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JBR

The Journal of Biomedical Research, 2014, 28(3):178-193

Invited Review

Apolipoprotein B100 quality control and the regulation of hepatic very low density lipoprotein secretion

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Received 15 January 2014, Accepted 15 February 2014, Epub 28 March 2014

Abstract

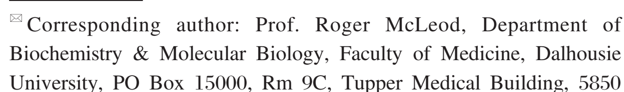
Apolipoprotein B (apoB) is the main protein component of very low density lipoprotein (VLDL) and is necessary for the assembly and secretion of these triglyceride (TG)-rich particles. Following release from the liver, VLDL is converted to low density lipoprotein (LDL) in the plasma and increased production of VLDL can therefore play a detrimental role in cardiovascular disease. Increasing evidence has helped to establish VLDL assembly as a target for the treatment of dyslipidemias. Multiple factors are involved in the folding of the apoB protein and the formation of a secretion-competent VLDL particle. Failed VLDL assembly can initiate quality control mechanisms in the hepatocyte that target apoB for degradation. ApoB is a substrate for endoplasmic reticulum associated degradation (ERAD) by the ubiquitin proteasome system and for autophagy. Efficient targeting and disposal of apoB is a regulated process that modulates VLDL secretion and partitioning of TG. Emerging evidence suggests that significant overlap exists between these degradative pathways. For example, the insulin-mediated targeting of apoB to autophagy and postprandial activation of the unfolded protein response (UPR) may employ the same cellular machinery and regulatory cues. Changes in the quality control mechanisms for apoB impact hepatic physiology and pathology states, including insulin resistance and fatty liver. Insulin signaling, lipid metabolism and the hepatic UPR may impact VLDL production, particularly during the postprandial state. In this review we summarize our current understanding of VLDL assembly, apoB degradation, quality control mechanisms and the role of these processes in liver physiology and in pathologic states.

Keywords: apolipoprotein B, very low density lipoprotein, proteasome, ubiquitin, autophagy, steatosis

APOLIPOPROTEIN B AND THE ASSEMBLY OF VERY LOW DENSITY LIPOPROTEINS

Apolipoprotein (apo) B provides the structural framework for the assembly of triglyceride (TG)-rich lipoproteins in the liver and the intestine (reviewed in[1–8]). Each apoB-containing lipoprotein particle contains a single molecule of apoB, either apoB100, or apoB48 (the N-terminal 48% of apoB100[2]). Increased hepatic secretion of apoB or decreased removal from the plasma can increase the plasma concentration of apoB[9], leading to an increased risk for developing atherosclerosis[10–13]. Therefore, interventions aimed at reduction of particle number may be even more important than reduction of the lipid contents (including cholesterol) of the particles[14,15].

Although the details of assembly and secretion of apoB-containing lipoproteins are not completely



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The authors reported no conflict of interests.

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doi: 10.7555/JBR.28.20140019

defined, three components are recognized as necessary for efficient assembly: apoB, an adequate supply of lipids and the microsomal TG transfer protein (MTP)[3,16,17]. ApoB is unique in its ability to associate with neutral lipids from cellular stores, and high levels of fatty acid substrates for TG biosynthesis can increase hepatic very low density lipoprotein (VLDL) assembly[18–23]. Mutations in the amino terminus of apoB that prevent the initiation of assembly also reduce hepatic VLDL secretion[24,25]. Recent studies showed that reduction of apoB mRNA by antisense oligonucleotide (ASO) techniques reduced VLDL production in HepG2 cells[26] , in mice[27,28], in cynomolgus monkeys[29] and in humans[30–32]. Thus, gene silencing approaches to treat dyslipidemias at the level of hepatic VLDL assembly have now reached clinical application[33].

Assembly of hepatic VLDL has been the subject of intense research for more than 25 years and experiments in hepatoma cells in culture have added greatly to our knowledge of liver biology. When exogenous fatty acid (usually oleate)[34–37] is provided in the culture medium, rat hepatoma cells assemble and secrete authentic VLDL. This model has suggested that VLDL assembly occurs in two steps, one co-translational (the first step) and another post-translational (the second step)[36,38]. The larger amount of TG is added during the second step, perhaps via fusion of a primordial apoB lipoprotein particle with an apoB-free TG droplet in the secretory pathway[39,40]. Although this is an attractive mechanism, it is also recognized that assembly in primary hepatocytes[41,42] may proceed without two discrete steps.

The microsomal TG transfer protein, MTP, facilitates apoB secretion[43–46] in part by addition of lipid to the polypeptide as apoB translocates into the lumen of the ER (***Fig. 1A***). The initial association of apoB with phospholipids may occur independently of MTP as MTP is not required for the secretion of the N-terminal apoB18[47]. MTP is particularly important for the translocation of full-length apoB100, but may not be required for translocation of the shorter apoB48 that is also synthesized by rodent hepatocytes[48–50]. Nevertheless, this does not exclude a role for MTP in the *lipidation* of apoB48. MTP appears to be required for VLDL maturation[35,51,52] and can be found in the Golgi apparatus in addition to the ER[53], consistent with a post-ER second step of lipidation[54]. MTP is subject to diurnal regulation[55] via the genes *Clock* and *Shp*. Mutation of these genes causes hypertriglyceridemia in mice, since Shp normally suppresses the MTP promoter and this is released when Clock is absent. Shp levels change in a diurnal manner and disruption of this cycle can cause increased VLDL output by the liver. Perhaps this is due to a mismatch in the normal feeding-fasting cycle with MTP levels. In apoB100-only *ob/ob* mice, adenovirus-mediated overexpression of MTP stimulated an increase in VLDL-TG secretion without increasing apoB100 secretion[56]. This suggests that MTP allows greater incorporation of TG into existing apoB-containing lipoproteins, thus yielding larger particles, rather than increasing the number of secreted particles. MTP can also transfer cholesteryl ester (CE) from its site of synthesis in the ER (by ACAT1, ACAT2) onto nascent VLDL and thereby relieve product inhibition to enhance cholesterol esterification[57].

MTP became an obvious pharmacologic target in attempts to reduce hepatic lipoprotein production. However, one of the concerns associated with pharmacological inhibition or knockout of *MTP* has been that this can cause hepatic lipid accumulation. Liver-specific knockout of *MTP* caused hepatic steatosis but did not affect insulin sensitivity or glucose tolerance[58]. Selective inhibition of the TG transfer activity of MTP has been suggested as an alternative approach to this type of therapy[59]. These authors restored MTP in knockout mice using adenoviruses with either drosophila MTP, which transfers only phospholipids, or with human MTP, which transfers both TG and PL. Both of the constructs restored some of the plasma lipoproteins and both attenuated liver TG accumulation by stimulating β-oxidation without enhancing lipogenesis. This experiment suggests that PL transfer alone may be sufficient to restore a low level of lipoprotein assembly. Thus, selective reduction of TG transfer may be an alternative approach to reducing VLDL output. MTP inhibition leading to TG accumulation in liver may not always occur with knockdown of the MTP mRNA. Combined therapy with MTP and DGAT2 siRNA were shown to reduce both plasma cholesterol and hepatic TG levels in mice[60].

VLDL assembly is regulated by apolipoproteins other than apoB[61], by lipid availability[62] and by signal transduction mechanisms reporting on organismal nutrition[8,63,64]. This review will focus on the elements of apoB regulation that affect protein folding, and the consequences of failed assembly. In our view, this process is in itself regulatory, and only becomes obvious when the fidelity of substrate production is compromised. The overwhelming evidence supports a surveillance process that repeatedly samples the assembly environment and only triggers apoB intracellular destruction after repeated attempts at proper assembly have failed. Indeed, we believe that the studies of apoB degradation have revealed a process at the interface between physiological regulation of a

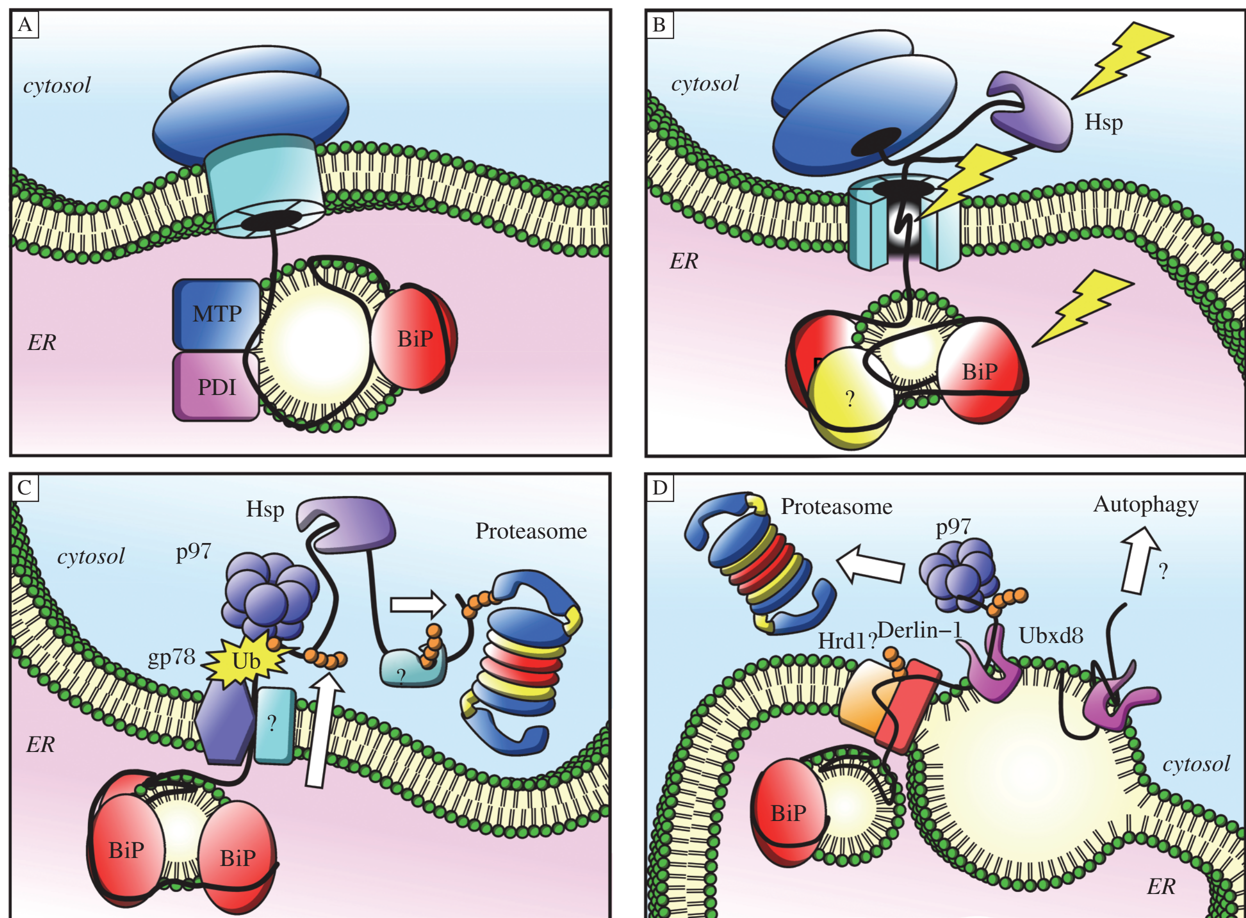

***Fig. 1* ER–associated degradation of ApoB.** A: Cotranslational lipid recruitment. Following engagement between the ribosome and translocation channel, apoB crosses the ER bilayer and enters the ER lumen. There, the growing polypeptide begins to acquire lipid ligands, forming a nascent lipoprotein. Lipidation of apoB is an MTP-dependent process. MTP is present in the lumen as a heterodimer with PDI. The ER chaperone BiP is associated with apoB regardless of apoB lipidation status. Efficient translocation allows the growing apoB peptide to begin forming a secretion-competent lipoprotein. Additional protein folding machinery associates with nascent apoB, as discussed in the text. B: Translocation arrest and substrate selection. If the supply of lipid ligands is inadequate to sustain the assembly of this apoB-containing lipoprotein, translocation of apoB into the ER lumen becomes delayed, or arrested. When inefficient lipidation occurs, hydrophobic regions of lumenal apoB may be exposed to the aqueous environment in the lumen. This leads to further BiP binding (and other luminal chaperones), prolonged exposure of apoB to the inside of the translocation channel, and sudden exposure of apoB motifs to the cytosol (''looping out''). Cytosolically exposed apoB associates with cytosolic chaperones such as Hsp70 and Hsp90. With regards to substrate selection for ERAD, one, two or all three of these events may contribute to triggering downstream events in ERAD. C: Ubiquitination and retrotranslocation of apoB. p97 has been shown to facilitate the proteasomal degradation of apoB. Gp78 (and possibly other ubiquitin ligases, including Hrd1) mediates the ubiquitination of apoB. The presence of a ''threshold'' number of ubiquitin moieties on apoB likely provokes the p97 complex to act on the target substrate. There may exist ''sub-ERAD'' levels of apoB ubiquitination, either in the form of short ubiquitin chains, or low number of chains per polypeptide. p97-dependent retrotranslocation of ERAD substrates appears to require temporary deubiquitination of the target peptide. The substrate then becomes ubiquitinated once more in order to stimulate further proteasomal substrate trafficking and processing events. It is unknown through which channel/mechanism apoB is removed from the ER, nor is it known which (if any) cytosolic ubiquitin ''shuttling'' proteins are involved in delivering apoB to the proteasome. D: Retrotranslocation via ER-associated lipid droplet. A secondary ERAD pathway has been for shown for apoB. It depends on MTP activity and the formation of a primordial apoB-containing lipoprotein. ''ApoB crescents'' have been described as an intersection of proteasomal and autophagic degradation of apoB. Movement of apoB from the ER membrane to the ER-associated lipid droplets requires Derlin-1. The membrane-associated hairpin protein Ubxd8 was found to mediate the extraction of apoB from these lipid droplet-associated crescents by p97. It is a remarkable discovery that lipidated apoB, found in an abnormal, cytosolic lipid droplet-associated state, is extracted in linear form and can be processed by the proteasome.

functional lipid transport entity and pathological destruction of a potentially cytotoxic macromolecular aggregate. Hepatic lipotoxicity is relevant to fatty liver disease, hepatic insulin resistance, inflammation and impaired ER function. Over the course of this review we will examine the consequences of apoB production in various models and speculate on the role of apoB in these assorted pathologies.

APOLIPOPROTEIN B PRODUCTION AND DEGRADATION

Factors promoting lipid availability during VLDL assembly

The pathways of neutral lipid mobilization for assembly into VLDL are still poorly understood. Lehner and colleagues have recently reviewed luminal lipid metabolism and lipoprotein assembly[62] and Sturley and Hussain have reviewed the formation of lipid droplets on either the cytoplasmic or luminal side of the ER membrane[65]. This is a very active area of current investigation, which will only be summarized here.

Several laboratories have explored the roles of TG hydrolases in mobilization of TG for VLDL assembly. Caviglia[66] showed that the TG hydrolase ABHD5/CGI-58 is involved in the mobilization of stored TG for VLDL. Knockdown of the enzyme in McA-RH7777 cells using RNAi reduced apoB, TG and CE secretion. The secreted TG were enriched in saturated fatty acids at the expense of oleic acid, suggesting that this hydrolase may be involved in selective fatty acid mobilization. Ces-1/Es-x is another member of the TG hydrolase family that appears to be involved in the partitioning of regulatory fatty acids[67]. Knockout of this gene in mice increased hepatic lipogenesis and increased secretion of VLDL. The knockout animals developed obesity, fatty liver, and insulin resistance, even on a chow diet. Furthermore, release of polyunsaturated fatty acids (PUFAs) from intracellular TG stores was decreased and sterol regulatory element-binding protein (SREBP) 1c-mediated lipogenesis was increased. Provision of dietary n-3 fatty acids was able to modulate the increased lipogenesis in this model. Ko *et al*[68] showed in McA-RH7777 cells that Ces1/Es-X prevents TG accumulation by enhancing β-oxidation, without having an effect on secretion of apoB and TG. Ectopic expression of hepatic lipase in the ER of McA-RH7777 cells decreased the incorporation of fatty acids into cellular TG and CE and decreased the secretion of apoB-containing lipoproteins[69], although the physiologic significance of this observation is not yet clear. The modified hepatocytes displayed an increase in PPARα expression and enhanced β-oxidation, suggesting that fatty acids generated by hydrolysis of TG in the ER may upregulate cellular utilization of energy. Thus, studies in these model systems indicate that intracellular lipolysis of TG can modulate lipid deposition and apoB secretion from hepatocytes.

Adipose TG lipase (ATGL) generates many of the FFAs found in the liver and may contribute to the characteristics of the ER stress response[70]. In this study, *ATGL* knockout or wild-type mice were treated with tunicamycin to induce ER stress. *ATGL* knockout animals accumulated more TG than wild-type animals even though lipogenesis, β-oxidation and VLDL secretion were decreased by tunicamycin in both groups. The knockout of ATGL prevented the increase in several ER stress and inflammatory markers, while fatty acid analysis indicated that absence of ATGL prevented the increase in the PA:OA ratio that was found in the wild-type mice. Thus, it appears that ER stress-associated changes in the hepatic ER fatty acid profile may contribute to inflammation.

In rats, overexpression of PGC-1α increased cellular mitochondrial content and function[71]. This was also observed in isolated hepatocytes, where increased fatty acid oxidation was detected. Consistent with a decrease in TG storage and secretion, apoB and MTP expression were also reduced. Thus, PGC-1α appears to regulate fatty acid availability for VLDL secretion through its effects on mitochondrial β-oxidation.

CideB is an ER and cytosolic lipid droplet (CLD)-associated protein that is involved in TG mobilization for VLDL assembly. When CideB was depleted in mice, hepatic TG was increased and VLDL secretion was reduced. In addition, the secreted VLDL particles were TG-poor[72]. Restoration of VLDL output was achieved by expression of CideB containing both ER and LD-associating domains, but not when either domain was absent. CideB also interacts directly with apoB, suggesting an integral role for CideB in providing effective lipid availability for assembly[72]. Recently, it was reported that CideB-null mice also displayed marked upregulation of hepatic perilipin 2/ADRP[73], which promotes storage, rather than mobilization, of TG in cytosolic lipid droplets. Knockdown of perilipin 2 in this model enhanced VLDL production and reduced TG accumulation, suggesting opposing roles for CideB and perilipin 2 in regulating hepatic lipid availability for VLDL assembly[73]. Gain and loss of function analysis has also established a role for the phosphatidic acid phosphohydrolase lipin-1 in hepatic TG metabolism[74]. Loss of lipin-1 function did not impair TG synthesis, nor did overexpression enhance TG synthesis. However, increasing lipin-1 decreased TG secretion and knockout increased TG secretion, suggesting that this enzyme plays a role in VLDL assembly, perhaps via modulation of TG availability.

Several of the soluble apolipoproteins have been implicated in the regulation of VLDL assembly and secretion, although the mechanisms of these effects are not yet clear. Studies in humans have indicated that

apoCIII plays a central role in the dysregulation that leads to hypertriglyceridemia and small dense LDL[75]. Particles secreted from the liver are enriched in apoCIII and deficient in apoE in hypertriglyceridemic subjects. These particles are also cleared more slowly from the plasma and the LDL particles that result from their catabolism are more dense, and by definition more atherogenic. Overexpression of apoCIII in transgenic mice also increased hepatic TG levels and induced insulin resistance[76]. Sundaram and colleagues showed that apoCIII has an intracellular role in VLDL assembly[77]. In McA-RH7777 cells they showed that overexpression of apoCIII increased apoB and VLDL secretion, but only in culture conditions where TG levels in the cells were also elevated. In this model system the enhancement was accompanied by an increase in the expression of MTP, and suggested that apoCIII was involved in the second step of lipidation.

ApoAIV has also been implicated in the assembly of VLDL[78]. In a model system where the apoAIV was trapped in the ER by appending to it the KDEL retention signal, secretion of apoB and TG were decreased. Interestingly, apoAIV increased the amount of secreted TG per particle without affecting MTP expression, suggesting that apoAIV may modulate apoB trafficking, allowing the particles to acquire additional TG. Plasma apoAII may also affect VLDL production rate[79]. Genetic polymorphisms in the apoA5 promoter that affect TG metabolism have been associated with coronary artery disease[80]. Thus, several of the soluble apolipoproteins may have an influence on VLDL production, but exactly how is not yet clear.

It has been known for some time that there is an inverse relationship between plasma HDL-C levels and TG levels in humans, and this is particularly apparent in the ABCA1-deficient state. The effect of HDL metabolism on hepatic VLDL production has been explored recently. Chung et al.[81] used a gene silencing technique to reduce ABCA1 levels in McA-RH7777 cells, which caused the accumulation of large HDL in the medium. This also caused an increase in the secretion of TG-rich $VLDL_1$, as a consequence of a decrease in the activation of PI3 kinase. These changes were corrected by the addition of normal HDL to the cell culture, suggesting that it is the characteristics of the HDL that affects the response of the hepatocyte. Wiersma showed that HDL can be taken up by hepatocytes in culture and components of HDL can be secreted in VLDL[82]. By overexpression or knockout of SRBI, VLDL-apoB and VLDL-TG secretion were increased or decreased, respectively. This was also found *in vivo*, where changes in MTP, SREBP-2 and liver cholesterol content were consistent with the changes in VLDL production. Thus, there is accumulating evidence of an effect of HDL metabolism on liver production of atherogenic lipoproteins.

Cellular trafficking events play an integral role during the assembly and secretion (or degradation) of nascent VLDL. Willnow has reviewed the role of sortilins in lipoprotein metabolism[83]. Sortilin (Sort1) is located on chromosome 1p13.3 and SNPs in this locus have been associated with hypercholesterolemia and myocardial infarction. Sort1 is an intracellular sorting receptor for apoB in the Golgi apparatus and knockout of Sort1 reduces the secretion of apoB-containing lipoproteins from the liver. This resulted in decreased plasma cholesterol levels and reduced atherosclerotic lesions in *LDLr*-null mice[84]. Conversely, overexpression of Sort1 increased plasma cholesterol and apoB-lipoprotein secretion.

The role of Sort1 and ER stress has been further explored by Ai and colleagues[85]. In contrast to the previous work these authors found that Sort1 expression was decreased in obese mice and that restoration of Sort1 expression decreased hepatic TG and apoB secretion. In addition, inhibition of mTOR reduced the level of ER stress and increased Sort1 expression, suggesting that induction of ER stress through the mTOR signaling pathway may contribute to dyslipidemia in obese states. Strong and colleagues have shown that sortilin reduces apoB secretion by binding to the secretory protein in the Golgi apparatus and delivering apoB to the lysosome for degradation[86].

Insulin stimulates an interaction between Sort1 and apoB via PI3 kinase in McA-RH7777 cells[87], suggesting that Sort1 may be involved in the normal insulin response in the hepatocyte. A direct interaction between Sort1 and apoB was detected by inhibition of lysosomal degradation, which also increased apoB secretion. This study suggests that the Sort1-apoB interaction and lysosomal degradation play roles in the insulin-dependent regulation of apoB secretion.

Trafficking of apoB in the secretory pathway may also be impacted by PCSK9[88], as overexpression of PCSK9 in multiple mouse models increased plasma apoB, cholesterol and TG due to increases in both LDL and VLDL. The authors suggested that interaction of PCSK9 with apoB in the hepatocyte may inhibit autophagy. In the secretory pathway PCSK9 may shuttle between the LDL receptor and apoB, thereby coordinately regulating lipoprotein import and export.

In intestinal Caco-2 cells, overexpression of Sar1b increased chylomicron production and enhanced the esterification of fatty acids into all lipids[89]. ApoB48 synthesis was increased as were the activities of MGAT, DGAT and MTP. Sec23 interactions with

Sar1b were demonstrated by immunoprecipitation. Since these proteins in turn interact with SCAP and SREBP1c, these complexes may be implicated in the enhancement of lipid biosynthesis. Although these observations may not be directly relevant to hepatic apoB metabolism, complementary pathways may be present in the hepatocyte and the enterocyte.

Mechanism(s) of apoB degradation

Although changes in the levels of apoB transcript[90] and translation of the mRNA[91–93] can play a role in apoB production, secretion of apoB is primarily regulated at the level of protein degradation[94]. Degradation of apoB can occur either co-translationally or after the full-length protein has entered the secretory pathway. Whether apoB is assembled into a secretory lipoprotein particle or is degraded before reaching the extracellular space depends on the interaction between apoB and components of the assembly and degradation systems. Degradation may be initiated when part of the apoB protein is recognized as malfolded by the quality control machinery of the ER due to incomplete lipidation, initiating delivery of the protein to the cytosolic proteasome[95]. The proteasome is often involved in the removal of abnormally folded cellular proteins in the process termed ER-associated degradation (ERAD)[96]. Although it may seem wasteful that normal, functional proteins such as apoB should be degraded before performing their primary function, perhaps as much as 25% of newly synthesized polypeptides may be removed by this surveillance system[97,98]. Surveillance can remove nascent proteins without initiating ER stress and the unfolded protein response (UPR)[99], which can compromise cell function and jeopardize cell survival[100–102]. ApoB can be degraded by autophagy when the nascent lipoprotein fails late-stage quality control surveillance and in response to hepatic insulin signaling[103]. Hepatic autophagy is highly integrated with lipid homeostasis[104]. In addition, selective autophagy removes and degrades ERAD-related protein complexes from the ER, by newly emerged mechanisms[105,106].

Since the first demonstration of apoB degradation in 1987[107], an enormous amount of effort has been expended in the study of this process. However, it was not until nearly 10 years later that the involvement of the cytosolic proteasome was demonstrated[108]. Much of the evidence for the role of the proteasome in apoB degradation has been derived from studies of the HepG2 cell because degradation of apoB by the ubiquitin-proteasome pathway is particularly active in this cell line due to inefficient VLDL assembly. Although this cell model does have shortcomings, other human liver cell lines do not appear to have significant advantages over HepG2[109,110]. ERAD of apoB is not unique to the HepG2 cell line and can also be demonstrated in the rat hepatoma McA-RH7777[111]. In primary hepatocytes, however, the role of the proteasome is less clear because the proteasome inhibitors (MG132, lactacystin and ALLN) used to demonstrate the involvement of the proteasome can be toxic to these cells[42].

Although apoB secretion is modulated by intracellular degradation, this may not always be equivalent to regulation. It is important to determine whether quality control surveillance machinery in the ER targets and destroys apoB in a reactive manner, in response to "malfolding" events during VLDL assembly, or whether apoB is removed by degradation via a proactive, and therefore potentially regulated process. Since multiple coordinated events are required for apoB to achieve its "native", secretion-competent conformation (including sustained translocation for the duration of apoB translation, disulfide bond formation and rearrangement, glycosylation, lipid ligand acquisition and intracellular trafficking) it is clear that the QC machinery will be reactive to the fidelity of these biosynthetic events. However, the modulation of apoB production in response to whole body metabolism in changing nutritional states suggests that there are proactive surveillance mechanisms that must remove nascent apoB when curtailing VLDL output is warranted. The remainder of this section will review apoB quality control and degradation mechanisms.

Luminal and cytosolic triggers for proteasomal degradation of apoB

Conditions that limit the assembly of VLDL such as reduced availability of lipid ligands[112] or MTP inhibition[113] can trigger the proteasomal degradation of apoB. The translocation efficiency of apoB is tied to its necessity for co-translational acquisition of lipid ligands. When lipidation is inadequate, apoB can undergo "translocation arrest." Considerable evidence supports the hypothesis that proteasomal degradation occurs as a consequence of apoB translocation arrest, causing regions of the polypeptide to become exposed to the cytosol[114–118]. Admittedly, this may reflect the relatively low efficiency of assembly in hepatoma cell systems. ApoB may exist transiently in a bitopic topology (exposed to both the ER lumen and the cytosol), simultaneously accessible to both assembly and degradation pathways[117]. Jiang and colleagues[119] have shown that the presence of MTP during apoB translation prolongs the window for lipid recruitment *in vitro*. Three "lesions" likely occur simultaneously, or in extremely

close succession: (1) exposure of poorly lipidated apoB regions in the ER lumen, (2) prolonged residence in the translocon, and (3) exposure of apoB motifs to the cytosol (depicted in ***Fig. 1B***). Given its bitopic topology, translocation arrested apoB may even behave as an ERAD substrate that is membrane-integrated, despite the final product of VLDL being a luminal, secreted lipoprotein[120]. These characteristics of apoB are important upon consideration that mechanisms of ERAD substrate recognition and processing are often determined by lesion location; specifically whether an unfolded or malfolded segment of the polypeptide is in the ER lumen (ERAD-L), within the bilayer (ERAD-M) or in the cytoplasm (ERAD-C)[121,122].

The ER chaperones BiP/Grp78, calreticulin, PDI and Grp94 have been found crosslinked to apoB in HepG2 cells, regardless of apoB lipidation status[123]. However, the ER-resident chaperone BiP was reported to increase binding to apoB when the interaction between nascent apoB and MTP was disrupted[124]. BiP is considered a principal indicator of ER stress, as its levels increase incrementally via transcriptional upregulation. Mild to moderate levels of fatty acid-induced ER stress increase both BiP levels and VLDL assembly[125]. Therefore, it seems that BiP levels may not regulate apoB ERAD but more likely contribute to stabilizing poorly lipidated apoB motifs prior to their removal from the ER. Low, medium and high levels of BiP do not correlate linearly, with respect to substrate fate. However, it appears that prolonged apoB-BiP binding may facilitate the ERAD of apoB[124].

There are ten different PDI species in mammals that are critical to the differences in ERAD substrate recognition[126]. This study in yeast, which have 5 PDIs, showed that PDI-1 interacted with exogenously expressed apoB proteins and its chaperone activity facilitated apoB degradation. In contrast, other ERAD substrates were not dependent on PDI-1 but were dependent on the redox activity in the ER lumen. Thus, chaperone interactions within the ER can determine substrate fates. It is currently unclear whether apoB is a client of the *N*-glycan sugar timing system that governs glycoprotein quality control[102]. While apoB does associate with calnexin and calreticulin under ''normal'' conditions, the difference between secretion and degradation of apoB is primarily determined by lipid availability. This does not preclude, however, the possibility that lipid-dependent folding events of the apoB protein might be connected to a glucose-trimming mechanism of quality control. ApoB also associates with the ER luminal protease ER-60 in HepG2 cells[127]. Subsequent studies of ER-60 overexpression and proteolytically inactive ER-60 suggested that ER-60 may cleave apoB translocation-arrested apoB in the ER lumen[128,129]. It is possible that luminal cleavage events are coordinated with retrotranslocation of poorly lipidated apoB polypeptides into the cytosol. Studies in HepG2 cells have revealed that the *N*-terminal region of apoB may not be removed from the ER into the cytosol[130], but may instead be cleaved and then either degraded or secreted[131].

Inefficiently translocated apoB is associated with the cytosolic chaperones Hsp70[118] and Hsp90[132]. It appears that cytosolic heat shock proteins are necessary to support degradation. Since increased Hsp70 expression enhances apoB ubiquitination when the lipid supply is unaltered, apoB may be stabilized by Hsp70 in the cytosol during stages where it is subject to quality control mechanisms. There are likely other ubiquitin proteasome system proteins in the cytosol that bind to apoB as well, beyond those of the heat shock family.

In some experimental systems, apoB chains that were degraded by the proteasome had already translocated into the ER lumen and interacted with calnexin[133], implying that they had attained full-length and acquired significant glycosylation prior to degradation. Nevertheless, if translocation arrest cannot be demonstrated *in vivo*, apoB can still be degraded from within the secretory pathway. Inhibition of the MEK-ERK pathway[134] increases the efficiency of VLDL assembly in HepG2 cells and decreases apoB ubiquitination[135]. In our experiments, cellular apoB is largely exposed to the cytosol[114,136] while smaller recombinant apoB proteins (such as apoB29) are not. Fusion proteins containing apoB29 and the apoB segment from B37-42 were partially arrested[114]. The $\beta 1$ domain (defined as between the C-termini of apoB22 and apoB42) has been implicated in translocation arrest, susceptibility to proteasomal degradation and LpB assembly[116,137]. Our studies suggest that not all $\beta 1$ regions increase the susceptibility of apoB to degradation, but that specific regions of the $\beta 1$ domain (including B37-42) may mediate proteasomal degradation and core lipid recruitment[114]. These unique sequences in apoB may mediate proteasomal degradation by increasing exposure to the cytosol or by slowing translocation sufficiently to initiate the ERAD. Intriguingly, intra-membrane cleavage of ERAD substrates has emerged as a novel proteolytic mechanism, and this process may yet have relevance to apoB[138].

Ubiquitination of apoB

ApoB could be targeted to ERAD by multiple ubiquitin ligases and with more than one means of escape from the ER. Overexpression of the ubiquitin ligase gp78 in HepG2 cells caused increased ubiquitination

and degradation of apoB combined with a requisite decrease in secretion[139]. Conversely, we observed apoB secretion to increase upon modest, siRNA-mediated depletion of gp78 in HepG2 cells[135]. Furthermore, our knockdown of gp78 paradoxically shifted the density of secreted apoB-containing lipo–proteins from HepG2 cells into the VLDL range, away from typically observed majority of LDL. Thus, in this model, ubiquitination is not only responsive to lipid recruitment during assembly but influences the ability of nascent apoB as it progresses through the secretory pathway. ApoB has also been found to associate with the ER-resident E3 ligase Hrd1, as well as the pur–ported retrotranslocation mediator Derlin-1[124]. Full length apoB is degraded by the proteasome, as are co-translationally truncated apoB polypeptides of many different sizes. E3 ligase overlap and/or coop–erativity is likely, and not without precedent. The cell may employ different machinery to detect and target these distinct apoB substrates to ERAD. The specifics of apoB ubiquitination are poorly understood and war–rant further investigation.

Retrotranslocation of apolipoprotein B

As the field of ERAD and substrate retrotransloca–tion advanced, so has our understanding of apoB as an ERAD substrate. For some time there has been an active (and technically challenging) search to identify a ‘‘retrotranslocon’’[140]. What has resulted from this search is an increased understanding of ER function, and an appreciation of the diverse nature of ERAD sub–strates and the means by which they are processed. Some substrates can be threaded through retrotranslo–cons as linear polypeptides, while others appear to move through to the cytosol while still folded or in multimeric complexes[141]. Presented below are data pertaining to apoB retrotranslocation and other relevant recent find–ings that have enhanced our understanding of the ER.

Partially translated apoB associates with both the Sec61 translocon and the proteasome[142], suggesting that retrotranslocation of arrested apoB could occur through the same pore that mediates translocation. Ubiquitinated apoB can found in the Sec61 complex in HepG2 cells[143]. It is tempting to speculate that the ‘‘hydrophobicity’’ threshold of the hydrophobic core in the Sec61 channel may play a role in apoB quality control[144]. This attribute of the Sec61 core was described in the context of nascent transmembrane domain integration into the bilayer. As apoB undergoes translocation arrest, its densely hydro–phobic epitopes could trigger a similar process that moves apoB laterally into the ER bilayer, perhaps into an ERAD-dedicated multimeric complex.

The ER-membrane spanning proteins Derlin-1 and the ubiquitin ligase Hrd1 facilitate movement of ERAD substrates to the cytosol, yet it has been unclear whether these proteins form a putative retrotranslocation channel themselves, or are required in another manner. Studies in mammalian cells and *C. Elegans* suggested that Derlin-1 proteins served as components in a retro-translocation channel[145,146]. More substrates for Derlin-1-dependent retrotranslocation have since emerged, lending support to this route out of the ER[147–149]. A unique photocrosslinking technique was used to show that amino acids of the Hrd1 protein within the trans–membrane domain come into direct contact with ERAD substrates[150,151]. The protein Usa1 (known as HERP in mammals) supports the interaction of multiple Hrd1 proteins, which may in turn facilitate the formation of relatively large, dynamic retrotranslocation channels capable of moving partially folded protein cargo through to the cytosol[152]. Recently, photocrosslinking and muta–genesis of yeast Der1 revealed that ‘‘recognized’’ sub–strates are transferred from luminal receptors Hrd3 and Yos9 and moved through the ER membrane in a manner that requires transmembrane domains of Der1. Further, Der1 TM domains facilitate the ubiquitination of the sub–strate (CPY*) in the cytosol by the Hrd1 ubiquitin ligase[153]. ApoB has been shown to associate with mam–malian Derlin-1 and Hrd1 at the ER in HepG2 cells, yet their requirement for nascent apoB ERAD is unclear[124].

The homo-hexamer AAA-ATPase p97 has been shown to facilitate removal of ubiquitinated apoB from the ER for proteasomal degradation (see ***Fig. 1C***)[124,154]. Reduction of p97 by siRNA in HepG2 cells delayed apoB turnover and accumulated apoB at the ER, while apoB secretion efficiency was unaltered. The effect of the p97 knockdown on apoB could be abolished by either knocking down the ubiquitin ligase gp78 or enhancing VLDL assembly pharmacologi–cally[135]. While these studies revealed a cotranslational role for p97 in apoB metabolism, Suzuki and collea–gues have found an alternative, lipid droplet-dependent pathway for retrotranslocation of lipidated ‘‘apoB-crescents’’ in Huh7 hepatoma cells[155]. Reduction of Derlin-1 caused apoB to accumulate only in the ER lumen, while depletion of tail-anchored protein Ubxd8 caused apoB to accumulate on ER-associated lipid droplets and the ER. This apoB accumulation was suppressed by inhibition of MTP activity, sug–gesting that these apoB ERAD substrates require at least partial lipidation to enter this particular pathway. Remarkably, Ubxd8 recruits p97 for the extraction of apoB from lipid droplets for proteasomal degradation (***Fig. 1D***)[155]. Membrane-embedded domains of Derlin-1 associate directly with ERAD substrates and

the ubiquitin ligase Hrd1, as shown by site-specific photocrosslinking[153]. Thus, it is possible that Hrd1 participates in processing apoB substrates as part of this ERAD protein complex. The role of p97 in apoB ERAD now appears to be twofold: retrotranslocation of nascent apoB from the ER and extraction of partially-lipidated apoB from lipid droplets. These are two distinct subclasses of apoB ERAD substrates that share some overlapping machinery.

Ubxd8 has been shown to regulate lipid droplet homeostasis in yeast[156]. Unsaturated fatty acids cause Ubxd8 to dissociate from ubiquitinated Insig-1 and oligomerize, which in turn increases Insig-1 levels at the ER and prevents the activation of SREBP. However, saturated fats do not provoke this response, resulting in inadequate regulation of lipids. The inability of Ubxd8 to "sense" saturated fats has been proposed as a means for hepatic metabolism dysregulation in the face of a Western-style diet[157]. Given that the Scap/SREBP proteins are essential for the progression of diabetic fatty liver[158] it would be fascinating to know whether Ubxd8 modulates neutral lipid availability for apoB production in a pathological setting, in addition to its involvement in trafficking apoB through the cytoplasmic lipid droplet (CLD) compartment during ERAD.

In addition to serving as storage organelles for excess fatty acids and cholesterol, which are stored as TG and cholesterol esters, respectively[159], CLDs are also protein storage depots[160]. Lipid droplets provide an intersection of lipid metabolism and protein quality control. Ancient ubiquitous protein 1 (AUP1) may facilitate the delivery of ERAD substrates to lipid droplets *en route* to the proteasome[161], while Ubxd8-depleted Huh7 cells accumulate many different ubiquitinated proteins in addition to apoB[155]. HMG CoA reductase is delivered to lipid droplets after it is dislocated from the ER membrane and before it is degraded by the proteasome[162].

How the biogenesis of lipid droplets is organized to support both assembly and degradation of apoB is unclear. At some point between substrate recognition and degradation, non-exchangeable apoB polypeptides must be stripped of their lipid ligands, which, *in vitro,* bind irreversibly to sequences in the β1 domain[163]. The answer to this question may emerge from probing VLDL assembly and lipid droplet dynamics in multiple hepatocyte models.

Role of deubiquitinating enzymes in modulating ERAD substrate fate

Although it was previously presumed that ubiquitin was only used to target non-functional proteins for disposal, ubiquitin-protein conjugates are now recognized to be dynamic structures. Deubiquitinating enzymes (DUBs) are responsible for the removal of ubiquitin from substrate proteins. The human genome encodes approximately 100 DUBs in 5 families[164]. Proteomic analysis of DUBs and their associated proteins by Sowa and colleagues revealed six DUBs likely to interact with the AAA-ATPase p97[165]. The role of DUBs in p97-dependent ERAD has recently been reviewed[166]. Dislocation of ERAD substrates requires p97 and a dimeric complex of Npl4 and Ufd1, which binds to ubiquitin in addition to p97[167]. Ubiquitin chain trimming on ERAD substrates may change the affinity for ubiquitin-binding effectors. This has been suggested to contribute to the transfer of the substrate from p97 to effectors such as the Bag6 holdase complex[168] to possibly facilitate transfer from the ER to the proteasome.

Ataxin-3 is a DUB associated with the neurodegenerative disorder spinocerebellar ataxia type 3[169]. Work by Zhong and Pittman in HEK293T cells suggested that ataxin-3 binds to p97 and reduces its interaction with Ufd1, therefore, causing accumulation of ERAD substrates in the ER[170]. In contrast, evidence has also shown that ataxin-3 may promote p97-associated deubiquitination to facilitate the transfer of ERAD substrates to the proteasome[171]. Expression of an inactive ataxin-3 mutant caused accumulation of ubiquitinated proteins associated with p97, suggesting that substrate transfer from p97 to the proteasome was blocked.

The mammalian DUB YOD1 is a component of the p97 complex by way of its UBX domain[172]. Expression of catalytically inactive YOD1 caused accumulation of a model ERAD substrate and of polyubiquitinated proteins. Interaction between YOD1 and other components of ERAD such as Derlin-1 and Ubxd8 were also reported. Recently, it was demonstrated that YOD1 also regulates the degradation of a nonubiquitinated ERAD substrate, cholera toxin A1 (CTA1)[173]. Using siRNA knockdown of YOD1 and a retrotranslocation assay, they concluded that YOD1, but not other DUBs (ataxin-3 and USP14), blocks CTA1 retrotranslocation. Since CTA1 is a nonubiquitinated substrate, they suggested that YOD1 inhibits retrotranslocation by deubiquitination of components of the ERAD machinery[173]. Notably, gp78-dependent ubiquitination of the ERAD-associated chaperone Bag6 cofactor Ubl4 caused inactivation of the Bag6 chaperone, while conversely the DUB Usp13 removed ubiquitin conjugates from Ub14 and preserved Bag6 function[174]. Liu et al. also demonstrated that Usp13 acts on ERAD substrates downstream of p97 since deglycosylation of TCR was not affected by Usp13 knockdown.

These data illustrate the emerging complexity and dynamics of ubiquitination in ERAD.

The DUB enzyme USP25 has been recently implicated in ERAD. Blount and colleagues showed that exogeneous USP25 interacts with the E3 ligase Hrd1 and with p97[175]. The catalytic activity of USP25 rescued an ERAD substrate (CD3) from proteasomal degradation, while knockdown of USP25 resulted in a decrease in levels of an ERAD substrate. Deubiquitination of ER membrane proteins has been suggested to "sharpen" substrate discrimination by modulating the affinity of E3 ligases for their substrates′ ubiquitin chains[176]. Taken together, ubiquitin chain remodeling appears to be prominent during ERAD substrate fate determination.

DUB activity may play a significant role in apoB quality control processes. Ubiquitination of apoB appears to be responsive to apoB folding and topology, which is governed by lipid recruitment. Removal of ubiquitin conjugates from apoB may rescue partially-assembled lipoproteins from degradation and allow apoB to reach secretion competence. Also, DUB activity is likely required to facilitate apoB retrotranslocation as well as efficient entry into the proteasome core. E3 ligases have an emerging role in regulating autophagy[177]. Indeed, broad spectrum DUB inhibition with PR619 has been reported to activate autophagy in oligodendroglial cells[178]. Further down the VLDL assembly line, DUB activity could participate in selecting and processing apoB for degradation by autophagy. As the ubiquitin landscape is revealed, it will be fascinating to observe the full extent to which deubiquitination is involved in VLDL assembly and protein quality control in general.

Clearance of nascent apoB-containing lipoproteins by autophagy

Autophagy is a rapidly growing area of cell biology. Dozens of proteins are actively involved in autophagy, across many cell types and tissues. The role of autophagy in hepatic apoB control quality control was recently reviewed[179], as has the broader role of autophagy in regulating lipid homeostasis in physiological and pathological settings[104]. Defective hepatic autophagy has been reported to associate with chronic ER stress and insulin resistance in diet-induced and genetic models of obesity in the mouse[180]. The relationship between hepatic autophagy, insulin signalling, lipid metabolism and apoB quality control is complex and incompletely understood. Autophagy is a recently appreciated mechanism for apoB disposal; however, it is unclear whether ERAD and autophagy are distinct processes, with different cues and physiological roles, or whether the two processes represent parts of a continuum of cellular degradation pathways. Summarized below are the observed mechanisms of apoB autophagy.

Omega-3 fatty acids were shown to induce hepatic autophagy of apoB and reduced VLDL secretion, in a process termed pre-secretory, post-ER proteolysis or PERPP[103]. Blocking lipid peroxidation in rat hepatoma and primary rodent hepatocytes restored VLDL secretion. It was later reported that PUFA-derived reactive oxygen species (ROS) were responsible for apoB aggregation and degradation by autophagy[181]. In McA-RH7777 cells, maturation of pre-VLDL to fully lipidated VLDL in the Golgi is impeded by providing the omega-3 fatty acid DHA, resulting in autophagy of apoB[182].

In primary mouse hepatocytes, the insulin-dependent degradation of apoB by autophagy was shown to require signaling through class II PI3-kinase[183]. However, in McA-RH7777 cells, class I and III PI3-kinases were apparently involved, with overexpression of the negative insulin regulator phosphatase and tensin homolog (PTEN) and mutant Vps34 (required for autophagy) showing that insulin-dependent apoB degradation involves a form of autophagy[184]. Interestingly, the flavonoid naringenin caused rapid, proteasome-independent apoB degradation and reduced apoB secretion from HepG2 cells via PI3K and MEK/ERK signalling, independent of the insulin receptor[185]. Although it remains unconfirmed, autophagy is the likely degradation mechanism elicited by naringenin. ApoB overproduction was prevented by naringenin treatment of LDL receptor knock-out mice with diet-induced insulin resistance[186]. These observations demonstrate that apoB autophagy is a complex, regulated and dynamic process. ***Fig. 2*** displays apoB as it moves from the ER to the Golgi and is either subject to degradation by autophagy or completes the second bulk lipidation event and is secreted from the cell. Also shown in ***Fig. 2***, in broad strokes, are the movement of lipids among the ER, Golgi, lipid droplets and autophagosomes that are involved in apoB metabolism.

Ohsaki and colleagues have described apoB "crescents"[187,188], which increase when autophagy or the proteasome are inhibited and decrease when MTP is reduced. The authors suggested that these structures are lipid droplets arrested in the ER membrane by binding to apoB and propose that apoB processing and lipid droplet formation are closely linked processes. Further, it has been proposed that these cytosolic lipid droplets are a site of convergence for proteasomal and autophagic apoB degradation. Thus, ERAD and autophagy may degrade apoB starting at

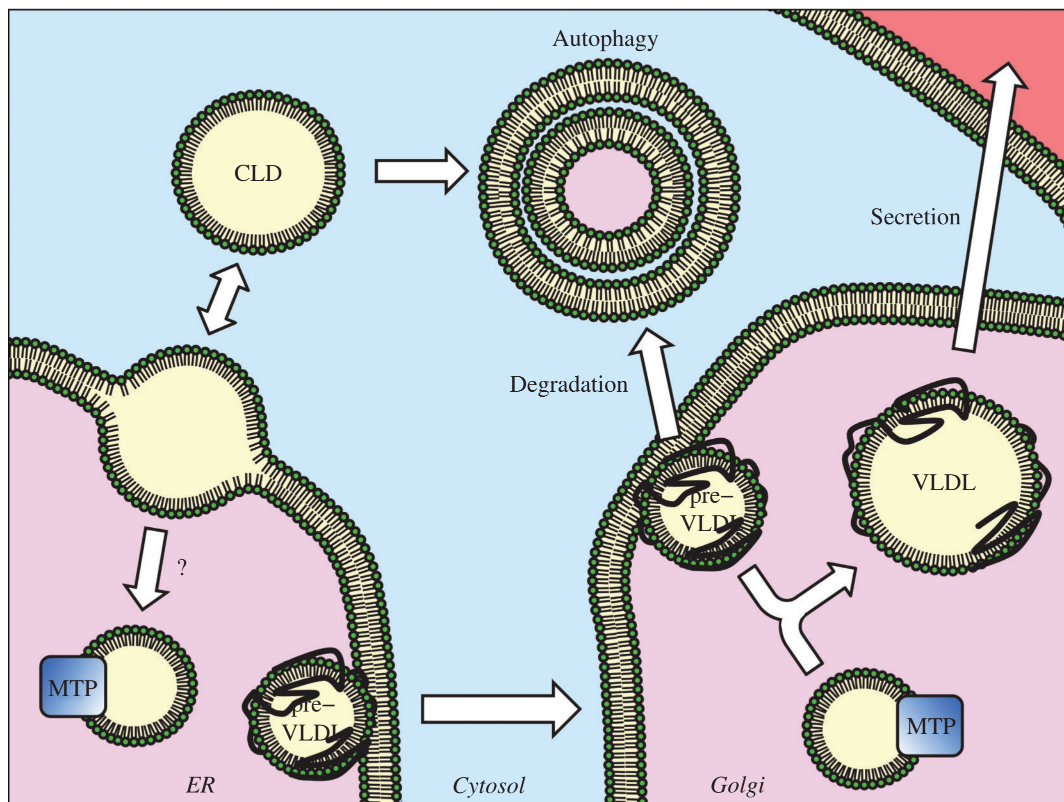

Fig. 2 **Schematic representation of VLDL production, apoB autophagy and lipid cargo movement.** Following synthesis, the apoB protein remains associated with the ER bilayer (left-hand side). This pre-VLDL particle is trafficked to the Golgi, where apoB then receives lipid ligands in bulk to form a full-sized VLDL particle (bottom, right). ApoB topology shifts and the VLDL particle is released from membrane association, followed by rapid secretion from the hepatocyte (top right). MTP is necessary for the formation of apoB-free lipid droplets (left, bottom), but MTP enzymatic activity is not required to facilitate the posttranslational bulk transfer of lipids onto apoB in the Golgi. ApoB is subject to late-stage quality control at the Golgi, where apoB can be removed from the secretory pathway by autophagy machinery. Movement of lipid metabolites is possible from cytosolic lipid droplets (CLD) to and from the ER bilayer, or to autophagosomes. Metabolic regulatory cues will determine the relative rate at which lipid metabolites are mobilized to the ER for export as VLDL, stored in the cytosol, oxidized in the mitochondria (not shown) or catabolized by autophagy. When apoB is destroyed via autophagy, its lipid cargo may become recycled and enter lipid pools that are oxidized, stored or re-mobilized to the ER.

temporally and spatially distinct exit points from the secretory pathway, yet both require the lipid droplet to process doomed apoB and its lipid cargo.

Glucosamine induces a PERK-dependent decrease in translation and an increase in autophagic and pro–teasomal degradation to clear apoB[189–191]. In McA-RH7777, apoB colocalized with the autophagy marker LC3 under stress conditions (tunicamycin or glucosa–mine)[191]. Signaling through the PERK arm of the UPR was required to remove apoB by ER stress-induced autophagy. Notably, this stress could be relieved with the small molecule chaperone 4-phenylbutyric acid (PBA). This pathway was present in primary rat and hamster hepatocytes at basal conditions, but was absent from HepG2 cells unless the proteasome was also inhibited and extracellular fatty acid was present. It is notable that different cell types dispose of apoB in response to a single stressor, glucosamine, by different mechanisms.

As with ERAD, the mechanism of substrate selection is unclear in the apoB autophagy (PERPP) pathway. LC3, a critical protein component of autophagosome formation, binds p62 that contains an ubiquitin associated (UBA) domain and is involved in ubiquitin-dependent substrate selection for autophagy[192]. Binding of p62 to apoB-polyubiquitin chains at the Golgi membrane could conceivably allow the regulated removal of apoB-containing lipoproteins from the secretory pathway. Intriguingly, p62 activates Nrf2[193], an ‘‘antioxidant response’’ transcription factor that can be activated by the UPR (and is proposed to be the ‘‘fourth arm’’ of the UPR)[194]. Perhaps the insulin and ROS-induced clearance of apoB are regulated by a p62-Nrf2 axis that connects oxidative stress, the UPR and VLDL assembly.

This concludes the overview of cellular degradative mechanisms that target nascent apoB. These protein quality control processes exhibit crosstalk between one another, as well as sharing regulatory cues with

other metabolic processes that impact apoB production. From an apoB-centric point of view, Section 3 will discuss the coordinated regulation of VLDL production by insulin signalling, lipid metabolism and the hepatic UPR in physiological and pathological settings.

APOLIPOPROTEIN B DEGRADATION – PHYSIOLOGY AND PATHOLOGY

The unfolded protein response (UPR) and the hepatic ER

The unfolded protein response (UPR) is a multi-pronged signalling response which was initially characterized as the ability of the ER to sense and adapt to the stress of an increased protein folding burden[195]. Activation of the UPR is observed in disease states affecting many cell types and tissues. However, it is not always clear whether the ER stress and resulting cellular response are the cause, or an effect, of the disease. Complicating matters is the emerging distinction between physiological and pathological ER stress[196,197]. The ER is a dynamic and finely-tuned organelle that serves as the major site of lipid and protein biosynthesis in the cell[198] and the UPR pathways are highly integrated into energy metabolism[197]. While the role of ER stress in apoB production *in vivo* is not fully known, recent studies have revealed that several components of the UPR impact VLDL output from the liver[199].

Activation of the UPR, generally speaking, reduces new protein synthesis while expanding the protein folding and degradative capacity of the ER. The IRE1α arm of the UPR stimulates lipid biosynthetic pathways to expand the ER membrane to provide space for the elevated protein folding/degradative requirements. IRE1α also has a constitutive role in regulating hepatic lipid homeostasis, particularly through modulating postprandial, insulin-mediated *de novo* lipogenesis[200]. Indeed, XBP1, directly downstream of IRE1α, has been described as a *bona fide* transcription factor in hepatic lipogenesis[201]. Thus, activation of IRE1α may provide additional lipids to support VLDL assembly. Enhanced degradation of ERAD substrates by the ubiquitin proteasome system and autophagy could degrade apoB and decrease the amount of apoB molecules reaching secretion competence. Thus, during ER stress, there is simultaneous activation of processes that either support, or limit, VLDL assembly. Consideration of the characteristics of each individual ER stressor being deployed experimentally (pharmacologic, diet-induced, or genetic) is essential to prediction of the net effect on ER homeostasis.

Free fatty acids (FFA) are the most physiologically relevant hepatic ER stressors. A widely cited example of reduced VLDL output is during FFA-induced hepatic ER stress[125]. While induction of ER stress decreased VLDL production in animal and cell models, the authors emphasized that apoB demonstrated a dose- and time-dependent parabolic response to FFA. At mild to moderate levels of FFA-induced ER stress, apoB secretion was increased, demonstrating a window of ER stress activation wherein lipid availability is able to outstrip the concomitant decline in protein folding capacity in the ER.

Recent studies have found connections between the hepatic IRE1α arm of the UPR and VLDL output. Increased IRE1α–XBP1s activation induced PDI expression, which in turn increased MTP activity and apoB secretion, while liver-specific deletion of IRE1α impaired VLDL assembly in mice[202]. The catalytic activity of PDI was dispensable for this particular function in lipoprotein assembly, suggesting that PDI increased VLDL assembly by modulating MTP activity rather than through its enzymatic function.

There is mounting evidence to suggest that the hepatic UPR is involved in maintaining normal glucose and lipid metabolism. An insulin-mediated, nutrient-sensing signalling node, has been described that involves mammalian Target Of Rapamycin Complex 1 (mTORC1)[203]. This analysis revealed a metabolic transcriptional program with a broad regulatory connections between insulin and the UPR. mTORC1 is activated in the fed state[204]. Meanwhile, mTORC1 inhibition by rapamycin blocked postprandial activation of hepatic IRE1α, and prevented XBP1-dependent lipogenesis in rats[205]. XBP1 is required for insulin-stimulated activities of SREBP1[200]. Nuclear levels of the lipogenic regulator SREBP1c were also decreased by the mTOR inhibition by rapamycin[158]. Proteolytic processing and nuclear translocation of the SREBP proteins is needed for development of diabetic fatty liver and hypertriglyceridemia in mice[158]. Mouse models of obesity showed that elevated hepatic ER stress, SREBP activation, increased lipid synthesis and overproduction of VLDL were mTORC1-dependent[85]. Moreover, hepatic mTORC1 is elevated in *ob/ob* mice[206]. Amazingly, a mouse model of liver-specific, inducible XBP1 revealed that XBP1 expression alone is sufficient to trigger a switch into the postprandial phenotype, even in the absence of caloric intake[207].

As mentioned in section 2a, repression of sortilin-1 by ER stress induction (via the UPR transcription factor named ATF3) caused increased VLDL output from the liver, providing an example of increased apoB

secretion during ER stress *in vivo*[85]. Further, the suppression of sortilin-1 in *ob/ob* mice was found to be induced via increased hepatic mTORC1 activity. Taken together, these data suggest that increased mTORC1 activity and hepatic ER stress can provide an environment suitable for elevated VLDL production, via decreased apoB degradation and elevated insulin-mediated synthesis of lipid ligands for apoB. The next section delves into the unfortunate bifurcation of hepatic insulin signalling and the resulting pathological impact on VLDL production during insulin resistance.

Reciprocal effects of insulin resistance and fatty liver on apoB metabolism

Haas *et al.* have reviewed the regulation of apoB metabolism by insulin[208]. Abnormalities of both intestinal and liver lipoprotein production underlie the complex hyperlipidemia that occurs in insulin resistance[64]. Verges reviewed the mechanisms behind VLDL overproduction during insulin resistance[209] and Sparks *et al.* have reviewed the role of selective hepatic insulin resistance[63]. Here, we shall examine how apoB production is affected by insulin resistance, accumulation of fatty liver and activation of the hepatic UPR.

A unique half insulin-sensitive/half insulin-resistant response arises in the livers of diabetic rats[210]. Hepatic glucose production normally ceases after the feeding-induced increase in circulating insulin. Liver insulin resistance results in sustained glucose production[211]. On the other hand, the lipogenic pathways in the liver remain insulin-sensitive[210], promoting lipid biosynthesis. *De novo* lipogenesis (DNL) increases production of fatty acids in response to insulin. When hepatic glucose production continues in parallel with insulin-sensitive DNL, TG accumulation increases production of VLDL[212]. Non-alcoholic fatty liver disease (NAFLD) and increased levels of VLDL-derived atherogenic lipoproteins result from these metabolic abnormalities in insulin-resistant individuals[213]. Nutrient excess contributes to the chronic over-production of liver lipids by frequently flooding the organ with dietary substrates, thus creating a self-sustaining, pathological pattern of gene regulation.

Pour and Adeli[214] explored how insulin decreases the apoB mRNA translation when insulin is a global inducer of translation. ApoB mRNA accumulates in cytoplasmic complexes called P bodies, which contain silenced mRNAs. ApoB mRNA within P bodies could be observed 4-16 h after insulin treatment and their presence correlated with the decrease in apoB translation. Insulin resistance may prevent the movement of apoB mRNA into this inactive pool and perhaps allow for the "over-translation" of apoB as a prelude to the overproduction of VLDL. Sparks and colleagues[215] have extensively studied hepatic insulin resistance and the production of VLDL. Recently, they showed that although both MTP expression and apoB secretion are decreased by insulin under normal conditions, insulin was still able to suppress apoB secretion when MTP was overexpressed. This suggests that the decrease in apoB secretion is, in part, independent of the insulin effect on MTP.

Tsai *et al*.[216] found that hepatic inflammation underlies the increased VLDL production during insulin resistance in the diet-induced, insulin-resistant hamster. Fructose feeding of hamsters for as little as 4 days caused a decrease in Inhibitor of NF-kappaB (IκB) levels in the liver, while activation of IκB kinase (IκK) increased apoB synthesis and decreased degradation. IκK lies upstream of NF-κB, and has been shown to inhibit insulin signaling. The postprandial state is associated with increased inflammation and oxidative stress markers in circulation. Human studies have shown specific dietary fats can affect the nature of this response[217,218]. Chronic inflammation and ER stress appear to be linked mechanistically in the liver during obesity and type 2 diabetes[219,220], with crosstalk amongst the UPR and inflammatory networks being relevant to several human diseases[221].

Basciano and colleagues[222] studied LXRα activation in hamsters with the agonist T0901317. After 7 days the agonist increased TG, apoB and VLDL-TG secretion via an increase in lipogenesis, promoted by stimulation of SREBP-1c, fatty acid synthase (FAS) and stearoyl-CoA desaturase (SCD). The agonist stabilized apoB for secretion without an effect on MTP. They suggested that dysregulation of hepatic insulin signaling can be a major mechanism for enhanced VLDL assembly and secretion with this drug. Interestingly, in a transgenic mice study, the LXR-SREBP1-PLTP regulatory axis was found to govern VLDL size by increasing TG synthesis and phospholipid availability for VLDL assembly[223]. The ratio of hepatic phosphatidyl choline (PC) to phosphatidyl ethanolamine (PE) has also been shown to modulate membrane integrity and steatosis[224]. LXR supports maintainence of ER membrane integrity by driving the expression of Lpcat3[225], whose enzymatic activity contributes unsaturated fatty acyl chains to the phospholipids comprising the ER bilayer. Moreover, restoring the imbalanced PC/PE ratio in ER stressed obese rodent livers improved calcium homeostasis, ameliorated ER stress and improved glucose homeostasis[226]. These data provide

an intriguing link between hepatic ER stress, lipid synthesis and apoB production.

Irrespective of the extent of obesity, the metabolic dysfunction characterized by insulin resistance and VLDL overproduction is reflective of hepatic TG content[227]. High levels of insulin, in the absence of insulin resistance, do not increase VLDL production in humans[228]. PTEN is a negative regulator of PI3 kinase signaling in the insulin pathway. Qiu and colleagues[229] showed that liver-specific knockout of PTEN in mice decreased hepatic apoB and MTP mass and increased liver TG levels, concomitant with a decrease in apoB secretion. These changes were mediated by an increase in the degradation of the nascent apoB protein. They suggested that inappropriate decreases in hepatic PTEN expression may contribute to hepatic steatosis. Liver specific knockout of PTEN leads to enhanced hepatic insulin signaling with increased secretion of TG and apoB[230]. If liver TG stores are normalized by inhibiting DGAT1, DGAT2 or SREBP1c, insulin signaling is retained but TG and apoB secretion are reduced. Acute reduction of PTEN does not reduce liver TG stores and also does not reduce apoB secretion despite enhanced insulin signaling. These observations suggest that hepatic TG is the dominant regulator of apoB secretion.

One report elegantly demonstrated the difference between excess *de novo* lipogenesis (high fructose feeding) and exogenous lipid oversupply (high fat feeding) in rats[231]. Both high fructose and high fat diets caused steatosis and blunted insulin sensitivity within days. Interestingly, ER stress was detected in the fructose-fed animals but not in the high fat-fed animals, suggesting that the stress was associated with the lipogenesis, and was not an immediate component of the early fatty liver disease or insulin resistance induced by the high-fat diet[231]. Increased mRNA expression of both MTP and apoB was observed in NAFLD subjects[232]. However, in this study the most severe insulin resistance was associated with reduced MTP, suggesting that as the liver disease worsens, this compensatory mechanism is lost. This data implies that VLDL secretion persists during pathological hepatic ER stress and NAFLD, perhaps until progressive liver damage impacts the capacity of the secretory pathway to produce VLDL.

While *in vivo* evidence does not support the existence an ER stress-dependent decrease in VLDL output as a causative mechanism of fatty liver, perturbed apoB protein quality control during the progression of insulin resistance and NAFLD may still have unique, deleterious consequences. Augmenting apoB production is sufficient to either worsen or improve ER homeostasis[233]. Su *et al.* proposed that increased intracellular apoB is a link between ER stress and hepatic insulin resistance. Dietary up-regulation of apoB, or its direct overexpression, caused ER stress and decreased insulin signaling in the liver, suggesting that apoB dysregulation in itself is sufficient to perturb ER homoeostasis. Furthermore, reduction of apoB using siRNA prevented ER stress and hepatic insulin resistance. Defective hepatic autophagy has been observed to promote ER stress and insulin resistance during obesity[180]. It is conceivable that inefficient turnover of apoB selected for autophagy contributes to the disruption of hepatic metabolism, perhaps by creating a backlog of lipid cargo.

Continuous apoB production inflicts a constant, unique metabolic burden on the hepatocyte and the loss of insulin-dependent suppression of hepatic apoB levels may confer an additional burden, on top of that already posed by chronic over-nutrition. Successful moderation of apoB production is dependent on ER homeostasis, while conversely, hepatic ER homeostasis is dependent, in part, on the moderate and unimpeded export of lipidated apoB. Abrogation of VLDL output will cause neutral lipids to accumulate[234]. Combined with β-oxidation, VLDL output serves as a means to consume and partition energy resources within hepatocytes such that lipotoxic intermediary metabolites do not accumulate (specifically diacylglycerol (DAG) and ceramide). It has been suggested that proper liver homeostasis via continuous export of TG (and apoB) is maintained at the expense of an atherogenic plasma lipid profile[235]. In this respect, apoB is perhaps a molecular "steatosis suppressor," to borrow semantics from cancer researchers.

The physiological UPR and metabolic homeostasis

The UPR transcription factors that maintain ER homeostasis and help elicit the hepatic feeding response also have a significant impact on VLDL output and the metabolic status of the liver. Postprandial, insulin-mediated suppression of VLDL output coincides with physiological activation of the UPR. However, hepatic insulin resistance is also associated with chronic UPR activation and VLDL overproduction during the postprandial period. These observations underscore the important differences between a physiological and pathological UPR. While the chronic, steady-state hepatic ER stress associated with metabolic diseases is well-documented, the short term effects of a single large meal on hepatic ER stress remain largely unexplored.

When the ''postprandial UPR'' is superimposed onto an already-stressed ER, the resulting UPR may represent a pathological insult, whereby increased inflammation and excessive lipogenesis ensue. The mammalian postprandial UPR is an intriguing concept requiring experimental characterization under physiological and pathological conditions.

The chaperone component of ER homeostasis is precisely regulated; likened to a ''double-edged sword,'' where too much or too little can be dangerous[235]. The enhancement of chaperone function is an intriguing potential therapeutic target for many human diseases, especially given the well-documented age-related decline in chaperone regulation[236,237]. The chemical chaperone 4-phenyl butyric acid (PBA) was able to normalize ER homeostasis and VLDL secretion in mice subjected to chronic oleate exposure[125]. Interestingly, PBA may improve insulin signalling and β-cell function in humans with lipid-induced insulin resistance[238]. Treatment of humans with the chemical chaperone tauroursodeoxycholic acid (TUDCA) improved insulin sensitivity in the liver and skeletal muscle[239]. Restoration of effective protein quality control may represent a point at which persistent cycles of pathological ER stress, inflammation and insulin-resistance might be interrupted[240]. Whether modulation of the UPR and chaperoning capacity in general will provide clinical benefits for metabolic diseases remains to be seen.

Concluding remarks

ApoB is a fascinating and complex substrate for cellular quality control. Since apoB production is continuous and regulated by many direct and indirect metabolic factors, the liver must be able to both maintain sufficient apoB to dispense with excess cellular TG when necessary, and to prevent the accumulation of apoB when hepatocellular nutrient levels are low. However, over time and in the presence of chronic levels of excess dietary carbohydrate and fat, the fidelity of insulin signalling can diminish, as does appropriate metabolite partitioning and protein quality control (***Fig. 3***). Overflow levels of cytosolic lipid stores in the liver as well as chronic inflammation in the organ can create an environment in which VLDL production is continuous, rather than episodic and can contribute to extrahepatic pathologies such as atherosclerosis. Further work on the hepatic quality control mechanisms will be required to delineate causality and to increase the potential for new and improved therapeutic interventions.

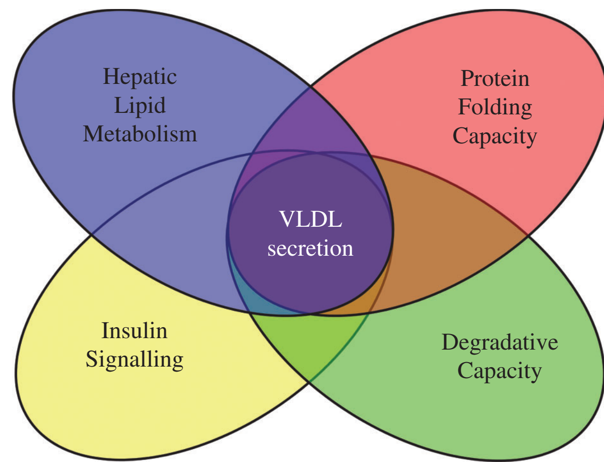

Fig. 3 **Factors affecting the net output of VLDL.** This diagram illustrates the distinct, yet interconnected, processes that combine to determine the net rate of VLDL output. The figure serves as a qualitative schematic to envision factors that influence the regulatory and quality control decisions during VLDL assembly. Each of these factors have been shown to impact the fate of nascent apoB independently, however under physiological conditions the status of insulin signalling, lipid homeostasis, protein folding capacity and degradative capacity act in concert. In pathological states, these four processes can become discordant such that hepatic VLDL output is inappropriate.

References

[1] Shelness GS, Ledford AS. Evolution and mechanism of apolipoprotein B-containing lipoprotein assembly. *Curr Opin Lipidol* 2005; 16: 325–32.

[2] Avramoglu RK, Adeli K. Hepatic regulation of apolipoprotein B. *Rev.Endocr.Metab Disord* 2004; 5: 293–301.

[3] Hussain MM, Shi J, Dreizen P. Microsomal triglyceride transfer protein and its role in apoB-lipoprotein assembly. *J Lipid Res* 2003; 44: 22–32.

[4] Davidson NO, Shelness GS. Apolipoprotein B: mRNA editing, lipoprotein assembly, and presecretory degradation. *Annu Rev Nutr* 2000; 20: 169–93.

[5] Shelness GS, Ingram MF, Huang XF, DeLozier JA. Apolipoprotein B in the rough endoplasmic reticulum: Translation, translocation and the initiation of lipoprotein assembly. *J Nutr* 1999; 129: 456S–62S.

[6] Davis RA. Cell and molecular biology of the assembly and secretion of apolipoprotein B-containing lipoproteins by the liver. *Biochim Biophys Acta* 1999; 1440: 1–31.

[7] Chan L. Apolipoprotein B, the major protein component of triglyceride-rich and low density lipoproteins. *J Biol Chem* 1992; 267: 25621–4.

[8] Rutledge AC, Su Q, Adeli K. Apolipoprotein B100 biogenesis: A complex array of intracellular mechanisms regulating folding, stability, and lipoprotein assembly. *Biochem Cell Biol* 2010; 88: 251–67.

[9] Havel RJ, Kane JP. Disorders of the biogenesis and secretion of lipoproteins containing the B apolipoproteins. In: Scriver CR, Beaudet AL, Sly WA, Valle D (Eds) *The Metabolic and Molecular Bases of Inherited Disease* Volume II. McGraw-Hill, Inc; 1995:1853–1885.

[10] Sniderman AD, Furberg CD, Keech A, Lennep JERv, Frohlich J, Jungner I, et al. Apolipoproteins versus lipids as indices of coronary risk and as targets for statin treat– ment. *Lancet* 2003; 361: 777–80.

[11] Sniderman AD, Pierre ACS, Cantin B, Dagenais GR, Despres JP, Lamarche B. Concordance/discordance between plasma apolipoprotein B levels and the choles– terol indexes of atherosclerotic risk. *Am J Cardiol* 2003; 91: 1173–7.

[12] Pierre ACS, Cantin B, Dagenais GR, Despres JP, Lamarche B. Apolipoprotein-B, low-density lipoprotein cholesterol, and the long-term risk of coronary heart dis– ease in men. *Am J Cardiol* 2006; 97: 997–1001.

[13] Benn M, Nordestgaard BG, Jensen GB, Tybjaerg-Hansen A. Improving prediction of ischemic cardiovascular dis– ease in the general population using apolipoprotein B: The copenhagen city heart study. *Arterioscler Thromb Vasc Biol* 2007; 27: 661–70.

[14] Sniderman A, Couture P, Graaf JD. Diagnosis and treat– ment of apolipoprotein B dyslipoproteinemias. *Nat.Rev.Endocrinol.* 2010; 6: 335–46.

[15] Sniderman AD, Graaf JD, Couture P, Williams K, Kiss RS, Watts GF. Regulation of plasma LDL: The apoB paradigm. *Clinical Science* 2009; 118: 333–9.

[16] Gordon DA, Jamil H. Progress towards understanding the role of microsomal triglyceride transfer protein in apoli– poprotein-B lipoprotein assembly. *Biochim Biophys Acta* 2000; 1486: 72–83.

[17] Wetterau JR, Lin MC, Jamil H. Microsomal triglyceride transfer protein. *Biochim Biophys Acta* 1997; 1345: 136–50.

[18] Zhang YL, Hernandez-Ono A, Ko C, Yasunaga K, Huang LS, Ginsberg HN. Regulation of hepatic apolipoprotein B-lipoprotein assembly and secretion by the availability of fatty acids. I. differential response to the delivery of fatty acids via albumin or remnant-like emulsion particles. *J Biol Chem* 2004; 279: 19362–74.

[19] Sniderman AD, Cianflone K. Substrate delivery as a determinant of hepatic apoB secretion. *Arterioscler Thromb* 1993; 13: 629–36.

[20] Taghibiglou C, Carpentier A, Iderstine SCV, Chen B, Rudy D, Aiton A, et al. Mechanisms of hepatic very low density lipoprotein overproduction in insulin resis– tance. evidence for enhanced lipoprotein assembly, reduced intracellular apoB degradation, and increased microsomal triglyceride transfer protein in a fructose-fed hamster model. *J Biol Chem* 2000; 275: 8416–25.

[21] Kummrow E, Hussain MM, Pan M, Marsh JB, Fisher EA. Myristic acid increases dense lipoprotein secretion by inhibiting apoB degradation and triglyceride recruitment. *J Lipid Res* 2002; 43: 2155–63.

[22] Zammit VA, Lankester DL. Oleate acutely stimulates the secretion of triacylglycerol by cultured rat hepatocytes by accelerating the emptying of the secretory compartment. *Lipids* 2001; 36: 607–12.

[23] Heath RB, Karpe F, Milne RW, Burdge GC, Wootton SA, Frayn KN. Dietary fatty acids make a rapid and substan– tial contribution to VLDL-triacylglycerol in the fed state. *Amer J Physiol Endo Metab* 2007; 292: E732–9.

[24] Chen Z, Fitzgerald RL, Schonfeld G. Hypobetali– poproteinemic mice with a targeted apolipoprotein (apo) B-27.6-specifying mutation: In vivo evidence for an important role of amino acids 1254–1744 of ApoB in lipid transport and metabolism of the apoB-containing lipopro– tein. *J Biol Chem* 2002; 277: 14135–45.

[25] Burnett JR, Zhong S, Jiang ZG, Hooper AJ, Fisher EA, McLeod RS, et al. Missense mutations in APOB within the betaalpha1 domain of human APOB-100 result in impaired secretion of ApoB and ApoB-containing lipo– proteins in familial hypobetalipoproteinemia. *J Biol Chem* 2007; 282: 24270–83.

[26] Liao W, Ning G. Knockdown of apolipoprotein B, an atherogenic apolipoprotein, in HepG2 cells by lenti– virus-mediated siRNA. *Biochem Biophys Res Commun* 2006; 344: 478–83.

[27] Crooke RM, Graham MJ, Lemonidis KM, Whipple CP, Koo S, Perera RJ. An apolipoprotein B antisense oligonucleotide lowers LDL cholesterol in hyperlipidemic mice without causing hepatic steatosis. *J Lipid Res* 2005; 46: 872–84.

[28] Soutschek J, Akinc A, Bramlage B, Charisse K, Constien R, Donoghue M, et al. Therapeutic silencing of an endo– genous gene by systemic administration of modified siRNAs. *Nature* 2004; 432: 173–8.

[29] Zimmermann TS, Lee AC, Akinc A, Bramlage B, Bumcrot D, Fedoruk MN, et al. RNAi-mediated gene silencing in non-human primates. *Nature* 2006; 441: 111–4.

[30] Akdim F, Visser ME, Tribble DL, Baker BF, Stroes ES, Yu R, et al. Effect of mipomersen, an apolipoprotein B synthesis inhibitor, on low-density lipoprotein cholesterol in patients with familial hypercholesterolemia. *Am J Cardiol* 2010; 105: 1413–9.

[31] Visser ME, Akdim F, Tribble DL, Nederveen AJ, Kwoh TJ, Kastelein JJ, et al. Effect of apolipoprotein-B synth– esis inhibition on liver triglyceride content in patients with familial hypercholesterolemia. *J Lipid Res* 2010; 51: 1057–62.

[32] Raal FJ, Santos RD, Blom DJ, Marais AD, Charng MJ, Cromwell WC, et al. Mipomersen, an apolipoprotein B synthesis inhibitor, for lowering of LDL cholesterol con– centrations in patients with homozygous familial hypercholesterolaemia: A randomised, double-blind, pla– cebo-controlled trial. *Lancet* 2010; 375: 998–1006.

[33] Norata GD, Tibolla G, Catapano AL. Gene silencing approaches for the management of dyslipidaemia. *Trends Pharmacol Sci* 2013; 34: 198–205.

[34] McLeod RS, Wang Y, Wang S, Rusi+a–Eol A, Links P, Yao Z. Apolipoprotein B sequence requirements for hepatic very low density lipoprotein assembly. *J Biol Chem* 1996; 271: 18445–55.

[35] Wang Y, McLeod RS, Yao Z. Normal activity of micro– somal triglyceride transfer protein is required for the ole– ate-induced secretion of very low density lipoproteins containing apolipoprotein B from McA-RH7777 cells. *J Biol Chem* 1997; 272: 12272–8.

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The Journal of Biomedical Research, 2014, 28(3)

Invited Review

Apolipoprotein B100 quality control and the regulation of hepatic very low density lipoprotein secretion

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Received 15 January 2014, Accepted 15 February 2014, Epub 28 March 2014

Supplementary References

References

[36] Boren J, Rustaeus S, Olofsson SO. Studies on the assem–bly of apolipoprotein B-100- and B-48-containing very low density lipoproteins in McA-RH7777 cells. *The Journal of biological chemistry* 1994; 269: 25879–88.

[37] Rustaeus S, Lindberg K, Boren J, Olofsson SO. Brefeldin A reversibly inhibits the assembly of apoB containing lipoproteins in McA-RH7777 cells. *J Biol Chem* 1995; 270: 28879–86.

[38] Innerarity TL, Boren J, Yamanaka S, Olofsson SO. Biosynthesis of apolipoprotein B48-containing lipopro–teins. regulation by novel post-transcriptional mechan–isms. *J Biol Chem* 1996; 271: 2353–6.

[39] Alexander CA, Hamilton RL, Havel RJ. Subcellular loca–lization of B apoprotein of plasma lipoproteins in rat liver. *J Cell Biol* 1976; 69: 241–63.

[40] Hamilton RL, Wong JS, Cham CM, Nielsen LB, Young SG. Chylomicron-sized lipid particles are formed in the setting of apolipoprotein B deficiency. *J Lipid Res* 1998; 39: 1543–57.

[41] Rusinol A, Verkade H, Vance JE. Assembly of rat hepatic very low density lipoproteins in the endoplasmic reticu–lum. *The Journal of biological chemistry* 1993; 268: 3555–62.

[42] Kulinski A, Rustaeus S, Vance JE. Microsomal triacyl–glycerol transfer protein is required for lumenal accretion of triacylglycerol not associated with ApoB, as well as for ApoB lipidation. *J Biol Chem* 2002; 277: 31516–25.

[43] Leiper JM, Bayliss JD, Pease RJ, Brett DJ, Scott J, Shoulders CC. Microsomal triglyceride transfer protein, the abetalipoproteinemia gene product, mediates the secretion of apolipoprotein B-containing lipoproteins from heterologous cells. *J Biol Chem* 1994; 269: 21951–4.

[44] Gordon DA, Jamil H, Sharp D, Mullaney D, Yao Z, Gregg RE, et al. Secretion of apolipoprotein B-containing lipoproteins from HeLa cells is dependent on expression of the microsomal triglyceride transfer protein and is regulated by lipid availability. *Proc Natl Acad Sci U S A* 1994; 91: 7628–32.

[45] Patel SB, Grundy SM. Interactions between microsomal triglyceride transfer protein and apolipoprotein B within the endoplasmic reticulum in a heterologous expression system. *J Biol Chem* 1996; 271: 18686–94.

[46] Wang S, McLeod RS, Gordon DA, Yao Z. The microso–mal triglyceride transfer protein facilitates assembly and secretion of apolipoprotein B-containing lipoproteins and decreases cotranslational degradation of apolipopro–tein B in transfected COS-7 cells. *J Biol Chem* 1996; 271: 14124–33.

[47] Liu Y, Manchekar M, Sun Z, Richardson PE, Dashti N. Apolipoprotein B-containing lipoprotein assembly in microsomal triglyceride transfer protein-deficient McA-RH7777 cells. *J Lipid Res* 2010; 51: 2253–64.

[48] Rusinol AE, Jamil H, Vance JE. In vitro reconstitution of assembly of apolipoprotein B48-containing lipoproteins. *J Biol Chem* 1997; 272: 8019–25.

[49] Herscovitz H, Kritis A, Talianidis I, Zanni E, Zannis V, Small DM. Murine mammary-derived cells secrete the N-terminal 41% of human apolipoprotein B on high density lipoprotein-sized lipoproteins containing a tria–cylglycerol-rich core. *Proc Natl Acad Sci U S A* 1995; 92: 659–63.

[50] Sellers JA, Shelness GS. Lipoprotein assembly capacity of the mammary tumor-derived cell line C127 is due to the expression of functional microsomal triglyceride transfer protein. *J Lipid Res* 2001; 42: 1897–904.

[51] Larsson SL, Skogsberg J, Bjorkegren J. The low density lipoprotein receptor prevents secretion of dense apoB100-containing lipoproteins from the liver. *J Biol Chem* 2004; 279: 831–6.

[52] Pan M, Js JSL, Fisher EA, Ginsberg HN. The late addition of core lipids to nascent apolipoprotein B100, resulting in

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doi: 10.7555/JBR.28.20140019

the assembly and secretion of triglyceride-rich lipoproteins, is independent of both microsomal triglyceride transfer protein activity and new triglyceride synthesis. *The Journal of biological chemistry* 2002; 277: 4413–21.
[53] Swift LL, Zhu MY, Kakkad B, Jovanovska A, Neely MD, Valyi-Nagy K, et al. Subcellular localization of microsomal triglyceride transfer protein. *J Lipid Res* 2003; 44: 1841–9.
[54] Gusarova V, Seo J, Sullivan ML, Watkins SC, Brodsky JL, Fisher EA. Golgi-associated maturation of very low density lipoproteins involves conformational changes in apolipoprotein B, but is not dependent on apolipoprotein E. *J Biol Chem* 2007; 282: 19453–62.
[55] Pan X, Zhang Y, Wang L, Hussain MM. Diurnal regulation of MTP and plasma triglyceride by CLOCK is mediated by SHP. *Cell Metab.* 2010; 12: 174–86.
[56] Chen Z, Newberry EP, Norris JY, Xie Y, Luo J, Kennedy SM, et al. ApoB100 is required for increased VLDL-triglyceride secretion by microsomal triglyceride transfer protein in ob/ob mice. *J Lipid Res* 2008; 49: 2013–22.
[57] Iqbal J, Rudel LL, Hussain MM. Microsomal triglyceride transfer protein enhances cellular cholesteryl esterification by relieving product inhibition. *J Biol Chem* 2008; 283: 19967–80.
[58] Minehira K, Young SG, Villanueva CJ, Yetukuri L, Oresic M, Hellerstein MK, et al. Blocking VLDL secretion causes hepatic steatosis but does not affect peripheral lipid stores or insulin sensitivity in mice. *J Lipid Res* 2008; 49: 2038–44.
[59] Khatun I, Zeissig S, Iqbal J, Wang M, Curiel D, Shelness GS, et al. Phospholipid transfer activity of microsomal triglyceride transfer protein produces apolipoprotein B and reduces hepatosteatosis while maintaining low plasma lipids in mice. *Hepatology* 2012; 55: 1356–68.
[60] Tep S, Mihaila R, Freeman A, Pickering V, Huyhn F, Tadin-Strapps M, et al. Rescue of mtp siRNA-induced hepatic steatosis by DGAT2 siRNA silencing. *J Lipid Res* 2012; 53: 859–67.
[61] Sundaram M, Yao Z. Intrahepatic role of exchangeable apolipoproteins in lipoprotein assembly and secretion. *Arterioscler Thromb Vasc Biol* 2012; 32: 1073–8.
[62] Lehner R, Lian J, Quiroga AD. Lumenal lipid metabolism: Implications for lipoprotein assembly. *Arterioscler Thromb Vasc Biol* 2012; 32: 1087–93.
[63] Sparks JD, Sparks CE, Adeli K. Selective hepatic insulin resistance, VLDL overproduction, and hypertriglyce ridemia. *Arterioscler Thromb Vasc Biol* 2012; 32: 2104–12.
[64] Xiao C, Hsieh J, Adeli K, Lewis GF. Gut-liver interaction in triglyceride-rich lipoprotein metabolism. *Am J Physiol Endocrinol Metab* 2011; 301: E429–46.
[65] Sturley SL, Hussain MM. Lipid droplet formation on opposing sides of the endoplasmic reticulum. *J Lipid Res* 2012; 53: 1800–10.
[66] Caviglia JM, Sparks JD, Toraskar N, Brinker AM, Yin TC, Dixon JL, et al. ABHD5/CGI-58 facilitates the assembly and secretion of apolipoprotein B lipoproteins by McA RH7777 rat hepatoma cells. *Biochim Biophys Acta* 2009; 1791: 198–205.
[67] Quiroga AD, Li L, Trotzmuller M, Nelson R, Proctor SD, Kofeler H, et al. Deficiency of carboxylesterase 1/esterase-x results in obesity, hepatic steatosis, and hyperlipidemia. *Hepatology* 2012; 56: 2188–98.
[68] Ko KW, Erickson B, Lehner R. Es-x/Ces1 prevents triacylglycerol accumulation in McArdle-RH7777 hepatocytes. *Biochim Biophys Acta* 2009; 1791: 1133–43.
[69] Erickson B, Selvan SP, Ko KW, Kelly K, Quiroga AD, Li L, et al. Endoplasmic reticulum-localized hepatic lipase decreases triacylglycerol storage and VLDL secretion. *Biochim Biophys Acta* 2013; 1831: 1113–23.
[70] Fuchs CD, Claudel T, Kumari P, Haemmerle G, Pollheimer MJ, Stojakovic T, et al. Absence of adipose triglyceride lipase protects from hepatic endoplasmic reticulum stress in mice. *Hepatology* 2012; 56: 270–80.
[71] Morris EM, Meers GM, Booth FW, Fritsche KL, Hardin CD, Thyfault JP, et al. PGC-1alpha overexpression results in increased hepatic fatty acid oxidation with reduced triacylglycerol accumulation and secretion. *Am J Physiol Gastrointest Liver Physiol* 2012; 303: G979–92.
[72] Ye J, Li JZ, Liu Y, Li X, Yang T, Ma X, et al. Cideb, an ER- and lipid droplet-associated protein, mediates VLDL lipidation and maturation by interacting with apolipoprotein B. *Cell metabolism* 2009; 9: 177–90.
[73] Li X, Ye J, Zhou L, Gu W, Fisher EA, Li P. Opposing roles of cell death-inducing DFF45-like effector B and perilipin 2 in controlling hepatic VLDL lipidation. *J Lipid Res* 2012; 53: 1877–89.
[74] Chen Z, Gropler MC, Norris J, Jr JCL, Harris TE, Finck BN. Alterations in hepatic metabolism in fld mice reveal a role for lipin 1 in regulating VLDL-triacylglyceride secretion. *Arterioscler Thromb Vasc Biol* 2008; 28: 1738–44.
[75] Zheng C, Khoo C, Furtado J, Sacks FM. Apolipoprotein C-III and the metabolic basis for hypertriglyceridemia and the dense low-density lipoprotein phenotype. *Circulation* 2010; 121: 1722–34.
[76] Lee HY, Birkenfeld AL, Jornayvaz FR, Jurczak MJ, Kanda S, Popov V, et al. Apolipoprotein CIII overexpressing mice are predisposed to diet-induced hepatic steatosis and hepatic insulin resistance. *Hepatology* 2011; 54: 1650–60.
[77] Sundaram M, Zhong S, Khalil MB, Links PH, Zhao Y, Iqbal J, et al. Expression of apolipoprotein C-III in McA-RH7777 cells enhances VLDL assembly and secretion under lipid-rich conditions. *J Lipid Res* 2010; 51: 150–61.
[78] Weinberg RB, Gallagher JW, Fabritius MA, Shelness GS. ApoA-IV modulates the secretory trafficking of apoB and the size of triglyceride-rich lipoproteins. *J Lipid Res* 2012; 53: 736–43.
[79] Chan DC, Watts GF, Ooi EM, Chan DT, Wong AT, Barrett PH. Apolipoprotein A-II and adiponectin as determinants of very low-density lipoprotein apolipoprotein B-100 metabolism in nonobese men. *Metabolism* 2011; 60: 1482–7.
[80] Triglyceride Coronary Disease Genetics Consortium and Emerging Risk Factors Collaboration, Sarwar N, Sandhu MS, Ricketts SL, Butterworth AS, Di Angelantonio E, et al. Triglyceride-mediated pathways and coronary disease: Collaborative analysis of 101 studies. *Lancet* 2010; 375: 1634–9.
[81] Chung S, Gebre AK, Seo J, Shelness GS, Parks JS. A novel role for ABCA1-generated large pre-beta migrating

nascent HDL in the regulation of hepatic VLDL triglyceride secretion. *J Lipid Res* 2010; 51: 729–42.

[82] Wiersma H, Nijstad N, Gautier T, Iqbal J, Kuipers F, Hussain MM, et al. Scavenger receptor BI facilitates hepatic very low density lipoprotein production in mice. *J Lipid Res* 2010; 51: 544–53.

[83] Willnow TE, Kjolby M, Nykjaer A. Sortilins: New players in lipoprotein metabolism. *Curr Opin Lipidol* 2011; 22: 79–85.

[84] Kjolby M, Andersen OM, Breiderhoff T, Fjorback AW, Pedersen KM, Madsen P, et al. Sort1, encoded by the cardiovascular risk locus 1p13.3, is a regulator of hepatic lipoprotein export. *Cell Metab* 2010; 12: 213–23.

[85] Ai D, Baez JM, Jiang H, Conlon DM, Hernandez-Ono A, Frank-Kamenetsky M, et al. Activation of ER stress and mTORC1 suppresses hepatic sortilin-1 levels in obese mice. *J Clin Invest* 2012; 122: 1677–87.

[86] Strong A, Ding Q, Edmondson AC, Millar JS, Sachs KV, Li X, et al. Hepatic sortilin regulates both apolipoprotein B secretion and LDL catabolism. *J Clin Invest* 2012; 122: 2807–16.

[87] Chamberlain JM, O'Dell C, Sparks CE, Sparks JD. Insulin suppression of apolipoprotein B in McArdle RH7777 cells involves increased sortilin 1 interaction and lysosomal targeting. *Biochem Biophys Res Commun* 2013; 430: 66–71.

[88] Sun H, Samarghandi A, Zhang N, Yao Z, Xiong M, Teng BB. Proprotein convertase subtilisin/kexin type 9 interacts with apolipoprotein B and prevents its intracellular degradation, irrespective of the low-density lipoprotein receptor. *Arterioscler Thromb Vasc Biol* 2012; 32: 1585–95.

[89] Levy E, Harmel E, Laville M, Sanchez R, Emonnot L, Sinnett D, et al. Expression of Sar1b enhances chylomicron assembly and key components of the coat protein complex II system driving vesicle budding. *Arterioscler Thromb Vasc Biol* 2011; 31: 2692–9.

[90] Sparks JD, Collins HL, Chirieac DV, Cianci J, Jokinen J, Sowden MP, et al. Hepatic very-low-density lipoprotein and apolipoprotein B production are increased following in vivo induction of betaine-homocysteine S-methyltransferase. *Biochem J* 2006; 395: 363–71.

[91] Sidiropoulos KG, Pontrelli L, Adeli K. Insulin-mediated suppression of apolipoprotein B mRNA translation requires the 5′ UTR and is characterized by decreased binding of an insulin-sensitive 110-kDa 5′ UTR RNA-binding protein. *Biochemistry (N Y)* 2005; 44: 12572–81.

[92] Sidiropoulos KG, Zastepa A, Adeli K. Translational control of apolipoprotein B mRNA via insulin and the protein kinase C signaling cascades: Evidence for modulation of RNA-protein interactions at the 5′UTR. *Arch Biochem Biophys* 2007; 459: 10–9.

[93] Pontrelli L, Sidiropoulos KG, Adeli K. Translational control of apolipoprotein B mRNA: Regulation via cis elements in the 5′ and 3′ untranslated regions. *Biochemistry (N Y)* 2004; 43: 6734–44.

[94] Olofsson SO, Boren J. Apolipoprotein B secretory regulation by degradation. *Arterioscler Thromb Vasc Biol* 2012; 32: 1334–8.

[95] Fisher EA, Ginsberg HN. Complexity in the secretory pathway: The assembly and secretion of apolipoprotein B-containing lipoproteins. *The Journal of biological chemistry* 2002; 277: 17377–80.

[96] Ellgaard L, Molinari M, Helenius A. Setting the standards: Quality control in the secretory pathway. *Science* 1999; 286: 1882–8.

[97] Schubert U, Anton LC, Gibbs J, Norbury CC, Yewdell JW, Bennink JR. Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. *Nature* 2000; 404: 770–4.

[98] Oyadomari S, Yun C, Fisher EA, Kreglinger N, Kreibich G, Oyadomari M, et al. Cotranslocational degradation protects the stressed endoplasmic reticulum from protein overload. *Cell* 2006; 126: 727–39.

[99] Schroder M, Kaufman RJ. The mammalian unfolded protein response. *Annu Rev Biochem* 2005; 74: 739–89.

[100] Brodsky JL. The protective and destructive roles played by molecular chaperones during ERAD (endoplasmic-reticulum-associated degradation). *Biochem J* 2007; 404: 353–63.

[101] Brodsky JL, Gusarova V, Fisher EA. Vesicular trafficking of hepatic apolipoprotein B100 and its maturation to very low-density lipoprotein particles; studies from cells and cell-free systems. *Trends Cardiovasc Med* 2004; 14: 127–32.

[102] Maattanen P, Gehring K, Bergeron JJ, Thomas DY. Protein quality control in the ER: The recognition of misfolded proteins. *Semin Cell Dev Biol* 2010; 21: 500–11.

[103] Pan M, Cederbaum AI, Zhang YL, Ginsberg HN, Williams KJ, Fisher EA. Lipid peroxidation and oxidant stress regulate hepatic apolipoprotein B degradation and VLDL production. *J Clin Invest* 2004; 113: 1277–87.

[104] Christian P, Sacco J, Adeli K. Autophagy: Emerging roles in lipid homeostasis and metabolic control. *Biochim Biophys Acta* 2013; 1831: 819–24.

[105] Fourn VL, Park S, Jang I, Gaplovska-Kysela K, Guhl B, Lee Y, et al. Large protein complexes retained in the ER are dislocated by non-COPII vesicles and degraded by selective autophagy. *Cellular and molecular life sciences : CMLS* 2013; .

[106] Bernasconi R, Galli C, Noack J, Bianchi S, Haan CAd, Reggiori F, et al. Role of the SEL1L:LC3-I complex as an ERAD tuning receptor in the mammalian ER. *Mol Cell* 2012; 46: 809–19.

[107] Borchardt RA, Davis RA. Intrahepatic assembly of very low density lipoproteins. rate of transport out of the endoplasmic reticulum determines rate of secretion. *J Biol Chem* 1987; 262: 16394–402.

[108] Yeung SJ, Chen SH, Chan L. Ubiquitin-proteasome pathway mediates intracellular degradation of apolipoprotein B. *Biochemistry (N Y)* 1996; 35: 13843–8.

[109] Samanez CH, Caron S, Briand O, Dehondt H, Duplan I, Kuipers F, et al. The human hepatocyte cell lines IHH and HepaRG: Models to study glucose, lipid and lipoprotein metabolism. *Arch Physiol Biochem* 2012; 118: 102–11.

[110] Meex SJ, Andreo U, Sparks JD, Fisher EA. Huh-7 or HepG2 cells: Which is the better model for studying human apolipoprotein-B100 assembly and secretion? *J Lipid Res* 2011; 52: 152–8.

[111] Cardozo C, Wu X, Pan M, Wang H, Fisher EA. The inhibition of microsomal triglyceride transfer protein activity in rat hepatoma cells promotes proteasomal and

nonproteasomal degradation of apoprotein b100. *Biochemistry (N Y)* 2002; 41: 10105–14.

[112] Dixon JL, Furukawa S, Ginsberg HN. Oleate stimulates secretion of apolipoprotein B-containing lipoproteins from hep G2 cells by inhibiting early intracellular degradation of apolipoprotein B. *J Biol Chem* 1991; 266: 5080–6.

[113] Benoist F, Grand-Perret T. Co-translational degradation of apolipoprotein B100 by the proteasome is prevented by microsomal triglyceride transfer protein. synchronized translation studies on HepG2 cells treated with an inhibitor of microsomal triglyceride transfer protein. *J Biol Chem* 1997; 272: 20435–42.

[114] Lapierre LR, Currie DL, Yao Z, Wang J, McLeod RS. Amino acid sequences within the beta1 domain of human apolipoprotein B can mediate rapid intracellular degradation. *J Lipid Res* 2004; 45: 366–77.

[115] Fisher EA, Zhou M, Mitchell DM, Wu X, Omura S, Wang H, et al. The degradation of apolipoprotein B100 is mediated by the ubiquitin-proteasome pathway and involves heat shock protein 70. *The Journal of biological chemistry* 1997; 272: 20427–34.

[116] Liang J, Wu X, Jiang H, Zhou M, Yang H, Angkeow P, et al. Translocation efficiency, susceptibility to proteasomal degradation, and lipid responsiveness of apolipoprotein B are determined by the presence of beta sheet domains. *The Journal of biological chemistry* 1998; 273: 35216–21.

[117] Mitchell DM, Zhou M, Pariyarath R, Wang H, Aitchison JD, Ginsberg HN, et al. Apoprotein B100 has a prolonged interaction with the translocon during which its lipidation and translocation change from dependence on the microsomal triglyceride transfer protein to independence. *Proc Natl Acad Sci U S A* 1998; 95: 14733–8.

[118] Zhou M, Wu X, Huang LS, Ginsberg HN. Apoprotein B100, an inefficiently translocated secretory protein, is bound to the cytosolic chaperone, heat shock protein 70. *J Biol Chem* 1995; 270: 25220–4.

[119] Jiang ZG, Liu Y, Hussain MM, Atkinson D, McKnight CJ. Reconstituting initial events during the assembly of apolipoprotein B-containing lipoproteins in a cell-free system. *J Mol Biol* 2008; 383: 1181–94.

[120] Baker BM, Tortorella D. Dislocation of an endoplasmic reticulum membrane glycoprotein involves the formation of partially dislocated ubiquitinated polypeptides. *The Journal of biological chemistry* 2007; 282: 26845–56.

[121] Sato BK, Schulz D, Do PH, Hampton RY. Misfolded membrane proteins are specifically recognized by the transmembrane domain of the Hrd1p ubiquitin ligase. *Mol Cell* 2009; 34: 212–22.

[122] Carvalho P, Goder V, Rapoport TA. Distinct ubiquitin-ligase complexes define convergent pathways for the degradation of ER proteins. *Cell* 2006; 126: 361–73.

[123] Linnik KM, Herscovitz H. Multiple molecular chaperones interact with apolipoprotein B during its maturation. the network of endoplasmic reticulum-resident chaperones (ERp72, GRP94, calreticulin, and BiP) interacts with apolipoprotein b regardless of its lipidation state. *The Journal of biological chemistry* 1998; 273: 21368–73.

[124] Rutledge AC, Qiu W, Zhang R, Kohen-Avramoglu R, Nemat-Gorgani N, Adeli K. Mechanisms targeting apolipoprotein B100 to proteasomal degradation: Evidence that degradation is initiated by BiP binding at the N terminus and the formation of a p97 complex at the C terminus. *Arterioscler Thromb Vasc Biol* 2009; 29: 579–85.

[125] Ota T, Gayet C, Ginsberg HN. Inhibition of apolipoprotein B100 secretion by lipid-induced hepatic endoplasmic reticulum stress in rodents. *J Clin Invest* 2008; 118: 316–32.

[126] Grubb S, Guo L, Fisher EA, Brodsky JL. Protein disulfide isomerases contribute differentially to the endoplasmic reticulum-associated degradation of apolipoprotein B and other substrates. *Mol Biol Cell* 2012; 23: 520–32.

[127] Adeli K, Macri J, Mohammadi A, Kito M, Urade R, Cavallo D. Apolipoprotein B is intracellularly associated with an ER-60 protease homologue in HepG2 cells. *The Journal of biological chemistry* 1997; 272: 22489–94.

[128] Qiu W, Kohen-Avramoglu R, Rashid-Kolvear F, Au CS, Chong TM, Lewis GF, et al. Overexpression of the endoplasmic reticulum 60 protein ER-60 downregulates apoB100 secretion by inducing its intracellular degradation via a nonproteasomal pathway: Evidence for an ER-60-mediated and pCMB-sensitive intracellular degradative pathway. *Biochemistry (N Y)* 2004; 43: 4819–31.

[129] Rutledge AC, Qiu W, Zhang R, Urade R, Adeli K. Role of cysteine-protease CGHC motifs of ER-60, a protein disulfide isomerase, in hepatic apolipoprotein B100 degradation. *Arch Biochem Biophys* 2013; 537: 104–12.

[130] Liang S, Wu X, Fisher EA, Ginsberg HN. The amino-terminal domain of apolipoprotein B does not undergo retrograde translocation from the endoplasmic reticulum to the cytosol. proteasomal degradation of nascent apolipoprotein B begins at the carboxyl terminus of the protein, while apolipoprotein B is still in its original translocon. *The Journal of biological chemistry* 2000; 275: 32003–10.

[131] Du EZ, Kurth J, Wang SL, Humiston P, Davis RA. Proteolysis-coupled secretion of the N terminus of apolipoprotein B. characterization of a transient, translocation arrested intermediate. *The Journal of biological chemistry* 1994; 269: 24169–76.

[132] Gusarova V, Caplan AJ, Brodsky JL, Fisher EA. Apoprotein B degradation is promoted by the molecular chaperones hsp90 and hsp70. *J Biol Chem* 2001; 276: 24891–900.

[133] Liao W, Yeung SC, Chan L. Proteasome-mediated degradation of apolipoprotein B targets both nascent peptides cotranslationally before translocation and full-length apolipoprotein B after translocation into the endoplasmic reticulum. *J Biol Chem* 1998; 273: 27225–30.

[134] Tsai J, Qiu W, Kohen-Avramoglu R, Adeli K. MEK-ERK inhibition corrects the defect in VLDL assembly in HepG2 cells: Potential role of ERK in VLDL-ApoB100 particle assembly. *Arterioscler Thromb Vasc Biol* 2007; 27: 211–8.

[135] Fisher EA, Khanna NK, McLeod RS. Ubiquitination regulates the assembly of very low density lipoprotein in HepG2 cells and is the committing step of the apoB100 ERAD pathway. 2011; .

[136] Cavallo D, McLeod RS, Rudy D, Aiton A, Yao Z, Adeli K. Intracellular translocation and stability of apolipoprotein B are inversely proportional to the length of the nascent polypeptide. *J Biol Chem* 1998; 273: 33397–405.

[137] Yamaguchi J, Conlon DM, Liang JJ, Fisher EA, Ginsberg HN. Translocation efficiency of apolipoprotein B is determined by the presence of beta-sheet domains, not pause transfer sequences. *J Biol Chem* 2006; 281: 27063–71.

[138] Fleig L, Bergbold N, Sahasrabudhe P, Geiger B, Kaltak L, Lemberg M. Ubiquitin-dependent intramembrane rhom–boid protease promotes ERAD of membrane proteins. *Mol Cell* 2012; 47: 558–69.

[139] Liang JS, Kim T, Fang S, Yamaguchi J, Weissman AM, Fisher EA, et al. Overexpression of the tumor autocrine motility factor receptor Gp78, a ubiquitin protein ligase, results in increased ubiquitinylation and decreased secre–tion of apolipoprotein B100 in HepG2 cells. *J Biol Chem* 2003; 278: 23984–8.

[140] Hampton RY, Sommer T. Finding the will and the way of ERAD substrate retrotranslocation. *Curr Opin Cell Biol* 2012; .

[141] Petris G, Casini A, Sasset L, Cesaratto F, Bestagno M, Cereseto A, et al. CD4 and BST-2/Tetherin proteins retro-translocate from endoplasmic reticulum to cytosol as partially folded and multimeric molecules. *J Biol Chem* 2014; 289: 1–12.

[142] Pariyarath R, Wang H, Aitchison JD, Ginsberg HN, Welch WJ, Johnson AE, et al. Co-translational interactions of apoprotein B with the ribosome and trans–locon during lipoprotein assembly or targeting to the pro–teasome. *The Journal of biological chemistry* 2001; 276: 541–50.

[143] Chen Y, Caherec FL, Chuck SL. Calnexin and other fac–tors that alter translocation affect the rapid binding of ubi–quitin to apoB in the Sec61 complex. *The Journal of biological chemistry* 1998; 273: 11887–94.

[144] Junne T, Kocik L, Spiess M. The hydrophobic core of the Sec61 translocon defines the hydrophobicity threshold for membrane integration. *Mol Biol Cell* 2010; 21: 1662–70.

[145] Lilley BN, Ploegh HL. A membrane protein required for dislocation of misfolded proteins from the ER. *Nature* 2004; 429: 834–40.

[146] Ye Y, Shibata Y, Yun C, Ron D, Rapoport TA. A mem–brane protein complex mediates retro-translocation from the ER lumen into the cytosol. *Nature* 2004; 429: 841–7.

[147] Dougan SK, Hu CC, Paquet ME, Greenblatt MB, Kim J, Lilley BN, et al. Derlin-2-deficient mice reveal an essen–tial role for protein dislocation in chondrocytes. *Mol Cell Biol* 2011; 31: 1145–59.

[148] Moore P, Bernardi KM, Tsai B. The Ero1alpha-PDI redox cycle regulates retro-translocation of cholera toxin. *Mol Biol Cell* 2010; 21: 1305–13.

[149] Greenblatt EJ, Olzmann JA, Kopito RR. Derlin-1 is a rhomboid pseudoprotease required for the dislocation of mutant alpha-1 antitrypsin from the endoplasmic reticulum. *Nature structural & molecular biology* 2011; 18: 1147–52.

[150] Carvalho P, Stanley AM, Rapoport TA. Retrotranslo–cation of a misfolded luminal ER protein by the ubiqui–tin-ligase Hrd1p. *Cell* 2010; 143: 579–91.

[151] Stanley AM, Carvalho P, Rapoport T. Recognition of an ERAD-L substrate analyzed by site-specific in vivo photocrosslinking. *FEBS Lett* 2011; 585: 1281–6.

[152] Horn SC, Hanna J, Hirsch C, Volkwein C, SchA¼tz A, Heinemann U, et al. Usa1 functions as a scaffold of the HRD-ubiquitin ligase. *Mol Cell* 2009; 36: 782–93.

[153] Mehnert M, Sommer T, Jarosch E. Der1 promotes move–ment of misfolded proteins through the endoplasmic reti–culum membrane. *Nat Cell Biol* 2014; 16: 77–86.

[154] Fisher EA, Lapierre LR, Junkins RD, McLeod RS. The AAA-ATPase p97 facilitates degradation of apolipopro–tein B by the ubiquitin-proteasome pathway. *J Lipid Res* 2008; 49: 2149–60.

[155] Suzuki M, Otsuka T, Ohsaki Y, Cheng J, Taniguchi T, Hashimoto H, et al. Derlin-1 and UBXD8 are engaged in dislocation and degradation of lipidated ApoB-100 at lipid droplets. *Mol Biol Cell* 2012; 23: 800–10.

[156] Wang CW, Lee SC. The ubiquitin-like (UBX)-domain-containing protein Ubx2/Ubxd8 regulates lipid droplet homeostasis. *J Cell Sci* 2012; 125: 2930–9.

[157] Ye J. Cellular responses to unsaturated fatty acids mediated by their sensor Ubxd8. *Frontiers in Biology* 2012; 7: 397–403.

[158] Moon YA, Liang G, Xie X, Frank-Kamenetsky M, Fitzgerald K, Koteliansky V, et al. The Scap/SREBP pathway is essential for developing diabetic fatty liver and carbohydrate-induced hypertriglyceridemia in ani–mals. *Cell metabolism* 2012; 15: 240–6.

[159] Martin S, Parton RG. Lipid droplets: A unified view of a dynamic organelle. *Nature reviews.Molecular cell biology* 2006; 7: 373–8.

[160] Cermelli S, Guo Y, Gross SP, Welte MA. The lipid-dro–plet proteome reveals that droplets are a protein-storage depot. *Current biology : CB* 2006; 16: 1783–95.

[161] Klemm EJ, Spooner E, Ploegh HL. Dual role of ancient ubiquitous protein 1 (AUP1) in lipid droplet accumulation and endoplasmic reticulum (ER) protein quality control. *The Journal of biological chemistry* 2011; 286: 37602–14.

[162] Hartman IZ, Liu P, Zehmer JK, Luby-Phelps K, Jo Y, Anderson RGW, et al. Sterol-induced dislocation of 3-hydroxy-3-methylglutaryl coenzyme a reductase from endoplasmic reticulum membranes into the cytosol through a subcellular compartment resembling lipid droplets. *J Biol Chem* 2010; 285: 19288–98.

[163] Wang L, Martin DD, Genter E, Wang J, McLeod RS, Small DM. Surface study of apoB1694-1880, a sequence that can anchor apoB to lipoproteins and make it nonex–changeable. *J Lipid Res* 2009; 50: 1340–52.

[164] Komander D, Clague MJ, Urbe S. Breaking the chains: Structure and function of the deubiquitinases. *Nature reviews.Molecular cell biology* 2009; 10: 550–63.

[165] Sowa ME, Bennett EJ, Gygi SP, Harper JW. Defining the human deubiquitinating enzyme interaction landscape. *Cell* 2009; 138: 389–403.

[166] Liu Y, Ye Y. Roles of p97-associated deubiquitinases in protein quality control at the endoplasmic reticulum. *Curr Protein Pept Sci* 2012; 13: 436–46.

[167] Meyer HH, Shorter JG, Seemann J, Pappin D, Warren G. A complex of mammalian ufd1 and npl4 links the AAA-ATPase, p97, to ubiquitin and nuclear transport pathways. *EMBO J* 2000; 19: 2181–92.

[168] Wang Q, Liu Y, Soetandyo N, Baek K, Hegde R, Ye Y. A ubiquitin ligase-associated chaperone holdase maintains polypeptides in soluble states for proteasome degradation. *Mol Cell* 2011; 42: 758–70.

[169] Takiyama Y, Nishizawa M, Tanaka H, Kawashima S, Sakamoto H, Karube Y, et al. The gene for machado-

joseph disease maps to human chromosome 14q. *Nat Genet* 1993; 4: 300–4.

[170] Zhong X, Pittman RN. Ataxin-3 binds VCP/p97 and regulates retrotranslocation of ERAD substrates. *Hum Mol Genet* 2006; 15: 2409–20.

[171] Wang Q, Li L, Ye Y. Regulation of retrotranslocation by p97-associated deubiquitinating enzyme ataxin-3. *J Cell Biol* 2006; 174: 963–71.

[172] Ernst R, Mueller B, Ploegh HL, Schlieker C. The otubain YOD1 is a deubiquitinating enzyme that associates with p97 to facilitate protein dislocation from the ER. *Mol Cell* 2009; 36: 28–38.

[173] Bernardi KM, Williams JM, Inoue T, Schultz A, Tsai B. A deubiquitinase negatively regulates retro-translocation of nonubiquitinated substrates. *Mol Biol Cell* 2013; 24: 3545–56.

[174] Liu Y, Soetandyo N, Lee JG, Liu L, Xu Y, Clemons WM Jr, et al. USP13 antagonizes gp78 to maintain functionality of a chaperone in ER-associated degradation. *Elife* 2014; 3: e01369.

[175] Blount JR, Burr AA, Denuc A, Marfany G, Todi SV. Ubiquitin-specific protease 25 functions in endoplasmic reticulum-associated degradation. *PLoS One* 2012; 7: e36542.

[176] Zhang ZR, Bonifacino JS, Hegde RS. Deubiquitinases sharpen substrate discrimination during membrane protein degradation from the ER. *Cell* 2013; 154: 609–22.

[177] Kuang E, Qi J, Ronai Z. Emerging roles of E3 ubiquitin ligases in autophagy. *Trends Biochem Sci* 2013; 38: 453–60.

[178] Seiberlich V, Borchert J, Zhukareva V, Richter-Landsberg C. Inhibition of protein deubiquitination by PR-619 activates the autophagic pathway in OLN-t40 oligodendroglial cells. *Cell Biochem Biophys* 2013; 67: 149–60.

[179] Fisher EA. The degradation of apolipoprotein B100: Multiple opportunities to regulate VLDL triglyceride production by different proteolytic pathways. *Biochim Biophys Acta* 2012; 1821: 778–81.

[180] Yang L, Li P, Fu S, Calay ES, Hotamisligil GS. Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. *Cell metabolism* 2010; 11: 467–78.

[181] Pan M, Maitin V, Parathath S, Andreo U, Lin SX, Germain CS, et al. Presecretory oxidation, aggregation, and autophagic destruction of apoprotein-B: A pathway for late-stage quality control. *Proc Natl Acad Sci U S A* 2008; 105: 5862–7.

[182] Maitin V, Andreo U, Guo L, Fisher EA. Docosahexaenoic acid impairs the maturation of very low density lipoproteins in rat hepatic cells. *J Lipid Res* 2014; 55: 75–84.

[183] Andreo U, Guo L, Chirieac DV, Tuyama AC, Montenont E, Brodsky JL, et al. Insulin-stimulated degradation of apolipoprotein B100: Roles of class II phosphatidylinositol-3-kinase and autophagy. *PLoS One* 2013; 8: e57590.

[184] Sparks JD, O′Dell C, Chamberlain JM, Sparks CE. Insulin-dependent apolipoprotein B degradation is mediated by autophagy and involves class I and class III phosphatidylinositide 3-kinases. *Biochem Biophys Res Commun* 2013; 435: 616–20.

[185] Allister EM, Mulvihill EE, Barrett PH, Edwards JY, Carter LP, Huff MW. Inhibition of apoB secretion from HepG2 cells by insulin is amplified by naringenin, independent of the insulin receptor. *J Lipid Res* 2008; 49: 2218–29.

[186] Mulvihill EE, Allister EM, Sutherland BG, Telford DE, Sawyez CG, Edwards JY, et al. Naringenin prevents dyslipidemia, apolipoprotein B overproduction, and hyperinsulinemia in LDL receptor-null mice with diet-induced insulin resistance. *Diabetes* 2009; 58: 2198–210.

[187] Ohsaki Y, Cheng J, Fujita A, Tokumoto T, Fujimoto T. Cytoplasmic lipid droplets are sites of convergence of proteasomal and autophagic degradation of apolipoprotein B. *Mol Biol Cell* 2006; 17: 2674–83.

[188] Ohsaki Y, Cheng J, Suzuki M, Fujita A, Fujimoto T. Lipid droplets are arrested in the ER membrane by tight binding of lipidated apolipoprotein B-100. *J Cell Sci* 2008; 121: 2415–22.

[189] Qiu W, Avramoglu RK, Rutledge AC, Tsai J, Adeli K. Mechanisms of glucosamine-induced suppression of the hepatic assembly and secretion of apolipoprotein B-100-containing lipoproteins. *J Lipid Res* 2006; 47: 1749–61.

[190] Qiu W, Su Q, Rutledge AC, Zhang J, Adeli K. Glucosamine-induced endoplasmic reticulum stress attenuates apolipoprotein B100 synthesis via PERK signaling. *J Lipid Res* 2009; 50: 1814–23.

[191] Qiu W, Zhang J, Dekker MJ, Wang H, Huang J, Brumell JH, et al. Hepatic autophagy mediates endoplasmic reticulum stress-induced degradation of misfolded apolipoprotein B. *Hepatology* 2011; 53: 1515–25.

[192] Komatsu M, Kageyama S, Ichimura Y. p62/SQSTM1/A170: Physiology and pathology. *Pharmacological research : the official journal of the Italian Pharmacological Society* 2012; 66: 457–62.

[193] Komatsu M, Kurokawa H, Waguri S, Taguchi K, Kobayashi A, Ichimura Y, et al. The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. *Nat Cell Biol* 2010; 12: 213–23.

[194] Digaleh H, Kiaei M, Khodagholi F. Nrf2 and Nrf1 signaling and ER stress crosstalk: Implication for proteasomal degradation and autophagy. *Cell Mol Life Sci* 2013; 70: 4681–94.

[195] Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nature reviews.Molecular cell biology* 2007; 8: 519–29.

[196] Rutkowski DT, Hegde RS. Regulation of basal cellular physiology by the homeostatic unfolded protein response. *J Cell Biol* 2010; 189: 783–94.

[197] Walter P, Ron D. The unfolded protein response: From stress pathway to homeostatic regulation. *Science (New York, N.Y.)* 2011; 334: 1081–6.

[198] Rutkowski DT, Kaufman RJ. That which does not kill me makes me stronger: Adapting to chronic ER stress. *Trends Biochem Sci* 2007; 32: 469–76.

[199] Sparks CE, Sparks JD. Hepatic postprandial transition and very low-density lipoprotein biogenesis. *Curr Opin Lipidol* 2013; 24: 450–2.

[200] Ning J, Hong T, Ward A, Pi J, Liu Z, Liu HY, et al. Constitutive role for IRE1alpha-XBP1 signaling pathway in the insulin-mediated hepatic lipogenic program. *Endocrinology* 2011; 152: 2247–55.

[201] Lee AH, Glimcher LH. Intersection of the unfolded protein response and hepatic lipid metabolism. *Cellular and molecular life sciences : CMLS* 2009; 66: 2835–50.

[202] Wang S, Chen Z, Lam V, Han J, Hassler J, Finck B, et al. IRE1I±-XBP1s induces PDI expression to increase MTP activity for hepatic VLDL assembly and lipid homeosta– sis. *Cell Metabolism* 2012; 16: 473–86.

[203] Duvel K, Yecies JL, Menon S, Raman P, Lipovsky AI, Souza AL, et al. Activation of a metabolic gene regulatory network downstream of mTOR complex 1. *Mol Cell* 2010; 39: 171–83.

[204] Cnop M, Foufelle F, Velloso LA. Endoplasmic reticulum stress, obesity and diabetes. *Trends Mol Med* 2012; 18: 59–68.

[205] Pfaffenbach KT, Nivala AM, Reese L, Ellis F, Wang D, Wei Y, et al. Rapamycin inhibits postprandial-mediated X-box-binding protein-1 splicing in rat liver. *J Nutr* 2010; 140: 879–84.

[206] Tremblay F, Brule S, Um SH, Li Y, Masuda K, Roden M, et al. Identification of IRS-1 ser-1101 as a target of S6K1 in nutrient- and obesity-induced insulin resistance. *Proc Natl Acad Sci U S A* 2007; 104: 14056–61.

[207] Deng Y, Wang ZV, Tao C, Gao N, Holland WL, Ferdous A, et al. The Xbp1s/GalE axis links ER stress to post– prandial hepatic metabolism. *J Clin Invest* 2013; 123: 455–68.

[208] Haas ME, Attie AD, Biddinger SB. The regulation of ApoB metabolism by insulin. *Trends Endocrinol Metab* 2013; 24: 391–7.

[209] Verges B. Abnormal hepatic apolipoprotein B metabo– lism in type 2 diabetes. *Atherosclerosis* 2010; 211: 353–60.

[210] Li S, Brown MS, Goldstein JL. Bifurcation of insulin sig– naling pathway in rat liver: MTORC1 required for stimu– lation of lipogenesis, but not inhibition of gluconeogenesis. *Proc Natl Acad Sci U S A* 2010; 107: 3441–6.

[211] Meshkani R, Adeli K. Hepatic insulin resistance, meta– bolic syndrome and cardiovascular disease. *Clin Biochem* 2009; 42: 1331–46.

[212] Kammoun HL, Chabanon H, Hainault I, Luquet S, Magnan C, Koike T, et al. GRP78 expression inhibits insulin and ER stress-induced SREBP-1c activation and reduces hepatic steatosis in mice. *J Clin Invest* 2009; 119: 1201–15.

[213] Kumashiro N, Erion DM, Zhang D, Kahn M, Beddow SA, Chu X, et al. Cellular mechanism of insulin resistance in nonalcoholic fatty liver disease. *Proc Natl Acad Sci U S A* 2011; 108: 16381–5.

[214] Pour NK, Adeli K. Insulin silences apolipoprotein B mRNA translation by inducing intracellular traffic into cytoplasmic RNA granules. *Biochemistry (N Y)* 2011; 50: 6942–50.

[215] Sparks JD, Chamberlain JM, O′Dell C, Khatun I, Hussain MM, Sparks CE. Acute suppression of apo B secretion by insulin occurs independently of MTP. *Biochem Biophys Res Commun* 2011; 406: 252–6.

[216] Tsai J, Zhang R, Qiu W, Su Q, Naples M, Adeli K. Inflammatory NF-kappaB activation promotes hepatic apolipoprotein B100 secretion: Evidence for a link between hepatic inflammation and lipoprotein production. *American Journal of Physiology.Gastrointestinal and Liver Physiology* 2009; 296: G1287–98.

[217] Peairs AD, Rankin JW, Lee YW. Effects of acute inges– tion of different fats on oxidative stress and inflammation in overweight and obese adults. *Nutrition journal* 2011; 10: 122.

[218] Pellis L, Erk MJv, Ommen Bv, Bakker GC, Hendriks HF, Cnubben NH, et al. Plasma metabolomics and proteomics profiling after a postprandial challenge reveal subtle diet effects on human metabolic status. *Metabolomics : Official journal of the Metabolomic Society* 2012; 8: 347–59.

[219] Hotamisligil GS. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell* 2010; 140: 900–17.

[220] Rath E, Haller D. Inflammation and cellular stress: A mechanistic link between immune-mediated and metabo– lically driven pathologies. *Eur J Nutr* 2011; 50: 219–33.

[221] Garg AD, Kaczmarek A, Krysko O, Vandenabeele P, Krysko DV, Agostinis P. ER stress-induced inflamma– tion: Does it aid or impede disease progression? *Trends Mol Med* 2012; .

[222] Basciano H, Miller A, Baker C, Naples M, Adeli K. LXRalpha activation perturbs hepatic insulin signaling and stimulates production of apolipoprotein B-containing lipoproteins. *American Journal of Physiology. Gastro– intestinal and Liver Physiology* 2009; 297: G323–32.

[223] Okazaki H, Goldstein JL, Brown MS, Liang G. LXR-SREBP-1c-phospholipid transfer protein axis controls very low density lipoprotein (VLDL) particle size. *The Journal of biological chemistry* 2010; 285: 6801–10.

[224] Li Z, Agellon LB, Allen TM, Umeda M, Jewell L, Mason A, et al. The ratio of phosphatidylcholine to phosphatidy– lethanolamine influences membrane integrity and steato– hepatitis. *Cell metabolism* 2006; 3: 321–31.

[225] Rong X, Albert CJ, Hong C, Duerr MA, Chamberlain BT, Tarling EJ, et al. LXRs regulate ER stress and inflamma– tion through dynamic modulation of membrane phospho– lipid composition. *Cell Metab* 2013; 18: 685–97.

[226] Fu S, Yang L, Li P, Hofmann O, Dicker L, Hide W, et al. Aberrant lipid metabolism disrupts calcium homeostasis causing liver endoplasmic reticulum stress in obesity. *Nature* 2011; 473: 528–31.

[227] Magkos F, Fabbrini E, Mohammed BS, Patterson BW, Klein S. Increased whole-body adiposity without a con– comitant increase in liver fat is not associated with aug– mented metabolic dysfunction. *Obesity (Silver Spring)* 2010; 18: 1510–5.

[228] Duvillard L, Florentin E, Pont F, Petit JM, Baillot-Rudoni S, Penfornis A, et al. Endogenous chronic hyperinsuline– mia does not increase the production rate of VLDL apo– lipoprotein B: Proof from a kinetic study in patients with insulinoma. *J Clin Endocrinol Metab* 2011; 96: 2163–70.

[229] Qiu W, Federico L, Naples M, Avramoglu RK, Meshkani R, Zhang J, et al. Phosphatase and tensin homolog (PTEN) regulates hepatic lipogenesis, microsomal triglyceride transfer protein, and the secretion of apolipo– protein B-containing lipoproteins. *Hepatology* 2008; 48: 1799–809.

[230] Moon BC, Hernandez-Ono A, Stiles B, Wu H, Ginsberg HN. Apolipoprotein B secretion is regulated by hepatic triglyceride, and not insulin, in a model of increased hepatic insulin signaling. *Arterioscler Thromb Vasc Biol* 2012; 32: 236–46.

[231] Ren LP, Chan SM, Zeng XY, Laybutt DR, Iseli TJ, Sun RQ, et al. Differing endoplasmic reticulum stress response

to excess lipogenesis versus lipid oversupply in relation to hepatic steatosis and insulin resistance. *PloS one* 2012; 7: e30816.

[232] Higuchi N, Kato M, Tanaka M, Miyazaki M, Takao S, Kohjima M, et al. Effects of insulin resistance and hepatic lipid accumulation on hepatic mRNA expression levels of apoB, MTP and L-FABP in non-alcoholic fatty liver disease. *Experimental and therapeutic medicine* 2011; 2: 1077–81.

[233] Su Q, Tsai J, Xu E, Qiu W, Bereczki E, Santha M, et al. Apolipoprotein B100 acts as a molecular link between lipid-induced endoplasmic reticulum stress and hepatic insulin resistance. *Hepatology* 2009; 50: 77–84.

[234] Yao Z, Zhou H, Figeys D, Wang Y, Sundaram M. Microsome-associated lumenal lipid droplets in the regulation of lipoprotein secretion. *Curr Opin Lipidol* 2012; .

[235] Fu S, Watkins SM, Hotamisligil GS. The role of endoplasmic reticulum in hepatic lipid homeostasis and stress signaling. *Cell metabolism* 2012; 15: 623–34.

[236] Calderwood SK, Murshid A, Prince T. The shock of aging: Molecular chaperones and the heat shock response in longevity and aging–a mini-review. *Gerontology* 2009; 55: 550–8.

[237] Morimoto RI. The heat shock response: Systems biology of proteotoxic stress in aging and disease. *Cold Spring Harb Symp Quant Biol* 2011; 76: 91–9.

[238] Xiao C, Giacca A, Lewis GF. Sodium phenylbutyrate, a drug with known capacity to reduce endoplasmic reticulum stress, partially alleviates lipid-induced insulin resistance and beta-cell dysfunction in humans. *Diabetes* 2011; 60: 918–24.

[239] Kars M, Yang L, Gregor MF, Mohammed BS, Pietka TA, Finck BN, et al. Tauroursodeoxycholic acid may improve liver and muscle but not adipose tissue insulin sensitivity in obese men and women. *Diabetes* 2010; 59: 1899–905.

[240] Imrie D, Sadler KC. Stress management: How the unfolded protein response impacts fatty liver disease. *J Hepatol* 2012; 57: 1147–51.