Impact of visceral adipose tissue on liver metabolism and insulin resistance.
Part II: Visceral adipose tissue production and liver metabolism

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Abstract

Excess visceral adipose tissue is associated with anomalies of blood glucose homeostasis, elevation of plasma triglycerides and low levels of high-density lipoprotein cholesterol that contribute to the development of type-2 diabetes and cardiovascular syndromes. Visceral adipose tissue releases a large amount of free fatty acids and hormones/cytokines in the portal vein that are delivered to the liver. The secreted products interact with hepatocytes and various immune cells in the liver. Altered liver metabolism and determinants of insulin resistance associated with visceral adipose tissue distribution are discussed, as well as, determinants of an insulin-resistant state promoted by the increased free fatty acids and cytokines delivered by visceral adipose tissue to the liver.

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Résumé


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Abbreviations: ChREBP, carbohydrate response element-binding protein; DAG, diacylglycerol; FFA, free fatty acids (such as non-esterified fatty acids); HGP, hepatic glucose production; 11β-HSD1, 11β-hydroxysteroid dehydrogenase-1; IkB-α, inhibitor of kappa B-alpha; IKK-β, inhibitor of kappa B kinase-beta; IL-6, IL-1β, interleukin-6, -1β; IRS-1, IRS-2, insulin receptor substrate-1 and -2; JNK, c-jun NH2-terminal kinase; MCP-1, monocyte chemoattractant protein-1; NAFLD, nonalcoholic fatty liver disease; NF-κB, nuclear factor kappa B; NKT, natural-killer T cells; PEPCK, phosphoenolpyruvate carboxykinase; PGC-1-alpha, PPARγ coactivator-1-alpha; PKB/Akt, protein kinase B/Akt; PKC-delta, protein kinase C-delta; p38 MAP kinase, p38 mitogen-activated protein kinase; SREBP-1c, sterol regulatory element-binding protein-1c; T2D, type 2 diabetes; TG, triglyceride; TNF-α, tumor necrosis factor-alpha; VAT, visceral adipose tissue.

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Visceral adipose tissue (VAT) releases a large amount of free fatty acids (FFA) and hormones/cytokines into the portal vein that are directly delivered to the liver. We discuss here the influence of an increase in FFA and cytokines delivered by VAT to the liver via the portal vein on the induction of an insulin-resistant state.

1. Impact of increased FFA in the portal vein on hepatic metabolism

The liver both takes up and releases glucose. After carbohydrate ingestion (hyperglycaemia and hyperinsulinaemia), hepatic glucose uptake and glycogen storage are increased. Hepatic glucose production (glycogen breakdown and gluconeogenesis) prevails in the fasted state.

1.1. FFA reduce hepatic glucose uptake in the postprandial period

Splanchnic (hepatic) glucose uptake plays a major role in the disposal of an oral glucose load. Indeed, the splanchnic circulation takes up 40% of ingested glucose. Hepatic glucose uptake occurs only when hyperglycaemia and hyperinsulinaemia are present. Most studies have reported an impaired hepatic glucose uptake in type-2 diabetes (T2D) [1–3], but few studies have examined the effects of elevated FFA on hepatic glucose uptake in vivo. An acute elevation in plasma FFA concentrations impairs splanchnic glucose uptake in T2D patients [4,5], but not in nondiabetic subjects [6]. The mechanism behind the effects of elevated FFA on hepatic glucose uptake could involve decreases in glucokinase activity [7] and glycolytic flux [8].

1.2. FFA increase hepatic glucose production in the post-absorptive period

Early evidence supported the view that FFA regulate gluconeogenesis [9]. More recently, a relationship has been demonstrated between plasma FFA and hepatic glucose production [10]. Several lines of evidence show that FFA are important in the control of hepatic glucose production [11]. Thus, it is expected that an increase in FFA to the liver, given their unique role as a signal regulating hepatic glucose output, will result in overproduction of glucose. This combination of visceral adipose tissue depot and the liver could be viewed as a regulatory axis of both PKC-delta and inhibitor of kappa B kinase-beta (IKK-beta) [35]. DAG is a potent allosteric activator of PKC, and the increased activity of both PKC-delta and inhibitor of kappa B kinase-beta (IKK-beta) [35]. DAG is a potent allosteric activator of PKC, and the two-serine kinases phosphorylate insulin receptor substrate-1 (IRS-1) on serine residues and, thus, decrease tyrosine phosphorylation of IRS-1 and interrupt insulin signalling [36] (Fig. 1). It has also been shown that tyrosine phosphorylation of IRS-2 was blocked by co-culture of primary hepatocytes with fat cells [37]. This was mediated by the release of tumour necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6) by fat cells, activation of the nuclear factor-kappa B (NF-kB) pathway in the hepatocytes and blockade of the insulin-signalling pathway [38].

1.3. FFA reduce insulin suppression of hepatic glucose production

A number of studies have shown that elevated plasma FFA can impair hepatic insulin activity in both T2D and non-diabetic subjects. This is supported by in vitro studies of the effects of FFA on hepatic glucose metabolism. In vivo studies, in animals and humans have demonstrated both a correlation and a temporal relationship between changes in plasma FFA and changes in hepatic glucose production, while interventional studies have established that an increase or decrease in plasma FFA results in a decrease or increase in hepatic insulin activity. Excess VAT has been associated with hepatic insulin resistance [16], but this was nonspecific as excess subcutaneous adipose tissue (SCAT) was also correlated with insulin resistance [17].

FFA can cause hepatic insulin resistance by inhibiting insulin suppression of hepatic glucose production. FFA also led to an increase in gluconeogenesis in vitro [18,19], and the proposed mechanism involves an increased production of ATP and NADH, and activation of pyruvate carboxylase by the acetyl-CoA generated by fatty acid oxidation [18]. When plasma FFA are raised in vivo during a glucose clamp, insulin suppression of hepatic glucose production (HGP) was partially inhibited [20–24].

Insulin is a potent inhibitor of HGP mainly via inhibition of gluconeogenesis [25–29]. However, insulin is much less efficient in the inhibition of hepatic gluconeogenesis [30,31]. Thus, in obese T2D patients in whom overproduction of glucose by the liver is due to an enhanced gluconeogenesis, insulin is less efficient in inhibiting HGP, so that the liver is insulin-resistant [32]. FFA-induced hepatic insulin resistance is also associated with an increase in intrahepatic accumulation of triglycerides (TG) [33].

1.4. Cellular and molecular mechanisms of FFA-induced insulin resistance

It has been reported that infusion of heparinized lipid or increased plasma FFA resulted in the activation of protein kinase C-delta (PKC-delta) in rat liver [34]. During a euglycaemic hyperinsulinaemic clamp, with or without infusion of lipid, the induction of hepatic insulin resistance was associated with increased hepatic diacylglycerol (DAG), and increased activity of both PKC-delta and inhibitor of kappa B kinase-beta (IKK-beta) [35]. DAG is a potent allosteric activator of PKC, and the two-serine kinases phosphorylate insulin receptor substrate-1 (IRS-1) on serine residues and, thus, decrease tyrosine phosphorylation of IRS-1 and interrupt insulin signalling [36] (Fig. 1). It has also been shown that tyrosine phosphorylation of IRS-2 was blocked by co-culture of primary hepatocytes with fat cells [37]. This was mediated by the release of tumour necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6) by fat cells, activation of the nuclear factor-kappa B (NF-kB) pathway in the hepatocytes and blockade of the insulin-signalling pathway [38].
1.5. p38 Mitogen-activated protein kinase (p38 MAP kinase)

It is currently known that p38 MAP kinase is activated by hormones that increase intracellular cAMP [39], cytokines [40] and FFA [38,41,42]. P38 MAP kinase stimulates transcription of the key gluconeogenic enzymes phosphoenolpyruvate carboxykinase (PEPCK) and G-6-Pase [38–41], as well as, expression of the PPARγ coactivator-1-alpha (PGC-1-alpha) gene, and phosphorylation (activation) of PGC-1-alpha protein. P38 MAP kinase also promotes the transcription of PEPCK and G-6-Pase genes through phosphorylation of cAMP response element-binding protein (CREB) [43], and hepatic beta-oxidation through increases in PPARα activity by phosphorylation [44].

1.6. Mixed-lineage kinases

Saturated FFA are a major source of metabolic stress that activates the c-Jun NH2-terminal kinase (JNK). This FFA-stimulated JNK pathway is relevant to hallmarks of the metabolic syndrome, including insulin resistance. Studies in mice demonstrate a central role for mixed-lineage protein kinases (MLK) in this signalling pathway [45]. Saturated FFA cause PKC-dependent activation of MLK3, which subsequently leads to increased JNK activity by a mechanism that involves the MAP kinases MKK4 and MKK7. Loss of PKC, MLK3, MKK4 or MKK7 expression prevents FFA-stimulated JNK activation. These data establish a signalling pathway that mediates effects of metabolic stress on insulin resistance.

1.7. Liver fat accumulation is associated with insulin resistance

Lipids accumulate in hepatocytes in a number of situations, including obesity and T2D, and excess fat in the liver is associated with insulin resistance. The positive association between hepatic and visceral fat in T2D is in agreement with the important role of VAT in liver metabolism [46]. Liver fat is highly significantly and linearly correlated with all components of the metabolic syndrome independent of obesity. This is supported by the fact that the decrease in hepatic fat content observed after glitazone treatment of T2D and leptin treatment of lipodystrophic patients is accompanied by improvements in insulin sensitivity and splanchnic glucose uptake. Individuals with a fatty liver are more likely to have excess intra-abdominal fat and inflammatory changes in adipose tissue. Interventional studies have shown that liver fat can be decreased by weight loss, and the use of PPARγ agonists and insulin therapy [47]. Excess levels of FFA (Fig.1) also stimulate the accumulation of sphingolipid ceramide and ceramide metabolites that reduce insulin signal transduction by inhibiting protein kinase B/Akt (PKB/Akt) phosphorylation and activation ([48] for a review) (Fig. 2).

There is an inverse relationship between hepatic TG stores and insulin sensitivity [49–51]. Recent experiments have suggested a mechanistic link between intrahepatic lipids and insulin resistance. Mice in which lipoprotein lipase is overexpressed in the liver showed an accumulation of TG in the liver [52]. In addition, several alterations of insulin signalling were seen in these mice, including decreased tyrosine phosphorylation of IRS-2 and reduced downstream activation of PKB/Akt. Feeding the rats with a high-fat diet for three days induced hepatic fat accumulation, but did not alter peripheral insulin sensitivity [53]. However, hepatic glucose production during a clamp was decreased, demonstrating insulin resistance. At the molecular level, insulin-stimulated phosphorylation of IRS-2 was blunted. The proposed mechanism involves stimulation of PKC and JNK by increased intracellular acyl-CoA [53]. Thus, fat accumulation alone appears able to induce hepatic insulin resistance, but cannot alter basal glucose production, nor induce glucose intolerance or peripheral insulin resistance.

In obesity, an increased adipocyte mass and insulin resistance, especially in VAT, contributes to elevated FFA through lipolysis. As the rate of FFA uptake is directly related to plasma FFA concentration, increased lipolysis appears to be a major contributor to intrahepatic lipid accumulation. In addition, de novo lipogenesis is activated by glucose, and hyperinsulinaemia, by activation of carbohydrate response element-binding protein (ChREBP) and sterol regulatory element-binding protein-1c (SREBP-1c) [54], and low-grade inflammation and increased TNF-α. Indeed, increased de novo lipogenesis in hepatocytes is observed in NAFLD patients with insulin resistance [55]. Although in normal subjects the contribution of hepatic de novo lipogenesis to the pool of hepatic fatty acids is less than 5%, this increases to as much as 25% in NAFLD patients.

**Fig. 1.** Mechanisms of FFA-induced insulin resistance in the liver.

**Fig. 2.** Mechanisms of cytokine-induced insulin resistance in the liver.
Thus, fat accumulation in hepatocytes is a direct consequence of insulin resistance in adipose tissue and hyperinsulinaemia. Hepatic insulin resistance contributes to hepatic steatosis by increasing glucose levels and worsening hyperinsulinaemia. Hepatic insulin resistance has been correlated with hepatic fat content [56]. A number of studies have shown an association between NAFLD and hepatic insulin resistance, and inhibition of PKCδ has been shown to prevent insulin resistance [57].

1.8. Defect in mitochondrial metabolism

Obesity and T2D are characterized by lipid accumulation in skeletal muscles and liver, and lipid accumulation in the liver is the result of both esterification of fatty acids taken up from the circulation and de novo lipogenesis [58]. The inability of adipose tissue in obese people to buffer the postprandial influx of fatty acids plays a role in lipid accumulation in skeletal muscles and liver. In addition, there is a decreased capacity for mitochondrial fatty acid oxidation [59,60], due to an alteration in the expression of several nuclear genes involved in the regulation of mitochondrial biogenesis (PGC-1-alpha, the coactivator of PPARg, AMP kinase and calmodulin IV kinase) [61]. Lipid accumulation in the liver and skeletal muscle promotes a state of insulin resistance due to alteration of insulin signalling [61].

2. Impact of increased adipokines in the portal vein on hepatic metabolism

There is growing evidence that obesity is an inflammatory state, and that the release of inflammatory cytokines from adipose tissue may be an additional cause of insulin resistance. As discussed above, it is now well established that adipose tissue is not only a storage site for fat, but that it also produces a large number of cytokines and chemokines — collectively called “adipokines”. In fact, mice fed with a high-fat diet developed hepatic insulin resistance, and subacute hepatic inflammation associated with the increased production and secretion of several inflammatory cytokines (Fig. 2) [62]. When plasma FFA levels were acutely elevated by an intravenous infusion of heparinized lipid, hepatic and peripheral insulin resistance was consequently seen. FFA also increased the activities of IKK-β and NF-κB, and decreased inhibitor of kappa B-alpha (IkB-α) [35]. This was accompanied by increased expression of several NF-κB-dependent inflammatory cytokines — namely, interleukin-1β (IL-1β), TNF-α and IL-6 — and an increase in plasma monocyte chemoattractant protein-1 (MCP-1). Thus, elevated FFA either as a result of obesity or a high-fat diet can produce insulin resistance as well as chronic low-grade inflammation that, over time, lead to steatohepatitis.

Hepatocytes represent approximately two-thirds of the total cells in the liver. The remaining cells include biliary epithelial cells, sinusoidal endothelial cells, Kupfer cells (resident macrophages), stellate cells (also called Ito or fat-storage cells), dendritic cells and several types of lymphocytes [63–65]. Blood from the gastrointestinal tract passes via the afferent portal vein to the hepatic sinusoids, and interacts with the sinusoidal endothelial cells, and hepatic immune cells on its way to the efferent central vein. Sinusoidal endothelial cells represent approximately 50% of the nonparenchymal cells and, unlike endothelial cells in many other organs, form a sieve-like, fenestrated epithelium, and participate in antigen presentation. Kupfer cells occupy the sinusoidal space where they ingest microorganisms and other debris. Lymphocytes are mostly T cells, and natural-killer (NKT) cells, with B-lymphocytes making up a much smaller percentage. NKT cells account for up to 30% of the total lymphocytes in the liver, which is a higher percentage than is typically found in other organs.

Besides parenchymal cells, the liver contains sinusoidal cells (35% of total liver cells). These cells play important roles in liver morphology, function, defence and wound healing. Kupfer cells are liver-resident macrophages with key actions in innate immunity and parenchymal inflammation. As already mentioned, sinusoidal endothelial cells form the fenestrated endothelium in the liver parenchyma, and secrete a wide array of cytokines. Hepatic stellate cells occupy the space of Disse, lining the sinusoidal endothelial cells, and are important in storage and homeostasis. Data concerning insulin resistance in sinusoidal cells are scanty. The accumulation of lipid in the liver often accompanies, and parallels weight gain and obesity. Hepatic steatosis, the accumulation of lipid in hepatocytes, has negative effects on liver function that may be mediated by inflammation. For example, expression of proinflammatory cytokines, including IL-6, TNF-α and IL-1β, increases in the liver with increasing adiposity [62]. This suggests that steatosis could induce a subacute inflammatory response in the liver similar to that seen with the accumulation of lipid into adipocytes (see above). In addition, proinflammatory cytokines, lipids and other substances produced by abdominal fat and carried to the liver through the portal circulation may also contribute to hepatic inflammation. Proinflammatory substances activate Kupfer cells, which are abundant in the liver and account for more than 5% of total liver cells, and the activation of Kupfer cells, but not their number, increases with obesity [62]. The many additional immune cell types in the liver may also be involved in inflammation-induced insulin resistance. For example, the number of NKT cells is selectively reduced in liver steatosis in obese, leptin-deficient ob/ob mice and in mice receiving a high-fat diet [66,67]. Given that NKT cells have a regulatory role in immune function [68], such significant changes in their number could have important ramifications in terms of both physiology and pathology. Interestingly, adoptive transfer of NKT cells into ob/ob mice resulted in reductions in hepatic fat content and steatosis, and improvement in glucose intolerance [69].

3. Impact of cortisol from VAT on hepatic glucose production and insulin resistance

Recently, it was reported that visceral obesity was induced in transgenic mice overexpressing 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) in adipose tissue. Such obesity led to an increase in corticosteroids in the portal vein (+60%), and was accompanied by an hepatic insulin-resistant state, dyslipidaemia and T2D [70]. In the liver, glucocorticoids induced key enzymes of gluconeogenesis (PEPCK). 11β-HSD1
knockout [71,72] reduced the induction of key enzymes of gluconeogenesis in response to fasting in the liver [71]. These mice were also resistant to the development of obesity and diabetes. In humans, carbonoxolone, a nonselective inhibitor of 11β-HSD1, improved insulin sensitivity without modification of muscle glucose uptake, suggesting an effect on the liver [73]. In lean T2D patients, the stimulating effect of glucagon on hepatic glucose production was reduced with carbonoxolone treatment [74]. These effects were probably due to inhibition of the expression of key enzymes of gluconeogenesis (PEPCK and G-6-Pase) [75,76]. The bulk of the evidence suggests that the increased release of cortisol by VAT could play a role in hepatic insulin resistance by maintaining the expression of key enzymes of gluconeogenesis and, thus, hepatic glucose production, at a high level.

4. Conclusion and future avenues of research

Our understanding of the relative influence of metabolic (hepatic effects of VAT-released FFA) and humoral (adipokines and cytokines synthesized by VAT) factors on the abnormalities observed in the liver has been greatly improved. Studies in humans and in rodents have demonstrated that the mechanisms leading to an excess accumulation of hepatic TG are mainly linked to increased delivery of FFA from peripheral adipose tissue to the liver and enhanced de novo lipid synthesis via the lipogenic pathway in the liver itself, while lipid disposal via β-oxidation and VLDL export are only moderately affected. To date, no study has refuted the role of VAT-derived FFA in the pathogenesis of hepatic insulin resistance; the mechanisms have been clearly established and are discussed above. Studies conducted on rodents or dogs fed with high-fat diets [77], have solidly confirmed that FFA play an important role in the induction of hepatic insulin resistance. However, VAT is not the only source of systemic FFA; depending on the respective degrees of VAT and SCAT distribution, the FFA delivered by nonvisceral abdominal adipose tissue cannot be ignored in the development of insulin resistance, especially when they affect skeletal muscle [78].

NAFLD is associated with insulin resistance. The pathogenesis of NAFLD consists of hepatic fat accumulation and oxidative stress, with formation of free radicals. Despite the established correlation between fatty liver and insulin resistance, it remains unclear as to whether or not insulin resistance causes excess accumulation of TG in liver, or whether or not, the increase in TG itself or of its metabolite intermediates play a causal role in the development of hepatic or systemic insulin resistance. Recent studies have favoured the idea that the accumulation of intrahepatic lipids precedes insulin resistance, while others have shown that hepatic TG in itself is not toxic and may, in fact, protect the liver from lipotoxicity by buffering the accumulation of fatty acids, suggesting that hepatic steatosis may not necessarily be associated with insulin resistance [79]. In rodents, key enzymes of fatty acid synthesis have been shown, when knocked down, to reverse many of the metabolic defects associated with hepatic steatosis and/or insulin resistance, indicating that decreased TG synthesis in the liver is a potential and interesting target for the treatment of NAFLD. Although rodent models of hepatic steatosis and/or insulin resistance do not always perfectly reproduce the human pathology of NAFLD, the use of transgenic, knockout and knockdown mouse models has helped to better our understanding better of the molecular determinants of NAFLD over the past few years.

Thus, a more complete knowledge of the function and/or regulation of the transcription factors that control the activity of the enzymes modulating fatty acid synthesis in the liver may prove helpful in the development of potential therapeutic approaches in future.

References


