Insulin resistance is a key feature of the metabolic syndrome and can lead to the development of type 2 diabetes.1,2 These conditions are today increasingly common, primarily because of the increased prevalence of a sedentary lifestyle and obesity.3 Insulin resistance and type 2 diabetes are characterized by dyslipidemia, which is an important and common risk factor for cardiovascular disease. Diabetic dyslipidemia is a cluster of potentially atherogenic lipid and lipoprotein abnormalities that are metabolically interrelated. Recent evidence suggests that a fundamental defect is an overproduction of large very low-density lipoprotein (VLDL) particles, which initiates a sequence of lipoprotein changes, resulting in higher levels of remnant particles, smaller LDL, and lower levels of high-density lipoprotein (HDL) cholesterol. These atherogenic lipid abnormalities precede the diagnosis of type 2 diabetes by several years, and it is thus important to elucidate the mechanisms involved in the overproduction of large VLDL particles. Here, we review the pathophysiology of VLDL biosynthesis and metabolism in the metabolic syndrome. We also review recent research investigating the relation between hepatic accumulation of lipids and insulin resistance, and sources of fatty acids for liver fat and VLDL biosynthesis. Finally, we briefly discuss current treatments for lipid management of dyslipidemia and potential future therapeutic targets. (Arterioscler Thromb Vasc Biol. 2008;28:1225-1236)

Key Words: apolipoprotein B ▪ VLDL ▪ insulin resistance ▪ metabolic syndrome ▪ nonalcoholic fatty liver disease ▪ stable isotopes ▪ kinetics
lipemia.1,2,5–7 Diabetic dyslipidemia frequently precedes type 2 diabetes display qualitatively similar lipid abnormalities.2 Indeed, patients with insulin resistance both with and without type 2 diabetes display qualitatively similar lipid abnormalities.2

It is now recognized that the different components of diabetic dyslipidemia are not isolated abnormalities but are closely linked to each other metabolically,1,2,5 and are mainly initiated by the hepatic overproduction of large triglyceride-rich very low–density lipoproteins (VLDL1).13 It is thus of key importance to elucidate the mechanisms involved in the overproduction of VLDL1 in diabetic dyslipidemia. Here, we review the pathophysiology of VLDL metabolism in the metabolic syndrome and discuss how increased liver fat induces overproduction of VLDL1.

**Formation and Metabolism of VLDL**

**Production of Triglyceride-Poor VLDL in the Liver**

The assembly of VLDL involves a stepwise lipidation of the structural protein apolipoprotein B100 (apoB100) in the liver (Figure 1).8,9 The initiating step is lipidation of apoB by microsomal triglyceride transfer protein (MTP) in the rough endoplasmic reticulum.10,11 This results in the formation of a primordial pre-VLDL lipoprotein particle,12 which is converted to a triglyceride-poor VLDL particle by additional lipidation.13

**Maturation of VLDL in the Liver**

The triglyceride-poor VLDL particle can either be secreted from the cell as VLDL2 or further lipidated to form a mature, triglyceride-rich VLDL (ie, VLDL3).13,14 The lipidation is dependent on the small GTP-binding protein ADP-ribosylation factor 1 (ARF-1).15 This protein plays a central role in membrane trafficking between the ER and the Golgi apparatus, which is consistent with recent results showing that the late steps of VLDL formation occur in the Golgi apparatus.14,16–18 However, there are data to suggest that the ER is the site of maturation.19 The conversion of triglyceride-poor to triglyceride-rich VLDL requires a bulk addition of triglycerides and thus differs from the stepwise lipidation of apoB to form pre-VLDL.13 VLDL formation is highly dependent on the accumulation of triglycerides in the cytosol of lipid droplets. These lipid droplets are formed as small primordial droplets from microsomal membranes (12) and increase in size by fusion (13). The triglycerides within the droplets undergo lipolysis and are re-esterified (14) before they lipidate the triglyceride-poor VLDL to form triglyceride-rich VLDL.

**Delipidation of VLDL**

VLDL, intermediate-density lipoprotein (IDL), and LDL all contain 1 apoB100 per particle and are linked in a delipidation cascade. Triglyceride-rich VLDL is released from the liver and converted to IDL by lipoprotein lipase (LPL)-catalyzed hydrolyzation of the lipids. LPL can be inhibited by apoCIII.23 IDL can be further hydrolyzed by hepatic lipase to cholesterol-rich LDL, which is catabolized mainly by hepatic uptake of LDL through LDL receptors.24 Results from kinetic studies suggest that the liver also secretes smaller particles such as IDL and LDL25,26 and that IDL can be catabolized through LDL receptor-mediated uptake.27,28 During these processes, apoB100 remains with the particle.

**Dyslipidemia in Insulin Resistance and Type 2 Diabetes**

Abnormal concentrations of lipids and apolipoproteins can result from changes in the production, conversion, or catabolism of lipoprotein particles. Thus, although static measurements are important, they do not reveal the underlying mechanisms involved in the dysregulation of lipid disorders. To infer this information, it is necessary to perform in vivo tracer/tracere studies in which the rates of synthesis or catabolism of a particular lipoprotein or apolipoprotein can be determined.

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**Figure 1.** Assembly and secretion of apoB100-containing lipoproteins. ApoB is synthesized and translocated into the lumen of the endoplasmic reticulum (ER) (1). The growing apoB molecule is cotranslationally lipidated by MTP to form the primordial VLDL particle (pre-VLDL) (2). Alternatively, apoB fails to be lipidated and is incorrectly folded (3) and sorted to proteasomal degradation (4). Late in the ER compartment, the pre-VLDL particle is converted to a triglyceride-poor VLDL particle (5). Triglyceride-poor VLDL exits the ER by Sar1/CopII vesicles that bud off (6) from specific sites on the ER membrane.16 The vesicles fuse to form the ER Golgi intermediate compartment (ERGIC) (7), which then fuses with the cis-Golgi (8). The triglyceride-poor particles are either transported through the secretory pathway and then secreted (9), or further lipidated (10) to form triglyceride-rich VLDL (VLDL1) particles, which are then secreted (11). The formation of triglyceride-rich VLDL is highly dependent on the accumulation of triglycerides in cytosolic lipid droplets. These lipid droplets are formed as small primordial droplets from microsomal membranes (12) and increase in size by fusion (13).
Figure 2. Changes in lipoprotein metabolism in type 2 diabetes and the metabolic syndrome. Subjects diagnosed with the metabolic syndrome display, most noticeably, an increased production of VLDL (1), and there is a reduction in the catabolic rate of apoB-containing lipoproteins, in particular IDL and LDL (2).50–62 Together, these result in increased concentrations of apoB-containing lipoproteins.50–62 The catabolism of apoA1, the main apolipoprotein of HDL, is increased by 48% but apoA1 production is increased by 25%, probably because of some compensatory effect (3).52 This results in a 16% reduction in the concentration of HDL-apoA1.62

Kinetic Studies

Today, the majority of in vivo lipoprotein kinetic studies are performed using infusion of stable isotopes. The metabolism of lipoprotein particles can be followed by injecting amino acids labeled with stable isotopes that are then incorporated into proteins such as apoB100.29 The triglyceride content can be followed by infusion of labeled glycerol or free fatty acids.30 Several multicompartmental models have been proposed over the years to provide estimates of protein secretion and catabolism,31 and have been designed to analyze either the VLDL-apoB32,33 or the VLDL-triglycerides.34,35 Indeed, in recent years, much has been learned about the metabolic changes that contribute to dyslipidemia (Figure 2). To enhance the understanding of the pathways leading to VLDL subpopulations, we developed a combined multicompartmental model that allows the kinetics of triglyceride and apoB100 in VLDL1 and VLDL2 to be simultaneously assessed (Figure 3).36 Previous models combined infusion of [3H]glycerol and [3H]acetate tracers, a glycerol-to-triglyceride conversion subsystem, and a tracers, a glycerol-to-triglyceride conversion subsystem, and a subsystem for the assembly and secretion of the lipoprotein particles. Secreted particles are either cleared by the system or transferred down the delipidation chain, before being converted to IDL.

Increased Levels of VLDL

Increased levels of VLDL in the metabolic syndrome are associated with excess hepatic production of VLDL.32–45 We have observed that this increase is in the VLDL1 fraction.46,47 By contrast, hepatic secretion of VLDL2 is comparable in insulin-resistant and insulin-sensitive subjects.5,46,48,49 In subjects with type 2 diabetes, hepatic uptake of VLDL, IDL, and LDL is decreased, resulting in increased plasma residence time of these lipoproteins.50–52 which further contributes to the increased accumulation. There are also reports of increased production of IDL and LDL in insulin-resistant women without diabetes,53 and in men with mild but not severe diabetes.54

Formation of sdLDL

The formation of sdLDL is closely associated with insulin resistance and hypertriglyceridemia,6 and the VLDL1-triglyceride level is the major predictor of LDL size in individuals with or without type 2 diabetes.1,7,49,55 The mechanism that leads to the formation of sdLDL is well elaborated, and both cholesteryl ester transfer protein (CETP) and hepatic lipase are involved: (1) CETP facilitates the transfer of triglycerides from VLDL1 to LDL; (2) the resulting triglyceride-rich LDL is a preferred substrate for hepatic lipase; and (3) increased lipolysis of triglyceride-rich LDL results in the formation of sdLDL.7,16 Thus, it seems that the presence of large triglyceride-rich VLDL particles is a prerequisite for sdLDL formation, and such correlations have been observed.7,49,55 However, sdLDL are also observed in patients with type 2 diabetes and insulin resistance with close to normal triglyceride levels.56 This might be explained by increased hepatic lipase activity.

Several studies have shown that the presence of sdLDL particles is associated with increased cardiovascular risk.57–60 However, it is still under debate whether sdLDL levels add independent information on risk assessment over standard risk factors.61

Decreased Levels of HDL

Increased levels of VLDL1 also alter the composition of HDL through the actions of CETP and hepatic lipase, leading to the formation of small dense HDL and increased catabolism of these particles.62 Thus, there is an inverse correlation between HDL and liver fat.69

Postprandial Lipemia

Intestinal-derived apoB48-containing chylomicrons contribute to the large triglyceride-rich lipoproteins in the postprandial state. Although approximately 80% of the increase in triglycerides after a fat load meal comes from apoB48-containing lipoproteins,63 approximately 80% of the increase in particle count is from apoB100.64,65 Moreover, the area under the curve (AUC) for apoB100 is 10-fold higher than that of apoB48,66 and the production rate of apoB100 is 15 to 20 times higher than that of apoB48.67,68 ApoB48 and apoB100-containing particles are
cleared from the circulation by a common pathway and therefore compete for clearance. Thus, the major contribution to an atherogenic lipoprotein profile from chylomicrons is likely their interference with apoB100 catabolism.

The Lipid Triad
Collectively, the key components of the diabetic dyslipidemia can be attributed to increased accumulation of VLDL particles, predominantly caused by overproduction of VLDL. A similar dyslipidemia is also present in other syndromes, such as familial combined hyperlipidemia, hyperapobetalipoproteinemia, familial dyslipidemic hypertension, LDL subclass pattern B, and the Reaven syndrome, which are all characterized by increased hepatic apoB overproduction and insulin resistance.

Regulators of VLDL Assembly
Fatty Acids Increase VLDL Formation
An increased delivery of fatty acids increases the secretion of VLDL-triglycerides and apoB100 from human liver and from hepatocytes and HepG2 cells. Our turnover studies in vivo have demonstrated the importance of hepatic triglycerides for the assembly and secretion of VLDL. These studies have also confirmed the stepwise lipidation of VLDL and demonstrated that the secretion of VLDL1-apoB100 increases with increasing concentrations of liver lipids. Importantly, the relationship between triglyceride and apoB production rates for VLDL1 showed that subjects with type 2 diabetes secrete more—not larger—VLDL1 particles than non-diabetic controls. Thus, the amount of lipid added to an individual VLDL2 particle to produce VLDL1 is equal in subjects with type 2 diabetes and nondiabetic controls, but the rate of conversion is increased in subjects with type 2 diabetes.

Insulin Shifting the Balance From VLDL1 to VLDL2
Only a few studies have investigated the acute effect of insulin on VLDL kinetics in humans in vivo. Although the modeling approaches differ, they all show decreased secretion of VLDL-triglycerides and VLDL-apoB. Furthermore, insulin infusion has a greater effect on the secretion of VLDL-triglycerides than VLDL-apoB, and it has been shown to suppress mainly VLDL1-apoB production, with little effect on VLDL2-apoB100 production. Thus, insulin not only reduces the number of overall VLDL particles, but also shifts the balance between VLDL2 and VLDL1, to reduce the relative proportion of VLDL1 particles. However, the acute effect of insulin on lipolysis of circulating lipoproteins still remains to be fully elucidated.

Insulin has been shown to decrease VLDL formation by at least 2 mechanisms: (1) by regulating the amount of fatty acids in the circulation; and (2) by direct suppression of the production of VLDL1 in the liver, independent of the availability of fatty acids. The molecular mechanisms involved in the direct suppression of VLDL1 are elusive, and several mechanisms have been proposed. Sparks and coworkers have shown that activation of phosphatidylinositol 3-kinase (PI3-K) is necessary for the insulin-stimulated decrease in apoB secretion from rat hepatocytes. In addition, insulin downregulates MTP expression via activation of the mitogen-activated protein kinase (MAPK) pathway. Insulin may also decrease VLDL secretion by inhibiting the activity of the transcription factor Foxa2. Recent studies in ob/ob mice have shown that Foxa2 and its coactivator peroxisome proliferator-activated receptor gamma (PPARγ) coactivator β (Pgc-1β) promote fatty acid oxidation and stimulation of MTP in livers, resulting in increased VLDL secretion. However, these results require further investigation as it is surprising to observe the combination of increased fatty acid oxidation and VLDL secretion.

PPARα Agonists Decrease VLDL-Triglyceride Secretion
Fibrates activate PPARα, which plays a key role in the regulation of energy homeostasis and inflammation. Fibrates have been used since the 1970s for their lipid-modifying properties, which include reducing plasma triglycerides and VLDL and increasing HDL cholesterol.

Experiments in rat liver cells have shown that the triglyceride-lowering effect of fibrates is partly explained by increased oxidation of free fatty acids, diverting them away from triglyceride synthesis and thus reducing the hepatic synthesis of triglyceride-rich lipoproteins. However, in humans, data on direct effects of fibrates on VLDL production rate are inconclusive. The effect on lowering plasma triglycerides seems to be caused by an increased clearance rate, which is supported by the observations that PPARα induces expression of LPL and inhibits the synthesis of apoCIII.

Brain Glucose Controls VLDL Secretion
Rossetti and coworkers have recently shown that hypothalamic glucose-sensing mechanisms regulate liver, but not intestinal, VLDL-triglyceride production, and that this regulation is lost in diet-induced obesity. The cross-talk between the brain and the liver couples carbohydrate sensing to lipoprotein secretion by curtailing the activity of stearyl-coenzyme A (CoA) desaturase-1 (SCD1) in the liver and by interfering with a late step in the hepatic assembly and secretion of VLDL particles. These findings are consistent with a homeostatic loop in which the increased availability of carbohydrates limits the endogenous output of lipids into the circulation. This mechanism would, together with insulin, acutely downregulate VLDL secretion after a meal. Glucose-induced hyperglycemia-hyperinsulinemia lowers VLDL-triglyceride secretion by 50%, but the effects of insulin and glucose cannot be separated from each other.

Further Evidence for Independent Regulation of VLDL1 and VLDL2
In addition to independent regulation of VLDL1 and VLDL2 production by insulin and PPARα agonists (as described above), there is evidence to indicate a primary effect of ethanol on the stimulation of production of VLDL1 particles in humans. In addition, endogenous cholesterol synthesis correlates with VLDL2-apoB but not VLDL1-apoB production. This finding provides further support for independent regulation of VLDL1 and VLDL2, and may explain why VLDL2 but not VLDL1 is increased in patients with increased plasma cholesterol, as in moderate hypercholesterolemia and familial hypercholesterolemia.
Which Factors Predict Overproduction of VLDL?

We recently analyzed which features of type 2 diabetes and insulin resistance correlate with VLDL production, and revealed strong correlations with plasma glucose that are not apparent in the normal range of plasma glucose. By extending our study to monitor liver fat, intra-abdominal fat, subcutaneous fat, and adiponectin, we showed that fasting insulin, plasma glucose, intra-abdominal fat, and liver fat and HOMA-IR are predictors of VLDL-apoB and VLDL-tri-glyceride production. However, in a multiple regression analysis, only liver fat and plasma glucose remain significant. Moreover, the key predictors of liver fat are intraabdominal fat, adiponectin, and plasma glucose.

Liver Fat Correlates with Reduced Effect of Insulin

Nonalcoholic fatty liver disease (NAFLD) is defined as fat accumulation in the liver that exceeds 5% to 10% of liver weight in individuals who do not consume significant amounts of alcohol. Recent data show that NAFLD strongly associates with type 2 diabetes, obesity, and hyperlipidemia.

We tested the relationship between liver fat and VLDL suppression in subjects with a broad range of liver fat content. This study confirmed that liver fat predicts baseline VLDL production, and it also showed that liver fat is associated with lack of VLDL suppression in response to insulin: insulin downregulates VLDL secretion in subjects with low liver fat but fails to suppress VLDL secretion in subjects with high liver fat, resulting in overproduction of VLDL. The reason for this lack of effect is not known. Insulin suppresses the nonesterified fatty acid (NEFA) pool to a similar extent regardless of liver fat level, and thus the high VLDL production in individuals with high liver fat must be facilitated either by using a greater portion of systemic NEFA or recruiting other sources of triglycerides, such as from hepatic stores.

Does Liver Fat Cause Insulin Resistance?

Is the observed association between high liver fat and reduced suppression of VLDL by insulin the result of fatty liver, or is fatty liver merely a consequence of hepatic insulin resistance? The existence of a causal relationship between liver fat and hepatic insulin resistance is controversial with conflicting results: (1) Fatty liver and insulin resistance may be separate manifestations of metabolic derangements, and hepatic insulin resistance may reflect inflammation rather than lipid accumulation in this tissue. Some studies suggest that insulin resistance is an essential requirement for the accumulation of hepatocellular fat. It is indeed possible that hepatic steatosis results from insulin resistance because the insulin signaling pathways that drive fatty acid biosynthesis in the liver are relatively sensitive to the high levels of portal insulin flux to the liver that accompany systemic insulin resistance. (3) Several lipid intermediates (eg, ceramides, GM3 ganglioside, and diacylglycerol) appear capable of inactivating components of the insulin signaling pathway. The levels of such metabolites are elevated in individuals with insulin resistance, and it is also likely that yet to be identified lipids or lipid-derived metabolites affect insulin sensitivity. However, the existence of a causative association between accumulation of specific lipid species and insulin resistance remains controversial.

Hepatic Insulin Resistance Induces Dyslipidemia Without Fatty Liver

Liver insulin receptor knockout (LIRKO) mice develop hyperinsulinemia but their livers do not respond to it, and thus they can be used to investigate the effects of pure hepatic insulin resistance. These mice develop a proatherogenic lipid profile (low HDL cholesterol and VLDL particles enriched in cholesterol) but not fatty liver. Normally, insulin promotes apoB degradation, but the insulin-resistant LIRKO mice over secrete apoB100. In contrast to insulin-resistant humans who over secrete both apoB and triglycerides as VLDL, LIRKO mice secrete less VLDL-triglyceride than control mice. This is probably attributable to low expression of SREBP-1c and SREBP-2 and their targets, and suggests that apoB is secreted as denser particles in LIRKO mice. These data suggest that pure insulin resistance drives changes in cholesterol homeostasis whereas other factors, such as hyperinsulinemia, drive increased production of triglycerides and the development of fatty liver. The significance of these results for humans with the metabolic syndrome remains to be determined.

Models of Fatty Liver With and Without Insulin Resistance

Shulman and coworkers have presented data supporting the hypothesis that hepatic steatosis leads to hepatic insulin resistance (see review). In a rat model of NAFLD, lipid accumulation stimulates gluconeogenesis and activates protein kinase C epsilon (PKC-ε) and c-Jun N-terminal kinase-1 (JNK1), which may interfere with tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) and IRS-2 and impair the activation of glycogen synthase by insulin. Furthermore, recent data from this rat model show that antisense oligonucleotide (ASO)-mediated inhibition of PKC-ε reverses hepatic insulin resistance. PKC-ε has also been shown to be activated in the liver in patients with type 2 diabetes.

Further work in transgenic animals has also directly tested and confirmed the hypothesis that hepatic steatosis can lead to insulin resistance: (1) Overexpression of glyceraldehyde-3-phosphate acyl transferase (GPAT) in rat liver results in major accumulation of triglycerides and in insulin resistance. (2) Expression of LPL in mouse liver causes hepatic steatosis and hepatic insulin resistance as manifested by increased hepatic glucose output. (3) Expression of malonyl-CoA decarboxylase in liver of rats with diet-induced insulin resistance resolves hepatic steatosis and improves whole-animal, liver, and muscle insulin sensitivities.

Choi et al showed that ASO-mediated inactivation of acyl CoA diacylglycerol acyltransferase 2 (DGAT2) reverses diet-induced hepatic steatosis and insulin resistance by lowering hepatic diacylglycerol content and PKC-ε activation. However, Yu et al showed that ASO-mediated inhibition of DGAT2 reduces liver triglyceride content but does not improve insulin or glucose tolerance in mice fed a high-fat diet. Furthermore, Monetti et al showed that DGAT2-mediated lipid accumula-
tion in the liver does not cause insulin resistance, indicating that hepatic steatosis can occur independently of insulin resistance. It is known that hepatic fat does not always associate with insulin resistance in humans: for example, liver steatosis without hepatic insulin resistance is observed in patients with familial heterozygous hypobetalipoproteinemia.

**How Could Storage of Neutral Lipid Droplets Cause Insulin Resistance?**

The storage of lipids in mammalian cells was long considered to be a relatively simple process in which excess fatty acids were converted to neutral lipids and deposited in cytoplasmic lipid droplets. In recent years, the cell biology of the lipid droplet has begun to be understood in more detail. Accumulation of lipids in cells is a balance between the formation of lipid droplets and the hydrolysis of lipids in these droplets, catalyzed by hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL), and seems to be regulated by droplet-associated PAT proteins. Combined knockdown of the PAT proteins adipose differentiation-related protein (ADRP) and tail interacting protein of 47 kDa (TiP47) in cultured liver cells results in large lipid droplets with high turnover of triglycerides and insulin resistance. These data support an important structural role for ADRP and TiP47 as surfactant proteins at the surface of the lipid droplet, packaging lipid in smaller units, protecting lipid droplets against endogenous lipases, and facilitating triglyceride formation. Furthermore, by promoting lipid packaging into lipid droplets, the proteins may protect against the development of insulin resistance in hepatic cells.

A number of other enzymes and signaling proteins have also been shown to be associated with lipid droplets, and include fatty acid metabolic enzymes, eicosanoid-forming enzymes, specific kinases, and small GTPases. Thus, lipid droplets may integrate lipid metabolism, inflammatory mediator production, membrane trafficking, and intracellular signaling consistent with the hypothesis that a disturbed interplay between these pathways may link liver fat and insulin resistance. However, the mechanisms involved remain to be elucidated. Recent studies identifying the SNARE protein SNAP23 as a molecular link between lipid droplets and insulin resistance in muscle cells support the hypothesis that it is not lipid droplets per se that promote the development of insulin resistance but other molecular mechanisms.

**Sources of Fatty Acids for Liver Fat and VLDL-Triglycerides**

Potential sources of fatty acids for liver fat and VLDL-triglycerides include: (1) peripheral fats stored in adipose tissue that flow to the liver via the plasma NEFA pool; (2) fatty acids synthesized within the liver through de novo lipogenesis (DNL); (3) dietary fatty acids that are transported via chylomicrons from the intestine to the NEFA pool and then to the liver; and (4) uptake of chylomicron remnants by the liver (Figure 4).

Parks and coworkers have recently developed methodology to determine the fate of dietary fatty acids during the postprandial state and combined this technique with liver biopsy to simultaneously measure all 4 pathways of fatty acid delivery to the liver in patients suspected of having NAFLD. These quantitative metabolic data demonstrate that both elevated peripheral fatty acid flux and DNL contribute to liver fat and lipoprotein-triglycerides in NAFLD. Furthermore, a significant similarity was observed between the contributions of fatty acid sources for the liver triglyceride pools and the VLDL-triglyceride pool.

**NEFA Pool**

The hepatic uptake of fatty acids is not regulated and, as a result, the plasma NEFA concentration is directly related to the influx of fatty acids to the liver. Adipose tissue contributes approximately 80% of fatty acid content to the plasma NEFA pool in the fasted state, and even in the fed state it contributes approximately 60%. Thus, the most likely explanation for excess triglyceride accumulation in NAFLD is increased release of fatty acids from adipose tissue, which flow to the liver via the NEFA pool. In insulin-resistant states, insulin fails to suppress the activity of HSL and results in enhanced lipolysis and flux of fatty acids to the plasma NEFA pool.

Visceral adiposity has been identified as an independent risk factor for cardiovascular disease and the metabolic syndrome, and the severity of NAFLD has been shown to be positively related to the visceral fat accumulation regardless of body mass index. We found that the insulin-induced suppression of NEFA was inversely related to intraabdominal fat volume but not to subcutaneous fat, which supports the important role of visceral fat as a source of NEFA flux to the liver. Indeed, the contribution of visceral adipose tissue lipolysis to NEFA increases as a function of visceral fat. However, even in visceral obesity, 50% to 60% of NEFA entering the liver is from the systemic circulation.

**De Novo Lipogenesis**

In fasting healthy human subjects, DNL in the liver contributes less than 5% to VLDL-triglyceride content. By con-
trast, DNL has been shown to account for approximately 25% of liver and VLDL-triglyceride content in hyperinsulinemic subjects with NAFLD.\textsuperscript{151} In healthy subjects, DNL is elevated after meals,\textsuperscript{160} which can be accounted for by elevations in the circulating levels of lipogenesis precursors. However, in NAFLD, DNL is already elevated in the fasted state, and further postprandial elevation is not observed.\textsuperscript{151} Indeed, constant elevation of DNL was also observed in control subjects fed a diet high in simple carbohydrates for 25 days.\textsuperscript{161} A recent study showed that insulin-resistant lean subjects produce 60% less glycerol than insulin-sensitive lean subjects after receiving 2 high-carbohydrate meals.\textsuperscript{162} The energy is diverted to increased hepatic DNL and results in increased plasma triglyceride and reduced HDL cholesterol levels.\textsuperscript{162} These observations reflect the sustained elevation of factors involved in hepatic DNL,\textsuperscript{150,151} such as SREBP1-c,\textsuperscript{163,164} the carbohydrate response element-binding protein (ChREBP),\textsuperscript{165} and PPARγ.\textsuperscript{166–168}

**Chylomicron Synthesis and Other Sources**

After entering the blood stream through chylomicron synthesis in the intestine, dietary fatty acids can be taken up by liver as chylomicron remnants.\textsuperscript{150} Alternatively, LPL catalyzes the release of fatty acids from the chylomicrons at a rate that exceeds tissue uptake, resulting in spill over of these fatty acids into the plasma NEFA pool.\textsuperscript{150}

The contribution of dietary fatty acids to liver triglycerides depends on the fat content of the diet. Patients with NAFLD often consume significantly more saturated fats compared with control subjects matched for age, sex, and body mass index (BMI).\textsuperscript{169} However, studies of patients with NAFLD who had consumed a standardized 30% fat diet for the preceding 4 days showed that only 15% of the liver triglycerides were derived from dietary fatty acids.\textsuperscript{151}

During the same 4-day study, the source of 10% to 20% of VLDL-triglycerides was not accounted for by 1 of the 4 recognized pathways.\textsuperscript{151} The source of these fatty acids has been proposed to be either hepatic storage or visceral fat that is transported to the liver through the portal vein.\textsuperscript{151} Indeed, liver biopsies in these subjects showed that only 38% of liver triglycerides could be accounted for by the recognized pathways during the 4-day study period.\textsuperscript{151} The estimated turnover rate of liver fat in these subjects was 38 days,\textsuperscript{151} compared with estimates of 1 to 2 days in normal individuals.\textsuperscript{170} Interestingly, Vedala et al have presented kinetic evidence for a significant contribution from a slow-turnover hepatic cytosolic triglyceride storage pool to fasting VLDL-triglycerides.\textsuperscript{171} The turnover time for this pool was longer in hypertriglyceridemic (HPTG) subjects with diabetes compared with nondiabetic subjects with and without HPTG, and it is likely that the contribution of the slow-turnover hepatic cytosolic triglyceride storage pool can explain the lack of accountability of liver triglycerides in NAFLD subjects.\textsuperscript{151}

**Lipid Management of Dyslipidemia**

Compared with nondiabetic individuals, patients with type 2 diabetes are at a much greater risk for CVD. Consequently, the treatment of CVD risk factors is a healthcare priority in this patient population. A number of clinical trials with 3-hydroxy-3-methylglutaryl (HMG) CoA (HMG-CoA) reductase inhibitors (statins) have shown significant CVD risk reduction through LDL cholesterol lowering in patients with diabetes,\textsuperscript{172–177} mainly through increased LDL-receptor activity.\textsuperscript{178} Increased LDL-receptor activity may also correct chylomicron metabolism.\textsuperscript{179} Indeed, the recently published Collaborative Atorvastatin Diabetes Study (CARDS), a placebo-controlled trial of patients with type 2 diabetes, was terminated 2 years earlier than its anticipated length owing to the significant reduction in number of CVD events observed in patients randomized to receive low-dose atorvastatin versus placebo.\textsuperscript{172} The statin therapy in this trial resulted in significant reduction of CVD events in patients with type 2 diabetes without previous CVD or high levels of LDL cholesterol and indicated that patients with type 2 diabetes may be candidates for statin therapy regardless of LDL cholesterol level or absence of a previous CV event. Thus, statins are safe and efficacious in reducing CVD events in patients with type 2 diabetes, but recent clinical trials have demonstrated that statins fail to completely reverse the increased risk of CVD in subjects with type 2 diabetes.\textsuperscript{172,173}

Fibrates are associated with positive lipid-modifying properties (discussed earlier), and it was anticipated that fibrates would significantly reduce CVD risk in high-risk people with features of the metabolic syndrome. Indeed, results of posthoc analyses from the Veterans Affairs High-Density Lipoprotein Intervention Trial (VA-HIT) and the Bezafibrate Infarction Prevention (BIP) study indicated that treatment with fibrates is beneficial in individuals with diabetes and the metabolic syndrome.\textsuperscript{180} However, results from the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study were not as favorable as expected.\textsuperscript{181,182} The current recommendations for fibrates in the management of dyslipidemia in the metabolic syndrome are the topic of a separate review in this series.\textsuperscript{180}

Niacin (nicotinic acid) lowers VLDL, LDL, and sdLDL and raises HDL cholesterol levels, and thus has an overall beneficial effect on the lipoprotein profile.\textsuperscript{183} This effect results from inhibition of lipolysis in adipose tissue via G protein–coupled receptors, leading to a reduction of plasma NEFA\textsuperscript{183,184} and thus limiting the substrate for VLDL secretion. Clinical trials suggest that niacin is a powerful strategy for raising HDL cholesterol.\textsuperscript{185,186} However, higher doses of niacin (>1.5 g/d) may worsen glycemic control in individuals with type 2 diabetes, and recommendations are to use lower doses and combine with statins.\textsuperscript{187}

Diabetic dyslipoproteinemia is exacerbated by hepatic overproduction of large triglyceride-rich VLDLs, but neither statins nor fibrates reduce the flux of NEFA to the liver or reduce liver fat. Interestingly, the PPARγ agonist pioglitazone has been reported to reduce plasma triglyceride levels by increasing VLDL-triglyceride clearance rate with no effect on hepatic secretion of VLDL apoB.\textsuperscript{188} The effect on clearance rate can be explained by an increase of LPL mass and a reduced apoCIII production in response to pioglitazone.\textsuperscript{188} Recently, PPARδ agonists have been reported to markedly improve dyslipidemia and also reduce liver fat in obese men.\textsuperscript{189} Potential future targets include several transcription factors (eg, Foxa2 and Pgc-1b),\textsuperscript{87,88} key regulatory enzymes...
in hepatic lipid metabolism (eg, PKC-ε, DGAT2 and SCD1), lipid intermediates that interfere with insulin signaling, and the hypothalamic glucose-sensing mechanisms that regulate liver VLDL-triglyceride production. Novel targets also include the cannabinoid type 1 (CB1) receptor in the liver; the CB1 receptor antagonist rimonabant has been reported to suppress lipogenesis, lower plasma triglycerides, and raise HDL cholesterol.

### Conclusion

Several kinetic studies support the view that production of VLDL₁ and VLDL₂ can be independently regulated, and these lipoproteins have different effects on metabolism.

Overproduction of VLDL₁ alters the composition of HDL, which ultimately leads to an increased catabolism of these particles, and is closely associated with formation of sdLDL. Thus, it is important to realize that VLDL is not a homogenous pool of lipoprotein particles. We are beginning to understand the molecular mechanism(s) involved in the inability of insulin to suppress VLDL₁ production in the metabolic syndrome. Hopefully, clarification of these molecular mechanisms will be translated into targeted treatment for dyslipidemia, which is of key importance given the high risk for CVD in patients with the metabolic syndrome.

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Overproduction of Very Low-Density Lipoproteins Is the Hallmark of the Dyslipidemia in the Metabolic Syndrome
Martin Adiels, Sven-Olof Olofsson, Marja-Riitta Taskinen and Jan Borén

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