Ectopic fat accumulation: an important cause of insulin resistance in humans

Hannele Yki-Järvinen

INTRODUCTION

Although obesity is the major environmental cause of type 2 diabetes, studies performed in patients with various forms of lipodystrophy have demonstrated that subcutaneous fat is not a prerequisite for the development of severe insulin resistance or type 2 diabetes\(^1\). In patients with lipodystrophy, fat accumulates in insulin-sensitive tissues such as the liver\(^2\). In animal models of lipodystrophy, subcutaneous fat transplantation reverses insulin resistance and removes excessive fat from major insulin target tissues, e.g. skeletal muscle and liver\(^3\). In apparently healthy individuals, it is well established that the relationship between obesity and insulin resistance is characterized by marked interindividual variation at a given body mass index, implying that factors other than the total amount of fat contribute to variation in insulin sensitivity (Figure 1). This review focuses on data suggesting that ectopic fat accumulation in the liver and skeletal muscle in humans are critical determinants of insulin resistance and may also predispose to the development of type 2 diabetes. This area of investigation has been greatly facilitated by the recent development and validation of spectroscopic techniques, which enable reliable quantitation of fat in key insulin target tissues, such as skeletal muscle and liver.

SKELETAL MUSCLE

Methods

Lipids are either stored subcutaneously or in interstitial adipose tissue (extramyocellular lipid [EMCL]), or as intramyocellular lipid (IMCL) in the form of lipid droplets in the cytoplasm of muscle cells\(^6\). EMCL and IMCL can be quantitated non-invasively using proton magnetic resonance spectroscopy to monitor triglyceride (TG) methylene proton signals at 1.4 and 1.6 ppm\(^6,7\). In subjects with generalized lipodystrophy, no signal can be detected at 1.6 ppm, while a strong signal is observed at 1.4 ppm\(^7\). Intramyocellular fat can also be quantitated by electron microscopy\(^8\) and by staining biopsy specimens with oil red O\(^9\).

IMCL associates with insulin sensitivity independently from other factors

Table 1 summarizes human studies examining whether IMCL associates with insulin sensitivity, and whether the association can be attributed to overall obesity, body fat distribution, or physical fitness\(^10-14\). In each of the studies, the relationship between IMCL and insulin sensitivity was independent of overall obesity and fat distribution. Maximal aerobic power was measured in two studies and did not influence the relationship between IMCL and insulin sensitivity\(^12,14\).

Possible mechanisms relating IMCL to insulin action

The exact mechanism(s) by which IMCL might impair insulin action are uncertain. In fatless mice, IMCL accumulation is associated with defects in insulin activation of insulin receptor substrate 1 (IRS-1)-associated phosphatidylinositol (PI) 3-kinase, which is important for insulin activation of glucose transport and glycogen synthesis\(^8\). A similar defect has been shown recently to characterize non-diabetic, non-obese men with ‘high’ compared with ‘low’ IMCL (Figures 2 and 3). In this study, the men were divided into two groups based on their median IMCL and were comparable with respect to age, body mass index,
waist-to-hip ratio and maximal aerobic power\textsuperscript{14}. The molecular mechanisms linking IMCL to insulin signalling pathways are unclear. It has been suggested that long-chain acyl coenzyme A (LCACoA) esters may provide a link, as the concentration of LCACoA esters is six times higher in human skeletal muscle than fat, and is closely inversely correlated with insulin sensitivity in normal men\textsuperscript{15}. LCACoA esters appear to directly inhibit activities of enzymes such as hexokinase in human skeletal muscle\textsuperscript{16}. In addition, LCACoA esters have been shown to increase protein kinase C activity\textsuperscript{17}, either directly or through conversion to diacylglycerol (DAG). An increase in the activity of protein kinase C\textsubscript{\gamma}, a serine kinase, which is associated with decreased tyrosine phosphorylation of IRS-1, could provide a link between increased LCACoA and impaired insulin signalling in skeletal muscle\textsuperscript{18}.

### Regulation of IMCL

An inverse correlation between plasma free fatty acids (FFA) and insulin action has been observed in most human studies where the insulin sensitivity of glucose uptake has been measured in vivo\textsuperscript{19,20}. A defect in insulin suppression of serum FFA has also been observed in individuals with high rather than low IMCL, independent of body weight (Figure 4)\textsuperscript{14}. This is not unexpected, given that intramuscular TG originate from circulating FFA. Indeed, IMCL can be increased acutely (within hours) by raising plasma FFA using infusions of heparin and a lipid emulsion\textsuperscript{21,22}. Exercise is also able to acutely change muscle TG content. For example, heavy resistance exercise for 30 min has been reported to decrease muscle TG by 30\%\textsuperscript{23}. An increase in dietary fat intake from 43\% to 54\% of total energy for 4 weeks increases muscle lipoprotein lipase (LPL) activity and leads to an increase in muscle TG content\textsuperscript{24}. Although IMCL was not measured in the latter study, an increase in muscle TG is strongly correlated with increased IMCL measured in vivo\textsuperscript{25,26}.

### Table 1

Recent studies examining relationship between muscle lipid accumulation and insulin sensitivity using the euglycaemic insulin clamp technique in non-diabetic subjects

<table>
<thead>
<tr>
<th>Reference</th>
<th>n*</th>
<th>Subjects</th>
<th>Method</th>
<th>Association independent of</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pan et al. (Ref 10)</td>
<td>38</td>
<td>Non-diabetic Pima Indians</td>
<td>Total muscle TG in muscle biopsy’</td>
<td>Yes</td>
</tr>
<tr>
<td>Perseghin et al. (Ref 11)</td>
<td>14</td>
<td>Lean offspring\textsuperscript{1}</td>
<td>H spectroscopy soleus</td>
<td>Yes</td>
</tr>
<tr>
<td>Jacob et al. (Ref 12)</td>
<td>13</td>
<td>Lean insulin-sensitive offspring\textsuperscript{**}</td>
<td>H spectroscopy soleus</td>
<td>Yes</td>
</tr>
<tr>
<td>Krssek et al. (Ref 13)</td>
<td>23</td>
<td>Normal-weight adults</td>
<td>H spectroscopy soleus</td>
<td>Yes</td>
</tr>
<tr>
<td>Virkamäki et al. (Ref 14)</td>
<td>20</td>
<td>Non-diabetic men</td>
<td>'H spectroscopy versus lateralis</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*\(n\) = number of subjects studied.

\textsuperscript{1}method does not allow distinction between intramyocellular lipid (IMCL) and extramyocellular lipid; \textsuperscript{**}offspring of type 2 diabetic parents. The offspring in the study of Perseghin et al. (see Ref 11) had higher IMCL than controls, as did the insulin-resistant offspring compared with sensitive offspring in the study of Jacob et al. (see Ref 12)

---

**Figure 2** Intramyocellular lipid (IMCL) content (a), body mass index (b), insulin sensitivity (c), and maximal oxygen consumption (d) in ‘low’ IMCL (LoIMCL) and ‘high’ IMCL (HiIMCL) groups; NS (not significant), *\(P<0.05\) and **\(P<0.01\) refer to the statistical significance of the difference between the groups

**Figure 3** Serum FFA (free fatty acid) concentrations in ‘low’ intramyocellular lipid (IMCL) [open circles] and ‘high’ IMCL [filled circles] groups during the euglycaemic hyperinsulinaemic clamp study; NS (not significant), *\(P<0.05\) and **\(P<0.01\) refer to the statistical significance of the difference between the groups at the given timepoint [reproduced with permission from Virkamäki et al. (see ref 14)]
study, serum TG were similar before and after the high-fat diet, implying that the change in muscle TGs was due to change in IMCL. Unexpectedly in light of the inverse correlation between IMCL and insulin action in sedentary individuals (see Table 1), muscle TG stores and insulin sensitivity are increased by training25–29. This paradox has not been explored in depth, but one possibility is that training induces formation of lipid deposits that are in close proximity to the intracellular site of FFA oxidation, while lipids accumulate elsewhere in sedentary people30.

THE LIVER

Methods

In the liver, there is no adipocyte-associated TG, and all fat is located within hepatocytes7. Liver fat can therefore be quantified non-invasively simply by monitoring TG methylene proton to water signals using proton spectroscopy (Figure 5). Liver fat measured with this technique correlates with that measured chemically by liver biopsy and with computerized tomography (CT)31. As in skeletal muscle, imaging techniques do not allow cellular characterization of the TG deposits. In the liver, TG may accumulate either as small or large lipid deposits (micro- or macrovesicular steatosis). The size of the TG deposits, and the presence of inflammation and other abnormalities, can be determined only by histological analysis of liver biopsies.

Causes of fat accumulation in the liver

Accumulation of TG in hepatocytes reflects an imbalance between hepatic TG synthesis and utilization. Utilization includes mitochondrial β-oxidation, production of ketone bodies, and secretion of TG in very-low-density lipoprotein (VLDL) particles. Histologically, the fatty liver can be classified as micro- or macrovesicular32. Causes of microvesicular steatosis, such as fatty liver during pregnancy, Reye’s syndrome and certain drugs and toxins, are thought to share a common mechanistic feature—impairment of mitochondrial β-oxidation32. Microvesicular steatosis is often accompanied by severe hepatic dysfunction. The causes of macrovesicular steatosis include alcohol, non-alcoholic fatty liver disease (NAFLD) associated with features of insulin resistance, total parenteral nutrition, protein-calorie malnutrition, and jejunoileal bypass. NAFLD is a term describing a large spectrum of conditions ranging from fat alone to fat plus inflammation, fat plus ballooning degeneration, and non-alcoholic steatohepatitis (NASH)33. By definition, NAFLD can only be diagnosed in patients who do not consume significant amounts of alcohol (20 g ethanol/day in women; 80 g/day in men)34. The patients should also not have clinical or laboratory evidence of autoimmune, viral or drug- or toxin-induced liver disease or of congenital metabolic disorders34. NASH cannot be distinguished from other types of NAFLD without liver histology.
Fat accumulation in the liver and insulin resistance

According to the Third National Health and Nutrition Examination Survey (NHANES III), a population-based sample of 13,500 US adults aged 17–74 years, the prevalence of NAFLD is 23.5% when defined as an abnormal aspartate amino transferase (AST), alanine amino transferase (ALT) or γ-glutamyl transferase (GGT) value without evidence for hepatitis B or C, increased transferrin saturation (>50%) or excessive alcohol intake (<2 drinks for women and <3 drinks for men). In this survey, adults with NAFLD were twice as likely to have type 2 diabetes as those without NAFLD after adjustment for age, gender, race and body mass index using logistic regression. If the relationship were proven to be causal, then up to 13.7% of diabetes cases in the USA might be attributable to NAFLD. Similar data were reported earlier from the Hispanic Health and Nutrition Examination Survey (1982–1984). In this survey, data from 2999 men and women aged 20–74 years representative of the Mexican American population were analysed. An elevated ALT (43 IU/L) was reported in 6% of men and 2% of women. The odds ratio for diabetes as a predictor of elevated ALT was 4.1 after adjustment for age and sex, and decreased to 3.0 after adjustment for age, sex, body mass index and alcohol consumption. These epidemiological data require confirmation from prospective studies, but they suggest that NAFLD increases the risk of type 2 diabetes independent of obesity.

NAFLD appears to predispose to type 2 diabetes because it is associated closely with insulin resistance and its consequences, including hyperinsulinaemia, glucose intolerance, hypertriglyceridaemia, decreased high-density lipoprotein (HDL) cholesterol concentration, and hypertension. Although the prevalence of NAFLD increases with increasing obesity, it may also be found in non-obese subjects. Indeed, in a study of over 2000 Japanese individuals, examining the relationship between fatty liver, as determined by ultrasonography, hypertension and a low HDL cholesterol concentration, non-obese subjects with a fatty liver had the highest odds ratio for having hypertension and a low HDL cholesterol concentration. Fatty liver was related to hypertension, dyslipidaemia and glucose intolerance in women, independent of age, obesity and alcohol consumption. These data suggest that fat accumulation in the liver is a hitherto underestimated component of the insulin resistance syndrome.

Causes and mechanisms of ectopic fat accumulation

As in subjects with TG accumulation in skeletal muscle, patients with NAFLD have elevated FFA concentrations that are poorly suppressed by exogenous insulin. Whether this reflects insulin resistance of lipolysis and contributes to fat accumulation in the liver is unknown, since FFA or glycerol fluxes have not been compared between NAFLD patients and weight-matched controls without NAFLD. The flux of FFA to the liver can originate from three sources: peripheral adipocytes, dietary TG in the form of chylomicrons, and FFA synthesized via de novo lipogenesis from carbohydrates. The latter possibility is not likely to be of importance since de novo lipogenesis does not occur in humans unless carbohydrate energy intake exceeds total energy intake. A high-fat (83% fat), low-carbohydrate (2% carbohydrate) diet was shown recently to reduce the ability of insulin to suppress both endogenous glucose production and serum FFA concentrations when compared with a eucaloric low-fat (0%), high-carbohydrate (85%) diet. The liver fat content was not measured in this study. It is unknown whether more moderate variation in dietary fat content will influence hepatic insulin sensitivity.

Fatty liver: a cause or consequence of hepatic insulin resistance?

There are no prospective human data that have tried to define whether a fatty liver precedes features of insulin resistance or vice versa, or whether they develop simultaneously. Murine models have shown that selective hepatic insulin resistance by tissue-specific deletion of the hepatic insulin receptor leads to glucose intolerance and diabetes and also to a fatty change in the liver, but this does not exclude the possibility that excessive storage of TG in the liver also can cause insulin resistance in the liver. This is exemplified by fatless mice, in which removal of hepatic fat by transplanting adipose tissue subcutaneously abolishes all signs of hepatic insulin resistance.

Clinical implications

Inhibition of hepatic glucose production both between and during meals is a key action of insulin. During insulin
therapy or lowering of glucose concentrations by metformin, inhibition of hepatic glucose production lowers plasma glucose concentrations, which counteracts the insulin stimulation of glucose to promote its own utilization. The net result is an unchanged rate of glucose utilization, despite improved insulin sensitivity in patients with type 2 diabetes. This implies that the sensitivity of liver glucose production is a major determinant of insulin requirements. To investigate this, we determined insulin absorption and actions of both subcutaneous and intravenously administered insulin in weight-stable type 2 diabetic patients treated with bedtime insulin and metformin. Hepatic insulin sensitivity was correlated very closely with liver fat content (Figure 6) and also with the daily insulin dose (Figure 7). Insulin antibodies and interindividual variation in insulin absorption were only minor contributors to the variation in insulin requirements.

The amount of fat in the liver might also influence the interindividual responses to glitazone therapy. In ob/ob, db/db and lipodystrophic A-ZIP/F1 mice, a fatty liver is associated with a marked increase in expression of peroxisome proliferator activated receptor (PPARγ) and PPARα genes. Treatment with the PPARγ agonist rosiglitazone in ob/ob, db/db and A-ZIP/F1 mice has been reported to increase the size of the fatty liver further via induction of PPAR-dependent lipogenic enzymes but to slightly decrease liver size in control mice. Troglitazone has been reported to increase liver fat in ob/ob and db/db mice but not to change liver fat content in lipodystrophic A-ZIP/F1 mice with a fatty liver. In high-fat-fed rats, the PPARγ agonist pioglitazone does not appear to change liver fat content but decreases IMCL. In this model, a PPARγ agonist was reported to decrease both liver and muscle TG content. Human data regarding effects of PPAR activation on hepatic fat content are sparse and limited to two studies where troglitazone (now withdrawn because of severe idiosyncratic liver reactions) was used to treat type 2 diabetic patients or patients with various types of lipodystrophies. In these human studies, liver volume and fat content were reported to decrease. However, it is unknown whether liver fat was normal or increased before treatment in these studies. Taken together, it seems that in animal models, pure PPARγ agonists are unable to reduce liver fat content. At present, human data regarding effects of PPARγ agonists are limited. Interestingly, and consistent with deleterious effects of PPARγ agonists on a fatty liver, inhibition of RXR and PPARγ decreases TG content of the liver by increasing FA combustion and energy dissipation.

CONCLUSIONS

The evidence presented in this document suggests that ectopic fat accumulation in insulin-sensitive tissues is associated with insulin resistance independent of overall obesity. However, our understanding of the causes and mechanisms underlying fat accumulation in skeletal muscle and the liver are limited. Identifying why some individuals store fat in insulin-sensitive tissues, but others do not, may be of great importance for the development of new insulin-sensitizing agents and for optimal use of current therapies.

Acknowledgments The author wishes to acknowledge the entire research group working with ectopic fat accumulation, including Takashi Goto MD, Leena Ryysy MD, Anneli Seppälä-Lindroos MD, Satu Vehkavaara MD, and Jukka Westerbacka at the Department of Medicine, Division of Diabetes; Elena Kosheninnikova MD and Antti Virkamäki MD at the Minerva Institute for Medical Research; Anna-Maija Häkkinen PhD, at the Department of Oncology; and Juha Halavaara MD at the Department of Radiology, University of Helsinki, Helsinki, Finland.
Research supported by grants from the Academy of Finland, Sigrid Juselius Foundation and the Finnish Diabetes Research Society.

REFERENCES

32. Fong DG, Nehra V, Lindor KD, Buchman AL. Metabolic and nutritional considerations in nonalcoholic fatty liver. Hepatology 2000; 32:3–10
40. Wanless IR, Lentz JS. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. Hepatology 1990; 12:1106–10


Cruickshank EWH. On the production and utilization of glycogen in normal and diabetic animals. *J Physiol* 1913; 47:1–14


