Non-alcoholic fatty liver disease (NAFLD) is now recognised as the hepatic component of metabolic syndrome (MetS). NAFLD is an example of ectopic fat accumulation in a visceral organ that causes organ-specific disease, and affects risk of other related diseases such as type 2 diabetes and CVD. NAFLD is a spectrum of fat-associated liver conditions that can culminate in end stage liver disease, hepatocellular carcinoma and the need for liver transplantation. Simple steatosis, or fatty liver, occurs early in NAFLD and may progress to non-alcoholic steatohepatitis, fibrosis and cirrhosis with increased risk of hepatocellular carcinoma. Prevalence estimates for NAFLD range from 2 to 44% in the general population and it has been estimated that NAFLD exists in up to 70% of people with type 2 diabetes. Although many obese people have NAFLD, there are many obese people who do not develop ectopic liver fat. The aim of this review which is based on a presentation at the Royal Society of Medicine, UK in December 2012 is to discuss development of NAFLD, ectopic fat accumulation and insulin resistance. The review will also describe the relationships between NAFLD, type 2 diabetes and CVD.

Ectopic fat: Energy balance: Non-alcoholic fatty liver disease: Cardio-metabolic risk factors: Type 2 diabetes: CVD

Obesity is a risk factor that is common to type 2 diabetes and CVD. However, increasing evidence suggests that BMI, as used to define obesity, may be less important than other risk factors, such as visceral fat mass or visceral fat accumulation in the liver (ectopic fat), in contributing to both diseases.

In support of the notion that BMI is an imprecise measure of cardio-metabolic risk, there may be marked differences in cardio-metabolic risk factors, among individuals with similar BMI, contributing to diseases such as type 2 diabetes, CVD and non-alcoholic fatty liver disease (NAFLD). Some obese people have few other cardio-metabolic risk factors, whereas other similarly obese individuals may have many of the metabolic risk factors associated with insulin resistance and metabolic syndrome (MetS). It has been suggested that 30% of obese patients are metabolically healthy, and have excellent insulin sensitivity, low levels of liver fat and lower intima media thickness of the carotid artery than the majority of metabolically unhealthy obese patients. Furthermore, although both subcutaneous and visceral adipose tissues are correlated with metabolic risk factors, visceral adipose tissue remains more strongly associated with an adverse metabolic risk profile even after accounting for standard anthropometric indexes.

Waist:hip ratio shows a graded and highly significant association with myocardial infarction risk worldwide, whereas hip circumference is not associated with an

Abbreviations: ABSI, a body shape index; acyl-CoA, acyl-coenzyme A; DAG, diacylglycerol; LCFA, long chain fatty acid; LPA, lysophosphatidic acid.; MetS, metabolic syndrome; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; NAFLD, non-alcoholic fatty liver disease; PA, phosphatidic acid; rictor, rapamycin insensitive companion of TOR.

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increased risk of myocardial infarction, emphasising that abdominal fat accumulation *per se* may be particularly important in mediating the association between body fat mass and CVD[7]. Abdominal obesity assessed by waist, or waist:hip ratio, are both related to increased risk of all-cause mortality throughout the range of BMI. However, the relative risks seem to be stronger in younger than in older adults and in those with relatively low BMI compared with those with high BMI[7]. Current cut-points as recommended by the World Health Organisation seem appropriate, although it may be important that age-specific and BMI-specific and ethnic-specific waist cut-points, may be warranted. Waist circumference alone could replace both waist:hip ratio and BMI, as a single risk factor for all-cause mortality; waist circumference and waist:hip ratio, seem to be better indicators of all-cause mortality, than BMI[8]. Recent evidence also suggests that different patterns of body shape are associated with variable risks of death. For example, within a sample of 14 105 non-pregnant adults (age ≥18 years) from the National Health and Nutrition Examination Survey 1999–2004, with follow-up for mortality averaging 5 years (828 deaths), investigators developed A Body Shape Index (ABSI) based on waist circumference adjusted for height and weight: ABSI = waist circumference/(BMI/2/3/height(1/2))[9]. ABSI had little correlation with height, weight or BMI. However, death rates increased approximately exponentially with above average baseline ABSI. Moreover, the authors concluded that body shape, (as measured by ABSI), appeared to be a substantial risk factor for premature mortality in the general population derivable from basic clinical measurements. Thus, these data illustrate evidence is accumulating that emphasises the importance of abdominal fat accumulation, manifest as a rounder body shape, as an important risk factor for premature death.

Accumulation of visceral adipose tissue fat is often associated with fat accumulation in other ‘ectopic’ sites such as the liver. Ectopic fat accumulation in the liver that is associated with other features of the MetS, is referred to as NAFLD[10,11]. It is now clear that NAFLD is not only a potential risk factor for chronic liver disease[10], but also is a risk factor for type 2 diabetes and CVD[12]. Although the mechanisms by which NAFLD[13] increases risk of type 2 diabetes and CVD are unclear, and need to be better elucidated[14,15], recent evidence suggests important insights into how different products of the hepatic lipid synthesis pathway adversely affect insulin signalling and cause hepatic insulin resistance.

**How does ectopic fat accumulation in liver (hepatic steatosis) cause insulin resistance and affect cardio-metabolic risk factors?**

Although obesity is strongly associated with hepatic steatosis, body fat accumulation is not a *sine qua non* for developing NAFLD. Patients with lipodystrophy commonly develop hepatic steatosis, strongly suggesting that it is not body fat mass *per se* that is important, but it is adipose tissue function that is key in the pathogenesis of NAFLD[16]. Specifically, increased fatty acid fluxes from the adipose tissue pool throughout the day, increase the availability of long chain fatty acyl-coenzyme A (acyl-CoA) in the liver, particularly in physically inactive individuals[11]. It is likely that fatty acid fluxes from the adipose tissue pool to the liver will be decreased if individuals are engaging in high levels of physical activity, since high levels of aerobic physical activity increase the demand for skeletal and cardiac muscle long chain fatty acid (LCFA) oxidation and for generation of ATP to enable muscle contraction. High levels of muscle mitochondrial β oxidation of LCFA may provide an important buffer to protect the liver from an excessive supply of LCFA that may overwhelm the liver’s capacity to export fatty acids (as VLDL, or conjugated to cholesterol derivatives in bile).

With an increasingly sedentary population, increased concentrations of postprandial fatty acids promote lipid synthesis. Within adipose tissue and liver parenchymal cells, fatty acyl-CoA are esterified with glycerol 3-phosphate (derived from glycolysis) to form monoacylglycerol, diacylglycerol (DAG) and TAG (Fig. 1(a)). In insulin sensitive tissues, lipid synthesis may increase production of intermediates such as DAG, di-palmitoyl phosphatidic acid (PA) and other lipid products such as ceramides. Increased production of these lipid products may be very important in causing “resistance” in the hepatic insulin signalling pathway[17] (Fig. 1(b)). In liver, ceramides are another lipid product which utilise LCFA[18]. Ceramides are a family of waxy lipid molecules that are found in high concentrations within cell membranes and are one of the component molecules within the lipid bilayer. Ceramides can accumulate in cells via three main routes: the hydrolysis of the membrane phospholipid sphingomyelin, which is coordinated by the enzyme sphingomyelinase; *de novo* synthesis from LCFA such as palmitate and serine; and a salvage pathway that utilises sphingosine and forms ceramide[19,20].

Although in the past it was thought that ceramide was simply a structural molecule, it is now evident that increases in membrane ceramide have the potential to increase insulin resistance (see review[21]; Fig. 2).

Increased concentrations of fatty acids that are not utilised in oxidative metabolism pathways for energy production during catabolism (e.g. skeletal muscle activity in the fasted state) are used in lipogenesis. *De novo* lipogenesis generates TAG and phospholipids from glycerol 3-phosphate and LCFA (see Fig. 2). Acyl-CoA:glycerol-sn-3-phosphate acyltransferase catalyses the acylation of sn-glycerol-3-phosphate with acyl-CoA to generate lysophosphatic acid (LPA). LPA is thought to be the rate-controlling step in TAG synthesis. Subsequently, the enzymes acyl-CoA:1-acylglycerol-sn-3-phosphate acyltransferase, PA phosphatase and DAG:acyl-CoA acyltransferase generate PA, DAG and TAG. In the liver, TAG is either deposited in intracellular vacuoles or exported in VLDL particles. LPA and PA require translocation through the cytosol for TAG synthesis at the endoplasmic reticulum if they are not synthesised in the endoplasmic reticulum[22]. DAG can be hydrolysed to monoacylglycerol by hormone-sensitive lipase and subsequently to glycerol by monoglyceride lipase. These reactions release fatty acids. Glycerol can then be used as a substrate for...
gluconeogenesis (Fig. 1) but cannot be re-esterified with LCFA to form adipose tissue TAG.

The production of DAG has been suggested as a cause of hepatic insulin resistance and inflammation. Adipose tissue ‘inflammation’, generation of long chain fatty acids (LCFA) and the potential role of lipid intermediates (ceramide, di-palmitoyl phosphatidic (di-PPA) and diacylglycerol (DAG)) in hepatic insulin resistance. Liver (b): (1) Acyl-coenzyme A (acyl-CoA); glycerol 3 phosphate acyl transferase (GPAT) catalyses the formation of lysophatidic acid (LPA) the rate limiting step in TAG synthesis. (2) Comparative Gene Identification-58 (CGI-58) is an activator of adipose TAG lipase (ATGL) that converts TAG to DAG. (3) Membrane-associated DAG activates protein kinase C\textsubscript{e} (PKC\textsubscript{e}) membrane translocation to inhibit the insulin receptor kinase. DAG can also be released from membrane lipids by the action of phospholipase C.

Fig. 1. (colour online) Synthesis of lipid intermediates and TAG in adipose (a) and liver (b) tissue: links with insulin resistance and inflammation. Adipose tissue ‘inflammation’, generation of long chain fatty acids (LCFA) and the potential role of lipid intermediates (ceramide, di-palmitoyl phosphatidic (di-PPA) and diacylglycerol (DAG)) in hepatic insulin resistance. Liver (b): (1) Acyl-CoA synthesis catalysed by glycerol 3 phosphate acyl transferase (GPAT) is the rate limiting step in TAG synthesis. (2) Comparative Gene Identification-58 (CGI-58) is an activator of adipose TAG lipase (ATGL) that converts TAG to DAG. (3) Membrane-associated DAG activates protein kinase C\textsubscript{e} (PKC\textsubscript{e}) membrane translocation to inhibit the insulin receptor kinase. DAG can also be released from membrane lipids by the action of phospholipase C.
this class of drug will ever be licensed for NAFLD because of unacceptable side effects such as increased fracture risk, oedema and cardiac failure, weight gain and possibly an increase of bladder cancer.

Recently, there have been further studies that have added insight as to the role of PPARγ in affecting insulin signalling. PPARγ activity is regulated by nuclear co-repressor and the hepatokine fibroblast growth factor 21. It has been shown that nuclear co-repressor prevents PPARγ activity by facilitating cyclin-dependent kinase 5 binding. Cyclin-dependent kinase 5 phosphorylates PPARγ Ser273 thereby blocking PPARγ transcriptional activity. Thus nuclear co-repressor may play a role in causing insulin resistance.

The Ser/Thr protein kinase mammalian target of rapamycin (mTOR) is also important in regulating insulin signalling. In combination with other molecules, mTOR forms two complexes; mTOR complex 1 (mTORC1) and mTORC2. mTORC1 contains the protein regulatory associated protein of TOR, whereas mTORC2 contains the protein rictor (rapamycin insensitive companion of TOR). Both complexes are capable of regulating insulin sensitivity (Fig. 3). AMP-activated protein kinase negatively regulates mTORC1 in response to low cellular energy and thereby prevents the negative feedback loop that down regulates insulin signalling (Fig. 3). Overexpression of a dominant negative form of regulatory associated protein of TOR in the liver of insulin-resistant mice improved glucose tolerance and restored insulin sensitivity by reducing the negative feedback loop. mTORC1 inhibits insulin signalling via the effects of its substrate S6 kinase. These findings suggest that inappropriate mTORC1 activity in the liver contributes to glucose intolerance, at least in part. In contrast, mTORC2 has a positive effect on glucose uptake and glucose tolerance. Lipid synthesis in the liver may also cause insulin resistance via disruption of mTORC2 signalling. These authors showed that overexpression of acyl-CoA:glycerol 3 phosphate acyl transferase that catalyses the formation of LPA and which is the rate limiting step in TAG synthesis (Fig. 1), suppresses insulin signalling and insulin-mediated suppression of hepatic gluconeogenesis. They showed that mTOR- rictor binding was decreased resulting in diminished mTORC2 phosphorylation of Akt-Ser473 and Akt-Thr308. To determine which lipid intermediate was responsible for inactivating mTORC2, the investigators overexpressed glycerol-sn-3-phosphate acyltransferase 1, acyl-CoA: 1-acylglycerol-sn-3-phosphate acyltransferase or lipin to increase the cellular content of LPA, PA, or DAG, respectively. The inhibition of mTOR/rictor binding and mTORC2 activity coincided with the levels of PA and DAG species that contained 16:0, the preferred substrate of glycerol-sn-3-phosphate acyltransferase 1. Furthermore, di-16:0-PA strongly inhibited mTORC2 activity and
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disassociated mTOR/rictor in vitro (Fig. 2). Taken together, these data reveal a signalling pathway by which PA synthesised via the glycerol-3-phosphate pathway inhibits mTORC2 activity by decreasing the association of rictor and mTOR, thereby down-regulating insulin action and potentially causing insulin resistance (31). In support of these findings, to assess the role of hepatic mTORC2, liver-specific rictor knockout mice have been generated (32). Chow fed liver-specific rictor knockout mice displayed loss of Akt-Ser473 phosphorylation and reduced glucokinase and sterol regulatory response element binding protein 1c activity in the liver, leading to constitutive gluconeogenesis, and impaired glycolysis and lipogenesis. These liver-specific defects resulted in systemic hyperglycaemia, hyperinsulinaemia and hyperlipidaemia. Expression of constitutively active Akt2 in mTORC2-deficient hepatocytes restored both glucose flux and lipogenesis, whereas mTORC2 regulates both hepatic glucose and lipid metabolism via insulin-induced Akt signalling to control whole-body metabolic homoeostasis. Thus, these data demonstrate a potential important link between nutrient excess, TAG synthesis and hepatic insulin resistance (Fig. 3).

Ectopic fat, hepatic steatosis and non-alcoholic fatty liver disease progression
NAFLD is not a single disease and NAFLD describes a spectrum of fat-related liver conditions. The spectrum ranges from simple fatty liver (steatosis) to more severe steatosis coupled with marked inflammation, termed non-alcoholic steatohepatitis which is often progressive with development of fibrosis (40–50%) liver cirrhosis (15–17%), liver failure (3%) and potentially hepatocellular carcinoma. Current estimates are that 40% of people with NAFLD develop non-alcoholic steatohepatitis (33) and there is increased incidence of coronary (10.8%), cerebrovascular (37.3%) and peripheral (24.5%) vascular disease in individuals with NAFLD (34).

NAFLD is defined by the accumulation of liver fat >5% per liver weight, in the presence of <10 g daily alcohol consumption. The characteristic histology of NAFLD resembles that of alcohol-induced liver injury, but occurs in people who consume minimal alcohol. The reported prevalence of NAFLD is 2–44% in the general European population (including obese children) and 42.6–69.5% in people with type 2 diabetes (35). In routine clinical practice, most cases of fatty liver disease are attributable to alcohol excess; however, fatty liver disease can also occur in association with a wide range of toxins, drugs and diseases, such as morbid obesity, cachexia, type 2 diabetes, hyperlipidaemia and after jejunooileal bypass surgery. As important risk factors for NAFLD such as obesity and type 2 diabetes are increasing in prevalence, it is likely that there will be a marked increase in prevalent NAFLD. For people who are free from disease initially, the development of NAFLD will have important implications not just for the patients themselves but also for health care providers, responsible for patients with chronic liver disease, type 2 diabetes and CVD (36).
**Ectopic fat and non-alcoholic fatty liver disease**

The benefits of high levels of physical activity in association with lower risk of mortality have been known for over 50 years \(^{(37)}\). Over 20 years ago it was shown that a one S.D. increase in free-living energy expenditure (1200-808 kJ/d (287 kcal/d)) was associated with a 32% lower risk of mortality after adjusting for age, sex, race, study site, weight, height, percentage body fat and sleep duration \(^{(38)}\). Changes in lifestyle that promote weight loss, or increase physical activity, to induce a net negative energy balance, can be very effective in treating NAFLD \(^{(39,40)}\). Although the most effective strategy for treating NAFLD with lifestyle change is unclear, dietary energy restriction can be very effective in decreasing liver fat \(^{(39,41)}\), and 80% decreases in liver fat have been recorded over relatively short periods of time (e.g. 3 months) \(^{(40)}\). When energy restriction is severe (2510.4–3347.2 kJ/d (600–800 kcal/d)), marked decreases in liver fat are often noted \(^{(39,41)}\). That said, most of the evidence of benefit comes from trials of energy restriction that have tested the intervention in relatively small numbers of individuals, and most of the durations of intervention have often been very short (e.g. 8 weeks) \(^{(42)}\), but are on average approximately 6 months. In combination with an improvement in liver fat, energy restriction producing weight loss, is also very effective ameliorating many of the features of MetS, such as increased serum TAG, and glucose concentrations and decreased serum HDL-cholesterol concentration and increased blood pressure \(^{(41)}\).

Increases in physical activity energy expenditure tend to induce a net negative energy balance, although it is unlikely that all the benefit of physical activity (or exercise), is mediated by inducing this change in energy balance. It is presently uncertain as to the best form of physical activity energy expenditure (or exercise) to decrease ectopic fat accumulation in visceral organs such as the liver. Current evidence suggests high intensity activity may have a particularly beneficial effect to decrease hepatic fat and possibly also has a beneficial effect in decreasing risk of progression to liver fibrosis \(^{(41,46)}\). For example, vigorous physical activity at a metabolic equivalent of task (or measure of physical activity energy expenditure) equivalent of greater than or equal to six was associated with decreased odds for having non-alcoholic steatohepatitis (OR 0.65, 95% CI 0.43, 0.98) and also a decreased risk of progression to fibrosis \(^{(44)}\). In addition, interesting recent data suggest that resistance training may be particularly beneficial in decreasing hepatic fat content \(^{(43)}\). These authors showed that in nineteen patients with NAFLD, there was a significant 13% decrease in hepatic steatosis, as measured by proton magnetic resonance spectroscopy, after 8 weeks of resistance training (comprising eight different exercises on three non-consecutive days of the week for 45–60 min duration). There were also improvements in systemic lipid oxidation, glucose tolerance and insulin sensitivity. More importantly, these exercise-induced beneficial effects were noted to be independent of weight loss.

However, further evidence is needed before recommending physiologically stressful resistance training for this group of patients, not least because adherence to such a strenuous programme is likely to be low, and many patients with NAFLD have existing co-morbidities, such as CVD. Nevertheless, given that many people with NAFLD have obesity and features of MetS, and there is evidence of a benefit of resistance training in people with MetS \(^{(45,46)}\), it is plausible that there could be a substantial benefit in NAFLD. A meta-analysis of thirteen randomised controlled trials testing the effect of resistance training on MetS features found that this intervention improved most features of MetS \(^{(45)}\). Since adipose tissue secretes many adipokines and inflammatory cytokines (e.g. adiponectin, leptin, resistin, TNF-α, IL-6 and plasminogen activator inhibitor 1) and resistance training has been shown to produce a beneficial effect on these bioactive molecules \(^{(46)}\), it is plausible that resistance training may have a benefit in NAFLD. Table 1 summarises the benefits of lifestyle interventions on ectopic fat and related cardio-metabolic risk factors.

### Table 1. Summary of benefits of lifestyle intervention on ectopic fat (liver) and related cardio-metabolic risk factors

<table>
<thead>
<tr>
<th>Treatment/Intervention</th>
<th>Benefit</th>
<th>Risk factor modification and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lifestyle intervention (e.g., weight loss)</strong></td>
<td>↓ Liver enzymes, ↓ hepatic fat (MRS and US), ↑ insulin sensitivity, improved or unchanged NAFLD histological staging NAFLD improvement with ∼9% weight loss. Rapid weight loss can lead to ↑ hepatic fat</td>
<td>↓ Blood pressure, plasma TAG and glucose concentrations. ↑ HDL-C concentrations.</td>
</tr>
<tr>
<td><strong>Increased physical activity energy expenditure and resistance training</strong></td>
<td>↓ Liver enzymes, ↓ hepatic fat (MRS and US), ↑ insulin sensitivity, ↓ inflammation</td>
<td>↓ Blood pressure, plasma TAG and glucose concentrations. ↑ HDL-C concentrations.</td>
</tr>
<tr>
<td>Regular exercise showed improvements in NAFLD independent of body weight or visceral fat changes. Resistance training beneficial in decreasing liver fat, independent of changes in body weight</td>
<td>Improved cardio-respiratory fitness with more intense physical activity. Preliminary studies suggest a marked benefit of resistance training. BUT type of activity needs to be tailored to the individual patient</td>
<td></td>
</tr>
</tbody>
</table>

MRS, magnetic resonance spectroscopy; US, ultrasound; NAFLD, non-alcoholic fatty liver disease; HDL-C, HDL-cholesterol.
Conclusions

NAFLD is now recognised as the hepatic component of the MetS. NAFLD is an example of ectopic fat accumulation in a visceral organ that causes not only organ-specific disease, but also affects risk of other related diseases such as type 2 diabetes and CVD. Increasing evidence shows that the intermediates of lipid synthesis may promote hepatic insulin resistance and may explain, at least in part, the increase in risk of type 2 diabetes conferred by hepatic fat accumulation. Changes in lifestyle that promote a net negative energy balance are effective in decreasing liver fat. To date, it is uncertain whether weight loss per se or increases in physical activity are the best lifestyle changes to ameliorate liver fat in NAFLD. However, initial data suggest that resistance training may be a particularly important therapeutic strategy to treat liver fat. Whether this intervention or weight loss prevents NAFLD progression to liver fibrosis and cirrhosis requires more research. Nevertheless, given that changes focused on producing a net negative energy balance are effective in ameliorating features of the MetS, producing such a change by modifying lifestyle should be advocated for patients with NAFLD. Such a strategy, if implemented early in the development of NAFLD, is likely to decrease hepatic fat. This effect should also decrease risk of progression to chronic liver disease, decrease risk of type 2 diabetes and possibly also CVD.

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