Increased Very Low Density Lipoprotein Secretion, Hepatic Steatosis, and Insulin Resistance

Sung Hee Choi[1] and **Henry N Ginsberg**[2,*]

[1]Internal Medicine, Seoul National University College of Medicine, Seoul National University Bundang Hospital, Seoul, Korea

[2]Columbia University College of Physicians and Surgeons, New York, NY, USA

Abstract

Insulin resistance (IR) not only affects regulation of carbohydrate metabolism, but all aspects of lipid and lipoprotein metabolism. IR is associated with increased secretion of very low density lipoproteins (VLDL) and increased plasma triglycerides, as well as hepatic steatosis, despite the increased VLDL secretion. Here, we link IR with increased VLDL secretion and hepatic steatosis at both the physiologic and molecular levels. Increased VLDL secretion, together with the downstream effects on high density lipoprotein cholesterol and low density lipoprotein size is pro-atherogenic. Hepatic steatosis is a risk for steatohepatitis and cirrhosis. Understanding the complex inter-relationship between IR and these abnormalities of liver lipid homeostasis may provide insights relevant to new therapies for these increasing clinical problems.

Insulin resistance and dysregulated metabolism: not just a problem of carbohydrate metabolism

IR, a major abnormality underlying type 2 diabetes mellitus, is defined as the pathophysiological condition of reduced insulin responsiveness in liver, muscle, and adipose tissue (1;2). Although IR is typically considered in the context of carbohydrate metabolism, it is also a major driving force for the metabolic syndrome and the concomitant high risk for cardiovascular diseases (CVD) (2;3). Increased CVD in the metabolic syndrome is linked significantly to the lipid and lipoprotein abnormalities that are characteristic of individuals with IR (4). The dyslipidemia in IR is characterized by a triad of lipid abnormalities: 1) increased plasma concentrations of very low density lipoprotein (VLDL)-triglyceride (TG) and apolipoprotein B100 (apoB100; the full-length product of translation of the *apob* gene in the liver); 2) low levels of high density lipoprotein (HDL) cholesterol and apolipoprotein A1; and 3) relatively normal levels of low density lipoproteins (LDL) cholesterol with increased numbers of predominantly small, dense LDL particles. The latter two abnormalities are associated with the increased plasma VLDL levels via the actions of cholesterol ester transfer protein (CETP), which mediates the exchange of core lipids (TG and cholesterol ester (CE)) between VLDL and HDL or VLDL and LDL (5). These pathways will not be addressed in this review.

*whom correspondence should be addressed. hng1@columbia.edu.

Here we focus on the current understanding of why there is increased hepatic assembly and secretion of VLDL particles in IR. Additionally, we review current understanding of the hepatic steatosis that also occurs in IR, despite the increased secretion of VLDL particles. Integral to these questions, we believe, is apoB100, which is required for the assembly and secretion of hepatic-derived VLDL, intermediate density lipoproteins (IDL), and LDL particles in humans. ApoB48 is a truncated translation product of the *apob* gene that is synthesized only in the small intestine in humans and is required for the assembly and secretion of chylomicrons, the very large TG-rich lipoproteins that transport dietary fat and cholesterol (apoB48 is also synthesized and secreted from rodent livers). Although we will touch on abnormalities in apoB48 secretion in IR, this review will focus mainly on apoB100. When we are referring to pathways and processes that affect both apoB100 and apoB48, we will simply use the term apoB.

The complexity of apoB100 and apoB48, both in terms of their structures (6) and itineraries in the secretory pathways of the hepatocyte and small intestine, respectively, (7–9) enables these proteins to efficiently and effectively carry out their major role: to transport lipids, particularly TG, to peripheral tissues. Unfortunately, as described in detail below, these same complexities lay the foundation of both the increased secretion of VLDL (and chylomicrons) and the predisposition to hepatic steatosis in people with IR.

Sources of hepatic TG in normal and IR states

If the role of VLDL is to transport excess energy, mainly in the form of TG, out of the liver, it is not surprising that VLDL assembly and secretion is a substrate-dependent process that is highly regulated by the availability of TG (7;8;10). Hepatic TG derives from: 1) uptake of circulating albumin-bound fatty acids (FA); 2) uptake of circulating remnants of VLDL and chylomicron (the product of lipoprotein lipase (LpL)-mediated hydrolysis of the core TG in chylomicrons), with their remaining TG-FA; and 3) de novo synthesis of FA in the liver (lipogenesis). [FIGURE 1]

Impaired insulin signaling and increased FA flux into the liver

It is well established from both *in vitro* and *in vivo* studies over several decades that moderate increases in FA delivery to hepatocytes or the liver, respectively, stimulate assembly and secretion of VLDL (Figure 1A) (9–14). It is generally accepted that FA levels in blood and FA flux to the liver are increased in insulin resistant humans with and without type 2 diabetes (14;15), although this finding is not universal (16). The higher amounts of FA are thought to result from the failure of insulin to suppress lipolysis of adipose tissue TG (17); insulin-mediated suppression may vary, however, in different tissue depots, with visceral adipose tissue being more resistant to insulin than lower limb adipose tissue (18). Visceral adipose tissue has been suggested to play a key role in the abnormalities of hepatic lipid metabolism observed in IR. However, the large majority of FA flux to the liver derives from upper body non-visceral fat, although it reaches the liver mostly via the portal vein (19).

Recent studies have demonstrated important nuances in the role of FA in stimulating VLDL secretion. Intravenous infusions of oleic acid (OA) bound to albumin stimulate apoB secretion in mice without stimulating TG secretion, whereas delivery of a similar absolute amount of OA derived from endocytosis of remnant-like particles during infusion of Intralipid (an emulsion of TG and phospholipids [PL]) did not affect apoB secretion (20). Several fold more OA had to be delivered via Intralipid to stimulate apoB secretion, and at those levels of OA, TG secretion was also increased (20). The mechanism whereby small quantities of OA bound to albumin stimulate apoB secretion without increasing TG secretion is unclear, but may explain why, in people with IR, increased apoB100 secretion

by the liver may occur with little or no increase in TG secretion (21). Additionally, the type of FA may be crucial as palmitic acid (PA) and decosahexanoic acid (DHA) may have different effects from OA (discussed later in this review). An area that needs further exploration are the pathways whereby FA are taken up by the liver; both diffusion and receptor mediated mechanisms are likely to be important (22–24). The status of these pathways in IR has not been studied in depth, although recent reports suggest upregulation of some of the involved receptors in humans with hepatic steatosis (25;26).

Impaired metabolism of VLDL and chylomicron remnants in IR

Increased fasting and increased postprandial TG levels are characteristic abnormalities in IR (27). It was assumed for many years that increased postprandial TG was due to the combination of defective lipolysis of chylomicrons and VLDL, and increased VLDL secretion (27;28). Recent studies have, however, demonstrated that increased assembly and secretion of apoB48-containing chylomicrons in fructose fed hamsters (29) and humans during periods of hyperinsulinemia (30) is an additional factor. Increased FA flux to the small intestine, as well as IR, appears to play key roles in the intestine, just as they do in the liver (30;31). Thus, the small intestine appears to respond to IR in a manner similar to the liver (32). In addition to increased secretion of VLDL and chylomicrons, moderate reductions in LpL activity and increased secretion of apolipoprotein CIII (apo CIII), an inhibitor of LpL, into plasma in IR both contribute to less efficient lipolysis of VLDL and chylomicron TG (33), resulting in TG-enriched VLDL and chylomicron remnants that are removed by the liver (Figure 1B). Based on *in vivo* work with Intralipid (20) and early studies with cultured hepatocytes (34), this would be expected to result in increased assembly and secretion of VLDL apoB100 and TG.

Recent work on the "spillover" of dietary-derived chylomicron TG-FA has added further complexity to the role of postprandial lipids in the regulation of hepatic lipid metabolism (35;36). If modest, but significant quantities of FA generated by the lipolysis of chylomicron TG escapes "local" re-esterification in adipose tissue and are taken up, instead by the liver, they would act similarly to any other albumin-bound FA, and could stimulate apoB100 secretion irrespective of the stimulation of TG secretion.

Increased de novo FA synthesis in insulin resistance—The third source of hepatic TG is de novo lipogenesis (DNL) (Figure 1C). Although most of the detailed studies that we will refer to regarding the regulation of hepatic DNL have been conducted in rodents, lipogenesis does contribute significantly to VLDL secretion in humans, where it is increased in individuals with obesity and IR (37;38). Regulation of hepatic DNL by the transcription factor, sterol response element binding protein-1c (SREBP-1c), has been defined in great detail (39). SREBP-1c regulates nearly all genes involved in FA and TG synthesis, including acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), long-chain elongase (ELOVL6), stearoyl-CoA desaturase 1 (SCD1), and mitochondrial glycerol 3-phosphate acyltransferase (GPAT) (40). A major regulator of SREBP-1c is the liver x receptor (LXR) (41), which stimulates *SREBP-1c* transcription by binding to a LXR-response element in the promoter. LXR also directly stimulates transcription of the genes for both FAS gene (42) and the carbohydrate responsive element binding protein (ChREBP) (43), which regulates hepatic lipogenesis in a way that is complementary to SREBP-1c (44) (see below).

Insulin, at least in part through stimulation of LXR plays a central role in the regulation of hepatic *SREBP-1c* gene expression (40;41;43;45). Insulin also increases the cleavage of SREBP-1c to its mature, nuclear form via an LXR-independent mechanism (46). The role of insulin is best exemplified by the fasting–re-feeding paradigm in which mice that have been fasted 24 hrs are re-fed with high carbohydrate chow and show a dramatic stimulation of the

key genes and proteins in the lipogenic pathway; insulin is critical to this response (47;48). Of great interest and importance to the pathophysiology of increased VLDL secretion and hepatic steatosis is the selective IR that occurs in the liver regarding insulin's ability to regulate hepatic glucose and lipid metabolism (49). The basis for the continued ability of insulin to stimulate DNL at the same time that the hormone is unable to suppress hepatic gluconeogenesis has been a focus of research for the past decade. Although a detailed description of the studies done is beyond the scope of this review, it is clearly important to summarize this body of work and the models that have been proposed.

Differential insulin signaling through IRS-1 and IRS-2 has been proposed as a basis of selective insulin hepatic insulin resistance, where IRS-1 signaling through SREBP-1c remains active while IRS-2 mediated suppression of gluconeogenesis via deactivation of FoxO1 is lost (50). However, recent studies indicate that both IRS-1 and IRS-2 play important roles in regulating both glucose and lipid metabolism in the liver (51;52). Another proposal for selective insulin resistance is that while AKT plays a central role in transmitting insulin signaling to the carbohydrate pathway, other kinases are important for the regulation of DNL, particularly the atypical PKClambda(zeta) (53–55) and PKCbeta (53). Other studies, however, have provided strong evidence that AKT2 is critical for insulin stimulation of DNL and the development of hepatic steatosis (56;57). If the latter studies are correct, then how does selective IR occur if AKT2 is central to both insulin-mediated suppression of gluconeogenesis and insulin-stimulated DNL?

Several groups have studied the potential role of FoxO1 in DNL and hepatic steatosis, but at this point in time, genetic manipulations with gain and loss of FoxO1 function have produced an unclear picture (58–60). Thus, while FoxO1 may play a role in VLDL secretion via regulation of microsomal triglyceride transfer protein (see below), its role as a direct regulator of DNL remains to be determined. A potentially important alternative insulin signaling pathway for DNL is the mammalian target of rapamycin complex 1 (mTORC1) pathway. mTORC1 is a protein complex centrally involved in protein synthesis and cell growth that is sensitive to nutrient flux. This could be the pathway for the selective insulin-stimulated induction of SREBP-1c expression in an otherwise insulin resistant liver (61;62).

Another area of recent interest involves the role of micro-RNAs in lipid metabolism (63). In particular, both *in vitro* (64) and *in vivo* (65) studies have implicated miR-122 as an inhibitor of genes in the DNL pathway. In contrast, miR-33, which is transcribed from an SREBP2 intron, inhibits transcription of several genes involved in FA oxidation as well as the gene for cholesterol transporter, ABCA1 (66). Most recently, *Trib1*, a gene identified by GWAS as potentially involved in lipid metabolism, has been shown to regulate DNL, VLDL secretion, and plasma lipid levels in mice (67). How IR might affect micro-RNAs or *Trib1* is unknown. Lastly, several groups have provided evidence linking endoplasmic reticulum (ER) stress to increased lipogenesis; this will be discussed later in this review.

In addition to SREBP-1c, two other transcription factors play roles in hepatic fat metabolism, particularly in states of IR. ChREBP, noted earlier, is regulated mainly by glucose, but is thought to play a complementary role in the regulation of hepatic DNL (44). The fact that LXR, which is a key regulator of SREBP-1c, also regulates ChREBP (43) provides further evidence for a network of regulation in which both glucose and insulin are important. In the insulin resistant *ob/ob* mouse model, ChREBP is required for complete activation of *SREBP-1c, Acc, Fas, Elovl6*, and *Scd1* activation (68). In this model, ChREBP knockdown not only reduced the rate of DNL but also increased FA oxidation. ChREBP-deficient mice have reduced rates of both glucose and FA synthesis (69). Overall, several studies suggest that ChREBP can be responsible for as much as 50% of DNL under certain

conditions. Most relevant to this review, in IR, especially when glucose flux through the liver is increased, the role of ChREBP in stimulating DNL may be exaggerated.

Hepatic peroxisome proliferators activated receptor gamma (PPARγ) might be another important regulator of DNL and TG synthesis (70–72). I Increased hepatic DNL in the apoB/BATless mouse, a model of IR, increased VLDL secretion, and hepatic steatosis, was associated with increased hepatic expression of PPARγ2, but not SREBP-1c or ChREBP (73). Furthermore, after knockdown of *Pparγ* expression with anti-sense oligonucleotide, *Fas* and *Acc* mRNA levels as well as biochemically measured DNL and hepatic TG content were reduced, indicating a role for hepatic expression of PPARγ2 in the regulation of hepatic DNL and TG synthesis. Of note, neither *Fas* nor *Acc* are known to have PPARγ response elements in their promoters, suggesting an indirect effect of PPARγ2 on DNL. Relevant to states of IR, *Pparγ2* gene expression can be stimulated in primary mouse hepatocytes by insulin and OA (74).

Disposition of hepatic TG and IR

We have reviewed the three major pathways for delivery to and synthesis within the liver of FA and TG, as well as the dysregulation of each of those pathways in states of IR. Once TG begins to accumulate in the liver, there are also three pathways that handle its disposition: oxidation, storage, and secretion. It is clear that dysregulation of any of these pathways can result in abnormalites in both VLDL secretion and hepatic lipid content. We will examine each of these pathways, with focus on their roles in IR-associated increases in VLDL secretion and in hepatic steatosis. (FIGURE 2)

Hepatic FA oxidation and IR

Genetic or pharmacologic manipulation of hepatic FA oxidation results in altered hepatic TG content in numerous studies. However, there is minimal evidence that reduced FA oxidation is important for the increased rates of VLDL secretion and hepatic steatosis in diet-induced obesity in mice or in humans with IR (16). In fact, several studies point to increased (75) or normal (76) levels of hepatic FA oxidation in obesity and IR states. This, of course, does not rule out approaches to increase FA oxidation as potential therapies for the dyslipidemia/steatosis of IR.

Lipid droplets, FA oxidation, and VLDL secretion

The past decade has witnessed an explosion of new information regarding lipid droplets, first in adipocytes and more recently in hepatocytes. Rather than simply being passive depots for storage of excess energy, lipid droplets are complex and active components of the metabolic network that regulates energy metabolism in cells (77;78). The physiology of lipid droplets is mostly dependent on a family of proteins with similar structures; these include perilipin, adipocyte differentiation related protein (ADRP), tail interacting protein of 47Kda (TIP-47), S3–12, and oxidative tissue-enriched PAT protein (OXPAT). Recently, these have been renamed as members of the Perilipin Family (PLIN 1–5) (79). Other proteins of interest include members of the Cide family (80) and the FIT proteins (81). Genetic manipulations of several droplet coat proteins have been shown to affect hepatic lipid metabolism. For example, loss of PLIN 2 (ADRP) is associated with lower hepatic TG and increased VLDL secretion (82) whereas overexpression of hepatic ADRP has the opposite effect (83). Genetic manipulation of Cidec, also known as fat specific protein (FSP27) was associated with increased steatosis (forced Cidec expression) or decreased steatosis (Cidec knockdown) in hepatocytes. These observations are concordant with the increased amounts of FSP27 in fatty livers of ob/ob mice as well as the reduced FSP27 levels in livers of PPARγ-deficient mice.

In addition to lipid droplet-associated proteins, intracellular lipases play roles in regulating hepatic TG content. ATGL-deficient mice (84) have hepatic steatosis, in addition to increased adipose tissue and significant cardiac fat accumulation. Increased expression of ATGL in cultured hepatocytes results in increased FA oxidation, while knockdown of expression is associated with decreased FA oxidation (85). In these experiments, however, VLDL secretion was unaffected. Overexpression of ATGL in the liver was associated with reduced hepatic fat and increased FA oxidation and secretion (86). The role of HSL in the liver, where it is expressed at low levels, is less clear. In the whole body HSL-deficient mouse, although hepatic TG content was reduced, plasma FA levels were also decreased, making it difficult to identify the basis for less hepatic fat (13). Overexpresssion of HSL in the liver, however, results in increased FA oxidation and secretion along with lower hepatic TG content, suggesting a possible role for this enzyme (86). Even less information is available regarding how much droplet proteins and lipases are expressed in livers that are IR and/or steatotic. It is obvious, however, that this will be an emerging area of important new insights regarding the relationship between IR, VLDL secretion, and hepatic steatosis.

Recent studies of adiponutrin/patatin-like phospholipase domain-containing protein 3 (PNPLA3) are an example of the increasing importance of lipid droplet biology. A common SNP in *PLNPA3* was identified in the Dallas Heart Study as being associated with significantly greater prevalence of non-alcoholic fatty liver disease (NAFLD) (87). This finding has been confirmed in several populations (88), but although gene expression is regulated by SREBP-1c and possibly ChREBP (89), there is no relationship between the presence of the SNP and either IR or the metabolic syndrome (87;90). The fact that PNPLA3 appears to be a TG-hydrolase and the presence of the SNP abolishes that activity in cultured cells (91), suggests that PNPLA3 plays a role as a facilitator of hepatic steatosis when other factors such as IR or alcohol drive TG synthesis (92). The recent finding that PNPLA3-deficient mice do not develop a fatty liver supports this view (93).

Assembly and secretion of VLDL and IR

Regulation of the assembly and secretion of VLDL

The assembly and secretion of VLDL, a large macromolecular aggregate of thousands of lipid molecules and several proteins, is extremely complex (7–9;94). Much of this complexity derives from the structure of apoB, but the physiologic basis for complex regulation is that VLDL is a vehicle for transporting energy from the liver to the periphery and, therefore, should be regulated by the metabolic status of the liver. There is general, but not complete, agreement that assembly of VLDL occurs in two-steps. The first step involves targeting of nascent, lipid-poor apoB100 for complete translocation into the lumen of the endoplasmic reticulum (ER) and away from co- or early post-translational degradation by the proteasome (95;96); this first step is dependent on some minimal lipidation and microsomal triglyceride transfer protein (MTP) (8). Exogenous FA-albumin uptake by the liver may play a stimulatory role at this point that is independent of their role as substrate for core lipid synthesis (20) (Figure 1A). The second step involves the addition of core lipids, mainly TG. Existing experimental data support models with either a continuous addition of core lipids while apoB100 is associated with the ER membrane, or a single fusion of a nascent apoB100 particle with a lipid droplet in the lumen of the ER (9). During the second step, MTP appears necessary for the transfer of TG from the ER membrane into the lumen (97) where lipid droplets can form without apoB (98;99). In contrast, MTP may not be necessary for the "fusion" of luminal lipid droplets into nascent VLDL (100;101). How this fusion occurs is unclear, although an intra-ER luminal lipase, triglyceride-hydrolase 1 (TGH1) has been proposed as the catalyst (102). The site of later addition of lipid is also debated, with some data (including our own) (103) indicating the ER as the site, while other data support post-ER compartments, including the Golgi, as the site where mature VLDL are

formed (104). Although second-step lipid loading can provide some protection for apoB100 from degradation, this process mainly determines the size of the VLDL that is secreted. There are a number of other pathways that can degrade apoB post-translationally in the ER and post-ER (8), including autophagy (105–107). Of note, several lines of evidence suggest that TG derived from DNL increase the size but not the number of VLDL secreted by the liver (108–110); this contrasts with the potential for FA taken up from the circulation to directly stimulate apoB secretion (20).

Of interest is the finding in cultured rat hepatocytes that overexpression of apoCIII, which can occur in states of IR, may facilitate secretion of apoB and TG (111). However, reduced apoCIII production during treatment with pioglitazone did not lower in vivo measures of apoCIII secretion in humans although lower apoCIII secretion was associated with better lipolysis of VLDL (112).

Regulation of *VLDL* secretion by insulin in normal and IR states

The role of insulin in the regulation of hepatic VLDL secretion is central both to the normal regulation of nutrient flux into and out of the liver and to the abnormalities present in IR. Thus insulin is a key regulator of:1) FA flux to the liver; 2) hepatic DNL; 3) FA oxidation in the liver; 4) MTP synthesis; and 5) apoB degradation. We have addressed the first three already and will focus now on insulin's effects on MTP and on apoB degradation, particularly in IR states.

Regulation of MTP by insulin

MTP is an 88 kDa ER resident chaperone protein that, when heterodimerized with protein disulfide isomerase (PDI), catalyzes the transfer of neutral lipids (TG, Pl, CE) to nascent apoB, a rate-limiting step in hepatic VLDL production (113). MTP gene expression is negatively regulated by insulin in a dose-, and time-dependent manner in HepG2 cells (114). Suppression of MTP by insulin occurs, at least partly, via FoxO1; insulin normally leads to phosphorylation of FoxO1 and its exclusion from the nucleus, resulting in the inhibition of hepatic MTP expression. However, in IR, FoxO1 phosphorylation is reduced and this transcription factor localizes preferentially in the nucleus, enhancing MTP expression (115). The loss of insulin-mediated inhibition of MTP gene expression could play a role in VLDL overproduction in states of IR. Indeed, expression of a constitutively active FoxO1 increased VLDL secretion in mice (116). Insulin may also inhibit MTP via a MAPK pathway (117). Foxa2 has also been suggested, along with PGC-1β, as a regulator of MTP gene expression (118); there is controversy regarding insulin-mediated signaling to Foxa2, however. Other suggested regulators of MTP, which could be altered by IR, including hepatocyte nuclear factor 4 (HNF4) and peroxisome proliferator-activated receptor alpha (PPARα) (119; 120). The complex nature of the relationship between insulin signaling, MTP expression and VLDL secretion is exemplified in our studies of the liver specific insulin receptor knockout mouse (LIRKO). In this model, FoxO1 should reside completely in the nucleus; however, MTP gene expression was only minimally increased and VLDL TG secretion was reduced by 50% (121). Furthermore, there were no changes in *Mtp* mRNA or protein in the apoB/ BATless mouse, a model of IR and increased VLDL secretion (73). In contrast, M gene expression and protein are increased in ob/ob mice (122).

Regulation of apoB secretion by insulin

In addition to the role of insulin in the regulation of FA oxidation, DNL and MTP, insulin can directly affect the secretion of apoB100 by targeting it for degradation; apoB48 appears to be much less sensitive to insulin, at least in cultured liver cells. During the past 25 years, studies in cells, small animals, and humans have demonstrated a key role for hepatic insulin

signaling in the short-term regulation of VLDL assembly and secretion (9; 123). In cultured hepatocytes, acute exposure of cells to insulin inhibits the secretion of both triglyceride (TG) and apoB despite concomitant stimulation of TG synthesis (123;124). Acute inhibition of VLDL secretion from hepatocytes by insulin results from direct targeting of apoB100 for degradation, at least in part, via PI3-kinase (PI3-K) mediated mechanisms (125–127), although recent data suggests this is not via AKT1 (128). The recent identification of the autophagy pathway as a site of post-ER degradation of aberrantly folded or aggregated apoB100 (94;105; 129) suggest that this may be a mechanism for insulin-mediated degradation as well. In addition to cellular studies, *in vivo* acute glucose-stimulated hyperinsulinemia suppressed secretion of VLDL in rats (130), while short-term hyperinsulinemia with euglycemia inhibited the secretion of VLDL in normal humans (131;132). Hyperinsulinemia also inhibits intestinal secretion of apoB48 (133), although under the conditions of this experiment, suppression of circulating FA also had a significant effect.

If insulin targets apoB for degradation, why is there increased VLDL and chylomicron assembly and secretion in states of IR (134)? The answer can probably be found in a combination of loss of responsiveness to insulin over time and the countervailing stimulation of apoB secretion by hepatic TG. Thus, chronic exposure of cultured hepatocytes to insulin causes a loss of its inhibitory effects on apoB secretion (135;136). Similarly, VLDL secretion was not inhibited by short-term hyperinsulinemia in obese individuals (132) or people with type 2 diabetes mellitus (137), two states characterized by IR. These results suggest the development of resistance to insulin signaling in the pathway for apoB degradation when hyperinsulinemia is chronic. Mice with liver-specific loss of PTEN have both excessive insulin signaling and severe hepatic steatosis: TG. secretion is increased in these mice (138). Increased VLDL secretion has also been observed in several other in vivo mouse models where chronic hyperinsulinemia exists together with increased hepatic TG (73;122). It is difficult, therefore, to separate IR in the pathway to apoB degradation from a stronger stimulatory force to assemble and secrete VLDL arising from steatosis (139). Increased FA flux to the liver in some of these models or disease states may also counteract the inhibitory effects of insulin on apoB secretion. Relevant to this point, insulin-mediated inhibition of VLDL secretion is attenuated by pre-treatment with an LXR agonist, which increases hepatic TG content (140). Studies of LIRKO mice (121), showed that chronic, complete loss of insulin signaling was associated with increased apoB secretion, supporting to some extent a chronic role for insulin in the degradation of apoB, at least in livers with normal TG content. Overall, the results of these many studies indicate that acute hyperinsulinemia, such as in the immediate postprandial state, can inhibit VLDL secretion, whereas in states of IR and chronic systemic hyperinsulinemia, a combination of loss of responsiveness to insulin-targeting of apoB for degradation and/or the stimulation of apoB secretion by increased hepatic TG and/or increased FA flux to the liver, results in increased VLDL secretion and dyslipidemia.

Hepatic steatosis in IR

The remaining question is why does TG accumulate in insulin resistant livers even though VLDL assembly and secretion are increased (134;139)? VLDL apoB and TG secretion rates are increased 3–5 times in humans with IR compared to normal individuals (141;142); shouldn't this range of secretion be adequate to prevent accumulation of hepatic TG, or is there a limitation to the secretion of VLDL in states of IR? One limitation would be that increased hepatic TG, rather than stimulating increased assembly of VLDL particles, would stimulate the assembly of larger particles, but not more particle; this would relieve some of the stress but there is probably a limit to the size of VLDL that can be produced. As noted earlier, TG derived from DNL appears to increase particle size without increasing the

number of particles secreted (108–110), and DNL is increased in states of IR (37), providing more TG for VLDL secretion (38).

An additional basis for the counter-intuitive association of hepatic steatosis and increased VLDL secretionis ER stress. Detailed descriptions of ER stress and the associated uncoupled protein response (UPR) are beyond the scope of this review and are readily available (143;144). Several recent reports have examined the increasing evidence for a critical role of ER stress and the UPR in metabolic diseases, including IR and hepatic steatosis (145–148). Relevant to this review, FA-induced ER stress can inhibit apoB assembly and secretion directly (149). In both *in vitro* and *in vivo* studies, induction of ER stress by OA-targeted apoB100 for cotranslational and posttranslational degradation and decreased its secretion, (Figure 3). Importantly, the association between OA-induced ER stress and apoB secretion was parabolic in nature: modest ER stress was actually associated with increased apoB secretion (because OA-mediated stimulation of apoB was greater than ER stress-mediated inhibition) whereas more significant ER stress led to a loss of OA stimulation and actual inhibition of secretion. In a glucosamine-induced model of ER stress apoB is targeted for both ER-associated and post-ER degradation (150) as well as reduced synthesis (151). Adding further complexity is the finding that increased apoB entry into the ER lumen, as would occur if the first step in VLDL secretion was increased, may trigger ER stress (152). In any event, these findings are compatible with the concomitant occurrence of hepatic steatosis and increased VLDL secretion: ie, people with fatty livers typically secrete more VLDL than normal individuals but, because of ER-stress and the UPR (149), they do not secrete enough to meet the demand for TG transport. Additionally, there may be a point, particularly if non-alcoholic steatohepatitis replaces hepatic steatosis, that apoB secretion will fall even further, exacerbating the process (153). On the other hand, if autophagy is a pathway for the degradation of apoB (105–107), there may be concomitant protection from hepatic steatosis (154)

If the complexity reviewed above was not adequate, it now appears that ER stress and the UPR can also significantly affect hepatic lipid synthesis and steatosis (145;147;148;155;156). The rationale for a link between ER dysfunction, the UPR, and increased DNL can seem at first, to be counterproductive, because a major lipid synthesized is TG, which would need to be secreted via a dysfunctional ER. However, the synthesis of phosphatidylcholine, a major ER membrane PL, at a time when more ER is required, does make sense (157). Most importantly, studies to date indicate that the degree of ER stress and the extent to which the UPR compensates for ER stress determine the outcome. Severe ER stress resulting from genetic deletion of components of the UPR, together with a stressor such as tunicamycin, suppresses both DNL and fatty acid oxidation, with steatosis resulting from the decreased oxidation. On the other hand, during more "physiologic" ER stress, the UPR may stimulate DNL (158;159). To add further complexity to these new findings, XBP1, a key player in the UPR, appears to also play a role in the regulation of DNL via mechanisms that are independent of ER stress (155;160). Clearly, more work is needed to better define the role of ER stress in livers of IR rodents and humans.

An area we have not discussed, but one that almost certainly plays an important role in hepatic steatosis, is the state of increased systemic inflammation that accompanies IR (145;161). Altered Kupfer cells (162), stellate cells (163) and circulating mononuclear cells that infiltrate the liver (164) have all been implicated as facilitators of TG accumulation in the liver. This rapidly evolving field adds an additional dimension to the associations that have been discussed here.

Concluding Remarks

In this overview we attempted to provide a foundation for increased understanding of the commonly observed combination of increased VLDL secretion and hepatic steatosis in states of IR. The regulation of assembly and secretion of VLDL comprises a complexity appropriate for the creation of a large, multimolecular transport vehicle whose "raison d'etre" is to help maintain nutrient homeostasis in the liver. It is not surprising, therefore, that IR, which results from, and causes, disturbances in nutrient metabolism, has complex effects on VLDL secretion. Unfortunately, the complexity inherent in these systems has made it difficult to translate targets identified in cells and animal models to treatments of humans. Future studies will hopefully begin to consolidate the present large body of knowledge to reveal central targets amenable to effective therapies. Until that time, and preferential to the use of pharmacologic approaches, reduced energy intake coupled with increased energy expenditure are our "best bets" to improve IR and its consequences.

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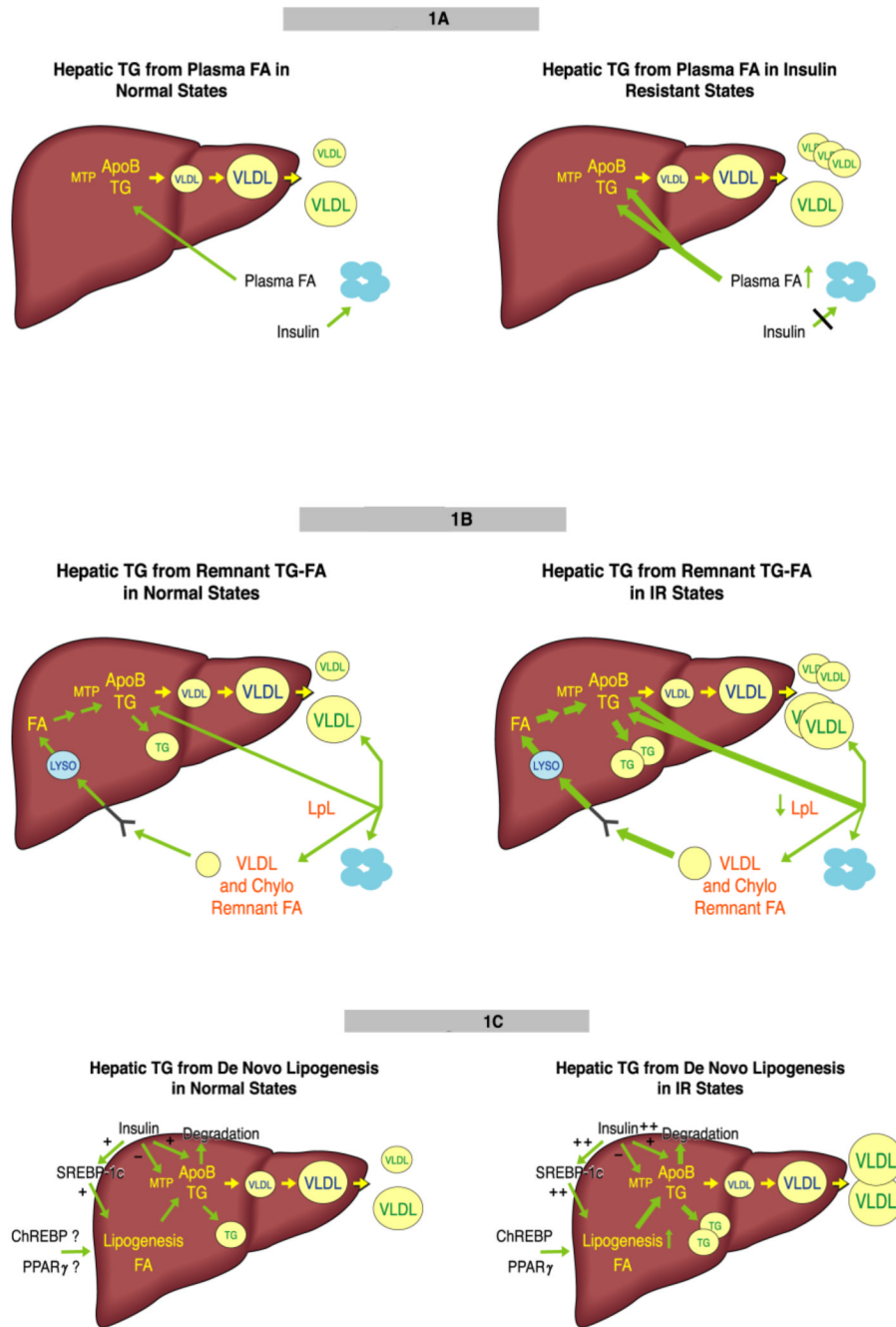
Fig 1.
Hepatic TG derives from three sources. **(a)** The major source is from lipolysis of adipose tissue TG. FA is released, bound to albumin, and circulates to all organs, with the large majority taken up by the liver through several pathways. The major regulation of adipose tissue lipolysis is via insulin signaling (**left**). In IR, there is increased release of FA throughout the day, with increased delivery to the liver. Studies in individuals with hepatic steatosis indicate that about 80% of hepatic and VLDL TG come from plasma FA. Studies in mice suggest that plasma FA can stimulate apoB secretion, even when the delivery of FA is not great enough to stimulate TG secretion. The result of IR-mediated perturbations is secretion of more VLDL that may also carry more TG (**right**). **(b)** VLDL and chylomicrons

(not shown) deliver TGFA to adipose tissue via the action of LpL. Approximately 80% of TG in these lipoproteins is removed during this process. The resulting remnant lipoproteins can deliver their remaining TGFA to the liver using several pathways, mainly the LDL receptor. The TGFA enters the lysosome and FA are released (**left**). In IR, LpL activity may be modestly reduced, leading to TGFA-enriched remnants that deliver more FA to the liver. The result of this IR-mediated perturbation is secretion of more VLDL that can also be larger. (**right**). (c) When the liver has more energy than it requires, glucose is converted to FA by DNL. Insulin plays the major regulatory role in this process via stimulation of SREBP-1c expression and maturation. Insulin may inhibit MTP expression, thus down-regulating VLDL assembly, as well as targeting apoB for degradation. ChREBP and PPARγ may also stimulate DNL (**left**). In IR, insulin levels are increased and, although the liver does not respond to insulin in terms of suppression of glucose production and release, DNL is increased. It is not clear if insulin's negative actions on MTP and apoB are maintained in IR. ChREPB and PPARγ probably add to the stimulation of DNL. The result of increased DNL is the secretion of the same number of larger VLDL (**right**).

**Disposition of Hepatic TG
in Normal States**

**Disposition of Hepatic TG
in IR States**
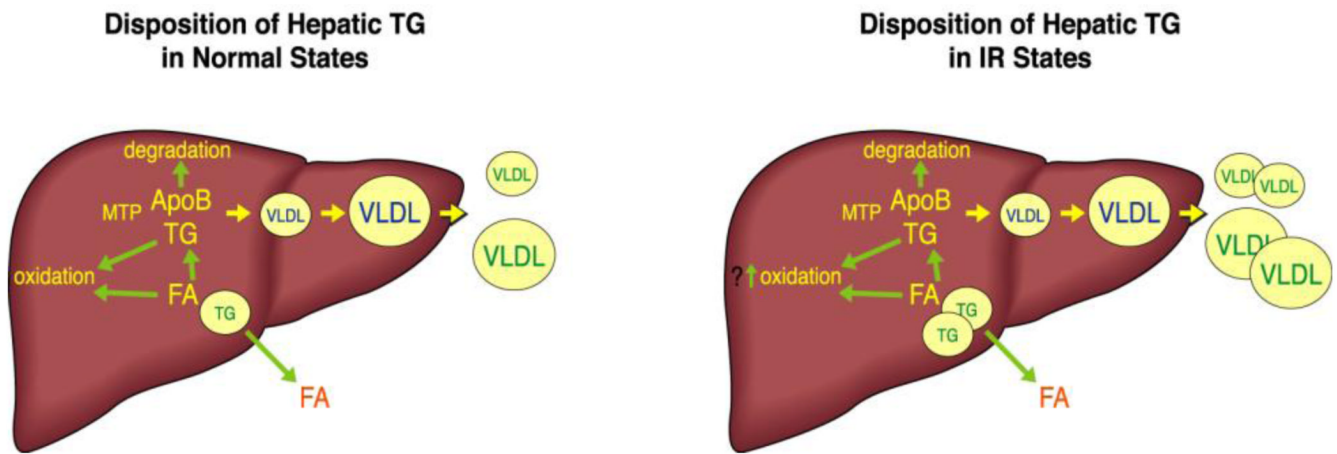
Fig 2.
TG that is synthesized in the liver can be disposed of by three processes. FA can be released from TG and oxidized: FA are a major source of energy for the liver. FA can be released from TG and then re-esterified into TG and assembled, with apoB, into VLDL for secretion. Recent studies in mice suggest that hepatocytes can also secrete FA into the blood; there is no evidence for this in humans. Finally, TG can be stored in lipid droplets (**left**). In IR, increased TG storage in lipid droplets results from increased availability of FA from plasma, remnants, and DNL. There is no evidence that steatosis results from decreased FA oxidation. In fact, the modest evidence that exists in humans suggests that FA oxidation is increased in people with IR. Secretion of TGFA is also increased in IR (**right**).

Effect of Mild ER Stress on the Assembly and Secretion of VLDL and Hepatic Steatosis
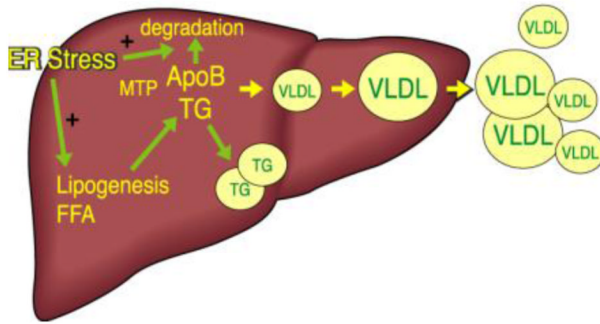
Effect of Severe ER Stress on the Assembly and Secretion of VLDL and Hepatic Steatosis
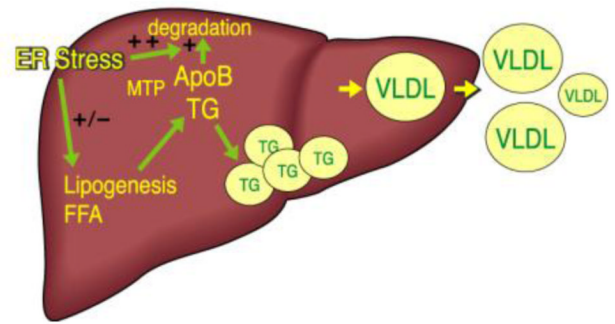
Fig 3.
ER stress and the associated UPR were first described as responses to inadequate energy in the cell leading to misfolding of proteins in the ER. Recent studies have demonstrated the ER stress can result from "over-nutrition" in the cell, particularly in terms of the delivery of FA. ER stress itself may stimulate DNL, leading to more FA in the hepatocytes. When ER stress is mild or moderate, the increased availability of TG and FA will stimulate both hepatic storage of TG and VLDL secretion (left). If ER stress is more severe, the UPR may actually inhibit VLDL secretion via either increasing co-translational degradation of apoB by the proteasome or stimulating post-ER degradation of apoB and VLDL, possibly via autophagy. In this scenario, which is often coincident with IR, VLDL secretion may be increased, but not enough to prevent significant steatosis, even though DNL may no longer be stimulated (right).