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Lipid accumulation in non-adipose tissue and lipotoxicity

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Abstract

Obesity is a well-known risk factor for the development of type 2 diabetes mellitus and cardiovascular disease. Importantly, obesity is not only associated with lipid accumulation in adipose tissue, but also in non-adipose tissues. The latter is also known as ectopic lipid accumulation and may be a possible link between obesity and its comorbidities such as insulin resistance, type 2 diabetes mellitus and cardiovascular disease.

In skeletal muscle and liver, lipid accumulation has been associated with the development of insulin resistance, an early hallmark of developing type 2 diabetes mellitus. More specifically, accumulation of intermediates of lipid metabolism, such as diacylglycerol (DAG) and Acyl-CoA have been shown to interfere with insulin signaling in these tissues. Initially, muscular and hepatic insulin resistance can be overcome by an increased insulin production by the pancreas, resulting in hyperinsulinemia. However, during the progression towards overt type 2 diabetes, pancreatic failure occurs resulting in reduced insulin production. Interestingly, also in the pancreas lipid accumulation has been shown to precede dysfunction.

Finally, accumulation of fat in the heart has been associated with cardiac dysfunction and heart failure, which may be an explanation for diabetic cardiomyopathy.

Taken together, we conclude that evidence for deleterious effects of lipid accumulation in non-adipose tissue (lipotoxicity) is strong. However, while ample human data is available for skeletal muscle and the liver, future research should focus on lipid accumulation in the pancreas and the heart.

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Keywords: Lipid accumulation; Non-adipose tissue; Lipotoxicity; Ectopic fat

1. Introduction

Obesity is due to a chronic positive energy balance, which increases the amount of triglycerides (TG) in adipose tissue. However, the TG can also be stored in non-adipose tissue, such as muscle, liver, pancreas and heart. Indeed, obesity has been shown to lead to excessive deposition of TG in these organs, which is termed ectopic fat deposition or steatosis. Lipid droplets accumulate in the cytoplasm of the cells and an excessive accumulation of these lipids may lead to cell dysfunction or cell death, a phenomenon known as lipotoxicity [1,2]. Obese individuals are at much greater risk of developing

type 2 diabetes and cardiovascular disease (CVD), it has been suggested that ectopic fat is a link between obesity and these diseases [3,4].

Indeed, human and animal data have shown that ectopic lipid accumulation is associated with insulin resistance in muscle and liver, and with functional losses in the pancreas and the heart in animal models. The combination of insulin resistance of skeletal muscle and liver on the one hand, and pancreatic insufficiency on the other hand, are well-known factors that lead to the development of type 2 diabetes mellitus. As stated above, obesity is also an important risk factor for CVD and also here may ectopic fat deposition play a role. Indeed, data from animal models suggest a causal relationship between cardiac lipid accumulation and dilated cardiomyopathy and ultimately heart failure [5–9].

Lipid accumulation in ectopic sites can occur either by increased uptake of fatty acids (FAs), increased synthesis within the tissue involved, or reduced FA oxidation/disposal [10].

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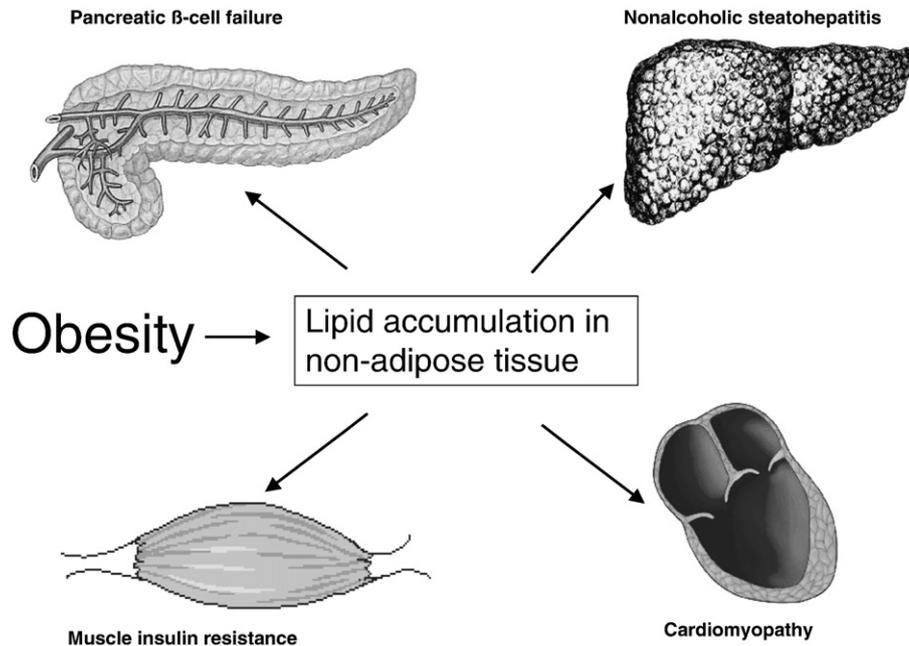


Fig. 1. Effects of obesity on lipid accumulation in non-adipose tissue.

However, the relative contribution of these factors to ectopic lipid accumulation varies in different physiological states and in different tissues. The purpose of this article is to review the evidence for lipotoxic mechanisms accompanying lipid storage in skeletal muscle, liver, pancreas and myocardium (see also Fig. 1).

2. Measurement of lipid content

Traditionally, the lipid content is quantified in a biopsy either biochemically or by electron- or light microscopy in combination with dedicated stainings. However, taking a biopsy is feasible in skeletal muscle, but lipid quantification of the human liver, the pancreas and the heart strongly depends on non-invasive methods. In these cases, very often magnetic resonance spectroscopy ($^1\text{H-MRS}$) is the method of choice. In contrast to the microscopic methods, where lipids in muscle tissue can be visualized within single fibers, $^1\text{H-MRS}$ measures lipid content of a larger muscle area (typically a few cm^3). In a $^1\text{H-MRS}$ spectrum, one can distinguish peaks originating from atoms at different chemical positions (due to a difference in shielding by electrons) and therefore one can quantify different chemical compounds if they contain mobile protons at sufficient concentrations. In muscle tissue, this is the case for e.g. water, creatine and fatty acids. Interestingly, in skeletal muscle, an additional effect plays a role (susceptibility effect). Due to slightly different magnetic properties of FA in lipid droplets inside myocytes (intramyocellular lipids, IMCL) and FA in surrounding adipose tissue (extramyocellular lipids, EMCL) and due to the parallel orientation of the adipose tissue layers in skeletal muscle relative to the magnetic field the resonance frequencies of IMCL and EMCL differ slightly, giving thus rise to two separate peaks, which allows quantifying these different lipid depots with $^1\text{H-MRS}$ (see also Fig. 2).

3. Skeletal muscle

As described above, lipid droplets in healthy skeletal muscle (also known as IMCL) can be quantified by $^1\text{H-MRS}$; and actually visualized by light microscopy using oil-red-O staining (see also Fig. 3) or by electron microscopy. The localization of the lipid droplets close to the mitochondria suggests that lipids inside the muscle are used as fuel for muscular activity. Based on earlier studies that have used biochemical quantification of muscular lipids there was some controversy about whether IMCL was decreased during exercise. Studies using electron microscopy and the more recent studies, using $^1\text{H-MRS}$ to

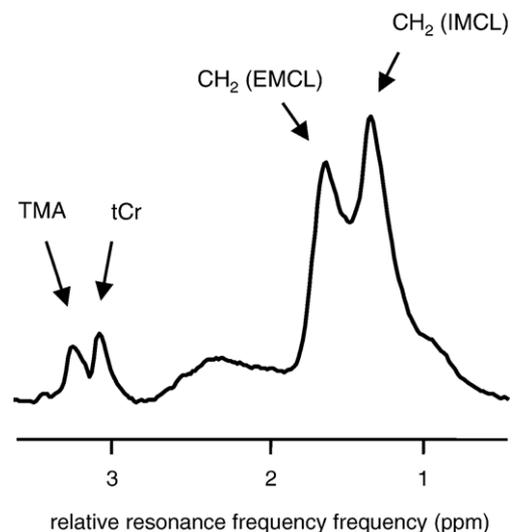


Fig. 2. Quantification of IMCL by MRS. The figure shows a spectrum from the human vastus lateralis muscle. TMA = trimethylammonium compounds, tCr = total creatine. The two arrows at the lower frequencies point out the peaks originating from the CH_2 -groups of EMCL and IMCL respectively.

quantify IMCL, agree however on a decrease of lipids inside the muscle after exercise [11–15]. The controversy resulting from earlier studies could have been due to the large variation of biochemical lipid analysis [16]. In line with a role of IMCL as substrate during physical activity, resting IMCL content is also increased after endurance training and in particular within the (oxidative) type I fibers, which rely more on fat oxidation [11]. Considering these findings, the physiological role of IMCL as substrate source during exercise seems well established and the increase of IMCL with training is a healthy physiological response. However, it has been reported that IMCL is also increased in subjects at risk for type 2 diabetes mellitus and strongly correlates with insulin resistance in sedentary subjects [17,18]. From these results, it has been suggested that IMCL content may impair insulin sensitivity. This seems paradoxical in the light of the high IMCL content in trained subjects [19,20], as it is well known that endurance trained subjects are very insulin sensitive [21–23].

Interestingly, while endurance training increases resting IMCL due to an increased demand of intramyocellular substrate, a high availability of FA can also result in a high resting IMCL content. Two days to 5 weeks of a high-fat diet increases IMCL stores and this accumulation of intramuscular fat may simply be due to a positive fat balance when changing to a high-fat diet [24–28]. Physically inactive humans consuming a high-energy, high-fat diet, may have a chronically positive energy and fat balance, resulting in fat accumulation in adipose tissue and probably also in skeletal muscle. Indeed, obesity is correlated with increased IMCL [29,30]. In accordance with the above suggestion, other conditions resulting in high circulating FA availability also increase IMCL content. For example, elevation of FFA by infusion or 72 h of fasting [31–33]. Also, submaximal exercise, which is well known to elevate plasma FFA, results in increased IMCL content in non-active muscle [34]. Therefore, the increase of

IMCL with training is a physiological adaptation that is demand-driven and paired with an increased fat oxidative capacity. Under the other conditions, fat in muscle is simply stored because the FA availability and oxidation are not in balance. These two situations can have different consequences concerning insulin resistance and strong evidence is accumulating that this is due to differential accumulation of lipid intermediates [10,35]. When fat oxidative capacity is high, concentrations of lipid intermediates such as diacylglycerol (DAG), ceramides, acyl-CoA will stay low, while situations with a high-fat availability but low fat oxidative capacity lead to increased concentrations of these intermediates [30]. In relation to insulin sensitivity, the capacity to use IMCL may be more important than IMCL levels *per se*. Indeed, it has been reported that correlations between acyl-CoA and insulin resistance are stronger than those between IMCL and insulin resistance [30].

For DAG it has been shown that the insulin resistance observed in human muscle when plasma FFA levels were elevated during euglycemic–hyperinsulinemic clamping was associated with increases in DAG [36]. *In vitro* was found that this metabolite can activate Protein Kinase C (PKC), thereby triggering a serine/threonine kinase cascade, leading to phosphorylation of serine/threonine sites on insulin receptor substrates (IRS-1 and IRS-2). This reduces the ability of the insulin receptor substrates to activate phosphatidylinositol 3-kinase, which ultimately results in a reduced GLUT4 translocation to the cell membrane. In this way, increased DAG concentrations may decrease insulin-stimulated muscle glucose uptake [37–41].

4. Liver

The liver plays a major role in a number of vital functions. Next to playing an important role in glycogen storage, plasma protein synthesis, and drug detoxification, it is a central organ for lipid metabolism. The liver synthesizes cholesterol and TG, and produces and takes up lipoproteins. Considering this role in the trafficking of lipids, it is not surprising that hepatocytes are capable of storing lipids in the form of small droplets of TG. However, usually the hepatic lipid content is low (below 5% of fat by wet weight) and when the liver lipid stores exceed this value, this is known as a fatty liver or liver steatosis. A well-known cause of a fatty liver is alcohol abuse and also an unfavorable genetic predisposition increases the risk of the developing the disease [42]. Importantly, overweight and obesity are strong risk factors for non-alcoholic fatty liver disease (NAFLD) and as the prevalence of obesity is reaching pandemic proportions, a significant part of the population will be affected by liver steatosis. Figures from the US and Japan show that nowadays already up to one third of the general population may have a fatty liver [43,44].

Fat accumulation in the liver is generally associated with the cluster of metabolic abnormalities related to the metabolic syndrome [45] and increased liver content is strongly correlated with insulin resistance [46–50]. It has been suggested that NAFLD, in its whole spectrum ranging from

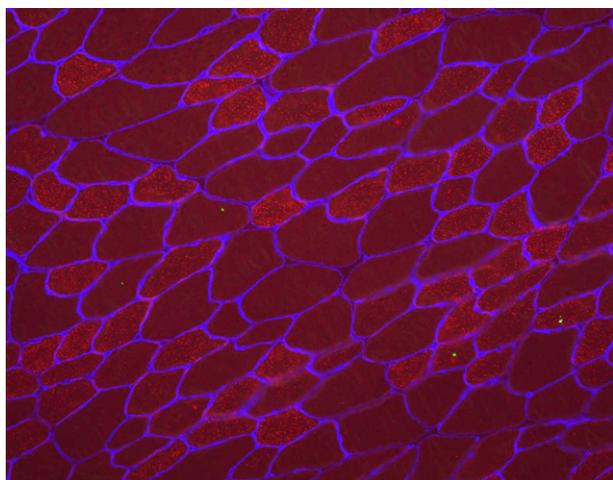


Fig. 3. Quantification of IMCL by histochemistry. The figure shows a stained section of rat tibialis anterior muscle. The red fluorescent signal originates from Oil Red O, showing IMCL; the blue fluorescent signal originates from a laminin staining, showing the cell membranes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pure fatty liver to non-alcoholic steatohepatitis (NASH) and cryptogenic cirrhosis, which in the end may lead to hepatocellular carcinoma, is simply the hepatic manifestation of the metabolic syndrome [51,52]. Therefore, it is not surprising that a fatty liver is associated with an increased risk of all-cause death [53].

The mechanisms underlying the development of NAFLD are not completely understood but it has been shown that FA in the liver come from several different sources: dietary fat, FA released from adipose tissue, and from *de novo* hepatic lipogenesis. An imbalance of any of the pathways involved in FA and TG delivery, synthesis, export or oxidation could contribute to lipid accumulation in the liver.

Regarding dietary fat, many studies in mice [54] and rats [55,56] have documented that a high-fat diet rapidly induces hepatic steatosis. Also in humans, it was shown that 2 weeks of high-fat diet (56% of energy as fat) increased lipid accumulation in the liver, while an isocaloric low fat diet (16% of total energy as fat) decreased liver fat content [57].

Interestingly, animal data suggest that saturated FA lead to more fat accumulation compared with unsaturated FA [58]. Especially, n-3 FA from fish oil appeared to be protective. As these n-3 FA are known to stimulate peroxisome proliferators-activated receptor- γ (PPAR- γ), this effect may be due to an increased FA oxidation [59]. However, these animal studies did often use very high doses of n-3 FA and it is questionable to what extent these results can be extrapolated to the human situation [59–61]. This has only been tested in one pilot study, in which was shown that supplementation with n-3 polyunsaturated fatty acids (PUFA) improves biochemical, ultrasonographic and haemodynamic features of liver steatosis. This study supports the efficacy of n-3 PUFA as a new therapeutic approach in the treatment of NAFLD [60].

In NAFLD patients, the postprandial suppression of adipose tissue lipolysis is blunted, leading to elevated plasma FA levels, thereby providing the liver with ample substrate for re-esterification of TG [62]. Therefore, adipose tissue can contribute considerably to the development of liver steatosis

by releasing FA. Additionally, adipokines released from this tissue may play an important role. For example, adiponectin (which is released from adipose tissue in quantities inversely related to adipose tissue mass) may have a protective effect, as plasma levels of adiponectin were significantly lower in subjects with NAFLD compared to body mass index (BMI) matched controls [63]. Adiponectin levels have also been found to correlate negatively with hepatic fat [63,64]. Further support for an important role of adiponectin comes from a mouse model, in which steatohepatitis was induced by a high-fat/alcohol diet. Adiponectin administration alleviated hepatic steatosis, and significantly attenuated hepatic inflammation and elevated levels of transaminases by increasing FA oxidation in the liver and decreasing the activities of enzymes involved in FA synthesis [65].

Next to dietary FA and FA released from adipose tissue, the liver is also capable of *de novo* lipogenesis. Although the *de novo* lipogenesis is probably not very important in healthy lean subjects [62], it has been shown in patients with NAFLD that *de novo* lipogenesis can contribute up to 24% of TG in the liver. This *de novo* lipogenesis was twice as high as the 10% reported previously in obese, hyperinsulinemic subjects [66] and is 4–5 times higher than the 5% observed in healthy populations [67,68]. In addition low fat/high carbohydrate diets have been shown to increase *de novo* lipogenesis in healthy subjects with body weights between 80 and 120% of their ideal body weight [69]. Therefore, not only a high-fat content, i.e. the above mentioned 56% of energy as fat [57], but also a high carbohydrate intake may be a concern.

It is not completely known how liver steatosis leads to insulin resistance. However, it is very likely that similarly as in skeletal muscle, not the TG *per se*, but the lipid intermediates are important. *In vitro* studies have indeed shown that increases in intracellular DAG activate PKC, which binds to and inactivates the insulin receptor kinase resulting in reduced insulin-stimulated IRS-1 and IRS-2 tyrosine phosphorylation. This in turn results in reduced insulin activation of PI 3-kinase and Akt, also known as protein kinase B (PKB). Reduced Akt/PKB activation results in lower glycogen synthase kinase-3 phosphorylation and lower forkhead box protein O (FOXO) phosphorylation. This in turn results in lower insulin-stimulated liver glycogen synthesis and decreased suppression of hepatic gluconeogenesis, which results in an increase in GLUT2 translocation and an increase in glucose release, respectively [40]. This decreased hepatic glucose storage and higher endogenous glucose production are clearly major factors in developing impaired glucose tolerance and diabetes.

Hepatic insulin resistance is also associated with increased insulin plasma concentrations, which in turn stimulates *de novo* lipogenesis and therefore enhances liver steatosis further. Animal studies have clearly shown that insulin activates the membrane-bound transcription factor sterol receptor binding protein 1-c (SREBP-1c), which transcriptionally activates most genes required for lipogenesis. In mice, even in the insulin resistant state, insulin stimulates hepatic SREBP-1c transcription and increases lipogenesis [70]. In addition, overexpression of SREBP-1c in transgenic mice leads to increased lipogenesis and

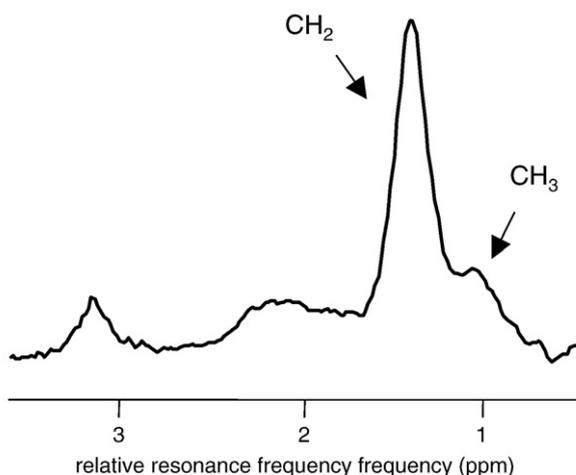


Fig. 4. Quantification of IHL by MRS. The figure shows a ^1H -MRS spectrum from a human liver. The two arrows indicate the CH_2 and the CH_3 peak.

the development of hepatic steatosis [71]. On the other hand, inactivation of the SREBP-1c gene in livers of *ob/ob* mice, a genetic model of leptin deficiency that develops obesity and hepatic steatosis, reduced the hepatic TG content by approximately 50% [72]. These data indicate that liver steatosis results in a vicious cycle by causing insulin resistance, which again favors hepatic lipid accumulation.

On the other hand, several interventional studies have demonstrated that normalization of liver fat content, which can also be measured by $^1\text{H-MRS}$ (see also Fig. 4) [73,74], normalizes insulin resistance and disturbed glucose metabolism. In this respect, the effect of weight loss has been investigated in several studies. In these studies, it has been found for example that moderate weight loss [75] due to a low-calorie diet [76] or dietary counselling combined with increased physical activity [77] strongly reduced hepatic lipid accumulation and simultaneously improved hepatic and whole body insulin sensitivity [77,78] and splanchnic glucose uptake [78].

Together, these data demonstrate that reduction of liver fat by moderate weight loss, can improve glucose metabolism in the liver. Furthermore, after treatment with pioglitazone for 16 weeks, the hepatic fat content in subjects with type 2 diabetes mellitus was decreased from 19.6 % to 10.4%, while the splanchnic glucose uptake increased from 33.0% to 46.2%. [79].

Besides hepatic insulin resistance, the inflammatory character associated with the development from liver steatosis to NASH is a concern. Although the pathogenesis of NASH has not yet been fully elucidated, it has been suggested that, next to the accumulation of FA in the liver, cytochrome P4502E1 (CYP2E1) has an important role. CYP2E1 plays a key role in the pathogenesis of alcoholic liver injury, including alcoholic steatohepatitis, because of the oxidative stress it generates [80,81]. Indeed, CYP2E1 concentrations increase not only in experimental animals which mimic human obesity [82], but also in morbid obese men with NAFLD or patients with NASH [83,84]. CYP2E1 concentrations are invariably elevated in the liver of patients with NASH [84] because FA and ketones, which are increased in diabetes, are also substrates for CYP2E1 [81]. An excess of these substrates upregulates CYP2E1. The resulting oxidative stress and liver injury are exacerbated by a diet low in carbohydrates and rich in fat, including unsaturated lipids, which promote CYP2E1 induction [85,86].

Oxidative stress causes also various types of functional and structural damage and commonly increases tumour necrosis factor (TNF)- α production in patients with NASH [87], in addition to the already increased release of TNF- α by adipose tissue. Adipose tissue produces and releases a variety of cytokines (interleukin-1, TNF- α), that appear to mediate the inflammatory response that accompanies obesity [88]. In addition, it is noteworthy that the expression of TNF- α is increased in adipose tissue of obese individuals and this expression correlates with BMI, percentage of body fat and hyperinsulinemia [89,90].

Besides the release of TNF- α from adipose tissue and the activation via oxidative stress, obese patients with NASH also have enhanced expression of TNF- α mRNA in hepatic tissue, whereas obese patients without NASH have not [91]. This

proinflammatory cytokine probably contributes to the inflammation and the steatosis as shown in alcoholic steatohepatitis [92]. Due to the inflammation that is associated with NASH, it is a risk factor for progressive liver disease and finally hepatocellular carcinoma.

5. Heart

There is increasing evidence for ectopic fat accumulation inside the heart to play a role in cardiomyopathy, and eventually heart failure, which may presently be an underestimated risk factor. Similarly as in skeletal muscle and liver, metabolic dysregulation in lipid-overloaded hearts may induce insulin resistance. Sharma et al. suggested that because failing hearts already exhibit impaired FA oxidation [93], a superimposed myocardial insulin resistance may impair glucose oxidation resulting in “energy starvation” of the heart, causing heart failure [8]. Furthermore, it has been suggested that lipid metabolites may be toxic to cardiomyocytes by inducing apoptosis [9,94]. Due to the limited accessibility of cardiac tissue, these cardiotoxic mechanisms have so far mainly been studied in rodents rather than in humans.

In one human study, the lipid content in hearts after heart failure was investigated [8]. A significantly increased intramyocardial lipid deposition was found in patients with heart failure and diabetes and/or obesity [8]. This lipid accumulation was associated with a gene expression profile similar to that in animal models of cardiac steatosis and heart failure [8]. In the Zucker diabetic fatty (ZDF) rat, a model of obesity secondary to genetic unresponsiveness to leptin, TG accumulated rapidly in the heart [9], as in other non-adipose tissues, when the animals became increasingly obese [9]. This build up of lipids was associated with a progressive increase in ceramide content and iNOS expression. By 14 weeks of age, DNA laddering was increased markedly, evidence of severe cardiac apoptosis [9]. These changes were accompanied by functional losses, as ZDF rats also developed eccentric left ventricular remodeling, increased left ventricular pressure and septal wall thickening [5,9].

The extreme obesity in the ZDF rat model made it difficult to determine whether the cardiac maladaptations are related to excessive myocardial lipid accumulation or to increased expression of conventional risk factors for CVD. To address this limitation, various lean genetic mouse models of cardiac-restricted steatosis have recently been developed [5–7,94–99]. These animals displayed increased myocardial lipid content in the absence of obesity or any other traditional cardiovascular risk factors. FA accumulation in these animals can be caused by either decreased FA oxidation or increased FA uptake.

Transgenic mice with decreased cardiac FA oxidation were generated to produce cardiomyocyte-restricted deletion of PPAR- δ . This down regulated constitutive expression of key FA oxidation genes and decreased basal myocardial FA oxidation. These mice had severe lipid accumulation in the heart and consequently, cardiac dysfunction, cardiac hypertrophy and congestive heart failure with reduced survival [6].

To test whether a mismatch between myocardial FA uptake and utilization may lead to the accumulation of cardiotoxic

lipid species, a transgenic mouse line which, under a myosin heavy chain (MHC) promoter, overexpressed long-chain acyl-CoA synthetase (ACS) in the heart was generated. This protein plays an important role in FA transport across the membrane. The mice showed severe cardiac steatosis, systolic dysfunction and hypertrophy. The results of this study further demonstrated, that an FA uptake/utilization mismatch leads to accumulation of lipid species which are toxic to cardiac myocytes [94]. Indications of the causal relationship between cardiac steatosis and cardiac dysfunction came from results showing that reversing steatosis also restored cardiac function. The same MHC-ACS mice were made hyperleptinemic by treatment with a recombinant adenovirus containing the leptin cDNA. The heart of the hyperleptinemic MHC-ACS-transgenic mice had normalized TG contents and exhibited normal echocardiograms [7].

Another study of the reversibility of cardiac lipid storage was performed in mice treated with streptozocin, a toxin inducing diabetes by affecting pancreatic β -cells. Treatment with streptozocin led to cardiac lipid accumulation. Interestingly, overexpression of apolipoprotein B (apoB), which increases the ability of cells to secrete TG-rich lipoproteins, reversed cardiac lipid accumulation in streptozocin treated animals. Mice, treated with streptozocin, overexpressing human apoB in the heart showed a decrease of 48% in TG content after 12 weeks with echocardiographic indexes of heart function being normal or only marginally affected, in comparison to the wild-type mice treated with streptozocin. These findings suggest that TG accumulation in the heart is important for the development of diabetic cardiomyopathy in mice, and that apoB formation by cardiomyocytes plays an integrated role in cardiac lipid metabolism [98]. Similarly, other treatments that ameliorated cardiac lipid accumulation, by downregulation or knocking out of PPAR- α also rescued the heart from dilated cardiomyopathy [96,97].

Taken together, there is strong evidence for lipotoxic mechanisms in rodents showing that lipid accumulation in the heart leads to heart failure. As mentioned earlier human cardiac tissue is not readily available and the study of cardiac lipid accumulation largely relies on non-invasive methods. $^1\text{H-MRS}$ has been developed for quantifying lipid content in skeletal muscle and liver [14,100,101]. Recently $^1\text{H-MRS}$ has been successfully adapted to also quantify lipid content in cardiac muscle of human subjects [102] (see also Fig. 5). The employment of this technique showed that TG was detectable in the myocardium of healthy human subjects even in those who are very lean. In overweight subjects myocardial TG content was elevated and was accompanied by increased left ventricular mass and a subtle reduction of septal wall thickening, which represents mild systolic dysfunction [102]. While it has been shown that the consumption of a single high-fat meal did not influence cardiac lipid content, despite of a 2-fold increase in serum TG, 48 h of fasting increased cardiac TG accumulation significantly [103]. In addition, myocardial fat was found to be higher in obese than in lean subjects and myocardial fat correlated with FFA levels, epicardial fat, and waist-to-hip ratio. Epicardial fat was positively associated with peripheral vascular

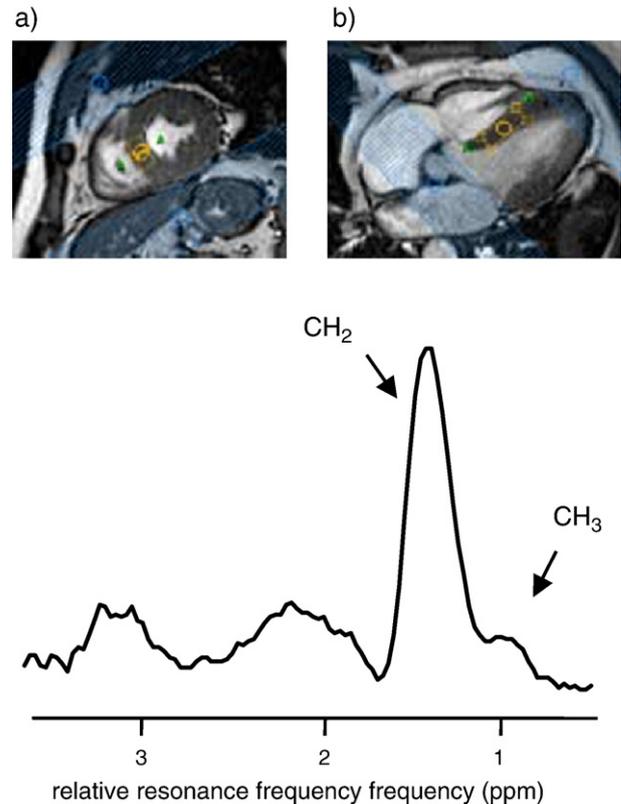


Fig. 5. $^1\text{H-MRS}$ spectrum acquired from a volume of $1 \times 2 \times 3$ cm positioned in the septum of the myocardium, as shown in the short axis view a) and four chamber view b).

resistance and negatively with the cardiac index. Together, these human data may indicate that the cardiac accumulation of TG is related to FFA exposure, generalized ectopic fat excess, and peripheral vascular resistance and that these changes precede left ventricle overload and hypertrophy [104]. Clearly, further human studies are needed to investigate this further.

6. Pancreas

As obesity develops, insulin secretion increases parallel to insulin resistance in order to maintain normal glucose homeostasis. Patients predisposed to diabetes, however, fail to compensate adequately for the greater insulin requirements [105]. It has also been suggested that the eventual impairment of insulin secretion in subjects with type 2 diabetes mellitus is related to ectopic fat accumulation inside the pancreas and high levels of FFA, which are both hallmarks of obesity [106,107]. Indeed, this relationship between obesity and lipid accumulation inside the pancreas, in addition to lipid accumulation inside the muscle and liver, was found *in vivo* in healthy Mexican-American girls, 14–17 years old and with a BMI ranging from 17.7 kg/m^2 to 46 kg/m^2 [108]. Support for the notion that chronic exposure of the β -cell to elevated FFA levels can be damaging to its function comes mainly from studies with isolated islets exposed to high concentrations of FFA for periods of 24–48 h [109,110]. Evidence for islet lipotoxicity *in vivo* comes from studies with the ZDF rat, which, as noted earlier,

shares many of the features of obesity-related type 2 diabetes mellitus in humans. When ZDF rats (*fa/fa*) were fed standard chow diet, the male ZDF rat developed marked hyperglycemia after 9 weeks of age [107]. This does not hold true for the fatty females or the lean littermates (*fa/+* or *+/+*) of the male animals. The obese ZDF females rarely exhibit hyperglycemia [111,112], although they have levels of obesity and insulin resistance comparable to males. In line with this, the male ZDF rat displayed a marked elevation of plasma FFA levels from 5 weeks of age onward, when compared to the female ZDF rat. Interestingly, just before the development of diabetes, an abrupt and massive increase in islet TG content appeared that coincided with severe disruption of islet morphology and β -cell function. Furthermore, energy restriction of these animals from 6 weeks of age greatly reduced their islet fat accumulation and under these circumstances islet function was largely restored and hyperglycemia did not develop. Taken together, this implicates that fat accumulation in the islet is an important contributor to β -cell failure in the ZDF rat, and probably in other rodent models of type 2 diabetes mellitus. Whether this is also true in humans is not yet clear, in part because of the scarcity of suitable pancreas specimens for histochemical analysis from individuals with increasing degrees of β -cell malfunction [113].

To show the direct relationship between fat accumulation in the pancreas and insulin secretion the reversibility of pancreatic dysfunction with normalization of TG content inside the pancreas was demonstrated in animal models. In PPAR- γ -deficient (*PPAR- γ +/-*) mice on a high-fat diet, insulin secretion was impaired and this was associated with increased islet TG content. Pioglitazone, a PPAR- γ agonist, decreased islet TG content and simultaneously restored the impaired insulin secretion in these animals [114].

Different mechanisms by which long-term exposure to FFA can lead to β -cell dysfunction have been proposed, including effects on insulin biosynthesis, preproinsulin gene expression and the expression of uncoupling proteins (UCP).

Firstly, FFA are shown to decrease insulin biosynthesis in isolated rat islets [115]. This study also indicated that at least some FFAs exert detrimental effects on pancreatic β -cell function by impairing preproinsulin gene expression. Therefore, long-term exposure to elevated FFA levels may be a factor in the development of type 2 diabetes mellitus due to their effects on preproinsulin gene expression.

In addition long-term exposure to FFA can also modify UCP expression and thereby changing glucose-stimulated insulin secretion by uncoupling the mitochondria and decreasing ATP production. A glucose-induced change in ATP/ADP ratio plays a crucial role in the coupling of glucose metabolism to insulin secretion and any factor uncoupling ATP formation from substrate oxidation will decrease glucose-stimulated insulin secretion. It has been shown that the pancreatic β -cells express the uncoupling protein UCP2 and that FFAs increase its expression and partly uncouple the β -cells [116].

Furthermore, long-chain acyl-CoA (LC-CoA) may mediate the effects of FFAs. This because FFA that cannot be oxidized,

may form LC-CoA and their effects on β -cell dysfunction are twofold. First, LC-CoA may activate the K_{ATP} channel and hyperpolarize the β -cell, rendering its depolarization by glucose more difficult and decrease glucose-stimulated insulin secretion in this way [117]. Second, a constitute reservoir of LC-CoA may downregulate specific PKC isoforms and suppress their incretin action on insulin secretion [118]. Incretin action means that with matched glucose concentrations the insulin secretion is greater following ingestion of glucose than it is following infusion of glucose.

In addition to the general hazardous effects of high concentrations of FFA on β -cell function, the saturated palmitic acid seems to be specifically toxic. Among all the FFAs studied *in vitro* on human and rodents pancreatic islets, palmitic acid rapidly induced β -cell apoptosis and reduced their proliferative capacity [119,120]. Increased levels of palmitic acid correlated with *de novo* synthesis of ceramides, known to activate the apoptotic pathway in several cell types, including the β -cells. The unsaturated oleic acid prevented apoptosis induced by palmitate, probably by mediating upregulation of the anti-apoptotic protein bcl-2 [120].

In summary, fat accumulation in the pancreatic islets leads to a decreased insulin secretion and might explain why insulin resistant people cannot meet the higher demands of insulin and ultimately develop type 2 diabetes mellitus [115–120]. However, in obese nondiabetic subjects a greater proportion of pancreatic fat was associated with increased, not decreased, insulin levels. This may indicate that the deleterious effect of pancreatic fat accumulation might require a long time before manifesting in impaired β -cell function. Indeed, it has been estimated that pancreatic β -cell damage is present for more than a decade before diabetes is diagnosed [121].

7. Conclusion

The Health Survey for England showed that in 2–10 year old children, 16% of boys and 12% of girls were obese (BMI \geq 95th percentile), and that in 11–15 year olds a staggering 24% of boys and 26% of girls were obese in 2004 [122]. Unfortunately, the alarming consequences of overweight and obesity are likely to increase as well as this new generation of overweight children with a longer duration of excess body weight, than any of their overweight predecessors, reaches adulthood.

It is well known that overweight and obesity are strong risk factors for cardiovascular disease and type 2 diabetes mellitus, which put a heavy load on the health care systems. As reviewed here, ectopic lipid accumulation may be a link between an increased body fat mass and these diseases. To summarize, there is strong evidence from human studies that hepatic and intramuscular lipid accumulation can cause insulin resistance in these tissues. Rodent data further suggest that pancreatic insufficiency may also occur due to excessive exposure to lipids. The combination of insulin resistance of skeletal muscle and liver on the one hand, and pancreatic insufficiency on the other hand, are the factors well known to lead to the development of type 2 diabetes mellitus. As stated above, obesity is also an important risk factor for CVD and also here, ectopic fat deposition may play an important role. Indeed,

data from animal models suggest a causal relationship between cardiac lipid accumulation and dilated cardiomyopathy and ultimately heart failure.

Taken together, we conclude that evidence for a hazardous role of lipid accumulation in non-adipose tissue (lipotoxicity) is strong. However, while quite a lot of human data is available from skeletal muscle and liver, future research should focus on lipid accumulation in the human pancreas and the heart. Newly available non-invasive methods, i.e. ^1H -MRS, make it possible to conduct interventions to investigate the causes, the time-course and the consequences of lipid accumulation in all of these tissues and to investigate the effectiveness of possible treatments.

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