Hepatic steatosis : metabolic consequences

Boer, A.M. den

Chapter 2

Hepatic Steatosis: a Mediator of the Metabolic Syndrome

Lessons from animal models

Arterioscler Thromb Vasc Biol. **2004; 24: 644-649**

Marion A.M. den Boer[1,2], P.J. Voshol[1,2], F. Kuipers[5], L.M. Havekes[1,3,4], J.A. Romijn[2]

[1]TNO Prevention and Health, Gaubius Laboratory Leiden, [2]Department of Endocrinology and Diabetes, [3]Department of General Internal Medicine, [4]Department of Cardiology, Leiden University Medical Center and [5]Center for Liver, Digestive and Metabolic Diseases, Department of Pediatrics, University Hospital Groningen, Groningen, Netherlands.

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 this metabolic syndrome obtained in rodent models are summarized. The current review focuses on non-alcoholic causes of steatosis, since 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 (FA) between different organs and the liver acts in concert with other organs, especially adipose tissue, in the orchestration of this inter-organ FA/TG partitioning. The amount of TG in an intrinsically normal liver is not fixed, but can readily be increased by nutritional, metabolic and endocrine interactions involving both TG/FA partitioning and TG/FA metabolism. Steatosis can also be induced by intrahepatic changes in glucose and FA/TG metabolism, independently of extrahepatic conditions. Steatosis is not merely a change in hepatic TG storage, but also reflects changes in the regulation of hepatic metabolic function. VLDL-TG production rates can be decreased, normal or increased in steatosis.

Several lines of evidence indicate that hepatic TG accumulation is also a causative factor involved in hepatic insulin resistance, defined by a decreased ability of insulin to suppress hepatic glucose production. Complex interactions between endocrine, metabolic and transcriptional pathways are involved in TG-induced hepatic insulin resistance. Therefore, the liver participates both passively and actively in the metabolic derangements of the metabolic syndrome. We speculate that similar mechanisms may also be involved in human pathophysiology.

Introduction

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, since the liver is not readily accessible in humans. Therefore, we focus in the present review on mouse models with variations in liver TG content induced by targeted interventions, in order 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, since the animal experiments we refer to, did not include alcohol as an experimental condition. We will briefly describe factors involved in body TG homeostasis, intra- and extrahepatic factors causing steatosis, the metabolic consequences of steatosis on 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, FA synthesis, and esterification on the one hand ("input") and hepatic FA oxidation and TG export on 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 FA/TG partitioning. Therefore, we will first describe whole body TG homeostasis.

In the absorptive state, dietary TG 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-, as well as VLDL-TG into FA.

Through the tissue-specific action of LPL the TG-derived FA are taken up mainly locally in peripheral tissues.[7] 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.[8,9]

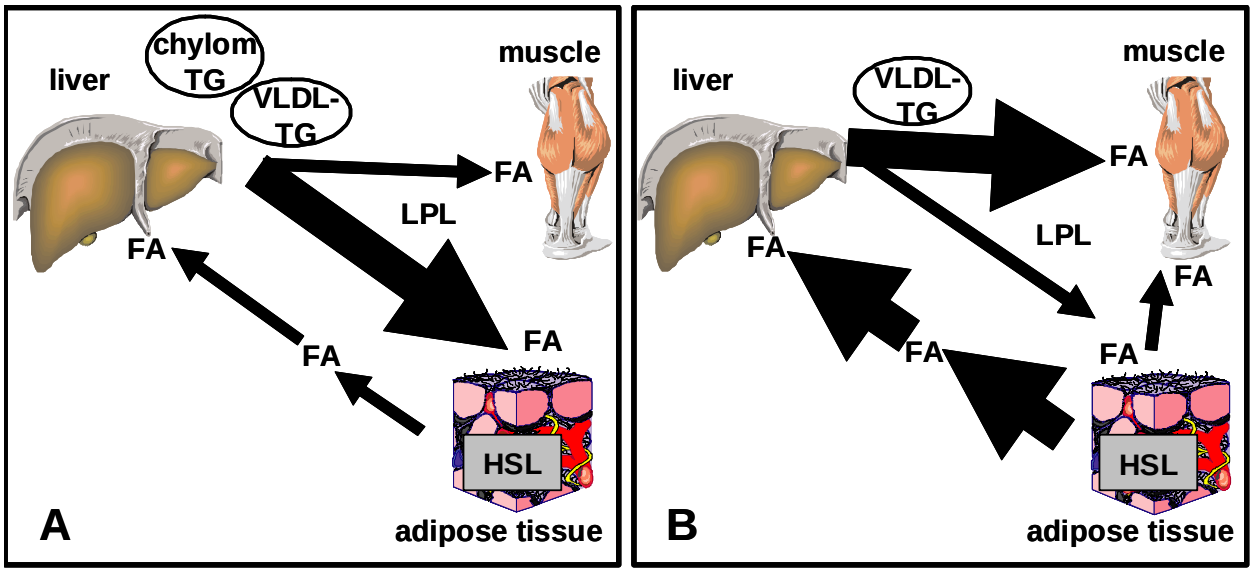

Figure 1. Diversion of fatty acids towards peripheral tissues. A. In the fed state chylomicron-triglycerides and VLDL-triglycerides are lipolyzed by lipoprotein lipase to generate fatty acids, that are mainly taken up by muscle and adipose tissue for oxidation and esterification into triglycerides, especially in the adipose tissue. **B.** In the fasting state triglycerides within the adipose tissue are lipolyzed by the enzyme hormone-sensitive lipase and fatty acids are released into the blood in excess of oxidative requirements. The excessive fatty acids can be taken up by the liver, for oxidation or for synthesis of VLDL-triglycerides. The arrows indicate the fluxes of fatty acids. FA = fatty acids, LPL = lipoprotein lipase, HSL = hormone-sensitive lipase, VLDL = very low density lipoprotein, chylom = chylomicrons derived from the intestine.

In the post-absorptive (fasting) state, whole-body TG metabolism differs from that of the absorptive state (Figure 1B). The TG contained within adipose tissue are continuously being hydrolyzed into FA and glycerol by the enzyme hormone-sensitive lipase (HSL).[10] Because HSL is inhibited by insulin, the activity of HSL increases in the low insulin state of fasting. Although some of the FA released by HSL are re-esterified within adipocytes, most FA are released into the blood and transported as free FA to other organs. In resting, i.e. non-exercise, conditions the amount of FA

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 FA. Within the liver these FA are either oxidized or re-esterified into TG, which can be secreted into the blood in the form of VLDL-TG. The FA re-esterified by the liver into TG are derived almost exclusively from the FA initially released by adipose tissue.[11] In turn, VLDL-TG are directed towards different tissues, depending on the tissue-specific availability of LPL.

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

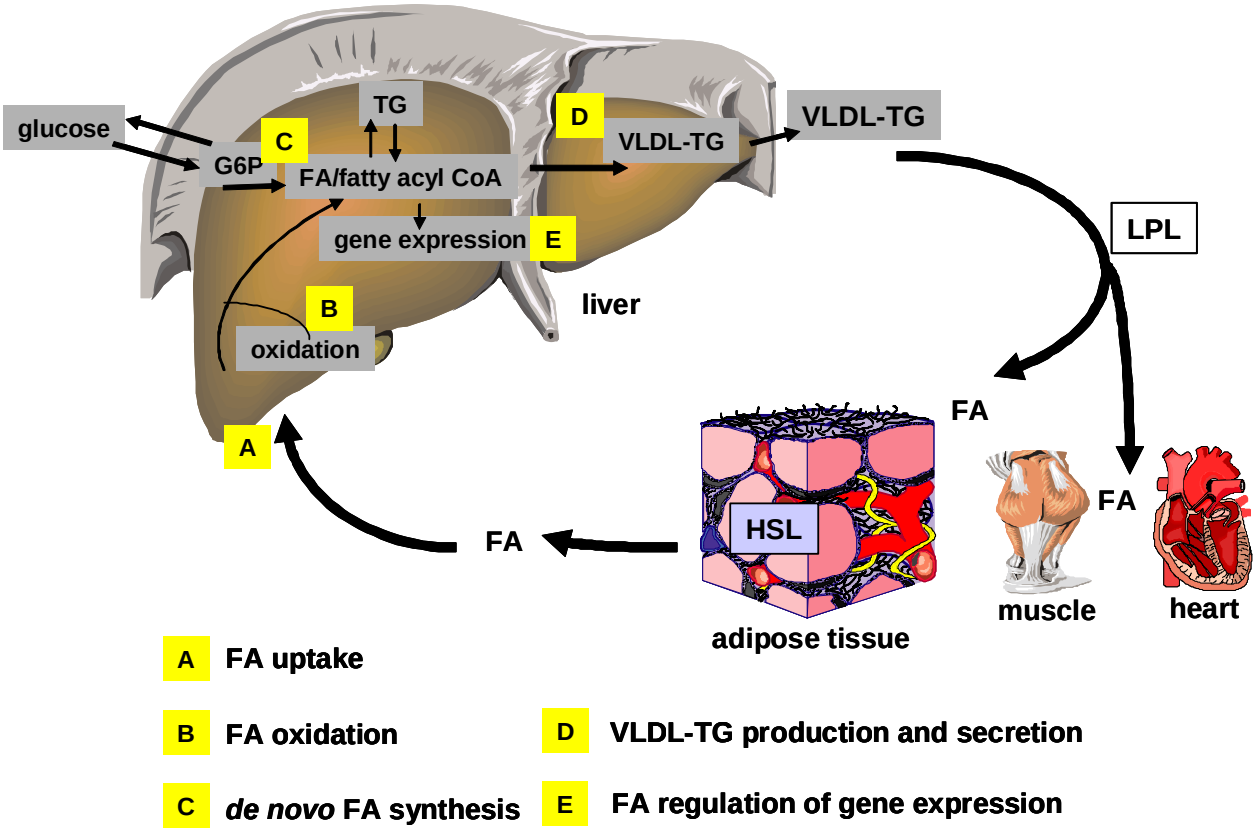

Figure 2. Major pathways of hepatic FA/TG metabolism in the liver. The liver plays a central role in lipid metabolism through **A** Uptake of fatty acids, **B** Fatty acid oxidation, **C** De *novo* fatty acid synthesis, **D** Assembly and secretion of VLDL-TG, **E** Effects of fatty acids on gene expression. FA = fatty acids, HSL = hormone sensitive lipase, LPL = lipoprotein lipase, G6P = glucose-6-phosphate.

Extrahepatic causes of steatosis

A major cause of steatosis is increased FA flux to the liver due to a high availability of plasma FA in relation to peripheral oxidative requirements. Several conditions increase the FA flux to the liver. An increase of exogenous fat, i.e. high-fat feeding, increases liver TG content.[12] This increase in hepatic TG content can occur within 10 days after starting the high fat diet in mice. Overnight fasting increases plasma FA to such an extent, that liver TG content increases in mice (unpublished observations). This flexibility of the liver to accommodate excessive plasma FA the form of hepatic TG after overnight fasting in 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.

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

The extrahepatic regulation of liver TG content is not merely a function of plasma FA 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 FA and TG levels. However, lipodystrophy and obesity are complex conditions, with changes other than those reflected merely in the FA/TG metabolism. Adipose tissue is not only an organ designed for passive storage and release of TG. 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 both obesity and lipodystrophy.[19] Leptin decreases the hepatic accumulation of TG 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 physiologic role of leptinemia in conditions of caloric excess is to protect non-adipose tissue from steatosis by preventing the up-regulation of lipogenesis and increasing FA oxidation.[21] These examples indicate that an intrinsically normal liver may develop steatosis due to nutritional, metabolic and endocrine interactions involving both inter-organ TG/FA partitioning and TG/FA metabolism.

Intrahepatic causes of steatosis

Several intrahepatic mechanisms induce steatosis. These changes involve alterations in hepatic glucose and/or FA metabolism. Increased *de novo* hepatic synthesis of FA and subsequent esterification into TG is an important cause of steatosis. This is illustrated by several examples. Firstly, high sucrose feeding induces liver steatosis by increased *de novo* lipogenesis.[11,22] Secondly, inhibition of glucose-6-phosphatase by S4048 results in hepatic entrapment of glucose and *de novo* lipogenesis, leading to massive steatosis within several hours.[23] Thirdly, inhibition of FA oxidation in the liver is another intra-hepatic cause of the development of liver steatosis. For instance, etomoxir, a carnitine O-palmitoyltransferase-1 (CPT-1)-inhibitor, inhibits FA oxidation and induces steatosis.[24] These observations indicate that steatosis can be caused by intra-hepatic 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 (MTP) impairs the assembly and probably the secretion of VLDL-TG particles and results in intrahepatic accumulation of TG.[25] Although the inverse relation between steatosis and VLDL production is self-evident in the case of MTP blockers, in other conditions the relation between steatosis and VLDL-production is not

straightforward, which is illustrated by several examples. In obese *ob/ob* mice, which have steatosis, hepatic VLDL production is not increased, but rather even decreased.[26] This decrease in VLDL production despite the high FA flux to the liver contributes to the massive steatosis that is observed in these animals. In CD36-deficient mice the flux of FA towards the liver is increased, precipitating steatosis, but there is no evidence of an increase in hepatic VLDL production (unpublished observations). Thus, availability of FA 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 FA ß-oxidation to get rid of the excess FA. 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.[23] In contrast, hamsters with increased *de novo* lipogenesis as a consequence of a diet high in fructose, have increased basal hepatic VLDL-TG production.[27] When lipogenesis is increased by pharmacological activation of the liver X receptor (LXR), hepatic VLDL-TG production is increased 2.5-fold and the liver produces large TG-rich VLDL particles.[28] 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-production.[29-32] If the ability of insulin to suppress the hepatic output of glucose and VLDL is decreased, this contributes to (postprandial) hyperglycemia and hyperlipidemia, 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 FA release from adipose tissue, as well as the direct hepatic effects of insulin on hepatic VLDL-TG assembly/secretion.[33] 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.

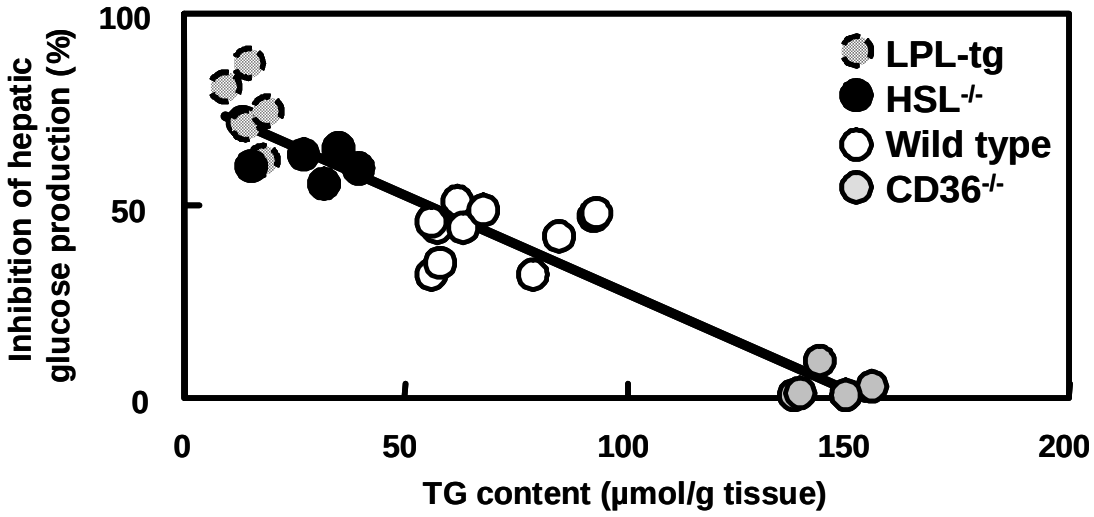

Figure 3. Insulin-mediated inhibition of hepatic glucose production is related to hepatic TG content. Muscle-specific LPL-overexpressing mice (LPL-tg) show increased TG content in the muscle, whereas liver TG content is decreased compared to wild-type mice. During a hyperinsulinemic euglycemic clamp the livers in these mice showed increased sensitivity to the suppressive effect of insulin on hepatic glucose production. Mice deficient in hormone-sensitive lipase ($HSL^{-/-}$) showed decreased hepatic TG content and increased inhibition of hepatic glucose production compared to wild-type mice. $CD36^{-/-}$ mice lacking the FA transporter that is normally present in muscle and adipose tissue, showed increased hepatic TG content and a decreased sensitivity of hepatic glucose production to insulin.[15-17]

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/FA partitioning. Interestingly, mice with decreased hepatic TG content compared to wild-type controls, such as mice with muscle-specific overexpression of LPL or $HSL^{-/-}$ mice, revealed increased insulin sensitivity.[16,17] Apparently, the relationship between hepatic TG content and insulin sensitivity holds true for both increased and decreased hepatic TG stores. The more complex mouse models of obesity, like the *ob/ob* mice, and its counterpart, the lipodystrophic mice, have steatosis with severe hepatic insulin resistance.[34-36] Adiponectin and leptin are not only capable of reversing 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.[37] 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.[38] 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.[39-41] Strong indications exist that alterations in hepatic FA/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 $^{-/-}$ mice.[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 in the HSL$^{-/-}$ mice.[16] 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 FA derivatives and components of the insulin-signaling cascade are involved in the FA-induced insulin resistance.[42] FA intermediates like diacylglycerols are known to stimulate certain protein kinase Cs (PKC). PKCs promote threonine phosphorylation of the insulin receptor and its substrates, thereby blocking the insulin cascade. Furthermore, FA derivatives act as agonists and antagonists for nuclear transcription factors like PPARs, SREBPs and LXR. In addition to their regulation by different FA 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.[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 FA 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. PPARα is mainly expressed in the liver. It is important in the regulation of several key enzymes in FA oxidation. PPARα$^{-/-}$ mice develop extensive hepatic steatosis after short-term fasting due to the considerably diminished hepatic oxidation capacity.[44] Drugs that activate PPARα, reduce liver TG content and improve hepatic insulin sensitivity in rodent models of liver steatosis.[45,46] Remarkably, PPARα$^{-/-}$ mice are protected against high fat induced insulin resistance.[47] This indicates that transcription factors like PPARα are involved in the interaction between hepatic FA metabolism and hepatic insulin resistance.

There are indications that inflammatory pathways are sub-clinically stimulated in insulin resistance. In tissues obtained from Zucker *fa/fa* rats, which have steatosis, basal IκB kinase β (IKKβ) activity was increased when compared to lean *fa/+* controls. IKKβ is a proximal activator of the transcription factor NF-κB. Inhibition of NF-κB by aspirin reverses hyperglycemia, 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 the IκB kinase β (IKKβ) attenuated insulin signaling in cultured cells, whereas IKKβ inhibition reversed insulin resistance.[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 FA/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 the metabolic syndrome reaches endemic proportions, it is important to investigate the causes and consequences of this syndrome both in human and in animal studies. The

combination of these studies may lead to a better prevention and treatment of the metabolic syndrome.

Ackowledgements

This work was supported by the Netherlands Organization for Scientific Research (NWO grants 903-39-291 and 916-36-071).

References

1. Arad Y, Newstein D, Cadet F, Roth M, Guerci AD. Association of multiple risk factors and insulin resistance with increased prevalence of asymptomatic coronary artery disease by an electron-beam computed tomographic study. *Arterioscler Thromb Vasc Biol.* 2001;21:2051-2058.
2. Laakso M, Lehto S. Epidemiology of risk factors for cardiovascular disease in diabetes and impaired glucose tolerance. *Atherosclerosis.* 1998;137 Suppl:S65-S73.
3. Tayama K, Inukai T, Shimomura Y. Preperitoneal fat deposition estimated by ultrasonography in patients with non-insulin-dependent diabetes mellitus. *Diabetes Res Clin Pract.* 1999;43:49-58.
4. Fujimoto WY, Bergstrom RW, Boyko EJ, Chen KW, Leonetti DL, Newell-Morris L, Shofer JB, Wahl PW. Visceral adiposity and incident coronary heart disease in Japanese-American men. The 10-year follow-up results of the Seattle Japanese-American Community Diabetes Study. *Diabetes Care.* 1999;22:1808-1812.
5. Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, Goto T, Westerbacka J, Sovijarvi A, Halavaara J, Yki-Jarvinen H. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J Clin Endocrinol Metab.* 2002;87:3023-3028.
6. Leevy CM. Fatty liver: a study of 270 patients with biopsy proven fatty liver and review of the literature. *Medicine (Baltimore).* 1962;41:249-276.
7. Farese RVJ, Yost TJ, Eckel RH. Tissue-specific regulation of lipoprotein lipase activity by insulin/glucose in normal-weight humans. *Metabolism.* 1991;40:214-216.
8. Brown MS, Kovanen PT, Goldstein JL. Regulation of plasma cholesterol by lipoprotein receptors. *Science.* 1981;212:628-635.
9. Rubinstein A, Gibson JC, Paterniti JRJ, Kakis G, Little A, Ginsberg HN, Brown WV. Effect of heparin-induced lipolysis on the distribution of apolipoprotein e among lipoprotein subclasses. Studies with patients deficient in hepatic triglyceride lipase and lipoprotein lipase. *J Clin Invest.* 1985;75:710-721.
10. Holm C, Osterlund T, Laurell H, Contreras JA. Molecular mechanisms regulating hormone-sensitive lipase and lipolysis. *Annu Rev Nutr.* 2000;20:365-393.

11. McDevitt RM, Bott SJ, Harding M, Coward WA, Bluck LJ, Prentice AM. De novo lipogenesis during controlled overfeeding with sucrose or glucose in lean and obese women. *Am J Clin Nutr.* 2001;74:737-746.
12. Gauthier MS, Couturier K, Latour JG, Lavoie JM. Concurrent exercise prevents high-fat-diet-induced macrovesicular hepatic steatosis. *J Appl Physiol.* 2003;94:2127-2134.
13. Basso LV, Havel RJ. Hepatic metabolism of free fatty acids in normal and diabetic dogs. *J Clin Invest.* 1970;49:537-547.
14. Coburn CT, Knapp FFJ, Febbraio M, Beets AL, Silverstein RL, Abumrad NA. Defective uptake and utilization of long chain fatty acids in muscle and adipose tissues of CD36 knockout mice. *J Biol Chem.* 2000;275:32523-32529.
15. Goudriaan JR, Dahlmans VE, Teusink B, Ouwens DM, Febbraio M, Maassen JA, Romijn JA, Havekes LM, Voshol PJ. CD36 deficiency increases insulin sensitivity in muscle, but induces insulin resistance in the liver in mice. *J Lipid Res.* 2003;
16. Voshol PJ, Haemmerle G, Ouwens DM, Zimmermann R, Zechner R, Teusink B, Maassen JA, Havekes LM, Romijn JA. Increased hepatic insulin sensitivity together with decreased hepatic triglyceride stores in hormone-sensitive lipase-deficient mice. *Endocrinology.* 2003;144:3456-3462.
17. Voshol PJ, Jong MC, Dahlmans VE, Kratky D, Levak-Frank S, Zechner R, Romijn JA, Havekes LM. In muscle-specific lipoprotein lipase-overexpressing mice, muscle triglyceride content is increased without inhibition of insulin-stimulated whole-body and muscle-specific glucose uptake. *Diabetes.* 2001;50:2585-2590.
18. Guerre-Millo M. Adipose tissue hormones. *J Endocrinol Invest.* 2002;25:855-861.
19. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med.* 2001;7:941-946.
20. Ebihara K, Ogawa Y, Masuzaki H, Shintani M, Miyanaga F, Aizawa-Abe M, Hayashi T, Hosoda K, Inoue G, Yoshimasa Y, Gavrilova O, Reitman ML, Nakao K. Transgenic overexpression of leptin rescues insulin resistance and diabetes in a mouse model of lipoatrophic diabetes. *Diabetes.* 2001;50:1440-1448.
21. Lee Y, Wang MY, Kakuma T, Wang ZW, Babcock E, McCorkle K, Higa M, Zhou YT, Unger RH. Liporegulation in diet-induced obesity. The antisteatotic role of hyperleptinemia. *J Biol Chem.* 2001;276:5629-5635.
22. Bacon BR, Park CH, Fowell EM, McLaren CE. Hepatic steatosis in rats fed diets with varying concentrations of sucrose. *Fundam Appl Toxicol.* 1984;4:819-826.
23. Bandsma RH, Wiegman CH, Herling AW, Burger HJ, ter Harmsel A, Meijer AJ, Romijn JA, Reijngoud DJ, Kuipers F. Acute inhibition of glucose-6-phosphate translocator activity leads to increased de novo lipogenesis and development of hepatic steatosis without affecting VLDL production in rats. *Diabetes.* 2001;50:2591-2597.

24. Koteish A, Diehl AM. Animal models of steatosis. *Semin Liver Dis.* 2001;21:89-104.
25. Letteron P, Sutton A, Mansouri A, Fromenty B, Pessayre D. Inhibition of microsomal triglyceride transfer protein: another mechanism for drug-induced steatosis in mice. *Hepatology.* 2003;38:133-140.
26. Li X, Grundy SM, Patel SB. Obesity in db and ob animals leads to impaired hepatic very low density lipoprotein secretion and differential secretion of apolipoprotein B-48 and B-100. *J Lipid Res.* 1997;38:1277-1288.
27. Avramoglu RK, Qiu W, Adeli K. Mechanisms of metabolic dyslipidemia in insulin resistant states: deregulation of hepatic and intestinal lipoprotein secretion. *Front Biosci.* 2003;8:d464-d476.
28. Grefhorst A, Elzinga BM, Voshol PJ, Plosch T, Kok T, Bloks VW, van der Sluijs FH, Havekes LM, Romijn JA, Verkade HJ, Kuipers F. Stimulation of lipogenesis by pharmacological activation of the liver X receptor leads to production of large, triglyceride-rich very low density lipoprotein particles. *J Biol Chem.* 2002;277:34182-34190.
29. Bacon BR, Farahvash MJ, Janney CG, B.A. Nonalcoholic steatohepatitis: an expanded clinical entity. *Gastroenterology.* 1994;107:1103-1109.
30. Lee RG. Nonalcoholic steatohepatitis: a study of 49 patients. *Hum Pathol.* 1989;20:594-598.
31. Powell EE, Cooksley WG, Hanson R, Searle J, Halliday JW, Powell LW. The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. *Hepatology.* 1990;11:74-80.
32. Wanless IR, Lentz JS. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. *Hepatology.* 1990;12:1106-1110.
33. Lewis GF, Steiner G. Acute effects of insulin in the control of VLDL production in humans. Implications for the insulin-resistant state. *Diabetes Care.* 1996;19:390-393.
34. Gavrilova O, Marcus-Samuels B, Graham D, Kim JK, Shulman GI, Castle AL, Vinson C, Eckhaus M, Reitman ML. Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. *J Clin Invest.* 2000;105:271-278.
35. Picard F, Richard D, Huang Q, Deshaies Y. Effects of leptin adipose tissue lipoprotein lipase in the obese ob/ob mouse. *Int J Obes Relat Metab Disord.* 1998;22:1088-1095.
36. Shimomura I, Bashmakov Y, Horton JD. Increased levels of nuclear SREBP-1c associated with fatty livers in two mouse models of diabetes mellitus. *J Biol Chem.* 1999;274:30028-30032.
37. Boelsterli UA, Bedoucha M. Toxicological consequences of altered peroxisome proliferator-activated receptor gamma (PPARgamma) expression in the liver: insights from models of obesity and type 2 diabetes. *Biochem Pharmacol.* 2002;63:1-10.
38. Shepherd PR, Withers DJ, Siddle K. Phosphoinositide 3-kinase: the key switch mechanism in insulin signalling. *Biochem J.* 1998;333 (Pt 3):471-490.
39. Azzout-Marniche D, Becard D, Guichard C, Foretz M, Ferre P, Foufelle F. Insulin effects on sterol regulatory-element-binding protein-1c (SREBP-1c) transcriptional activity in rat hepatocytes. *Biochem J.* 2000;350 Pt 2:389-393.

40. A.J., Considine RV, Jimenez-Linan M, Werman A, Pories WJ, Caro JF, Flier JS. Peroxisome proliferator-activated receptor gene expression in human tissues. Effects of obesity, weight loss, and regulation by insulin and glucocorticoids. *J Clin Invest.* 1997;99:2416-2422.
41. Zhang B, Berger J, Zhou G, Elbrecht A, Biswas S, White-Carrington S, Szalkowski D, Moller DE. Insulin- and mitogen-activated protein-mediated phosphorylation and activation of peroxisome proliferator-activated receptor gamma. *J Biol Chem.* 1996;271:31771-31774.
42. Shulman GI. Cellular mechanisms of insulin resistance. *J Clin Invest.* 2000;106:171-176.
43. Muller-Wieland D, Knebel B, Avci H, Lehr S, Laudes M, Ristow M, Krone W, Kotzka J. Insulin-regulated transcription factors: molecular link between insulin resistance and cardiovascular risk factors. *Int J Obes Relat Metab Disord.* 2001;25 Suppl 1:S35-S37.
44. Hashimoto T, Cook WS, Qi C, Yeldandi AV, Reddy JK, Rao MS. Defect in peroxisome proliferator-activated receptor alpha-inducible fatty acid oxidation determines the severity of hepatic steatosis in response to fasting. *J Biol Chem.* 2000;275:28918-28928.
45. Kim H, Haluzik M, Asghar Z, Yau D, Joseph JW, Fernandez AM, Reitman ML, Yakar S, Stannard B, Heron-Milhavet L, Wheeler MB, LeRoith D. Peroxisome proliferator-activated receptor-alpha agonist treatment in a transgenic model of type 2 diabetes reverses the lipotoxic state and improves glucose homeostasis. *Diabetes.* 2003;52:1770-1778.
46. Ye JM, Doyle PJ, Iglesias MA, Watson DG, Cooney GJ, Kraegen EW. Peroxisome proliferator-activated receptor (PPAR)-alpha activation lowers muscle lipids and improves insulin sensitivity in high fat-fed rats: comparison with PPAR-gamma activation. *Diabetes.* 2001;50:411-417.
47. Tordjman K, Bernal-Mizrachi C, Zemany L, Weng S, Feng C, Zhang F, Leone TC, Coleman T, Kelly DP, Semenkovich CF. PPARalpha deficiency reduces insulin resistance and atherosclerosis in apoE-null mice. *J Clin Invest.* 2001;107:1025-1034.
48. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, Shoelson SE. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science.* 2001;293:1673-1677.