Hepatic Steatosis: A Mediator of the Metabolic Syndrome: Lessons From Animal Models

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Abstract—Epidemiological studies in humans, as well as experimental studies in animal models, have shown an association between visceral obesity and dyslipidemia, insulin resistance, and type 2 diabetes mellitus. Recently, attention has been focused on the excessive accumulation of triglycerides (TG) in the liver as part of this syndrome. In this review, important principles of the pathophysiological involvement of the liver in the metabolic syndrome obtained in rodent models are summarized. We focus on non-alcoholic causes of steatosis, because the animal experiments we refer to did not include alcohol as an experimental condition. In general, there is continuous cycling and redistribution of non-oxidized fatty acids between different organs. The amount of TG in an intrinsically normal liver is not fixed but can readily be increased by nutritional, metabolic, and endocrine interactions involving TG/free fatty acid (FFA) partitioning and TG/FFA metabolism. Several lines of evidence indicate that hepatic TG accumulation is also a causative factor involved in hepatic insulin resistance. Complex interactions between endocrine, metabolic, and transcriptional pathways are involved in TG-induced hepatic insulin resistance. Therefore, the liver participates passively and actively in the metabolic derangements of the metabolic syndrome. We speculate that similar mechanisms may also be involved in human pathophysiology. (Arterioscler Thromb Vasc Biol. 2004;24:1-6.)

Key Words: lipoprotein metabolism ■ glucose metabolism ■ fatty acid metabolism ■ insulin resistance ■ mouse models

Epidemiological studies in humans have documented an association between visceral obesity and cardiovascular risk factors such as dyslipidemia, insulin resistance, and type 2 diabetes mellitus.1–4 Recently, attention has been focused on the excessive accumulation of triglycerides (TG) within the liver as part of this metabolic syndrome. It appears that fat accumulation in the liver is associated with several features of insulin resistance even in normal-weight and moderately overweight subjects.5 Nonetheless, from these observations in humans it remains unclear to what extent hepatic steatosis is a cause rather than a consequence of the metabolic syndrome. This issue is difficult to solve, because the liver is not readily accessible in humans. Therefore, in the present review we focus on mouse models with variations in liver TG content induced by targeted interventions to elucidate the role of liver steatosis in metabolic diseases like dyslipidemia, insulin resistance, and type 2 diabetes mellitus. Although alcohol-induced liver steatosis was already described by Thomas Addison in 1845, it is appreciated only since 1962 that steatosis can also occur without the use of alcohol, so-called non-alcoholic steatosis.6 The current review focuses on non-alcoholic causes of steatosis, because the animal experiments we refer to did not include alcohol as an experimental condition. We briefly describe factors involved in body TG homeostasis, intrahepatic and extrahepatic factors causing steatosis, the metabolic consequences of steatosis on very-low-density lipoprotein (VLDL) TG, and glucose production and potential molecular mechanisms mediating the effects of intrahepatic TG accumulation on hepatic metabolic function.

Whole-Body TG Homeostasis

The TG content of hepatocytes is regulated by the integrated activities of cellular molecules that facilitate hepatic TG uptake, fatty acid synthesis, and esterification through one mechanism (“input”), and hepatic fatty acid oxidation and TG export through the other (“output”). Steatosis occurs when “input” exceeds the capacity for “output.” The liver acts in concert with other organs in the orchestration of inter-organ fatty acid/TG partitioning. Therefore, we describe whole-body TG homeostasis.

In the absorptive state, dietary TGs are transported by the blood to peripheral organs in the form of chylomicrons (Figure 1A). Lipoprotein lipase (LPL) is required for the intravascular hydrolysis of plasma chylomicron TGs and VLDL TGs into fatty acids. Through the tissue-specific...
action of LPL, the TG-derived free fatty acids (FFA) are taken-up mainly locally in peripheral tissues. LPL is stimulated by insulin, especially in adipose tissue, and by exercise, especially in muscle. After the hydrolysis of a large part of the TGs in chylomicrons by LPL, remnant particles remain, which are transported to and taken-up by the liver.

In the post-absorptive (fasting) state, whole-body TG metabolism differs from that of the absorptive state (Figure 1B). The TGs contained within adipose tissue are continuously being hydrolyzed into fatty acids and glycerol by the enzyme hormone-sensitive lipase (HSL). Because HSL is inhibited by insulin, the activity of HSL increases in the low insulin state of fasting. Although some of the fatty acids released by HSL are re-esterified within adipocytes, most fatty acids are released into the blood and transported as FFAs to other organs. In resting, ie, non-exercise, conditions, the amount of fatty acids released by adipose tissue is considerably larger than the amount required for oxidative purposes. In this respect, the liver is of paramount importance, because the liver takes-up a considerable part of these fatty acids.

Within the liver, these fatty acids are either oxidized or re-esterified into TGs, which can be secreted into the blood in the form of VLDL TG. The fatty acids re-esterified by the liver into TG are derived almost exclusively from the fatty acids initially released by adipose tissue. In turn, VLDL TG are directed toward different tissues, depending on the tissue-specific availability of LPL.

Thus, there is a continuous cycling and redistribution of non-oxidized fatty acids between different organs, especially in the post-absorptive state, with a central role for the liver and the adipose tissue (Figure 2).

**Extrahepatic Causes of Steatosis**

A major cause of steatosis is increased fatty acid flux to the liver caused by a high availability of plasma FFA in relation to peripheral oxidative requirements. Several conditions increase the fatty acid flux to the liver. An increase of exogenous fat, ie, high-fat feeding, increases liver TG content. This increase in hepatic TG content can occur within 10 days after starting the high-fat diet in mice. Overnight
fasting increases plasma FFA to such an extent that liver TG content increases in mice (unpublished observations). This flexibility of the liver to accommodate excessive plasma fatty acids in the form of hepatic TGs after overnight fasting was demonstrated already in 1970 in dogs.13 These observations indicate that the amount of liver TG content is not fixed but can readily be modulated by nutritional conditions in otherwise normal livers.

Fatty acid delivery to the liver can also be increased because of disturbances in fatty acid/TG partitioning between different organs. This is illustrated by several observations. Mice lacking CD36, a fatty acid transporter in muscle and adipose tissue, have increased plasma fatty acid levels and show liver steatosis.14,15 Conversely, mice lacking HSL have low plasma FFA levels and low hepatic TG content.16 Finally, muscle-specific modulation of lipoprotein lipase may result in altered distribution of tissue TGs. In mice with muscle-specific LPL overexpression, muscle TG content is increased, whereas liver TG content is decreased compared with wild-type mice.17 These observations in mouse models without excessive changes in adipose tissue mass prove that alterations in whole-body FFA/TG partitioning inversely modulate TG content in the liver.

The extrahepatic regulation of liver TG content is not merely a function of plasma FFA delivery alone. Mouse models of lipodystrophy and models of its reverse condition, obesity, illustrate this. In both conditions, steatosis is present but can only partly be related to increased plasma FFA and TG levels. However, lipodystrophy and obesity are complex conditions, with changes other than those reflected merely in the fatty acid/TG metabolism. Adipose tissue is not just an organ designed for passive storage and release of TGs. In addition, adipose tissue also actively participates in the integration of whole-body energy and fuel metabolism by the secretion of many hormones. Important hormones, which are derived from adipose tissue and modulate hepatic TG content, are adiponectin, leptin, and resistin.18 Adiponectin decreases TG content in the muscle and liver of obese mice, and decreased adiponectin levels have been implicated in the development of steatosis in mouse models of obesity and lipodystrophy.19 Leptin decreases the hepatic accumulation of TGs in the A-ZIP/F-1 mouse, a model of severe lipodystrophy and low leptin levels.20 Finally, tissue-specific overexpression of wild-type leptin receptors in the steatotic livers of obese (fa/fa) Zucker rats, which have an inactivating mutation in the leptin receptor, reduced TG accumulation in the liver but not in other non-adipose tissues. It has therefore been proposed that the physiological role of leptinemia in conditions of caloric excess is to protect non-adipose tissue from steatosis by preventing the upregulation of lipogenesis and increasing fatty acid oxidation.21 These examples indicate that an intrinsically normal liver may develop steatosis because of nutritional, metabolic, and endocrine interactions involving interorgan TG/FFA partitioning and TG/FFA metabolism.

**Intrahepatic Causes of Steatosis**

Several intrahepatic mechanisms induce steatosis. These changes involve alterations in hepatic glucose and/or fatty acid metabolism. Increased de novo hepatic synthesis of fatty acids and subsequent esterification into TGs is an important cause of steatosis. This is illustrated by several examples. First, high-sucrose feeding induces liver steatosis by increased de novo lipogenesis.11,22 Second, inhibition of microsomal TG transfer protein by S4048 results in hepatic entrapment of glucose and de novo lipogenesis, leading to massive steatosis within several hours.23 Third, inhibition of fatty acid oxidation in the liver is another intrahepatic cause of the development of liver steatosis. For instance, etomoxir, a carnitine O-palmitoyltransferase-1 (CPT-1)-inhibitor, inhibits fatty acid oxidation and induces steatosis.24 These observations indicate that steatosis can be caused by intrahepatic alterations in glucose and fat metabolism, independently of extrahepatic conditions. For a detailed summary of other rodent models with steatosis, we refer to Koteish and Diehl.24

**Steatosis and VLDL TG Secretion**

A number of studies have addressed the relation between steatosis and basal VLDL TG production in mice and rats, and vice versa. Inhibition of microsomal TG transfer protein
creased. This decrease in VLDL production despite the high hepatic VLDL production is not increased but rather decreased. This decrease in VLDL production despite the high fatty acid flux to the liver contributes to the massive steatosis that is observed in these animals. In CD36-deficient mice, the flux of fatty acids toward the liver is increased, precipitating steatosis, but there is no evidence of an increase in hepatic VLDL production (unpublished observations). Thus, availability of fatty acids is not the only determinant of the rate of hepatic VLDL TG production.

In mice with increased de novo lipogenesis in the liver, VLDL TG production can be either unaltered or increased, probably depending on the cause of the increase in de novo lipogenesis and the capacity of the liver to increase fatty acid oxidation to get rid of the excess fatty acids. The inhibition of glucose-6-phosphatase by S4048 results in an increase in de novo lipogenesis and hepatic TG content without any stimulation of hepatic VLDL TG production. In contrast, hamsters with increased de novo lipogenesis as a consequence of a diet high in fructose have increased basal hepatic VLDL TG production. When lipogenesis is increased by pharmacological activation of the liver X receptor, hepatic VLDL production is increased 2.5-fold, and the liver produces large TG-rich VLDL particles. Therefore, it is likely that different molecular mechanisms are involved to explain the relation between steatosis and the rate of basal VLDL production in different conditions.

**Steatosis and Hepatic Insulin Resistance**

Steatosis is associated with hepatic insulin resistance, which means that the liver is less sensitive to the suppressive effects of insulin on hepatic glucose and VLDL TG production. If the ability of insulin to suppress the hepatic output of glucose and VLDL is decreased, then this contributes to (postprandial) hyperglycaemia and hyperlipidaemia, intrinsic features of the metabolic syndrome. As such, steatosis is not only a consequence of but also a major contributor to the metabolic syndrome.

The inhibitory effects of insulin on VLDL production involve peripheral effects, because insulin inhibits FFA release from adipose tissue, as well as the direct hepatic effects of insulin on hepatic VLDL TG assembly/secretion. Because the effects of steatosis on insulin sensitivity of hepatic VLDL TG production are complex and have been less extensively studied than those of glucose metabolism, we focus on insulin resistance of the hepatic glucose metabolism.

There is an inverse relationship between hepatic TG content and hepatic insulin sensitivity (Figure 3). We observed this inverse relationship in transgenic mice with targeted disruptions in TG/FFA partitioning. Interestingly, mice with decreased hepatic TG content compared with wild-type controls, such as mice with muscle-specific overexpression of LPL or HSL, revealed increased insulin sensitivity. Apparently, the relationship between hepatic TG content and insulin sensitivity holds true for increased and decreased hepatic TG stores. The more complex mouse models of obesity, like ob/ob mice, and its counterpart, lipodystrophic mice, have steatosis with severe hepatic insulin resistance. Adiponectin and leptin are capable of reversing not only steatosis but also hepatic insulin resistance in these mice. These observations further strengthen the notion that hepatic TG accumulation is a causative factor involved in hepatic insulin resistance.

Paradoxically, this relationship between steatosis and insulin resistance is dissociated in some mouse models by treatment with thiazolidinediones. These PPARγ-activators improve hepatic insulin resistance despite the augmentation of steatosis in obese and diabetic mice, but not in lean controls. The mechanisms that underlie this paradox have not yet been elucidated.

**Molecular Mechanisms Involved in Hepatic Insulin Sensitivity**

Insulin acts by stimulating the insulin receptor by sequential phosphorylation of proteins of the insulin-signaling pathway. Through these proteins, insulin exerts its metabolic effects, e.g., on glucose transport, glycogen synthesis, and lipid synthesis. In addition, the insulin-signaling pathway interacts
with transcription factors, resulting in altered transcription of a multitude of genes, involved in a variety of cellular functions.\textsuperscript{39-41} Strong indications exist that alterations in hepatic fatty acid/TG content modulate this insulin-signaling cascade. The expression of insulin receptors and phosphoinositol-3 kinase–mediated protein kinase B (PKB) phosphorylation are considerably decreased in a mouse model with steatosis and hepatic insulin resistance, such as CD36\textsuperscript{−/−} mice.\textsuperscript{15} Conversely, the expression of the insulin receptor and activation of phosphoinositol-3 kinase–mediated PKB phosphorylation are increased in a mouse model of decreased hepatic TG content and increased hepatic insulin sensitivity, like HSL\textsuperscript{−/−}.\textsuperscript{14} Apparently, the inverse relationship between hepatic TG stores and insulin sensitivity is linked to the activity of the insulin-signaling cascade at a molecular level.

There are indications that a direct interaction between fatty acid derivatives and components of the insulin-signaling cascade are involved in the fatty acid-induced insulin resistance.\textsuperscript{42} Fatty acid intermediates like diacylglycerols are known to stimulate certain protein kinase C (PKC). PKCs promote threonine phosphorylation of the insulin receptor and its substrates, thereby blocking the insulin cascade. Furthermore, fatty acid derivatives act as agonists and antagonists for nuclear transcription factors like PPARs, SREBs, and liver X receptor. In addition to their regulation by different fatty acid metabolites, these transcription factors are the targets for hormones, like insulin and leptin, growth factors, and inflammatory signals. Therefore, they appear to be a point of signaling convergence at a gene regulatory level.\textsuperscript{43} These transcription factors profoundly alter the expression of enzymes and proteins that are involved in glucose and lipid metabolism. We postulate that these effects on gene expression include alterations in the insulin-signaling cascade.

Therefore, the understanding of the extremely complex interaction between fatty acid derivatives and nuclear transcription factors is pivotal for understanding the relation between steatosis and the metabolic syndrome. This is illustrated by several observations in mice. PPARs are a family of nuclear receptors that have profound effects on gene expression and are involved in the modulation of glucose and lipid metabolism by complex mechanisms that are beyond the scope of this review. Nonetheless, several observations in mice point to a relationship between the activity of these receptors and hepatic insulin sensitivity. PPAR\textsubscript{α} is mainly expressed in the liver. It is important in the regulation of several key enzymes in fatty acid oxidation. PPAR\textsubscript{α}\textsuperscript{−/−} mice have extensive hepatic steatosis after short-term fasting because of the considerably diminished hepatic oxidation capacity.\textsuperscript{44} Drugs that activate PPAR\textsubscript{α} reduce liver TG content and improve hepatic insulin sensitivity in rodent models of liver steatosis.\textsuperscript{45,46} Remarkably, PPAR\textsubscript{α}\textsuperscript{−/−} mice are protected against high-fat–induced insulin resistance.\textsuperscript{47} This indicates that transcription factors like PPAR\textsubscript{α} are involved in the interaction between hepatic fatty acid metabolism and hepatic insulin resistance.

There are indications that inflammatory pathways are subclinically stimulated in insulin resistance. In tissues obtained from Zucker fa/fa rats, which have steatosis, basal IκB kinase\textsuperscript{α}(IKK\textsuperscript{α}) activity was increased when compared with lean fa/− controls. IKK\textsubscript{β} is a proximal activator of the transcription factor NF-κB. Inhibition of NF-κB by aspirin reverses hyperglycaemia, hyperinsulinemia, and dyslipidemia in obese rodents by sensitizing insulin signaling. The blunted insulin-stimulated phosphorylation of PKB in the livers of untreated Zucker rats was increased after salicylate treatment, providing a biochemical correlate for increased in vivo insulin sensitivity. Activation or overexpression of IKK\textsubscript{β} attenuated insulin signaling in cultured cells, whereas IKK\textsubscript{β} inhibition reversed insulin resistance.\textsuperscript{48} These observations suggest that NF-κB may be another transcription factor involved in steatosis-related hepatic insulin resistance.

To summarize, there are multiple endocrine, metabolic, and transcriptionally active factors involved in the interaction between hepatic fatty acid/TG metabolism and hepatic insulin sensitivity. The hierarchy between these different factors in modulating hepatic insulin sensitivity is at present unclear. Because the prevalence of metabolic syndrome reaches epidemic proportions, it is important to investigate the causes and consequences of this syndrome in human and in animal studies. The combination of these studies may lead to a better prevention and treatment of the metabolic syndrome.

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References


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