Insulin sensitivity of hepatic glucose and lipid metabolism in animal models of hepatic steatosis
Grefhorst, Aldo

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Chapter 8

The role of lipogenic transcription factors in diabetic dyslipidemia

Aldo Grefhorst, Folkert Kuipers

Laboratory of Pediatrics, University Medical Center Groningen

In preparation
Abstract

Diabetes mellitus type 2 is associated with hepatic fat accumulation (hepatic steatosis) and dyslipidemia, the latter a major risk factor for the development of diabetes-associated coronary heart disease. The components of diabetic dyslipidemia are elevated plasma triglyceride (TG) levels, low concentrations of high density lipoprotein (HDL) cholesterol and the presence of small, dense low density lipoprotein (LDL) particles. An increased production of large, TG-rich very low density lipoprotein (VLDL) particles is considered a major cause of all aspects of diabetic dyslipidemia. In this review, we will discuss the role of transcription factors that (indirectly) control the actions of insulin, glucose and lipids on hepatic fatty acid and TG metabolism and hence on VLDL production. Key “lipogenic” transcription factors that regulate expression of genes encoding enzymes and transporters involved in hepatic lipid and glucose metabolism are sterol-regulatory element-binding protein-1c (SREBP-1c), carbohydrate responsive element binding protein (ChREBP), and liver X receptor (LXR). However, only speculative data exist about their roles in controlling (insulin receptor of) VLDL secretion. Thus, only well-conducted in vivo studies, i.e. with knockout mice and pharmacological ligands, will give proper information about the role of lipogenic gene transcription in (insulin sensitivity of) VLDL production and hence diabetic dyslipidemia.
The metabolic syndrome, diabetes and dyslipidemia

Related to the increased incidence of obesity in the Western world, the combination of metabolic abnormalities collectively referred to as the metabolic syndrome shows a rapidly increasing prevalence. Components of the metabolic syndrome are hyperglycemia, insulin resistance, central obesity, dyslipidemia, and hypertension. According to the various guidelines (WHO 1999, European Group for the Study of Insulin Resistance 1999, and the ATP-III 2001), a person that has at least three of these components has attracted the metabolic syndrome (1). The guidelines agree that insulin resistance and/or diabetes mellitus type 2 are core components of the metabolic syndrome.

Diabetes mellitus type 2 is characterized by elevated blood glucose levels as a result of absent or decreased secretion of insulin by the pancreatic B-cells. In general, dysfunctional B-cells are a consequence of decreased sensitivity of the body for insulin (insulin resistance), for which the pancreas firstly compensated for by enhancing its insulin production. Insulin stimulates uptake of glucose by the peripheral tissues, i.e., muscle and adipose tissue, and inhibits hepatic glucose production (HGP). Patients with diabetes mellitus type 1 have elevated blood glucose levels because their pancreas does not produce insulin. In these patients, administration of exogenous insulin can lower blood glucose levels to normal values. In diabetes mellitus type 2 patients, however, liver and peripheral tissues are insensitive to the actions of insulin and exogenous insulin will not have the desired metabolic effects.

Diabetes mellitus type 2 is also associated with hepatic fat accumulation (hepatic steatosis) (2) and dyslipidemia, the latter a well-known risk factor for the development of cardiovascular disease (3). Studies have shown that patients with diabetes mellitus type 2 have a markedly increased risk for heart attacks (4,5). The components of diabetic dyslipidemia are increased plasma triglyceride (TG) levels, low concentrations of high density lipoprotein (HDL) cholesterol and the presence of small, dense low density lipoprotein (LDL) particles. An increased production of large, TG-rich very low density lipoprotein (VLDL) particles is considered a major cause of all aspects of diabetic dyslipidemia (6). In general, it is thought that diabetic patients secrete more large VLDL particles (7) and that insulin is not able to suppress the production rate of the large VLDL particles.

In this review, we will discuss the role of proteins that are able to regulate gene transcription (transcription factors) that can (indirectly) control the actions of insulin, glucose or lipids on fatty acid and TG metabolism and eventually VLDL production.

Whole-body fatty acid and triglyceride homeostasis

TG molecules are the most energy-dense molecules in mammalian physiology and consist of three fatty acids esterified to a glycerol backbone. Normally, a surplus of (dietary) energy is incorporated into TG and stored in adipose tissue. Upon fasting, the adipose TG store is used to deliver energy in the form of free fatty acids (FFA) to maintain whole-body energy balance. Transport of dietary and de novo synthesized lipids from the liver to the peripheral tissues (i.e., adipocytes), and from the adipocytes back to the liver is important in this balance. The liver plays a crucial role because it can synthesize, store, secrete and oxidize fatty acids.
Because TGs are very hydrophobic, they need to be transported in blood as lipoproteins, together with cholesterol, phospholipids and proteins. The core of a lipoprotein contains TG and esterified cholesterol whereas the surface consists of phospholipids and free cholesterol. Embedded in the lipoprotein surface are the so-called apolipoproteins, proteins needed for stabilisation of the particle and solubility of the core lipids (8). Moreover, apolipoproteins act as ligands for receptors and are required for the actions of specific enzymes (8).

**Triglyceride-rich lipoproteins**

In the enterocytes, dietary TGs are incorporated into lipoproteins called chylomicrons. Apolipoprotein B (apoB) is the main protein of TG-containing lipoproteins (chylomicrons and VLDL). In the surface of chylomicrons, a truncated form of apoB is present, consisting of only 48% of the N-terminal part and is therefore called apoB48. Editing of the apoB100 mRNA into apoB48 mRNA is regulated by the apoB editing complex-1 (apobec1) (9). In the circulation, the TG content of these particles is lipolysed primarily by lipoprotein lipase (LPL) secreted by muscle and adipose tissue. ApoC-III inhibits the actions of LPL while apoC-II enhances its lipolytic actions. The released fatty acids can be taken up and are reesterified into TGs (e.g., in adipocytes) or used as an energy source (e.g., in muscle). As a result of lipolysis, chylomicrons are depleted of TGs, become smaller and are referred to as chylomicron remnants. Both chylomicrons and chylomicron remnants are cleared by the liver upon binding to the LDL receptor, the LDL receptor related protein (LRP) or hepatic lipase (HL) (10).

TG transport from the liver to peripheral tissues induces their incorporation into VLDL particles. VLDL-TGs are lipolyzed by LPL in a similar way as chylomicrons and the fatty acids are taken up by the peripheral tissues. Upon lipolysis, the TG content of the VLDL particle becomes depleted and, as a result, the particle size decreases and the relative cholesterol concentration increases. The cholesterol-dense VLDL remnant particles are called intermediate density lipoprotein (IDL) or LDL particles, depending on their size and density. With increasing VLDL particles size, the ratio of TG over phospholipid will also increase (11) and the resulting LDL particle will contain relatively more cholesterol: small, dense LDL particles. It is known that small, dense LDL particles are associated with increased cardiovascular risk (3,12).

**Reverse fatty acid flux**

β-oxidation of fatty acids is considered the primary source of the energy and reducing equivalents (ATP, NADH) needed for de novo synthesis of glucose (gluconeogenesis) during fasting. Moreover, β-oxidation generates ketone bodies, an additional fuel source for the brain when glucose levels are low. In the β-oxidation process, fatty acyl-CoA’s are broken down into shorter chains by a series of dehydrogenases. For this process, FFAs are released from the adipose stores after lipolysis of TG by triglyceride hydrolase (TGH) (also called adipose TG lipase (ATGL) (13)) and hormone sensitive lipase (HSL) (14) and carried by serum albumin to the liver. Insulin inhibits lipolysis in peripheral tissues. Thus, upon fasting, when insulin levels are low, the insulin-mediated inhibition of lipolysis is absent and FFAs are released into the circulation, taken up by the liver and partly used in the β-oxidation process and partly reesterified to form TG. This latter process gives rise to the fasting induced hepatic steatosis (15).
Assembly of very low density lipoproteins

Lipidation of apolipoprotein B

Production of VLDL is considered a two-step process that takes place in two distinct parts of the liver cell (figure 8.1). Firstly, the apoB molecules become co-transcriptionally lipidated to form a small pre-VLDL particle in the rough endoplasmic reticulum (ER), a process catalyzed by the microsomal triglyceride transfer protein (MTTP) (16). When insufficient lipid is available, the apoB translocation is halted and the protein is degraded. The pre-VLDL particle is transported in Sar1/COPII vesicles to the smooth ER where the second step will take place. It is known that the ADP ribosylation factor-1 (ARF1) can control the formation of VLDL. It is thought that, because ARF1 is part of the COPI secretory complex involved in transport from the ER to the Golgi apparatus, it is able to influence the sorting procedure of pre-VLDL from the rough to the smooth ER (17).

Figure 8.1. Schematic representation of VLDL assembly and secretion, located in the endoplasmic reticulum (left part of the figure) and its membrane. ARF1, ADP ribosylation factor-1; CACT, carnitine acylcarnitine translocase; CPT, carnitine palmitoyl transferase; DAG, diacylglycerol; DGAT2, diacylglycerol acyltransferase; FA, fatty acid; FFA, free fatty acid; MTTP, microsomal triglyceride transport protein; PL, phospholipid; PLD, phospholipase D; TG, triglyceride; TGH, triglyceride hydrolase.
**Lipidation of pre-VLDL particles**

In the second step of VLDL assembly, the pre-VLDL particle is further lipidated and transported to the cellular membrane. This second step again involves the action of MTTP but also that of phospholipase D (PLD), the enzyme controlling conversion of phosphatidylcholine into phosphatidic acid, a TG precursor needed in VLDL assembly (18). Activation of PLD is regulated by ARF1 and recent data show that overexpression of ARF1 not only resulted in increased pre-VLDL translocation to the smooth ER, but, as a result, also in enhanced lipidation of pre-VLDL (17).

**Amount vs. size of VLDL particles**

The amount and size of VLDL particles depends on lipidation of apoB and pre-VLDL in the first and second step, respectively. Depending on the lipidation of the pre-VLDL particle, the liver can secrete VLDL particles that vary in size. The large VLDL particles (VLDL₁) and the smaller VLDL₂ particles are thought to be secreted via separate pathways by the liver and to follow separate routes within the VLDL-IDL-LDL pathway (reviewed by Taskinen (6)). In general, it is thought that diabetic patients secrete more VLDL₁ particles (7). Major issues that need to be resolved are whether insulin regulates lipidation of pre-VLDL rather than the assembly of pre-VLDL. And if this is the case, how insulin regulates VLDL lipidation and hence secretion. We will first briefly review how insulin controls sorting of apoB towards lipidation or degradation.

**Direct effects of insulin on apoB degradation and VLDL assembly**

*Effects of insulin on hepatic lipid metabolism are predominantly mediated via the phosphatidylinositol-3-kinase pathway*

Upon binding of insulin to the insulin receptor (a member of the tyrosine kinase receptor family), the receptor autophosphorylates and activates two distinct downstream signaling pathways: the phosphatidylinositol-3-kinase (PI3K) pathway and the MAP kinase/ERK kinase (MEK) pathway. The PI3K-pathway is mainly of relevance for the metabolic processes discussed in this review and will therefore be discussed.

Upon autophosphorylation of the insulin receptor, a downstream insulin receptor substrate (IRS) will be phosphorylated. Phosphorylated IRS has an increased affinity for the p85 subunit of PI3K. Activated PI3K will start the production of the second messenger phosphatidylinositol 2,4,5-trisphosphate (PIP₃) from the phospholipid PI₄,5P₂ (PIP₂). This second messenger activates, amongst others, protein kinase B (PKB) via phosphorylation, the protein involved in regulation of many steps in insulin-mediated hepatic glucose and lipid metabolism. For instance, PKB phosphorylates and hence inhibits activity of glycogen synthase kinase-3β (GSK3β), an inhibitor of glycogen synthase (GS). Thus, via its actions on PKB and GSK3, insulin stimulates conversion of glucose into glycogen (19). Nowadays, the PKB-mediated phosphorylation of a member of the forkhead transcription factor family, FoxO1, is intensively studied (20,21). Phosphorylation of FoxO1 blocks induction of expression of various genes involved in gluconeogenesis. In peripheral tissues, PKB stimulates the translocation of vesicles containing the glucose transporter-4 (GLUT4) to the membrane (22,23). Via this route, insulin stimulates peripheral glucose uptake.

**Direct effects of insulin**

Insulin regulates VLDL assembly in various ways. ApoB protein secretion is largely dependent on its intracellular degradation (24): increased or decreased apoB mRNA levels do not translate into similar changes in apoB protein secretion. Elegant *in vitro* studies showed...
that insulin is able to inhibit VLDL production via acceleration of the apoB degradation (25). This process mediated by PI3K (26) but probably independent of PKB (27). Via this route, insulin affects the number of VLDL particles secreted. On the other hand, insulin inhibits transcription of the gene encoding for MTTP (28,29), probably via activation of the transcription factor sterol regulator element binding protein-1c (SREBP-1c) (30). The role of SREBP-1c in insulin-mediated lipid metabolism will be discussed later in this review. Activation of PLD, important in the lipidation of VLDL, is prevented when PIP2 levels are low, thus under conditions of high insulin (26,31).

Taken together, these data show that insulin impairs the production of large VLDL particles via its effects on MTTP and PLD. Thus, the overall effect of insulin is the secretion of less and smaller VLDL particles. We recently confirmed this effect of insulin in vivo in mice in which we measured the VLDL-TG production rate under hyperinsulinemiac, euglycemic conditions (32): VLDL-TG production rate decreased during hyperinsulinemia, partly due to the secretion of smaller particles.

Because a VLDL particle contains a single apoB molecule, the amount of apoB secreted directly reflects the number of VLDL particles produced. As discussed, the amount of apoB secreted by the liver depends, in part, on its lipidation (33). Therefore, one can imagine that, when the amount of TG available for VLDL is increased to a larger extent than the amount of apoB, the size of VLDL particles will increase. Moreover, insulin regulates whole-body energy balance, including TG storage and fatty acid fluxes via its inhibitory effects on peripheral lipolysis. Thus, insulin might also affect VLDL secretion via its effects on hepatic lipid availability.

**VLDL production and hepatic lipid availability**

*Sources of fatty acids*

TGs are needed for lipidation of both the apoB and the pre-VLDL particle (33). The fatty acids that constitute the hepatic TG pool originate from three sources: from (i) the diet, (ii) the peripheral (adipose) stores, and (iii) *de novo* lipogenesis. Fatty acids taken up by the hepatocyte are not directly used for VLDL production but first esterified and stored as a cytoplasmic lipid droplet (33,34). Utilization of TG from this pool for VLDL assembly requires hydrolysis followed by re-esterification.

*De novo lipogenesis*

During *de novo* lipogenesis, fatty acids are made out of acetyl-CoA moieties (figure 8.2). The source of acetyl-CoA may be the glycolytic breakdown of glucose via pyruvate, a process in which pyruvate kinase (PK) is thought to be a major regulator (35). When glucose is taken up in the hepatocyte by the glucose transporter 2 (GLUT2), it is rapidly phosphorylated into glucose-6-phosphate (G6P) by glucokinase (GK). Then, G6P can either be used for production of glycogen, a process mediated by GS, or G6P can enter the glycolytic pathway to yield pyruvate, as mentioned above. The reverse pathway, *de novo* synthesis of glucose, is called gluconeogenesis. Herein, one G6P molecule is produced from two pyruvate molecules via intermediates such as oxaloacetate, phosphoenolpyruvate (PEP) and fructose 1,6-bisphosphate (for an exhaustive review, see (36)). The conversion of oxaloacetate into PEP is controlled by PEP carboxykinase (PEPCK), an enzyme considered rate-controlling in gluconeogenesis. The last step in gluconeogenesis, dephosphorylation of G6P, is controlled by the glucose-6-phosphatase complex.
In the flux from glucose to TG, pyruvate enters the tricarboxylic acid (TCA) cycle and the citrate produced herein can be converted into acetyl-CoA by ATP citrate lyase. Two acetyl-CoA’s are covalently linked to each other to form malonyl-CoA. This coupling of acetyl-CoA’s is regulated by acetyl-CoA carboxylase (ACC). Two different forms of ACC have been identified: activity of ACC1 produces malonyl-CoA that is used in de novo lipogenesis whereas ACC2 generates malonyl-CoA that inhibits carnitine palmitoyl transferase-1 (CPT1) (37), an enzyme that has been shown to be rate-controlling for hepatic β-oxidation (38). The two different functions of malonyl-CoA probably reflect the localization of ACC1 and ACC2 in the cytosol and anchored in the mitochondrial outer membrane, respectively (37). Upon actions of fatty acid synthase (FAS), the malonyl-CoA subunits are covalently linked to form fatty acids. The fatty acyl-CoA needed for esterification of the fatty acid into TG is generated by the actions of acyl-CoA synthase (ACS), while the subsequent esterification into TG is controlled by glycerol-phosphate acyltransferase (GPAT). More background about the role of various enzymes in TG synthesis can be found in the excellent review by Coleman et al. (39).
In healthy subjects fed a “normal” diet, de novo lipogenesis contributes only 6% to the TG found in both liver and VLDL (40). In patients with nonalcoholic fatty liver disease, however, the contribution of de novo lipogenesis to both TG pools was found to be increased to 26% (41). Yet, the major source of fatty acids found in hepatic and VLDL TGs originate from FFAs derived from peripheral tissues (40,41).

Novel aspects concerning the relationship between hepatic TG content and VLDL-TG production rate

Although it is commonly thought that the availability of TG at specific hepatocellular sites is a major determinant of number and size of the VLDL particle (33), a number of recent animal studies from our and other laboratories revealed that the hepatic TG content per se does not stimulate the VLDL-TG production rate. For instance, the commonly used leptin-deficient ob/ob mice have severe hepatic steatosis, high plasma FFA and increased hepatic de novo lipogenesis, but do not show an increased VLDL-TG production rate under basal conditions (42). Furthermore, hepatic steatosis associated with inhibition of glucose-6-phosphatase activity was also without effect on VLDL production (43). Acute hepatic steatosis upon inhibition of hepatic β-oxidation did not affect VLDL production nor impair hepatic insulin sensitivity (32). Various factors, apart from the accumulation of TG, might contribute to changes in VLDL production. TGs are not the sole components of VLDL: hepatic cholesterol and phospholipid availability or synthesis might also influence the secretion and assembly of VLDL. It has been suggested that de novo synthesis of cholesterol (44) and phospholipids (45), rather than their concentrations, are determinants of VLDL production. Moreover, both cholesterol and phospholipids are also secreted into bile by the hepatocyte. So far, it is not known whether cholesterol and phospholipids have different preferences for entry into VLDL or bile secretory pathways, but that might, in part, depend on the location of both processes in the liver and the availability of the lipids at these locations.

Studies show that in fasted conditions, the plasma FFA pool contributes the majority of fatty acids secreted by the liver in VLDL (46). Recently, its was shown in humans that fatty acids from the FFA pool are predominantly used by the liver for VLDL-TG synthesis in both fed and fasted state (47). CD36 is the fatty acid transporter in peripheral tissues. Therefore, CD36 knockout mice have an increased FFA flux to the liver (48), a condition resulting in increased hepatic TG concentrations. However, this condition is not associated with increased VLDL-TG production rates (49), thus suggesting that increased plasma FFA levels and hepatic steatosis per se do not necessary lead to increased VLDL production.

Lipogenic transcription factors

Hepatic de novo lipogenesis is, to a large extent, regulated by lipogenic transcription factors. Work in various animal models suggest, but did not yet prove, an independent role for these factors in the regulation of VLDL production. Transcription and/or activation of these factors is regulated by insulin or metabolites of glucose, fatty acids and cholesterol. Very important lipogenic transcription factors that regulate expression of genes encoding for enzymes involved in hepatic lipid and glucose metabolism are SREBP-1c, carbohydrate responsive element binding protein (ChREBP), and liver X receptor (LXR).
Transcription factors

Transcription of genes by the polymerase II complex results in production of RNA. Controlling transcription of a gene is the first step in controlling the production of a protein. The promotor regions of genes contain response-elements that can bind certain transcription factors depending on the sequences of the elements (Figure 3). Conformational changes of a transcription factor, *i.e.*, upon binding of its ligand or upon its phosphorylation/dephosphorylation, will change its affinity for co-activator and co-repressor complexes. These complexes consist of different proteins that can inhibit or induce activity of the polymerase-II complex and hence inhibit or induce gene transcription.

Thus, whether a transcription factor regulates transcription of a gene depends on the presence of its endogenous ligands, its phosphorylation state, its localisation (cytosolic vs. nuclear), the presence of specific response-elements in the promotor region and the availability of the corepressor or coactivator complexes (figure 8.3).

**SREBP-1c**

SREBP-1c is a member of the basic-helic-loop-helic-leucine zipper (bHLHLZ) transcription factor family that is synthesized as an inactive precursor protein embedded in the ER membrane. Upon decreasing cellular cholesterol contents, the SREBP-1c precursor protein is translocated to the Golgi membrane, escorted by the SREBP cleavage activation protein (SCAP) (50,51). In the Golgi, the SREBP-1c isoform is cleaved to form ‘mature’ SREBP-1c, a process mediated by the membrane-bound serine protease site 1 protease (S1P) (52,53) and the membrane-bound zinc-metallo-protease site 2 protease (S2P) (54,55) (figure 8.4). The mature SREBP-1c translocates to the nucleus where it binds to direct repeat sterol regulatory elements (SREs; consensus sequence: 5’-TCACnCCAC-3’) located in the promotor regions of several genes.

SREBP-1c induces transcription of almost all genes encoding for enzymes involved in both *de novo* fatty acid synthesis and esterification of fatty acids into TG (56). SREBP-1c knockout mice show markedly decreased mRNA levels of all of these genes (56,57). Upon fasting, when insulin levels are low, the expression of lipogenic genes is decreased. Hepatic
overexpression of SREBP-1c in mice prevented this fasting-induced effect on gene expression (58), suggesting that SREBP-1c is a key regulator of the early response of the liver to insulin. A recent study indeed confirmed that both transcription and activation of SREBP-1c is regulated by insulin (59), via a PI3K-dependent mechanism (60). The effect of insulin on nuclear translocation is, at least in part, due to the effect of reduced levels of the SCAP inhibitory protein Insig-2 (61). Additionally, insulin reduces the turnover rate of nuclear SREBP-1c (62).

The ob/ob mouse shows elevated liver TGs and increased blood glucose and insulin levels (63) but also increased nuclear SREBP-1c protein levels, despite severe hepatic insulin resistance (64). This suggests that SREBP-1c is very sensitive for the actions of insulin, even under conditions of reduced whole-body insulin sensitivity. This is, in part, explained by the fact that the insulin effects on glucose production are mediated via IRS1, which is downregulated in insulin-resistant animals whereas the effects of insulin on lipid metabolism are largely regulated via IRS2 (65). However, Shimomura et al. (66) showed that, despite IRS2-deficiency due to chronic hyperinsulinemia in ob/ob mice, insulin still stimulated transcription of SREBP-1c.

Figure 8.4. Schematic representation of SREBP-1c maturation and cleavage regulated by insulin. ER, endoplasmic reticulum; S1P, site 1 protease; S2P, site 2 protease; SCAP, SREBP cleavage activation protein; SREBP-1c, sterol-regulatory element-binding protein-1c.
In conclusion, insulin increases the nuclear activity of SREBP-1c in at least three manners: (i) via upregulation of SREBP-1c gene transcription, (ii) by stimulating production of the mature SREBP-1c form, and (iii) by decreasing the turnover rate of mature SREBP-1c. Of interest is the fact that the effects of insulin on SREBP-1c transcription and translocation are opposed by glucagon via increased intracellular cAMP levels which probably affect SREBP-1c activity via inhibition of the insulin-inhibited nuclear SREBP-1c turnover (62).

Although plasma TG levels are ~60% lower in SREBP-1c knockout mice (57), only speculative data exist about the role of SREBP-1c in the hepatic VLDL-TG production rate in basal and hyperinsulinemic states. Therefore, the precise role of SREBP-1c in the regulation of VLDL production and hence in development of diabetic dyslipidemia remains speculative. Moreover, the role of (insulin-mediated) SREBP-1c activity in the control of hepatic glucose metabolism is largely unknown, but is probably minor because lipid and glucose metabolism are controlled by insulin via distinct routes, i.e., IRS2 and IRS1, respectively (65).

**ChREBP**

When glucose is taken up by the liver and subsequently converted to G6P by GK, G6P is mainly directed into the glycogen pool or broken down in the glycolytic pathway, as mentioned before. However, when the flux through G6P is increasing, an ‘escape’ route called the pentose phosphate pathway becomes active (Figure 5). In this pathway, ribulose-5-phosphate is generated from G6P or from its glycolytic product fructose-6-phosphate via oxidative or non-oxidative routes, respectively (67). Via an epimerase, ribulose-5-phosphate is converted into xylulose-5-phosphate, an activator of protein phosphatase 2 A (PP2A). A few years ago, it was shown that the increased activity of PP2A upon increasing xylulose-5-P levels were associated with induced transcription of PK (68). Recently, the members of Uyeda’s laboratory clearly showed that a novel transcription factor, ChREBP, is responsible for this effect (69,70).

ChREBP is a member of the bHLHLZ transcription factor family and binds to the carbohydrate response element (ChoRE) present in promoters of several genes, that is composed of two E-box (5’-CACGTG-3’) or E-box-like sequences. The ChREBP protein probably binds as a heterodimer with Mlx (71) to its response elements. Upon increasing xylulose-5-phosphate levels, PP2A dephosphorylates ChREBP. ChREBP can be transported into the nucleus after dephosphorylation of Ser196 and dephosphorylation of a second amino acid (Thr666) enhances the binding activity of ChREBP to DNA (72). ChREBP phosphorylation is regulated by AMP-activated protein kinase (AMPK) and protein kinase A (PKA) (73). The activity of AMPK becomes induced upon increasing usage of energy, e.g., exercise. AMPK is considered to play a pivotal role in the control of lipid metabolism. For instance, AMPK phosphorylates and inhibits ACC1 and ACC2 (74) as well as SREBP-1c (75).

ChREBP induces transcription of various genes encoding enzymes involved in de novo lipogenesis (76). Remarkably, most lipogenic genes regulated by SREBP-1c are also regulated by ChREBP, i.e., ACC, FAS, ACS and GPAT. Thus, glucose not only regulates lipogenic gene expression via its effects on pancreatic insulin secretion and hence insulin-mediated SREBP-1c transcription and activation, but also via ChREBP.

Treatment of rats with the pharmacological glucose-6-phosphatase inhibitor S4048 resulted in elevated hepatic G6P levels (77) and increased lipogenesis (43), a process presumably mediated via ChREBP. Upon S4048 treatment, the VLDL-TG production rate was not affected (43), but hepatic glucose production was severely decreased (77). Because the data strongly suggest a role of ChREBP in the S4048-induced lipogenesis, more experiments are needed to elucidate the role of ChREBP on the VLDL production and particle size.
LXR

LXR is a member of the family of nuclear hormone receptors that needs to bind a ligand to become active and to induce or inhibit transcription of target genes. Other members of this family are the peroxisome proliferator-activated receptors (PPARs), the glucocorticoid receptor (GR) and many others (table 8.1). Two subtypes of LXR are known: LXRα is expressed mainly in the liver while LXRβ is ubiquitously expressed (78,79). Activated LXRs heterodimerize with the retinoid X receptor (RXR) and bind to the LXR response element (LXRE), consisting of two hexameric nucleotide direct repeats separated by four nucleotides (DR4; consensus sequence: 5’AGGTCAnnnCGGTCA-3’) (78,79). Oxysterols are oxygenated metabolites of cholesterol and they constitute the physiological ligands for LXR. The most potent LXR-activating oxysterols are 22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol and 24(S),25-epoxycolesterol (80). The first effects of activated LXRs were seen on genes encoding proteins involved in (reverse) cholesterol metabolism, but
pharmacological LXR activation studies showed that LXR also induces transcription of SREBP-1c (81-84), but not its translocation (59). Apart from indirect actions through SREBP-1c, LXR also directly influences transcription of Fas (85) and of genes encoding LPL (86) and stearoyl-CoA desaturase-1 (SCD1) (57). The latter protein is involved in the production of monounsaturated fatty acids, i.e., conversion of palmitic acid (C16:0) and stearic acid (C18:0) into palmitoleic acid (C16:1) and oleic acid (C18:1), respectively (87).

We previously showed that pharmacological LXR activation resulted in severe fatty liver and with 2-fold induced VLDL-triglyceride production rate in mice (84). This increased production was completely the result of the secretion of large, TG-rich particles, a condition similar to that of diabetic dyslipidemic patients. However, studies suggested anti-diabetic effects of LXR activation (88,89), presumably via the effects of LXR on the transcription of the gluconeogenic gene Pepck. In contrast, in vivo studies on insulin sensitivity showed no differences between LXR agonist treated and untreated lean and ob/ob mice (90). So far, no direct data exist on the direct role of LXR on the insulin sensitivity of VLDL production.

Table 8.1. The nuclear receptor superfamily

| Endocrine receptors: | Estrogen receptor (ER) α, β<br>Progestane receptor (PR)<br>Androstane receptor (AR)<br>Glucocorticoid receptor (GR)<br>Mineralocorticoid receptor (MR)<br>Retinoid acid receptor (RAR) α, β, γ<br>Thyroid receptor (TR) α, β<br>Vitamin D receptor (VDR)<br>Ecdysone receptor (EcR)1
| Adopted orphan receptors: | Retinoid X receptor (RXR) α, β, γ<br>Peroxisome proliferator-activated receptor (PPAR) α, β/δ, γ<br>Liver X receptor (LXR) α, β<br>Farnesoid X receptor (FXR) α, β<br>Pregnane X receptor (PXR)2<br>Constitutive androstane receptor (CAR)
| Orphan receptors: | Steroidogenic factor 1 (SF-1)<br>Liver related hormone-1 (LRH-1)<br>DAX-13<br>Short heterodimer partner (SHP)<br>TLX4<br>Photoreceptor cell-specific nuclear receptor (PNR)<br>NGFI-B α, β, γ4<br>Retinoid related orphan receptor (ROR) α, β, γ<br>Estrogen-related receptor (ERR) α, β, γ<br>RevErB α, β, γ5<br>Germ cell nuclear factor (GCNF)<br>TR 2,46<br>Hepatocyte nuclear factor-4 (HNF-4)<br>COUP-TF α, β, γ5

1, the EcR is found in insects and is the only nonvertebrate nuclear receptor with a known ligand; 2, in humans, the rodent PXR is called steroid X receptor (SXR); 3, abbreviation of: dosage-sensitive sex reversal-adrenal hypoplasia congenital critical region on the X chromosome, gene 1; 4, no full name; 5, abbreviation of: chicken ovalbumin upstream promoter-transcription factor.
Concluding remarks

Because the lipogenic transcription factors mentioned (SREBP-1c, ChREBP, and LXR) tightly control de novo lipogenesis and their activation and/or expression is controlled by insulin, carbohydrates and lipids (factors whose plasma levels are increased in type 2 diabetic subjects), one might expect that these factors play a role in the (insulin sensitivity of) VLDL production. However, proper studies relating these transcription factors with the insulin sensitivity of VLDL production have not been reported. Of course, one can question whether these factors play a huge role herein, because fatty acids produced in the de novo lipogenic pathway do not contribute that much to the VLDL-TG content (40,91). Only well-conduted in vivo studies, i.e. with knockout mice and pharmacological ligands, will give proper information about the role of lipogenic transcription in (insulin sensitivity of) VLDL production and hence diabetic dyslipidemia.

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150