Fructose consumption: potential mechanisms for its effects to increase visceral adiposity and induce dyslipidemia and insulin resistance

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Purpose of review

Based on interim results from an ongoing study, we have reported that consumption of a high-fructose diet, but not a high-glucose diet, promotes the development of three of the pathological characteristics associated with metabolic syndrome: visceral adiposity, dyslipidemia, and insulin resistance. From these results and a review of the current literature, we present two potential sequences of events by which fructose consumption may contribute to metabolic syndrome.

Recent findings

The earliest metabolic perturbation resulting from fructose consumption is postprandial hypertriglyceridemia, which may increase visceral adipose deposition. Visceral adiposity contributes to hepatic triglyceride accumulation, novel protein kinase C activation, and hepatic insulin resistance by increasing the portal delivery of free fatty acids to the liver. With insulin resistance, VLDL production is upregulated and this, along with systemic free fatty acids, increase lipid delivery to muscle. It is also possible that fructose initiates hepatic insulin resistance independently of visceral adiposity and free fatty acid delivery. By providing substrate for hepatic lipogenesis, fructose may result in a direct lipid overload that leads to triglyceride accumulation, novel protein kinase C activation, and hepatic insulin resistance.

Summary

Our investigation and future studies of the effects of fructose consumption may help to clarify the sequence of events leading to development of metabolic syndrome.

Keywords

dyslipidemia, free fatty acids, fructose consumption, hepatic steatosis, insulin resistance, metabolic syndrome

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Introduction

Studies investigating the effects of fructose consumption in humans and animals have been comprehensively reviewed [1–3,4••,5••]. These reviews are in agreement in their conclusions that, while there is strong evidence that diets high in fructose can produce obesity, insulin resistance/glucose intolerance, and dyslipidemia in animals, direct experimental evidence that consumption of fructose promotes the development of metabolic syndrome in humans is equivocal. We are currently conducting an investigation comparing the metabolic effects of consuming beverages sweetened with fructose or glucose providing 25% of energy requirements for 10 weeks in older, overweight and obese men and women. Based on interim results [6], we have reported that consumption of the high-fructose diet promotes the development of three of the pathological characteristics associated with metabolic syndrome: dyslipidemia, insulin resistance, and increased visceral adiposity. These observations have potentially important public health implications. In addition, these results suggest that such investigations may help to illuminate the causes and sequence of events leading to the development of metabolic syndrome. Rutledge and Adeli [7••] have recently outlined a potential sequence of events by which fructose consumption may contribute to development of the metabolic syndrome. They suggest that increased VLDL production induced by fructose increases visceral adiposity, which leads to insulin resistance in adipose tissue and, subsequently, to hepatic insulin resistance. We present this sequence of events, along with supporting evidence from the literature and from our current study. We then present another scenario in which overconsumption of fructose may result in a lipid overload within the liver that contributes to hepatic insulin resistance independently of visceral adiposity.

Fructose and hepatic lipogenesis/VLDL production

Both our current study [6] and earlier data [8] demonstrate that 10 weeks of fructose consumption markedly increases circulating postprandial triglyceride concentrations in older adults. In short-term studies in younger adults, we demonstrated that fructose consumption increases postprandial triglyceride concentrations within 24 h [9,10], which suggests that postprandial hypertriglyceridemia is the earliest metabolic perturbation associated with fructose consumption. The most likely mechanism for the postprandial hypertriglyceridemia is increased hepatic de-novo lipogenesis (DNL), which in turn upregulates VLDL production and secretion. Fructose consumption can promote hepatic lipogenesis because, first, the liver is the main site of fructose metabolism [11]; second, entry of fructose into glycolysis via fructose-1-phosphate bypasses the main rate-controlling step of glycolysis catalyzed by phosphofructokinase, thus providing unregulated amounts of lipogenic substrates acetyl-CoA and glycerol-3-phosphate [11], and, third, fructose can activate sterol receptor element binding protein-1c (SREBP-1c) independently of insulin, which then activates genes involved in DNL [12,13].

VLDL production and secretion are mainly regulated by the availability of lipid substrate [14]. Apolipoprotein B100 (ApoB) is essential for the intracellular assembly of triglyceride into VLDL. ApoB undergoes co-translational and posttranslational degradation, and its degradation is dramatically reduced when hepatic lipid content is increased [15]. In subjects consuming fructose, plasma ApoB concentrations were increased by more than 25% [6].

Recently it was reported that the contribution of de novo lipogenesis to fructose-induced hypertriacylglyceridemia is small [16•]. In this acute study of 14 healthy men and women, fructose contributed only 0.4% of the circulating VLDL-triglyceride measured 6 h after consumption of a high-fat (~60%), high-fructose (~40%) meal that contained 250 mg D-[U^{13}C]fructose. It is possible, however, that the 6-h measurement of the incorporation of the ^{13}C label into VLDL may not accurately reflect the rate of DNL. Fatty acids produced via DNL appear to be partitioned into the liver cytosolic triglyceride storage pool rather than immediately assembled into VLDL and secreted [17••]. Several studies have demonstrated that the measured contribution of DNL-derived free fatty acids (FFA) to VLDL-triglyceride increases progressively with longer periods of labeled precursor infusion (+24 h) that allows for equilibration of newly produced fatty acids into the liver triglyceride storage pool [17••,18–20].

Fructose and visceral adiposity

Rutledge and Adeli [7••] suggest that the increased VLDL production induced by fructose promotes obesity, although currently there is little experimental evidence to support this suggestion. In our current study, subjects consumed their usual ad-libitum diets along with either fructose-sweetened or glucose-sweetened beverages. Within 8 weeks, both groups gained an average of 1.5 kg. Intra-abdominal fat measured by computerized tomography, however, was significantly increased in subjects consuming fructose but unchanged in subjects consuming glucose (K. Stanhope, P. Havel, unpublished data). These results suggest that postprandial hypertriglyceridemia may specifically promote lipid deposition in visceral adipose tissue. As recently reviewed by Votruba and Jensen [21••], fat uptake is higher in abdominal subcutaneous fat than in subcutaneous fat in the thigh region [22,23], and higher in omental than in abdominal subcutaneous fat [24,25] following consumption of high-fat meals. Whether adipose uptake of meal-derived chylomicron-fatty acids differs from that of VLDL-fatty acids derived from fructose-induced DNL is unknown, however. It was recently shown that there was uptake of both chylomicron-fatty acids and VLDL-fatty acids by subcutaneous abdominal adipose following a mixed meal; however, the fractional extraction of chylomicron-derived fatty acids was greater, especially during the first 2 h after the meal [26••].

Visceral adiposity and portal free fatty acids concentrations

There is considerable evidence that visceral adiposity is associated with insulin resistance [27–32]. An important potential mediator of this association is the direct delivery of portal blood flow from visceral fat to the liver. Owing to the portal connection, FFAs released from visceral fat are more likely to contribute to disturbances in hepatic metabolism than FFAs released from other adipose depots [33–35]. Another important mechanism is the greater lipolytic capacity of visceral than peripheral adipocytes. Visceral adipocytes have been demonstrated to be more sensitive than subcutaneous fat cells to the lipolytic effect of catecholamines [36,37] and, importantly, less sensitive to the antilipolytic and fatty acid re-esterifying effects of insulin [38,39]. Furthermore, as visceral adiposity develops, visceral adipocytes enlarge. Large adipocytes are more insulin resistant than smaller adipocytes [40,41•], and therefore less sensitive to the effects of insulin to suppress lipolysis and promote re-esterification of fatty acids [42–44]. Visceral adiposity is also closely associated with reduced circulating levels of the adipocyte hormone adiponectin, perhaps because enlarged visceral adipocytes are also likely to produce less adiponectin. Adiponectin increases hepatic lipid

oxidation and improves insulin sensitivity by activating AMP kinase (see review, [45]).

Free fatty acid in the liver and hepatic triglyceride deposition

With increasing visceral adiposity, there is increased portal FFA delivery to the liver. It has been demonstrated that the fraction of FFA delivered to the liver from visceral fat is positively related to the visceral fat area, and is approximately 5–10% in normal-weight subjects and 20–30% in obese subjects [46,47]. Hepatic uptake of FFA is proportional to the rate of delivery [48–50]. In the liver, FFA is either oxidized or esterified to form triglyceride. The triglyceride is stored in the cytosol prior to being incorporated into VLDL and secreted [51]. Recent studies suggest that triglyceride turnover through the cytosolic pool and incorporation into VLDL can be rapid or delayed [17••]. It has been suggested that plasma FFA entering the liver may be routed through a more rapid turnover pool than fatty acids from dietary sources or those produced from DNL [18]. When triglyceride production exceeds FFA oxidation and VLDL production and secretion, triglyceride accumulates in the liver [51]. Triglyceride accumulation in the liver (i.e. non-alcoholic fatty liver disease—NAFLD) is positively associated with visceral adiposity [52]. Several studies of patients with type-2 diabetes and insulin resistance indicate that liver triglyceride content is also a strong correlate of hepatic insulin resistance [53–57] and the relationship is independent of visceral adiposity in both type 2 diabetic [56] and nondiabetic subjects [58].

Liver triglyceride content and hepatic insulin resistance

It has been suggested that hepatic triglyceride accumulation is a major mediator of hepatic insulin resistance [58,59••]. The Shulman group [60] has provided support for the hypothesis that lipid accumulation within the liver induces hepatic insulin resistance with evidence of a dose–response relationship between hepatic lipid content and insulin action and by demonstrating that prevention of hepatic fat accumulation abrogates the development of hepatic insulin resistance. Morino *et al.* [59••] suggest that the mechanism by which intracellular lipid causes insulin resistance in both liver and muscle is through diacylglycerol (DAG)-induced activation of novel protein kinase C (nPKC). DAG is a known activator of nPKC [61] and both DAG and nPKC are associated with lipid-induced insulin resistance in human muscle [62,63]. Several reports suggest that nPKC activation is associated with decreased insulin receptor or insulin receptor substrate 1 (IRS1) tyrosine phosphorylation [64–66], and other reports more specifically implicate nPKC in serine phosphorylation of insulin receptor, which impairs insulin signaling [67,68]. Studies conducted in 3T3-L1 adipocytes suggest that inhibitor kappa B kinase and c-JUN NH_2-terminal kinase (JNK1) may mediate the serine phosphorylation induced by nPKC [69].

Hepatic insulin resistance and lipogenesis

With impaired insulin signaling in the liver, there is decreased glycogen synthesis, and increased glycogenolysis and gluconeogenesis. As a compensatory response, insulin secretion increases. It has been suggested that the increased insulin secretion is a direct response to increased FFA levels rather than increased glucose production [70••]. Both fasting glucose and insulin concentrations were increased, however, in subjects consuming fructose within 2 weeks (K. Stanhope, P. Havel, unpublished data). As hyperinsulinemia develops, DNL is increased due to insulin activation of SREBP1-c [71]. Although the insulin-resistant liver is resistant to the effects of insulin to stimulate glycogen synthesis and inhibit gluconeogenesis and glycogenolysis, it does not appear to develop resistance to insulin's effect to promote lipogenesis [72].

Hepatic insulin resistance and VLDL production

VLDL production is also increased in the insulin-resistant liver due to mechanisms independent of hepatic lipid supply. With insulin resistance, there is reduced ApoB degradation and increased VLDL production [73]. The mechanism by which insulin directly inhibits VLDL production is unknown [15], but it has been suggested that insulin promotes ApoB degradation by inhibiting lipid transfer to VLDL-precursor ApoB [74] and by regulating a protease enzyme implicated in ApoB degradation [75]. Insulin also inhibits microsomal triglyceride transfer protein (MTP) expression via an insulin response element on the MTP gene [76]. MTP is essential for assembly of triglyceride and ApoB into VLDL and secretion of VLDL [77]. Lewis *et al.* [78] suggested that, in insulin-resistant states, there may be sustained upregulation of MTP expression and protein levels as a result of resistance to insulin's inhibitory effect on MTP.

Hypertriglyceridemia and cardiovascular disease risk

Upregulation of VLDL production leads to increased plasma triglyceride. Reduced clearance of triglyceride can also contribute to hypertriglyceridemia [79,80]. Insulin stimulates adipose lipoprotein lipase (LPL) and LPL activity is decreased in subjects with insulin resistance [81]. There is growing evidence linking postprandial hypertriglyceridemia with proatherogenic conditions [82••,83,84,85•,86••]. The relationship

between nonfasting triglyceride and cardiovascular disease is most likely mediated by effects of postprandial hypertriglyceridemia to promote lipid remodeling to a more atherogenic lipid profile consisting of increased concentrations of triglyceride-rich remnant lipoproteins and small dense LDL, and decreased concentrations of HDL [87,88••,89••]. In subjects consuming fructose, we have reported significantly increased circulating levels of remnant lipoproteins, small dense LDL, and oxidized LDL [6].

Peripheral insulin resistance

Elevated triglyceride, along with elevated levels of plasma FFA released from insulin-resistant adipose tissue, lead to increased flux of FFA and triglyceride to other tissues. In skeletal muscle, increased FFA availability can lead to increased muscle triglyceride content and intramyocellular lipid (IMCL) deposition. IMCL is closely related to insulin resistance in skeletal muscle [90–93]. IMCL, or associated lipid metabolites such as DAG, appear to inhibit insulin signaling, leading to a reduction in insulin-stimulated glucose transport [59••,94,95•] and systemic insulin resistance.

Free fatty acid: link between visceral adiposity and hepatic insulin resistance

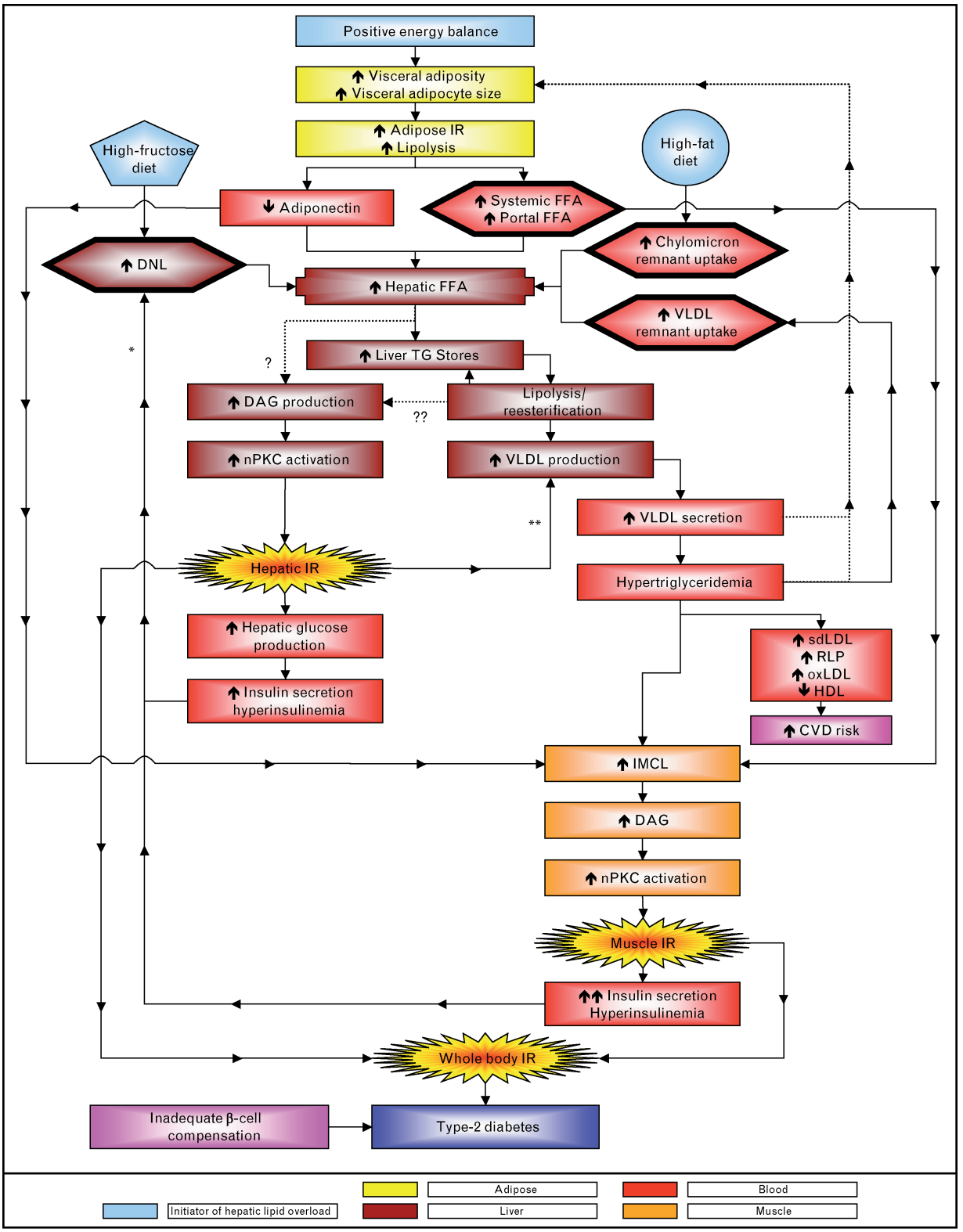
Strong support for the hypothesis that FFA release from enlarged visceral adipocytes is an important link between visceral adiposity and hepatic insulin resistance has been provided by Bergman *et al.* [96•], who conclude, first, that FFAs per se are among the most important products of the visceral adipocyte contributing to insulin resistance and hence metabolic syndrome; second, that the anatomical position of the visceral adipose depot (i.e. portal drainage to the liver) plays an important role in the pathogenesis of metabolic syndrome. When considering evidence that does not support these conclusions, it is important to be aware of the following. Bergman *et al.* [96•] reported that feeding dogs a 6-week hypercaloric high-fat diet resulted in a 76% increase in trunk fat, but fasting FFA concentrations were not affected. Twenty-four-hour systemic FFA profiles, however, determined from hourly blood sampling, were increased by 50% [96•]. This suggests that linking increases of FFA with visceral adiposity and insulin resistance may not be possible in studies that measure circulating metabolites only in the fasting state. In human subjects consuming a high-fructose diet for 10 weeks, we found no change in 24-h systemic FFA profiles (36 samples collected over 24 h, K. Stanhope, P. Havel, unpublished data), despite modest increases in visceral adiposity and insulin resistance. This does not exclude the possibility that portal concentrations and hepatic extraction of FFAs were increased in these subjects. Parallel measurements, however, of arterial and portal FFA concentrations in conscious dogs under experimental conditions that resulted in a wide range of FFA release demonstrated that, whereas portal vein FFA levels tended to be higher than arterial levels (~5–6%), the values obtained were highly correlated ($r^2 = 0.96$) [97]. These observations led us to consider the possibility that hepatic lipid overload, independent of visceral adiposity and FFA levels, may be an important mediator of insulin resistance in subjects consuming fructose.

Hepatic lipid overload may initiate liver triglyceride accumulation and hepatic insulin resistance independently of visceral adiposity and free fatty acid

The suggestion that fructose induces insulin resistance independently of visceral adiposity and FFA levels is supported by work from the Shulman group [59••,60]. These investigators have built on the work by Kraegen and colleagues [98], who demonstrated that 3 days of high-fat feeding results in hepatic insulin resistance prior to the development of peripheral insulin resistance. Shulman and colleagues also fed rats a high-fat diet (69%) for 3 days and reported a three-fold increase in liver triglyceride content without any significant changes in visceral fat weight [60]. The hepatic fat accumulation was associated with impaired IRS tyrosine phosphorylation, PKC-ε (a novel PKC) and JNK1 activation, decreased insulin stimulation of glycogen synthase and decreased insulin suppression of gluconeogenesis [60].

It has been proposed that obesity per se is not the main contributor to insulin resistance, but rather it is the accumulation of intracellular lipid metabolites (e.g. DAG) [58,99••]. As presented in Fig. 1, in addition to FFA, there are other additional sources of triglyceride that can lead to hepatic lipid accumulation: triglyceride generated by hepatic lipogenesis, and triglyceride derived from FFA released from VLDL and chylomicron remnants within hepatic lysosomes [51]. Therefore, by increasing the delivery of chylomicron remnant-triglyceride to the liver, feeding rats a high-fat diet for only 3 days resulted in impaired insulin signaling prior to increases in visceral adiposity [60]. We propose that a high-fructose diet, which provides substrate for de-novo lipogenesis, can also produce a lipid overload in the liver that results in hepatic insulin resistance independently of visceral adiposity and FFA levels. This suggestion is not mutually exclusive of the 'portal' FFA hypothesis. A sustained, moderate positive energy balance may indeed promote hepatic insulin resistance as a result of increased visceral fat accumulation and increased portal delivery of FFA. During consumption of a high-fructose diet, however, a contributing and possibly major mechanism may be a more direct intra-hepatic lipid oversupply via fructose-induced lipogenesis.

Figure 1 A high-fructose diet increases hepatic de-novo lipogenesis and a high-fat diet increases hepatic chylomicron remnant uptake



Either diet can produce a hepatic lipid overload along with, or independently of, visceral adiposity and increased portal free fatty acid (FFA) delivery. Visceral adiposity with adipocyte hypertrophy has been hypothesized to reduce adiponectin production and delivery to the liver which would be

Liver lipid accumulation and insulin resistance are not always associated

Although there is much support for the hypothesis that hepatic lipid accumulation initiates insulin resistance, there is also contradictory evidence. Lonardo *et al.* [100••] investigated the association of hepatic steatosis with insulin resistance in patients with, first, familial heterozygous hypobetalipoproteinemia (FHBL), second, NAFLD, third, hepatitis C virus infection (HCV), and fourth, healthy subjects without steatosis. Data from subjects with NAFLD and HCV supported the association between liver steatosis and insulin resistance. FHBL subjects, however, did not have significantly increased HOMA-IR compared with healthy subjects, and the 17 FHBL subjects with liver steatosis did not have higher HOMA-IR than the five FHBL subjects without liver steatosis. Subjects with FHBL have mutations in the ApoB gene that lead to triglyceride accumulation in the liver due to impaired VLDL production and secretion. The lack of insulin resistance in these subjects suggests that the mechanism by which hepatic triglyceride stores are increased is key to the development of insulin resistance [100••]. It also suggests that there may be steps downstream of liver triglyceride accumulation, for example VLDL production or secretion, that are associated with the induction of insulin resistance. The very low fasting triglyceride concentrations observed in the subjects with FHBL (mean = 34 mg/dl) are consistent with reduced rates of VLDL production and secretion. Conversely, it has been reported that subjects heterozygous for a mutation that increases ApoB transcription (−516C/T) exhibited increased postprandial triglyceride concentrations [101] and insulin resistance [102•].

Data from other studies also indicate a lack of association between liver triglyceride and insulin resistance. Patients with glycogen storage disease type 1 have severe steatosis without insulin resistance [103,104]. In mice lacking hepatic MTP [105] and in transgenic mice overexpressing acyl-CoA:diacylglycerol acyltransferase 2 (DGAT2) in the liver [106•], there were increased liver triglyceride accumulation and reduced circulating triglyceride levels in the absence of insulin resistance. Rats administered antisense to stearyl CoA desaturase-1 (SCD1) and fed a lard-supplemented diet had increased liver triglyceride, reduced circulating triglyceride, but normal insulin sensitivity. Control rats treated with scrambled antisense exhibited the expected decrease of insulin sensitivity on the lard diet, yet had only one-third the hepatic triglyceride content [107•]. The dissociation between hepatic triglyceride content and insulin resistance noted in these studies again suggests that mechanisms operating downstream of liver triglyceride accumulation, which are connected to the formation or secretion of VLDL, may be involved in the development of hepatic insulin resistance.

Linking hepatic insulin resistance with VLDL production

A detailed model for production of VLDL has been proposed [51,108]. To briefly summarize, the liver triglyceride synthesized from extracelluar and endogenous sources of FFA does not serve as a direct precursor of VLDL, but rather is stored in the cytosolic triglyceride pool. This cytosolic triglyceride is not incorporated into VLDL en bloc, but rather is first hydrolyzed to FFA, monoacylglycerol, and DAG. These lipolytic products are then re-esterified in the vicinity of ApoB-VLDL precursor. Not all of this resynthesized triglyceride is incorporated into VLDL; instead, as much as 50% is recycled back to the cytosolic pool [51,108]. A possible explanation for the disconnect between liver triglyceride accumulation and hepatic insulin resistance may be that the DAG production responsible for the induction of hepatic insulin resistance results from the triglyceride lypolysis, re-esterification and recycling associated with VLDL assembly, rather than from the DAG associated with the initial synthesis of triglyceride from extra-hepatic and endogenous sources of FFA. Accordingly, when the assembly of VLDL is inhibited, as in the examples described above (FHBL, MTP blockade and administration of SCD1 antisense), the resulting high liver triglyceride content does not result in DAG/nPKC-induced hepatic insulin resistance [100••,105,107•].

Possibly contradicting this suggestion is the report that the transgenic mice overexpressing DGAT2 in the liver,

Figure 1 (*Continued*)

expected to promote hepatic lipid accumulation. The esterification of hepatic FFA to triglycerides (TG) stored in hepatocytes can increase diacylglycerol (DAG) levels (?); as can the lipolysis, re-esterification, recycling of cytosolic TG that is associated with VLDL assembly (??). Either or both of these sources of DAG may lead to activation of nPKC and impaired hepatic insulin signaling/insulin resistance. VLDL production is regulated by the hepatic lipid supply and is further upregulated by insulin resistance. Hyperinsulinemia may increase DNL because of insulin's ability to activate SREBP1-c (*). Insulin promotes ApoB degradation and inhibits MTP(**), and both of these processes are likely to be downregulated in the insulin-resistant liver. Increased VLDL production and secretion lead to hypertriglyceridemia. Whether increased VLDL secretion and elevated triglyceride levels directly promote visceral adiposity warrants further investigation (???). Postprandial hypertriglyceridemia increases cardiovascular disease (CVD) risk by promoting lipid/lipoprotein remodeling leading to increased circulating concentrations of small dense LDL (sdLDL), remnant lipoproteins (RLP), and oxidized LDL (oxLDL) and decreased concentrations of HDL. Hypertriglyceridemia and increased levels of circulating FFA can promote accumulation of IMCL, DAG production, and nPKC activation and impaired insulin signaling in skeletal muscle. The end result is whole body insulin resistance, which, when accompanied by inadequate pancreatic beta cell compensation, leads to type 2 diabetes.

described above as having increased liver triglyceride accumulation and normal insulin sensitivity, also had increased hepatic DAG content [106•]. The increased DAG accumulation, however, may have resulted from the upregulation of the initial synthesis of triglyceride, which increased triglyceride stores, rather than from lipolysis, re-esterification and recycling associated with VLDL assembly. The circulating triglyceride concentrations of the DGAT2 transgenic mice were reduced compared with the wild-type control mice, which suggests VLDL assembly was not upregulated by DGAT2 overexpression [106•]. Another recent study also reported that mice overexpressing DGAT2, after injection of adenovirus containing DGAT2 transgene, had increased liver triglyceride, but levels of plasma triglyceride and the hepatic production rate of VLDL were not affected [109]. It has been recently reported that hepatic levels of DAG were increased in patients with NAFLD and nonalcoholic steatohepatitis compared with control subjects; however, the authors noted that the impact of the location of DAG within the hepatocyte requires investigation [110•].

Conclusion

A sustained and moderate positive energy balance is likely to promote metabolic syndrome by increasing visceral-fat accumulation, resulting in increased portal delivery of FFA to the liver. A high-fructose diet may more directly and rapidly produce a lipid oversupply within the liver via increased DNL. An oversupply of hepatic lipid results in liver triglyceride deposition and increased VLDL assembly and secretion. It has been proposed [59••] that the liver triglyceride accumulation is associated with increased levels of DAG that activate nPKC and disrupt insulin signaling. Several recent studies, however, reporting a disconnect between liver triglyceride accumulation and insulin resistance [100••,107•] provide support for our hypothesis that there may be steps downstream of liver triglyceride accumulation (for example, VLDL production or secretion) that are associated with the induction of hepatic insulin resistance.

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