Ectopic lipids and organ function
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Purpose of review
To summarize recent studies that shed more light on possible mechanisms by which ectopic lipid storage affects organ function.

Recent findings
Although ectopic lipids have been considered as biomarkers of lipotoxicity, adaptation of metabolic fluxes and of mitochondrial function seem to be more important than actual cellular fat contents in liver and muscle. Diabetic and obese humans have elevated myocardial lipid contents, which are associated with mitochondrial and contractile dysfunction and could even precede the development of heart failure. Although pancreatic fat content is negatively associated with insulin secretion, β-cell triglycerides are not easily accessible to measurement in humans rendering their role for β-cell function unclear. New approaches to quantify energy metabolism in various organs could help to identify novel biomarkers of organ function in humans.

Summary
Dietary intake of high-caloric high-fat diets and sedentary lifestyle lead to increased storage of triglycerides not only in adipose tissue but also ectopically in other tissues. Intracellular lipid contents in skeletal muscle and liver have been related to insulin resistance and inflammatory processes. Myocardial fat is increased in heart failure, whereas pancreatic fat could relate to insulin secretion.

Keywords
diabetes, fatty liver, heart failure, lipids, mitochondrial function

Introduction
Overnutrition and physical inactivity lead to increased availability of free fatty acids (FFAs) and storage of triglycerides in adipose tissue and subsequently also in nonadipose tissue [1–3]. This review aims to summarize mainly studies published over the past 2 years, which contribute to understanding the role of ectopic lipid deposition for organ function or dysfunction in humans. It focuses on organs in which ectopic lipids and tissue functionality can be exactly quantified such as skeletal muscle (glucose disposal and physical activity), liver (glucose disposal, gluconeogenesis and lipoprotein production), heart (contractile function) and pancreas (insulin secretion).

Methods for the quantification of ectopic fat
Determination of ectopic lipids in humans can be performed by analyses of tissue biopsies with limitations such as invasiveness and small sample size. Insufficient representation of the whole organ and contamination by surrounding adipose tissue contribute to high variability and overestimation of lipid contents. Noninvasive techniques achieve (semi)quantitative [ultrasound, computed tomography (CT)] or quantitative [proton magnetic resonance spectroscopy (1H MRS)] assessment of ectopic fat in various body regions. The latter allows for examination of extended tissue volumes and repeated measurements as recently described [4].

Intramyocellular lipids
Intramyocellular lipid (IMCL) contents relate to insulin resistance in various sedentary populations [5–7]. Ectopic lipid accumulation may result from lipid oversupply, which is associated with elevation of intracellular lipid metabolites and stimulation of inflammatory pathways both interfering with insulin signal transduction [8] (Fig. 1a). Alternatively, the primary cause of IMCL accumulation could reside in impaired lipid oxidation [9]. It was recently reported that insulin-resistant relatives of patients with type 2 diabetes mellitus (T2DM) with approximately 60% higher IMCL contents and approximately 60% lower glucose disposal than insulin-sensitive controls also feature approximately 38% reduced mitochondrial density [10]. Further, Petersen et al. [11,12] found that insulin-resistant relatives have approximately 80–100% higher IMCLs along with approximately 30% and approximately 85% lower fasting and insulin-stimulated flux through ATP synthase (fATP). fATP determines net ATP production, which results from nutrient oxidation to produce ATP (oxidative phosphorylation) and the prevailing energy...
increased VLDL and HCL. Enhanced glycolysis and activation of SREBP-1c, both of which stimulate lipogenesis, resulting in GLUT4 and phosphorylation of glucose to G-6-P. (b) Hepatocyte. Nine phosphorylation of IRS, resulting in decreased translocation of the synthesis of triglycerides and its storage as IMCL and increases signal- and giving rise to ROS production. The rising acyl-CoA pool feeds (a) Skeletal striated myocytes. FFA enter via the FATP and increase the synthesis of triglycerides and its storage as MYCL. PPARγ, its coactivator PGC-1α, and its coactivator-1α, can be downregulated. Depletion of high-energy phosphates leads to contractile dysfunction. β-ox, β oxidation; ChREBP, carbohydrate responsive element-binding protein; CoA, coenzyme A; DAG, diacylglycerol; FATP, fatty acid transport protein; FFA, free fatty acid; FoxO1, Forkhead box O1; G-6-P, glucose-6-phosphate; GLUT4, glucose-transporter 4; GNG, glu- coseogenesis; HCL, intrahepatocellular lipid; IMCL, intramyocellular lipid; IRS, insulin receptor substrates; MYCL, intramyocellular lipids; NFκB, nuclear factor kappa B; PGC-1α, PPARγ coactivator-1α; PKC, protein kinase C; ROS, reactive oxygen species; SREBP-1c, sterol regulatory element-binding protein; TCA, tricarboxylic acid cycle.

A recent study [13] demonstrated that exercise training concomitantly increased fat oxidation and insulin sensitivity and decreased IMCL contents in obese elderly suggesting that exercise training promotes lipid utilization in skeletal muscle, thereby reversing insulin resistance. On the contrary, physical fitness, assessed from maximal aerobic capacity (VO2max), increases both IMCL contents and insulin sensitivity according to the ‘training paradox’. Elevation of IMCL in endurance-trained athletes despite high insulin sensitivity indicates that oxidative capacity confounds the positive relationship between IMCL and insulin resistance [14,15]. Apparently, IMCL serve as energy source during intensive aerobic exercise in athletes [16] but reflect an imbalance between energy supply and demand in sedentary insulin-resistant humans. Analysis of a larger population revealed a threshold value of VO2max, which separates a positive and a negative relationship between IMCL contents and insulin resistance [15]. Insulin-sensitive obese humans, so-called ‘fit-fat individuals’ likely compensate their increased lipid availability by augmented lipid oxidation resulting in normal IMCLs content despite increased body weight [14,17**].

We recently summarized possible adaptive mechanisms of the skeletal muscle to fat overflow [19]. Mice lacking adipocyte-specific fatty acid-binding protein (FABP), which is responsible for intracellular FFA trafficking are protected against lipid-induced insulin resistance [20]. In line with the training paradox, endurance-trained humans exhibit upregulated muscular FABP, which possibly allows for increased lipid oxidation [21]. On the contrary, upregulation of myocellular diacylglycerol (DAG)–acyltransferase protects against lipid-induced insulin resistance though it augments triglyceride synthesis in skeletal muscle [22]. Channeling FFAs into triglycerides resulted in decreased DAG and ceramide levels indicating that the predictive value of IMCL...
contents strongly depends on lipid flux rates and that lipid metabolites are more important for the development of insulin resistance (Fig. 1a). Of note, 5 h of lipid infusion resulting in a physiological increase in plasma FFA of approximately 1 mmol/l induced insulin resistance with reduced glucose transport/phosphorylation without any alterations of IMCL, again supporting the concept that triglyceride storage is not prerequisite for insulin resistance [23]. Interestingly, plasma FFA elevation impaired fATP during insulin stimulation indicating that prolonged lipid oversupply also interferes with insulin sensitivity of mitochondrial function [23]. Furthermore, we demonstrated that even insulin-resistant nonobese individuals with T2DM can exhibit normal IMCLs and glucose phosphorylation/transport but reduced fATP during both fasting and insulin stimulation [24**]. This mitochondrial abnormality strongly related to plasma FFA concentrations and insulin sensitivity but not to IMCL. Taken together, metabolic patterns and particularly ectopic muscle fat differ in various human populations. The differences likely result from inherited metabolic (mitochondrial), secondary acquired alterations, such as glucolipotoxicity, or both. Glucolipotoxicity summarizes the detrimental effects of hyperglycemia and hyperlipidemia leading to insulin resistance and impaired function of muscle, liver and pancreatic β cells.

**Intrahepatocellular lipids**

In the absence of alcohol intake or other hepatotoxic agents, intrahepatocellular lipid (HCL) accumulation is termed nonalcoholic fatty liver (NAFL) [25,26]. Increased HCL contents correlate negatively with whole body and hepatic insulin sensitivity as demonstrated by impaired suppression of endogenous glucose production and decreased hepatic glycogen synthesis during hyperinsulinemic clamps [27,28]. The observation that insulin-resistant states are characterized by impaired insulin-mediated inhibition of gluconeogenesis, likely due to dysregulation of the transcription factor Fork-head box O1 (FoxO1), whereas insulin-mediated triglyceride synthesis remains elevated has been termed ‘selective insulin resistance’ [29] (Fig. 1b). Lipolysis in insulin-resistant adipose tissue or hypercaloric high-fat diets stimulate lipid/carbohydrate flux into the liver. Lipid flux along with hyperinsulinemia will drive lipogenesis via the transcription factors [peroxisome proliferator-activated receptor γ (PPARγ), sterol regulatory element-binding protein 1c (SREBP-1c) and carbohydrate responsive element-binding protein (ChREBP)], increased expression of rate-limiting enzymes of lipogenesis and of glycolysis [30–32] and elevated acetyl coenzyme A (CoA) entering the citrate cycle or de-novo lipogenesis [26] (Fig. 1b).

Postprandially, the liver converts FFA derived from chylomicron remnants via microsomal TG transfer protein (MTP) into triglycerides, which are secreted as VLDL. Other adaptive mechanisms to increased lipid availability involve activation of PPARγ, which stimulates the expression of mitochondrial enzymes of β oxidation and ketogenesis to metabolize increased acetyl-CoA and possibly increased VLDL secretion. Recently, patients with T2DM and NAFL were found to exhibit increased VLDL secretion during fasting and hyperinsulinemia, whereas patients with T2DM but without NAFL and nondiabetic individuals had lower VLDL production [33*]. It has been speculated that excessive VLDL production associated with insulin resistance is caused by the inability of insulin to regulate FoxO1 transcriptional activation of MTP [34**].

Although HCL are better predictor of insulin resistance than IMCL, it is yet unclear to which extent increased HCLs could induce skeletal muscle insulin resistance. One recent study [35**] tested the hypothesis that insulin resistance in otherwise healthy, young, lean and normoglycemic volunteers induces atherogenic lipoprotein production by altering the postprandial distribution of energy storage. They found that impaired skeletal muscle glycogen synthesis causes ingested carbohydrates to be shifted to the liver, giving rise to de-novo lipogenesis and subsequent hypertriglyceridemia. This would be in line with the concept that impaired skeletal muscle glucose disposal is the primary driving force in the pathogenesis of the metabolic syndrome including steatosis, dyslipidemia, hepatic and adipose tissue insulin resistance [35**]. On the contrary, a randomized controlled trial with the PPARγ agonist, pioglitazone, not only markedly reduced whole body and muscular insulin resistance and HCL contents but also improved histologic findings of NAFL [36]. In mice, pioglitazone might enhance enzymatic antioxidant defense and DNA repair mechanisms, thereby attenuating lipid-induced hepatic oxidative DNA damage [37]. Resveratrol, an activator of the sirtuin SIRT1, likewise delayed the development of NAFL in rodents via antioxidant and inflammatory activities and PPARγ coactivator-1α (PGC-1α) activation of mitochondrial biogenesis [38]. Of note, mitochondrial dysfunction, oxidative stress and imbalance of adipokine secretion are considered key factors for the development of NAFL and its progression to nonalcoholic steatohepatitis (NASH).

Thus, mitochondrial dysfunction could be elementary for hepatic steatosis. HCL stimulate mitochondrial lipid oxidation and concomitantly production of radical oxygen species (ROS) promoting lipid peroxidation and damage of mitochondrial proteins and mitochondrial DNA [39,40]. Although patients with NAFL show increased mitochondrial β oxidation rates [41–43], their livers’ ability to resynthesize ATP after fructose challenge is decreased and negatively related to BMI.
Accordingly, insulin-resistant humans exhibit doubled on heart [58]. Subsequently, cytoprotective processes activate inflammatory cytokines and the progression to cirrhosis and loss of organ function [42]. Hence, further development of tools for noninvasive in vivo assessment of liver mitochondrial function is of major importance. In elderly humans, prostate-deprived individuals and patients with liver metastases, application of 31P MRS showed increased ratios of phosphomonosesters (PME) and phosphodiesters (PDE) to ATP and reduced ATP/Pi ratios [47–49]. However, these studies used ratios of steady-state levels of phosphorus metabolites as surrogate marker of hepatic energy metabolism instead of using direct measures of energy turnover. Recently, our group reported application of absolute quantification and direct measurement of ATP synthesis in human liver [50], thus allowing for further investigation of hepatic energy metabolism, lipid accumulation and the relation of these alterations to liver function.

**Myocardial lipids**

Cardiovascular diseases (CVD) determine cardiac mortality in patients with the metabolic syndrome. Increased lipid availability and changes in cardiac energy metabolism might contribute to the development of contractile dysfunction. Myocardial lipids (MYCLs) correlate with BMI, concentric left ventricular hypertrophy, nonischemic heart failure and decreased regional systolic performance [52,53]. The term ‘diabetic cardiomyopathy’, describing ventricular dysfunction in patients with T2DM without CVD and hypertension, emerged from the observation that patients with T2DM experience heart failure more frequently despite the absence of classic risk factors [54]. Likewise, rodent models with T2DM and obesity and without atherosclerosis also show increased prevalence of contractile dysfunction, which relates to the inability to increase lipid oxidation with increased FFA supply and subsequent lipid accumulation [55,56] (Fig. 1c). Of note, there is evidence that cardiomyocytes can also develop insulin resistance, which could be mediated at least in part by lipid-induced protein kinase C (PKC) activation as reported for myocytes and hepatocytes [57]. Furthermore, cardiomyocytes of diabetic rodents showed increased oxidation and transport rates of palmitate despite near-normal myocardial contractile function, which supports the concept that lipotoxicity mediates adverse effects of diabetes on heart [58].

Accordingly, insulin-resistant humans exhibit doubled MYCL despite normal left ventricular ejection fraction suggesting that lipid accumulation in human cardiomyocytes could be an early feature of impaired cardiac function in the insulin-resistant state, preceding the onset of T2DM [59,60]. Hence, 6 months of pioglitazone treatment not only improved glycemic control but also decreased both MYCLs and HCLs in patients with T2DM [60]. In lean humans, MYCLs content was normal in failing heart, but increased significantly in obesity or T2DM. MYCLs correlate with BMI [52,61] and were also found to relate to FFA availability during 48 h of fasting, which concomitantly reduced left ventricular diastolic dysfunction [61,62]. To test effects of elevated plasma FFAs on HCLs, MYCLs and myocardial function, healthy men were examined before and after 3 days of high-fat high-energy diet [63]. The dietary intervention increased HCL but not MYCL and myocardial systolic function, suggesting tissue-specific partitioning of lipids for ectopic deposition in insulin-sensitive humans [63]. The same research group demonstrated that MYCL contents increase with aging, independent of BMI and blood pressure and relate to age-dependent decline in diastolic function [64]. A recent study [53] reported increased MYCL in obese humans and in patients with T2DM suffering from heart failure and suggested that FFAs uptake exceeding oxidative capacity, resulted in increased lipid storage and lipotoxicity. PPARα and its coactivator PGC-1α, a regulator of mitochondrial biogenesis and enzymes involved in lipid oxidation, have been reported to be downregulated in hypertrophied and failing hearts, thereby promoting MYCL accumulation [65–67]. On the contrary, PPARα-regulated gene expression was increased in humans with MYCL overload indicating FFAs-induced stimulation of lipid oxidation and suggesting insufficient adaptation of oxidation to FFA availability (Fig. 1). Moreover, increased PPARα activity and FFA oxidation relate to ROS production, which might impair contractile heart function [68]. High fat feeding, obesity and T2DM are associated with activation of PPARα and genes involved in fatty acid oxidation. Thus, a recent study [69] tested the hypothesis whether 8-week high-saturated-fat diet in a rodent model of heart failure following coronary artery ligation would result in improved contractile function and mitochondrial lipid oxidation via PPARα stimulation. Genes promoting FFA oxidation were downregulated, but the activity of acyl-CoA dehydrogenases and state-3 respiration using lipid substrates was increased, and contractile function was improved by high-fat feeding, which could result from improved substrate utilization. This is in line with the concept of adaptive enhancement of lipid oxidation by increased lipid availability as demonstrated in the liver and in skeletal muscle preceding detrimental alterations such as lipid peroxidation, ROS production and mitochondrial damage [43,70]. Accordingly, recent studies demonstrated higher myocardial FFA uptake ratios in patients with heart failure [71] and increased utilization
and accumulation of FFA [72,73]. Thus, in the long run, increased uptake, utilization and storage of excess lipids might be cytoxic to the myocardium [69]. In patients with obesity and T2DM, cardiac lipotoxicity due to increased myocardial FFA availability has been implicated in the pathogenesis of overt heart failure [74,75]. A recent study [76] aimed to investigate the effects of excessive amounts of palmitate–carnitine and palmitate–CoA on mitochondrial function and showed increased ROS production and mitochondrial permeability loss membrane potential. Using nuclear magnetic resonance (NMR) analysis of tissue extracts in a rodent model with early heart failure revealed decreased oxidative capacity but 30–40% lower MYC and impaired energy conversion, for example, increased carbohydrate rather than lipid oxidation during β-adrenergic challenge, suggesting impaired substrate utilization in this model [77]. Reduced cardiac phospho creatine (PCr)/ATP ratios suggesting cardiac energy deficit were found in patients with T2DM [78,79] and with heart failure due to other causes and related negatively to plasma FFAs in a group of individuals with T2DM [80]. Thus, mitochondrial and contractile dysfunction associated with augmented MYCL contents relate to insulin resistance and T2DM, similar to observations in skeletal muscle and liver of insulin-resistant states. It is noteworthy that high FFA levels predict sudden cardiac death and all-cause and cardiovascular mortality among 3315 patients scheduled for coronary angiography [81].

### Pancreatic lipids

Experimental data indicate that adipocyte infiltration of pancreatic islets could contribute to β-cell dysfunction. As both pancreatic lipid (PCL) and insulin secretion increase with BMI [82], pancreatic fat was not proven to be deleterious to β-cell function. Analyzing PCLs accumulation and β-cell function in patients with T2DM and age and BMI-matched controls revealed that the patients had doubled PCLs, which correlated negatively with β-cell function even after correction for BMI, plasma glucose and triglycerides. Thus, PCLs might contribute to β-cell dysfunction in addition to effects induced by glucolipotoxicity [83,84]. However, islet cell mass amounts to only less than 2% of pancreatic mass, and 1H MRS cannot discriminate between adipocytes and nonadipocytes. Thus, measured PCLs are mostly due to infiltrating adipose tissue. Employing CT scanning, PCLs were found to increase with BMI in 165 patients with T2DM and 660 age, sex and BMI-matched controls without any differences between the groups [85]. Histological analysis of the pancreas showed that most PCLs in humans are present in adipocytes within the exocrine tissue or in adipose tissue in the interlobular space so that PCLs are unlikely to serve as a biomarker of endocrine function of the pancreas.

### Conclusion

Ectopic lipid deposition might be a marker of impaired organ function, but numerous parameters influence this association. Adaptive processes to increased lipid availability might induce enhancement of lipid oxidation, oxidative stress and mitochondrial damage. On the contrary, functional or structural abnormalities of mitochondria coincide with accumulation of triglyceride and lipid metabolites along with lipotoxicity and insulin resistance. In addition to direct lipid-induced effects, genetic susceptibility, for example, family history of T2DM, determines cellular function.

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### References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 67).


This study reports on the ectopic lipids in a large well characterized population highlighting the relevance of lipid accumulation in skeletal muscle vs. liver.


This study explores in vivo fasting and insulin-stimulated mitochondrial function in patients with type 2 diabetes and analyses the relationships of mitochondrial impairment with insulin sensitivity, glucose transport, availability and ectopic deposition of lipids.


This study reports for the first time in vivo noninvasive quantification of ATP synthase flux in human liver.


**Nutrition and metabolism**


