet (goal weight loss 7 kg) with or without exercise (3 to 5 times weekly at 60-70% of maximal heart rate) in obese NGT (41) and obese T2DM patients (42), respectively. The obese NGT groups (diet-only and diet with exercise) both lost ~ 10% of body weight (9-10 kg) and had equal increases in glucose disposal rates. IMCL decreased in the diet-only group, not in the diet with exercise group. In the other study of the same group, 10 obese T2DM patients lost 7% of body weight (7 kg) and had an 54% increase in insulin-stimulated glucose disposal and a slight but significant increase in IMCL as measured by CT (42). Other studies in obese NGT
Table 1. Effect of diet and exercise on insulin sensitivity and intramyocellular lipid (IMCL) content.

| Ref | patients | n | BMI start kg/m² | age yrs | intervention | duration | body weight loss kg | effect on skeletal muscle | Effect on IMCL insulin sensitivity |
|-----|----------|---|-----------------|--------|--------------|----------|---------------------|--------------------------|---------------------------------|
| 30  | obese NGT| 12| 31±2            | unknown| 25% caloric restriction | 6 months | -8 kg               | Si, no change            | no change                      |
| 12  | obese NGT| 12| 33±2            | 12.5% caloric restriction + 12.5% exercise | -8 kg | Si 37±18%, p<0.01 | no change            |
| 11  | obese NGT| 11| 33±2            | 15% weight loss hypocaloric diet 1200 kcal/day | -11 kg | Si 70±34%, P<0.04 | no change            |
|     | controls | 11| 31±2            | controls | 0 kg         | Si, no change | no change            |
| 31  | morbid obese NGT| 9| 48±9            | 39±12  | diet 1200 kcal/day | 6 months | -14±12 kg           | M-value, no change         | no change                      |
|     | controls | 8| 51±8            | 39±12  | biliopancreatic diversion | unknown | -33±10 kg | M value 23±3 to 52±11 µmol/kgFFM/min, p<0.05 | 1.6±1.1 to 0.2±0.4, p<0.05 |
| 32  | obese NGT| 20| 34±1            | 42±2   | -700 kcal compared to normal diet followed by energy restriction + exercise | 15 weeks | -11 kg | OGGTT, no change     | no change                      |
|     | obese T2DM| 8| 30±1            | 47±3   | 1200 kcal/day 3% fat diet (until normoglycemia) studies after weight stabilisation period | 3-12 weeks (mean 7) | -8±1 kg | GDR, no change       | no change                      |
| 34  | obese NGT| 13| 33±2            | unknown| -522 kcal compared to normal diet | 3 months | -6 kg | M-value, no change   | no change                      |
| 35  | overweight T2DM| 7| 27±3            | 55±5   | -25-30 kcal/kgLBM | 2 weeks | BMI -1.5±0.0% | M-value, no change         | no change                      |
|     | overweight T2DM| 7| 27±3            | 46±3   | -25-30 kcal/kgLBM + advice to walk 2-3 td 5-6days/week | 2 weeks | BMI -2.3±0.1% | M-value 5.3±0.3 to 8.2±0.5 mg/kg/min, P< 0.001 | 3.8±0.4 to 3.1±0.4, p<0.03 |
| 36  | morbid obese NGT| 7| 44±6            | unknown| diet 1200 kcal/day | 6 months | -5±4 kg | M value, no change   | no change                      |
| 38  | obese T2DM| 13| 36±1            | 50±3   | VLCD 600-800 kcal/day | 8 weeks | -9±1 kg | HOMA-IR -0.9 is -44±7%, p<0.001 | no change                      |
| 37  | obese NGT| 5 | 36±5            | 38±12  | VLCD 700 kcal/day | 6 days  | -2.3 kg | GDR, no change       | -56%, p=0.006 (1H-MRS)       |
Table 1. (continued)

| Ref | patients | n   | BMI start | age yrs | intervention | duration | body weight loss kg | effect on skeletal muscle | Effect on IMCL insulin sensitivity |
|-----|----------|-----|-----------|---------|--------------|----------|---------------------|--------------------------|-----------------------------------|
|     | obese T2DM | 7   | 37±7      | 43±6    | VLCD 700 kcal/day | 6 days   | - 3.7 kg            | GDR, no change            | - 40%, p=0.04 (1H-MRS)             |
| 40  | obese T2DM | 10  | 40±2      | 55±3    | VLCD 500 kcal/day until 50% excess weight was lost | mean 17 weeks | - 22 kg            | GDR 18.8±2.0 to 39.1±2.8 umol/kgLBM/min, p<0.001 | 7±14 to 4±1, p<0.002 |
| 41  | obese NGT   | 7   | 33±1      | 46±2    | 25% caloric restriction, goal 7% weight loss | 18.6±0.7 weeks | -11±2%, ca - 8 kg | M value increased 29±7% p<0.05 | decreased, p<0.05 |
|     |           | 9   | 35±1      | 42±3    | 25% caloric restriction + 3/5 days/wk exercise at 60-70% MHR | 19.2±0.4 weeks | - 9±1%, ca - 9 kg | M value increased 38±9% p<0.05 | no change |
| 42  | obese T2DM | 10  | 34±1      | 44±3    | 25% caloric restriction + 3/5 days/wk exercise at 60-70% MHR | 16-20 weeks | -7.1±0.1% = ca 7 kg | GDR 4.1±0.6 to 6.3±0.9 mg/kgLBM/min, p<0.05 | 48±1 to 50±1, p<0.01 (CT) |
| 43  | obese NGT   | 21  | 33        | 40      | - 500-1000 kcal compared to normal diet with 4-6x/wk exercise at 65-75% MHR | 16 weeks | - 10 kg            | GDR 6.5 naar 9.7 mg/kgLBM/min p<0.05 | no change |
| 44  | obese IGT   | 11  | 34±1      | 67±1    | -600 kcal/day compared to normal diet and aerobic exercise 5days/wk exercise at 75% VO_{max} | 12 weeks | ca -8 kg            | M value 2.9±0.3 to 4.7±0.6 mg/kgLBM/min, p<0.01 | 3.9±0.6 to 2.5±0.3, p<0.05 |
|     |           | 12  | 35±2      | 66±1    | aerobic exercise 5days/wk exercise at 75% VO_{max} | 12 weeks | ca -3 kg            | M value 3.0±0.4 to 4.2±0.7 mg/kgLBM/min, p<0.05 | 3.9±0.6 to 3.0±0.4, p<0.05 |
| 45  | obese NGT   | 25  | 30±1      | 66±1    | 4-5days/wk supervised aerobic exercise at 75% MHR | 16 weeks | - 1.3 kg            | M-value, no change            | 21% increase, p<0.01 |
| 46  | obese NGT   | 20  | 30±1      | 59±1    | 2x/week 30 min aerobic + 1x/week resistance exercise both at 55% VO_{max} | 12 weeks | no change            | GDR, no change                  | no change |
|     | obese T2DM | 18  | 30±1      | 59±1    | 2x/week 30 min aerobic + 1x/week resistance exercise both at 55% VO_{max} | 12 weeks | no change            | GDR 18.4±1.4 to 21.0±1.4 umol/kgLBM/min, p<0.05 | no change |

NGT: normal glucose tolerant; GDR: glucose disposal rate; HOMA-IR: homeostatic model assessment of insulin resistance; IGT: impaired glucose tolerant; LBM: lean body mass; T2DM: Type 2 Diabetes Mellitus.
(31,43), obese impaired glucose tolerant (IGT) (44) or obese T2DM patients (38) consistently showed increased insulin sensitivity with no differences between diet-only or diet with exercise group (with the exception of (31) all measured with the hyperinsulinaemic euglycaemic clamps) The effect on IMCL in these studies was less consistent showing either no effect (31,43) or a decrease (38,44) in IMCL accumulation (Table 1).

**Effect of exercise on IMCL accumulation**

Few studies have addressed the effect of exercise-only on IMCL and insulin sensitivity. A 4-month exercise program (4-5 times weekly 45 minutes at 75% of maximal heart rate) in obese NGT subjects increased insulin-stimulated glucose disposal as well as IMCL (by 21%) as measured by muscle biopsy (45). Interestingly intramuscular ceramide and DAG levels decreased. No control group participated in the study. A study in obese subjects with IGT investigated the effect of exercise-only (5 days/week 60 min at 75% maximum aerobic capacity \(\text{VO}_{2\text{max}}\)) vs. diet (circa 1300 kcal/day) combined with exercise for 12-week. The diet with exercise group lost more body weight but the improvement in peripheral insulin sensitivity and the decline in IMCL were similar in the two intervention groups (44). Recently the effect of a 12-week combined aerobic and resistance exercise program was investigated in 18 T2DM (body mass index (BMI) 30 kg/m\(^2\), HbA1c 7.2%, age 59 years) and 20 healthy controls matched for age, body weight and BMI. The intervention had no effect on body weight (46). Exercise increased insulin-stimulated glucose disposal only in the T2DM patients but not to levels comparable to that in the control group. In addition, exercise had no effect on IMCL in the controls and increased IMCL in the T2DM patients along with an increase in insulin-stimulated glucose oxidation and suppression of fat oxidation (Table 1).

**Conclusion**

Moderate weight loss has no effect on IMCL but can improve peripheral insulin sensitivity if a VLCD is used. Larger weight losses are needed to improve both peripheral insulin sensitivity and deplete IMCL stores. With respect to the effect of exercise on IMCL the story is more complex. Athletes have increased IMCL stores that serve as a readily available energy supply and training further increases IMCL in healthy subjects. On the other hand, sedentary insulin-resistant subjects also have increased IMCL, caused by diminished oxidative capacity. These are associated with increased intramyocellular lipid intermediates that interfere with insulin signaling and other cellular processes. Exercise training in these subjects either increases, decreases or has no effect on IMCL accumulation. This is probably associated with the stage of disease (can fat oxidation and/or mitochondrial function be restored) as well as with the intensity of the exercise program and sort (aerobic/resistance) exercise. Overall, studies show that exercise improves peripheral insulin sensitivity. Factors involved are a reduction in lipid intermediates and hence improved insulin signaling and mitochondrial function, increased oxidative capacity and increased capillary blood flow. In addition, contraction and
hypoxia activate adenosine monophosphate-activated protein kinase (AMPK) which leads to increased GLUT4 translocation independent of insulin.

**LIVER**

**Intrahepatic lipids and insulin resistance of the liver**

Accumulation of fat in the liver in the absence of excessive alcohol ingestion is referred to as non-alcoholic fatty liver disease (NAFLD). The spectrum of liver abnormalities within this entity ranges from hepatic steatosis with or without mild increases in serum AST/ALT to non-alcoholic steatohepatitis (NASH) with or without fibrosis, cirrhosis and incidental hepatocellular carcinoma. The world-wide estimated prevalence in the general population is about 20%, with large differences across countries (47). There is a strong association with obesity. A cross-sectional study in 1515 severely obese NGT subjects showed abnormal liver biopsies in 90% of people (48). The majority had hepatic steatosis, but one third had portal inflammation and fibrosis. A prospective study demonstrated a 4-times increased risk to develop hepatic steatosis in obese persons as compared to controls (49).

Non-invasive methods for measuring hepatic TG content such as ultrasound, CT and \(^1\)H-MRS cannot distinguish NAFLD from NASH and fibrosis. A definite diagnosis can only be made by liver biopsy with histologic examination. The cut-off value for abnormal lipid accumulation in the liver has been defined as more than 5% of liver volume or when more than 5% of hepatocytes contain visible intracellular lipids (50). Two recent studies in respectively a NGT mixed (Hispanic, non-Hispanic, African American) population (51) and a lean Caucasian population (52) found that the 95\(^{th}\) percentile for hepatic TG content was 5.6% and 3% respectively, using \(^1\)H-MRS. The Pathology Committee of the NASH Clinical Research Network designed and validated scoring system of 14 histological features examining liver biopsy findings detailing steatosis, fibrosis, inflammation, and liver cell injury (53). An NAFLD activity score > 5 was universally associated with NASH.

Fat accumulation in the liver is associated with hepatic insulin resistance as well as with peripheral insulin resistance in skeletal muscle and adipose tissue (10,54-56). In a large European cohort of 1307 middle-aged NGT subjects, patients with a high fatty liver index (an estimate for hepatic steatosis based on an algorithm including BMI, waist circumference, TG, and gamma-glutamyltransferase) had a lower glucose disposal rate as measured by a euglycaemic hyperinsulinaemic clamp as well as higher FFA levels at the end of the insulin infusion (57). The latter is suggestive for decreased insulin sensitivity of adipose tissue. Korenblat et al. (54) found a negative correlation between hepatic insulin sensitivity measured by the hyperinsulinaemic euglycaemic clamp and hepatic TG content (measured by \(^1\)H-MRS) in 42 nondiabetic obese subjects. Indeed, a multivariate linear regression analysis found that hepatic TG content was the best predictor of insulin sensitivity in liver, skeletal muscle and...
adipose tissue, independent of BMI and percent body fat. Some claim that the amount of hepatic TG content is directly correlated with the severity of insulin resistance but this cannot be confirmed by others.

The mechanism behind hepatic TG accumulation and the development of hepatic insulin resistance is similar to that described for skeletal muscle (22,58). An increase in DAG in hepatic cells leads to activation of PKC, leading to decreased insulin receptor kinase activity and subsequently lower insulin-stimulated IRS2 tyrosine phosphorylation and lower IRS2–associated PI3K-activity which results in reduced insulin stimulation of glycogen synthase activity. This ultimately leads to decreased insulin-stimulated hepatic glucose uptake and reduced insulin suppressibility of hepatic glucose production. Furthermore, reduced activity of AKT2, a protein kinase downstream of IRS and PI3K, results in decreased phosphorylation of the forkhead box O (FOXO) transcription factor, allowing it to enter the nucleus and activate the transcription of the rate-controlling enzymes of gluconeogenesis. The result is increased hepatic glucose production and decreased hepatic glucose uptake, which both contribute to increased plasma glucose levels (Figure 3).

**Effect of diet on intrahepatic lipids**

Non-invasive techniques like CT and 1H-MRS have shown that weight loss by nutritional interventions can result in a large decrease in hepatic TG content in obese and T2DM subjects (33,37,38,59-63). Because of the different populations and differences in baseline hepatic TG content, studies are not well comparable with respect to the individual effect of level of caloric restriction and/or amount of weight reduction on loss of hepatic TG content. Nonetheless, it has been shown that even a relatively small drop in BMI of 3-6% is associated with a
considerable reduction in hepatic TG content of 34-40% (37,60,61). The main reduction in hepatic TG content already occurs in the first two weeks of dietary restriction (38,59). The percentual decline in hepatic TG content positively correlates with the hepatic TG content at baseline, as patients with a high hepatic TG content at start of the diet lose relatively more TG than patients with low hepatic TG content, with the same amount of weight loss (59,60,62). Two studies measured both hepatic TG content and hepatic insulin sensitivity with a hyperinsulinaemic euglycaemic clamp (33,63). In obese (BMI 30.1 kg/m²) T2DM patients, 7 weeks of a liquid formula diet (1200 kcal/day) led to a weight reduction of 8%. Hepatic TG content decreased by 81% and insulin mediated suppression of the endogenous glucose production (EGP) improved substantially (33). Viljanen et al. (64) found similar results in obese NGT subjects (BMI 33.7 kg/m²). In this 6-week study a VLCD (550 kcal/day) led to a weight reduction of around 11 kg, a decrease in hepatic TG content of 60% together with a 40% decrease in basal EGP and hepatic insulin resistance index as well as a diminished hepatic FFA uptake.

Although the abovementioned studies clearly show that diet-induced weight loss leads to a decrease in hepatic TG content, the effect of this decrease in hepatic TG content on liver histology (i.e with liver biopsies) has only been scarcely studied. In obese patients with NASH moderate weight loss over a 12-month period, obtained with dietary advices only, led to an improved steatosis score in 9 of the 15 subjects. The improvement was associated with greater weight loss as compared to the patients that showed no change in steatosis (64). Another study in 15 patients with obesity and NASH combined a hypocaloric diet with exercise during 12 weeks while 10 obese subjects with NASH served as controls. The BMI decreased by 3 kg/m² in the intervention group and the steatosis score decreased from 2.3 to 1.3 (30-50% steatosis to less than 30%) while no changes were observed in the control group (65). In contrast, severe caloric restriction during 8 months in 41 morbidly obese (BMI 43.3 kg/m²) subjects leading to an impressive median weight loss of 34 kg (-27% of BMI) showed normalization of liver architecture in 19 patients (66). However, they also found an increase in hepatic inflammation and fibrosis in some patients, this was associated with a greater weight loss and elevated FFAs.

**Effect of diet and exercise on intrahepatic lipids**

Studies on the effect of exercise on hepatic steatosis are scarce (review (67)). Most of these combine an exercise program with a hypocaloric diet. Both in obese as well as in T2DM patients these combined interventions led to a reduction of hepatic steatosis. Even a 2-week intervention with minimal weight reduction led to a significant 20% reduction in hepatic TG content in T2DM patients (38). However, since moderate weight loss alone already reduces hepatic steatosis the role of exercise is uncertain. Larson-Meyer studied a diet-only (25% calorie restriction (CR)) vs. a diet (12.5% CR) combined with exercise (12.5% CR) obese NGT group. In this 6-month study no additional effect of exercise upon the diet was found at an equal total amount of caloric restriction (31).
**Effect of exercise on intrahepatic lipids**

Only one study has investigated the influence of exercise alone on hepatic TG content in overweight (BMI 27.7 ± 0.5 kg/m²) sedentary men (68). After a 6-week aerobic exercise programme (60-85% of VO_{2max} for a minimum of 20 min. at least three times per week) without significant effect on body weight no changes were found in hepatic TG content as measured by ¹H-MRS, although both peripheral and hepatic insulin sensitivity (measured by the hyperinsulinaemic euglycaemic clamp) improved. In this study, the hepatic TG content was already low at the start of the intervention and the amount of exercise was modest. Nevertheless, this study suggests that exercise alone (without weight loss and/or dietary caloric restriction) has beneficial effects on hepatic insulin resistance. The mechanism by which exercise improves hepatic insulin resistance is probably different than that in muscle. Indirect signals via factors mediated by the muscle and adipose tissue and a decrease in circulating FFAs are thought to play a role.

**EPICARDIAL AND PERICARDIAL FAT**

Pericardial fat is the adipose tissue surrounding the heart. It consists of two layers: epicardial fat (visceral fat) originating from mesothelial cells and paracardial or mediastinal fat, originating from mesenchymal cells (69). For more in depth information on the anatomic and pathophysiologic role of pericardial fat we refer to two excellent reviews on this subject (69,70).

Several functions have been proposed for epicardial fat tissue (70). Scientific proof is however rather difficult to obtain since most animal species have very little epicardial fat (71). In guinea pigs, rates of lipolysis and lipogenesis were 2-fold higher in epicardial fat than in other fat depots. This led to the assumption that epicardial fat might act as a buffer to protect the myocardium from highly toxic fatty acid levels and to provide fatty acids as a direct energy source in times of energy demand (71,72). Coronary arteries are embedded in epicardial fat so that another putative function might be to protect the coronary arteries from the tension and torsion induced by the arterial pulse wave and provide an environment in which the coronary arteries can easily expand. This fat compartment also acts as a metabolically active organ, secreting cytokines (73,74).

Several cross-sectional studies have suggested a positive relation between an increased epicardial fat volume and coronary artery disease (14,75-77). Furthermore, an increased epicardial fat volume has been associated with insulin resistance in non-diabetic obese patients (13) and with the presence of T2DM in a Han Chinese population (78,79).

**Effect of diet on epicardial fat**

Two studies have examined the effect of diet-induced weight loss on epicardial fat. Kim et al. (80) studied 27 moderately obese NGT subjects, who lost 11% (9.5 kg) of weight during
a 12-week weight loss intervention study. Epicardial fat thickness measured over the right ventricle wall by echocardiography decreased by 17% from baseline. Iacobellis et al. (81) studied 20 severely obese (BMI 45 ± 5 kg/m²) subjects (probably including patients with IGT or T2DM) who followed a 6-month low calorie diet (900 kcal/day) and lost 20% (25 ±10 kg) of bodyweight. Epicardial fat decreased by 32% from baseline. This was accompanied by an improvement in left ventricular mass and diastolic cardiac function. The change in diastolic function was also positively correlated with the change in epicardial fat thickness. Moreover, in 15 obese patients with T2DM, we found a significant decrease in pericardial fat after weight loss with a 16-week VLCD, measured with MRI (unpublished data) (82).

**Effect of exercise on epicardial fat**

To date, only two studies examined the effect of (aerobic) exercise on epicardial fat (83). In one study, 24 obese NGT (BMI 30.7 ± 3.3 kg/m²) middle-aged Japanese men followed a supervised exercise program for 3 months. The exercise intensity was gradually increased in 4 weeks from 50-60 to 60-70% of the maximum heart rate 3 days/week 60 minutes, which was continued for the remainder of the study. Following the intervention the BMI decreased by 4.3 ± 3.0% (circa -1 kg/m²) and VO$_{2_{max}}$ increased by 20%. Epicardial fat thickness measured by echocardiography over the free wall of the right ventricle decreased significantly. The change in visceral adipose tissue (-15%) was significantly correlated with the change in epicardial adipose tissue (-8.6%).

In the other study, 32 obese postmenopausal women were randomized to diet-only or diet combined with moderate or intensive exercise for 20 weeks. All three groups had a similar 15% reduction in bodyweight and a 17% reduction in pericardial fat. However, no differences were observed between the diet-only or diet with exercise group (84).

**MYOCARDIAL TRIGLYCERIDE CONTENT**

In addition to the epicardial/pericardial fat depositions, TG can also be stored within the cardiomyocytes. This is referred to as myocardial TG content or myocardial steatosis. Myocardial TG accumulation can be measured with great sensitivity by 1H-MRS (85). Patients with IGT and T2DM have an increased myocardial TG content compared to obese and lean controls (12,86,87). This accumulation of myocardial TG is a result of excessive fatty acid uptake relative to the oxidation. If fatty acids are converted to myocardial TG, several intermediates are released (e.g. ceramide) which in animal models caused cardiac dysfunction (88,89). In T2DM patients, myocardial steatosis was associated with impaired left ventricular diastolic function (12). Indeed, an increased fatty acid uptake in the myocardium has been found in healthy obese and T2DM patients compared to healthy lean subjects (90,91). But in the one study that investigated the fate of the intracellular fatty acids in the resting state in insulin-naïve
T2DM patients as compared to controls an increase, not a decrease in fatty acid oxidation was found. Fatty acid re-esterification was negligible but lower in T2DM patients as compared to the controls (91). Interestingly, neither plasma FFA levels nor myocardial blood flow was increased both in subjects with obesity (90) and T2DM (91) suggesting another mechanism, for example at the (cellular) level of the FAT/CD36, to account for the increased fatty acid uptake (Figure 4).

The myocardial TG content is not static. Three days of severe caloric restriction (450 kcal/day to complete starvation) in healthy volunteers and patients with T2DM increases myocardial TG content, which is associated with a decrease in left ventricular diastolic function (92,93).

**Effect of diet on myocardial triglyceride content**

Thirty-four obese (BMI 33.7 ±0.7 kg/m²) healthy persons underwent a 6-week VLCD (550 kcal/day). Before and after the intervention intramyocardial TG were measured, a hyperinsulinaemic euglycaemic clamp was performed and either glucose uptake or fatty acid uptake was measured by positron emission tomography. The intervention led to a weight loss of 11.2 ± 0.6 kg and a non-significant decrease in myocardial TG content of 31% (n=8, p=0.076). Myocardial fatty acid uptake decreased significantly. Myocardial mass and work decreased significantly by 7 and 26% respectively (94).

Our group investigated the effect of a 16-week VLCD (450 kcal/day) on myocardial TG content in obese patients with T2DM (95). We showed that a decrease in BMI (from 35.6 ± 1.2 to 27.5 ± 1.3 kg/m²) was associated with a significant decrease in myocardial TG content and an improvement in left ventricular diastolic function.
Ectopic fat and insulin resistance

Effect of exercise on myocardial triglyceride content

No other studies regarding the effect of diet-induced weight loss and/or exercise are available. It would be interesting to study endurance trained athletes to see whether they have, like in skeletal muscle, higher levels of intramyocardial TG than healthy controls and whether this can be accounted for by impaired myocardial fatty acid oxidation, and is related with cardiac function.

DISCUSSION

T2DM is a multifactorial disease in which genetic, environmental and lifestyle factors induce insulin resistance and impaired insulin secretion, ultimately leading to chronic hyperglycemia and its complications. Given the association between T2DM and obesity the recent focus of research has been the link between them. Vague already described a link between visceral adipose tissue, insulin resistance and T2DM in 1947 (96). But it has not been until the start of the obesity and diabetic epidemic that further elaboration on his work started. This research revealed that adipose tissue is not merely a storage depot for TG but actively secretes a vast array of factors such as cytokines, metalloproteinases and adipokines that can induce inflammation and insulin resistance (8).

In addition, with the advancement of radiological techniques it has become apparent that patients with insulin resistance and T2DM not only have a higher visceral to subcutaneous fat ratio as compared to healthy subjects but that TG are also stored in other organs called ectopic fat depositions for example in the liver, skeletal muscle, heart, and perhaps the pancreas (97,98). TG in the cells of these organs disrupts normal metabolic processes leading to increased hepatic glucose production, decreased insulin-stimulated glucose disposal and impaired cardiac function respectively. The consequences of elevated fatty acids and/or lipid accumulation in the pancreas seem to be only in order when elevated glucose levels are already present (15). Whether these ectopic fat storage is cause or consequence of insulin resistance and T2DM is currently under investigation. If ectopic fat is the cause of insulin resistance than it should be present already before the onset of insulin resistance (primary steatosis). On the other hand, when ectopic fat is the consequence of insulin resistance than insulin resistance should always be present. To date only cross-sectional studies have been performed.

Several large lifestyle intervention studies such as the Diabetes Prevention Study (99), the Da Qing study (100) and the Diabetes Prevention Program (101) all showed that lifestyle changes aimed at weight loss decrease the risk of developing T2DM by 31-58%. In patients already affected by T2DM, weight loss also decreased insulin resistance and improved gluco-regulation (40). The data in this review show that substantial weight loss mobilizes ectopic fat stores in all organs and that this was associated with an improvement of the function
of that organ. Thus, a reduction in hepatic TG was accompanied by a decline in fasting EGP (33,63) and an improvement in the insulin-suppressibility of EGP. A decrease in myocardial TG (95) and epicardial fat (81) were both associated with improved diastolic cardiac function. Finally a decline in IMCLs leads to an improved insulin-stimulated glucose disposal (40). It should be noted however that the amount of weight loss and/or the severity of caloric restriction are of influence on the effect and that there seems to be a tissue-specific reaction. For example, around eight kilograms weight loss following a 1200 kcal/day diet for 7 weeks led to a decrease in hepatic TG and improved insulin sensitivity of the liver but had no effect on insulin-stimulated glucose disposal or IMCL in obese T2DM patients. When obese women with a history of gestational diabetes were subdivided in groups with high and low liver fat, a similar weight loss led to greater loss of hepatic fat in the high liver fat group while both groups lost an equal amount of visceral and subcutaneous fat (62). A prolonged VLCD in more severely obese insulin-dependent T2DM patients leading to around 22 kg of weight reduction also decreased IMCL and improved insulin-stimulated glucose disposal (40). We recently corroborated these findings in a similar group of patients who underwent a 16-week VLCD. The largest reduction occurred in hepatic TG content (-85%) whereas IMCL accumulation in the skeletal muscle decreased by 38%. The relative reduction in visceral fat was larger than the reduction in subcutaneous abdominal fat (-60%, -45% resp.) (82). The above studies suggest that hepatic TG content is the most easily mobilized, followed by visceral fat. The tissue-specific reaction to dietary interventions is also present when, vice versa, patients are subjected to high-fat feeding. Three days of a high-fat high-energy diet in health young males greatly increased hepatic TG stores but had no effect on myocardial TG (102).

Few studies have investigated the effect of exercise per se, that is exercise without weight loss and/or caloric restriction. The effect of exercise varies in the different organs with respect to TG accumulation. In muscle, exercise can even increase IMCL. However, when this is accompanied with increased fatty acid oxidation this is positive and in accordance with the athlete's paradox. The latter refers to the fact that endurance-trained athletes have increased IMCL but are very insulin sensitive. In these athletes the IMCL are a substrate source during exercise and the high turnover rate prevents accumulation of lipid intermediates that have a negative effect on insulin signaling and can form lipid peroxides. In the sedentary state, when metabolic flexibility is low, IMCL accumulate with the aforementioned deleterious effect on cellular processes. Exercise can either, increase, decrease or have no effect on IMCL but does improve insulin sensitivity. Apart from a decrease in lipid intermediates/increased fatty acid oxidation, an increase in capillary density and activation of AMPK with subsequently enhanced GLUT4 translocation are also involved. Exercise in combination with diet also depletes hepatic TG content and improves hepatic insulin sensitivity whereas the one study that investigated the effect of exercise alone found no effect on hepatic TG content but an increase in hepatic insulin sensitivity. The underlying mechanism is probably not direct but via a decrease in factors produced by adipose (8) and skeletal muscle tissue. Only one study
investigated the effect of exercise on epicardial fat: this was reduced but cardiac function was not measured. No studies on the effect of exercise on myocardial TG content have been published.

The clinical significance of measuring ectopic fat depositions after weight-loss interventions is limited. Performing an MRI/1H-MRS in every patient is not practical from a logistical and cost perspective, especially since it has no implications for treatment at this moment.

From a scientific point of view the most intriguing question is how and why ectopic fat storage begins and whether this process is genetically determined or can be modified by measures other than the always so difficult to obtain weight loss. Therefore, it is interesting that exercise leads to improvements in insulin sensitivity, whereas it does not lead to decreases in ectopic fat. For that matter it is interesting that Europeans are relatively protected from (diet-induced) obesity and insulin resistance. On the other hand, aboriginals adapting a Western lifestyle rapidly obtain obesity and diabetes (103). Whether this is associated with the deposition of ectopic fat is unknown although reasonable to assume. Also the sequence of affected organs is unknown. The fact that people adapting a Western lifestyle develop obesity and T2DM suggests however that food might induce an inflammatory process. This of course upon a genetic background that, from an evolutionary standpoint, is set to store calories. Dietary intervention studies in which radiologic techniques, metabolic studies and tissue biopsies are combined hopefully will shed more light upon the question what determines why people store fat in non-fat compartments. And more importantly, what is the most effective treatment in order to halt the increasing diabetes epidemic.
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