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MECHANISMS OF METABOLIC DYSLIPIDEMIA IN INSULIN RESISTANT STATES: DEREGULATION OF HEPATIC AND INTESTINAL LIPOPROTEIN SECRETION

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1. ABSTRACT

The growing epidemic of the metabolic syndrome is now well recognized and there is widespread effort to understand the pathogenesis of this complex syndrome and its major metabolic consequences. One of the severe complications accompanying insulin resistant states is the hypertriglyceridemia that appears to occur largely due to overproduction of triglyceride-rich, apolipoprotein B (apoB) containing-lipoproteins. As a result, mechanisms regulating the overproduction of these atherogenic apoB-containing lipoproteins have been the focus of much investigation in recent years. Both *in vitro* as well as *in vivo* models of insulin resistance are currently being used to further our understanding of the mechanisms involved in the deregulation of lipid metabolism in insulin resistant states. Evidence from these animal models as well as human studies has identified hepatic very low density lipoprotein (VLDL) overproduction as a critical underlying factor in the development of hypertriglyceridemia and metabolic dyslipidemia. In recent years, a dietary animal model of insulin resistance, the fructose-fed hamster model developed in our laboratory, has proven invaluable in studies of the link between development of an insulin resistant state, derangement of hepatic lipoprotein metabolism, and overproduction of apoB-containing lipoproteins. Evidence from the fructose-fed hamster model now indicates oversecretion of both hepatically-derived apoB100-containing VLDL as well as intestinal apoB48-containing triglyceride-rich lipoproteins in insulin resistant states. A number of novel intracellular factors that may be involved in modulation of VLDL have also been identified. This review focuses on these recent developments and examines the hypothesis that a complex interaction among enhanced flux of free fatty acids from peripheral tissues to liver and intestine, chronic up-regulation of *de novo* lipogenesis by hyperinsulinemia, and attenuated insulin signaling in the liver and the intestine may be critical to lipoprotein overproduction accompanying insulin resistance.

2. DEREGULATION OF LIPOPROTEIN METABOLISM IN INSULIN RESISTANT STATES

2.1. Metabolic dyslipidemia in insulin resistance

Insulin resistance results from the insensitivity of tissues to the normal effects of insulin, consequently leading to hyperglycemia, hypertriglyceridemia, and elevated plasma levels of free fatty acids (FFA) (1-5). As the body attempts to normalize plasma glucose levels, a compensatory mechanism by which insulin is oversecreted may ultimately lead to pancreatic failure. Prospective studies have shown that type 2 diabetes occurs once pancreatic B cells fail to compensate for the insulin resistant state. The pathophysiology of the insulin resistant state, in addition to the development of type 2 diabetes, includes obesity, atherosclerosis, hypertension, and dyslipidemia. The atherogenic dyslipidemia associated with insulin resistant states is characterized by hypertriglyceridemia, an increase in VLDL secretion from the liver (6-9), an increase in atherogenic small dense low density lipoprotein (LDL) (10), and a decrease in

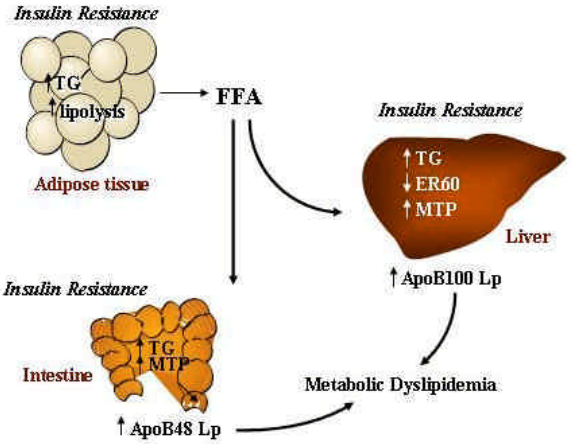

Figure 1. Insulin Resistance in Adipose, Liver, and Intestine Leading to Metabolic Dyslipidemia: Insulin resistance causes reduced FFA absorption and enhanced lipolysis by adipocytes resulting in enhanced FFA flux into peripheral tissues including liver and intestine. Enhanced core lipid availability due to higher FFA flux in combination with hepatic and intestinal insulin resistance appear to lead to a considerable overproduction of both hepatically- and intestinally-derived apoB-containing lipoprotein particles which cause fasting and postprandial metabolic dyslipidemia

antiatherogenic high density lipoprotein (HDL) cholesterol (11).

2.2. Insulin regulation of apoB-containing lipoproteins

Studies have shown that insulin acutely inhibits VLDL secretion both *in vitro* and *in vivo*, in animal studies, as well as in fasting human subjects (12). In partially hepatectomized rats, apolipoprotein B (apoB) secretion was found to be more responsive to the effect of insulin (13). In addition, studies in glucose injected rats confirmed the suppressive effects of insulin on VLDL triglyceride (TG) and apoB secretion (14). Studies in our own laboratory using a cell-free system have demonstrated an attenuation of apoB mRNA following insulin modulation (15).

In contrast, chronically hyperinsulinemic subjects appear resistant to the inhibitory effects of insulin on VLDL secretion (16). Upon fructose feeding, a mildly diabetic rat model showed elevated plasma levels of free fatty acids and triglycerides in addition to impaired clearance of VLDL (17,18). Much the same has been seen in obese diabetic human subjects. A significant improvement was seen in hyperinsulinemic and type 2 diabetic subjects upon administration of atorvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG coA) reductase inhibitor with TG lowering properties. Over a forty day treatment period, there was a significant decrease in plasma total cholesterol, LDL cholesterol, TG, and apoB. There was also an increase in LDL particle diameter (19). These results suggest that atorvastatin treatment may be beneficial in modifying the lipoprotein profile of these subjects and decreasing LDL particle density to more buoyant, less atherogenic LDL particles.

It has been postulated that there may be an acute effect of insulin directly on apoB synthesis, stability and subsequently secretion that is modulated by FFA availability (12). This effect has been attributed to increased lipolysis leading to reduced FFA uptake by adipocytes resulting in increased FFA flux to the liver and muscle (reviewed in (20). The increase in FFA can further attenuate insulin signaling and exacerbates insulin resistance (Figure 1). On the other hand, FFA may stimulate the secretion of apoB-containing lipoprotein in a number of hepatocyte model systems. Within hepatic tissue, FFA is accumulated and stored in the liver as TG, which correlates positively with VLDL secretion. Insulin may directly control the rate of hepatic VLDL production by influencing the rate of apoB synthesis and degradation (15,21-23), or indirectly *via* its effect on the supply of FFA to the liver for lipoprotein production (8,24). Therefore, an increased FFA flux as is observed in insulin resistance may cause increased TG availability that may, in turn, stimulate assembly and secretion of VLDL (25). In *in vitro* studies, this has been observed in HepG2 cells (26-28) and some primary hepatocyte experiments (29-31). Conversely, studies in other primary hepatocyte systems including rat (32,33), hamster (34,35), and human hepatocytes (36) have failed to demonstrate FFA-mediated stimulation of apoB secretion. More recently, treatment of HepG2 cells with anti-retroviral protease inhibitor compounds resulted in an increase of ubiquitinated apoB and prevented apoB degradation (37). There was also a significant accumulation of intracellular apoB and an impairment of apoB lipoprotein secretion that was attributed to a sharp decrease in intracellular synthesis of neutral lipids. Secretion could be restored by the addition of exogenous fatty acid suggesting that the intracellular pool of apoB could be secreted upon lipid availability as in the case of increased FFA flux to the liver in insulin resistance. It is possible that under insulin resistant conditions, all hepatocyte systems may respond to exogenous FFA by oversecreting VLDL. Studies in a fructose-fed model of insulin resistance have recently shown hepatic VLDL overproduction accompanied by elevated plasma FFA levels suggesting an enhanced FFA flux into the liver (38).

While it has been well established for some time that triglyceride protects apoB from degradation (39), the role of cholesteryl ester in apoB secretion has been controversial (40,41). Cholesteryl ester is believed to be an important substrate for microsomal triglyceride transfer protein (MTP) and plays an important role in the protection of newly synthesized apoB from degradation (42-44). *In vitro* studies have shown that acyl-CoA cholesterol acyl transferase (ACAT) inhibitors reduce apoB100 secretion in primary rabbit hepatocytes (45), primary rat hepatocytes (46,47), and HepG2 cells (40,48-50). Several *in vivo* studies in miniature pigs (50-53), rabbits (54,55), rats (55), hamsters (45,55), and monkeys (55,56), have confirmed these *in vitro* observations. In contrast, studies have argued against any regulatory role of cholesteryl ester in apoB secretion (41). Recent studies

have shown that citrus flavinoids cause a decrease in ACAT2 and MTP accompanied by a dramatic decrease in apoB-containing lipoprotein secretion (57). Studies in our own laboratory using the novel ACAT inhibitor avasimibe (CI-1011) have shown increased apoB stability in the presence of the inhibitor as measured by pulse-chase experiments as well as by trypsin sensitivity assay (49). However, poor lipidation in the presence of this inhibitor also suggest that it may exert its inhibitory effect through novel mechanisms that remain to be further studied.

Within the ER, the generation of lipoproteins destined for secretion is initially dependent on lipid transfer catalyzed by MTP and is followed by the MTP-independent incorporation of additional neutral lipid along the secretory pathway (58). It was recently shown using an MTP inhibitor (BMS-197636-01), an MTP and TG synthesis inhibitor (CP-10447), as well as the TG synthesis inhibitor (Triacsin C), that the late addition of neutral lipid to nascent lipoproteins within the ER lumen is independent of MTP activity and availability of newly synthesized TG (59). Interestingly, the hepatic expression of MTP appears to be modulated by insulin in rat hepatocytes suggesting an additional mechanism for the regulation of apoB by insulin (60).

Insulin treatment clearly ameliorates the lipoprotein profile of diabetic individuals, however, the ability of insulin to directly regulate apoB gene expression remains controversial. Thus, increased VLDL-triglyceride production in insulin resistance appears to directly or indirectly result from decreased sensitivity to the inhibitory effects of insulin on VLDL secretion. Treatment of rat hepatocytes with several specific inhibitors has shown that insulin-mediated inhibition of VLDL secretion may be PI-3-kinase dependent. A significant increase in insulin stimulated PI-3-kinase activity was observed within the same subcellular compartment containing apoB (61). This may suggest an insulin-mediated localization and activation of PI-3-kinase within a compartment also containing apoB. Current research in several laboratories has focused on understanding the intracellular events linking insulin resistance to hepatic VLDL overproduction.

2.3. The hepatic insulin signaling pathway and disruption in insulin resistance

Under normal conditions, insulin binding to its cell surface receptor initiates a cascade of events beginning with receptor autophosphorylation and activation of receptor tyrosine kinases (62,63). This, in turn, results in tyrosine phosphorylation of adaptor proteins such as members of the insulin receptor substrate (IRS) family (IRS-1/2/3/4), and Shc (64-68). Insulin signaling may be regulated by the activity of phosphotyrosyl-protein phosphatases (PTPases) through dephosphorylation of the insulin receptor, IRS-1, IRS-2, and Shc leading to modulation of insulin action downstream of the receptor (69). IRS-1 and IRS-2 function as adaptor proteins for src homology 2 (SH2)-domain containing signaling proteins including the Grb-2-SOS complex, SHP2, Nck, as well as the regulatory subunit of the lipid kinase, PI-3-kinase (70-73). Generation of 3'-phospholipids such as PI-3,4,5-P3 (PIP3) is dependent on activation of PI3-kinase. Binding of IRS to p85, the regulatory subunit of PI-3-kinase, activates the PI-3-kinase-PKB/Akt pathway, which is necessary for insulin action on glucose transport and glycogen synthesis (65,74,75).

Insulin resistance is thought to result predominantly from defects in the signaling pathway downstream of the insulin receptor. Among obese and type 2 diabetes subjects, there was an observed decrease in IRS-1-associated tyrosine phosphorylation and a decrease in PI3-kinase activity in skeletal muscle and adipocytes, the tissues predominantly targeted by insulin (76-79). Obese hyperinsulinemic human subjects exhibited decreased insulin receptor expression level and activity as well as decreased tyrosine kinase activity in skeletal muscle (80) and adipocytes (81). Patients with type 2 diabetes exhibited reduced insulin signaling in skeletal muscle and the liver (80). In lean, type 2 diabetes subjects, insulin-stimulated PI-3-kinase activity was also decreased. Similar findings have been reported in numerous genetic (82-86) and induced (87,88) rodent models of obesity. Studies involving targeted disruption of the insulin receptor, IRS-1 or IRS-2 (89-94) suggest that insensitivity to the effects of insulin in a combination of tissues including muscle, liver, adipose tissue, and pancreatic B cells may lead to insulin resistance and diabetes (75).

Protein tyrosine phosphatase-1B (PTP-1B) is a member of the PTPases family of enzymes that are expressed in insulin sensitive tissues and appears to be an important candidate for involvement in insulin signaling (95). Overexpression studies have shown that PTP-1B dephosphorylates the insulin receptor *in vitro* (96,97) as well as inducing the downregulation of IRS-1 and insulin stimulated PI-3-kinase activity (98). Increased PTP-1B mass and activity has been associated with carbohydrate-induced insulin resistance (99) and normalization of PTP-1B mass and activity results in the reversal of this type of insulin resistance (100). Elchebly *et al* (101) have generated PTP-1B knockout mice which exhibited increased sensitivity towards insulin-induced IR and IRS-1 tyrosine phosphorylation and were resistant to obesity. It has been shown in both transfection studies, as well as transgenic animals, that PTP-1B dephosphorylates the leptin receptor-associated kinase Jak2 (102-104). In PTP-1B deficient mice, there was an enhanced response toward leptin-mediated loss of body weight. Studies within our own laboratory have shown a significant increase in PTP-1B mass and activity with a concomitant decrease in PI-3-kinase and PKB/Akt phosphorylation in a fructose-fed hamster model of insulin resistance (105). Interestingly, a significant increase in PTP-1B protein mass was seen following two days of insulin treatment in primary hepatocytes. This was accompanied by a decrease in insulin receptor mass and phosphorylation suggesting a possible link between the impairment of intracellular signaling and overproduction of apoB-containing lipoproteins.

2.4. Animal models of insulin resistance

In animal models, insulin resistance may be induced by genetic alterations, changes in diet,

administration of pharmacological agents, or surgical procedures (reviewed in 106-109). In particular, genetically modified and diet-induced animal models have proven invaluable in furthering our understanding of mechanisms involved in insulin resistance and its associated dyslipidemia. These animal models almost always exhibit insulin resistance, obesity, dyslipidemia and in certain instances hypertension. The classic animal models used in the past include the *ob/ob* mouse, the *db/db* mouse, and the Zucker *fa/fa* (fatty) as well as the ZDF/Drt *fa* (diabetic/fatty) rat. These rodent models may develop moderate to severe insulin resistance or diabetes, obesity, and an increase in plasma lipid and lipoprotein levels.

While whole body disruption of the insulin receptor causes neonatal mortality in transgenic mice (92), successful studies using tissue targeted disruption of the receptor have been performed. Studies using mice expressing a dominant negative insulin receptor transgene in skeletal muscle and fat showed decreased insulin receptor phosphorylation, decreased insulin receptor signaling, and impaired insulin action in these tissues (110). Despite this, these mice failed to develop insulin resistance or chronic hyperglycemia. Liver-specific insulin receptor knockout mice developed severe insulin resistance and glucose intolerance at an early age (111). Surprisingly, the fasting hyperglycemia returned to normal levels as these animals matured. Taken together these observations suggest that a defect in insulin action within a tissue such as the liver may be critical to the development insulin resistance, but that defects in the liver alone are not sufficient to cause chronic fasting hyperglycemia. Furthermore, defects in molecules such as leptin, that are apparently not directly related to insulin signaling or lipoprotein metabolism, suggest that the mechanisms of lipid deregulation may be extremely complex and involve multiple metabolic pathways originating in several tissues. These mechanisms may involve tissues such as muscle, liver, and adipose, in addition to less well understood tissues such as the brain.

Streptozotocin (STZ) has been successfully used in rats to induce insulin deficiency, insulin resistance, and decreased plasma leptin concentrations through the destruction of pancreatic B cells (112). These animals also exhibit increased plasma FFA and TG concentrations. In STZ treated animals that were fat-fed, plasma glucose clearance by adipose tissue was impaired compared to controls (113). Transplantation with fetal pancreatic islets normalized blood glucose, plasma triglyceride, cholesterol, and VLDL-triglyceride turnover rate among other factors, suggesting a direct link between insulin availability and VLDL regulation (114).

A transgenic mouse has been developed that overexpresses the A1 adenosine receptor in adipose tissue (115). This receptor has been implicated *in vitro* in the metabolism of intracellular fat accumulation, FFA metabolism and plasma glucose regulation (116-118). Interestingly, although the control and transgenic animals were of the same size and body composition, the transgenics exhibited lower plasma FFA, and failed to develop insulin resistance as shown by oral glucose tolerance tests.

More recently, a model has been developed in the laboratory of Ginsberg and colleagues in order to directly study apoB overproduction associated with insulin resistance. This model, dubbed ApoB/BATless used a human apoB transgenic mouse crossed with a brown adipose tissue knockout mouse that exhibits peripheral insulin resistance (119). The resulting animal developed obesity, hypertriglyceridemia, hypercholesterolemia, and hyperinsulinemia when placed on a high fat diet. Although an increase in apoB was seen in the apoB/BATless mice, the mRNA levels of both MTP, as well as apoB were similar between these and control mice expressing only the human apoB transgene, suggesting VLDL assembly and secretion were regulated post-translationally. Future studies of these animals should aid in understanding the manner by which specific signaling mechanisms may be involved in lipoprotein regulation.

A significant concern when using rodent models has been that their lipoprotein profile differs significantly from that of humans. While humans produce primarily VLDL and LDL, rodent lipoprotein distribution is shifted primarily towards HDL. Several models have been developed that address this issue. The “sand rat” (*psammomys obesus*), a gerbil native to the desert regions of the eastern Mediterranean and northern Africa, spontaneously develops obesity and insulin resistance when taken off its habitual diet of succulent plants and fed standard rodent chow (106,120). It has recently been shown that elevated levels of protein kinase C epsilon in skeletal muscle may contribute to the development of insulin resistance seen in these animals (121). Reports of elevated leptin levels and possible leptin resistance in obese and diabetic animals suggest this factor may also be involved in the development of obesity. More recently, beacon, a novel factor was found to be differentially expressed in the brain of obese and control animals (122). A recent study characterizing the lipid distribution within these animals found significant increases in circulating VLDL and LDL in hyperinsulinemic as well as hyperinsulinemic and hyperglycemic animals (123).

2.5. The fructose-fed hamster model of insulin resistance

The Syrian golden hamster has been used with increasing frequency in recent years to study hepatic lipid metabolism as its lipoprotein metabolism closely resembles that of humans (124-127). Our laboratory has developed and extensively characterized a diet induced animal model of insulin resistance, the fructose-fed Syrian golden hamster (38).

Hamsters develop hyperlipidemia and atherosclerosis in response to a modest increase in dietary cholesterol and saturated fat (128,129) and can be made obese, hypertriglyceridemic, and insulin-resistant by fructose feeding (38). Fructose feeding for a two week period induced significant increases in plasma TG, cholesterol, FFA, and the development of whole body

insulin resistance. Induction of insulin resistance was accompanied by a considerable rise in the *in vivo* production of hepatic VLDL-apoB and –TG. These data suggest overall improved efficiency of VLDL assembly in fructose-fed animals. This may be due to the increased intracellular stability and availability of apoB, elevated levels of available neutral lipid or increased MTP mass or activity. Although increased apoB stability and MTP were observed, FFA flux to the liver was not determined in this study. The observed increase in VLDL production may be due to a direct effect of availability of these substrates. In addition to providing substrate, the elevated FFA levels may induce hepatic enzymes such as fatty acid synthase (FAS) and peroxisome-proliferator activated receptor (PPAR), thus favouring lipoprotein assembly over oxidative degradation. Further studies will be required to understand the interplay between all these factors in regulating VLDL secretion.

The MTP promoters of human and hamster are organized similarly (130) and contain a number of regulatory elements including an insulin response element (IRE), activating protein 1, hepatic nuclear factor 1, and hepatic nuclear factor 4 (130,131). Hepatic MTP mRNA levels can also be upregulated by a high-fat diet in hamsters (132) and decline in response to sterol depletion in HepG2 cells (133). This regulation may occur *via* activation of sterol response element binding proteins (SREBPs) which bind a putative sterol response element (SRE) within the -124 to -116 of the 5' promoter (133). There is also evidence that chronic modulation of apoB and VLDL secretion can be achieved via changes in MTP expression and activity. Hyperlipidemia in an animal model of type 2 diabetes with visceral fat obesity, the Otsuka Long-Evans Toskushima fatty rat, is also associated with elevated hepatic MTP mRNA (134).

2.6. Link between changes in hepatic insulin signaling and VLDL secretion in the fructose-fed hamster model

We have obtained evidence for a strong correlation between the insulin signaling pathway and regulation of VLDL secretion. Impairment of hepatic insulin signaling and insulin resistance including reduced tyrosine phosphorylation of the insulin receptor, IRS-1, IRS-2, and Akt, elevated protein mass and activity of PTP-1B, and suppressed activity of PI 3-kinase associated with IRS proteins (105). In the fructose-fed hamster model, we have observed a considerable decrease in MTP mass and IR, IRS-1, and IRS-2 phosphorylation and mass accompanying VLDL overproduction. Treatment of fructose-fed hamsters with the insulin sensitizer rosiglitazone restored these intracellular factors, as well as plasma lipoproteins back to near control levels (135). Interestingly, fructose treatment caused a dramatic increase in PTP-1B that was decreased to below control level upon treatment with rosiglitazone. The activity of PTP-1B has been linked to the attenuation of insulin signaling and knockout of PTP-1B results in enhanced insulin sensitivity (101). In addition, a single nucleotide polymorphism within the PTP-1B gene has been shown to correlate to protection from type 2 diabetes (136). Whether the effect observed in our model is due to a direct effect of PTP-1B on hepatic tissues or an indirect effect on peripheral tissues is currently unknown. However, these observations strongly implicate PTP-1B in the development of insulin resistance and potentially in the dyslipidemia associated with this condition.

2.7. Amelioration of insulin resistance reverses hepatic lipoprotein overproduction

Studies by Carpentier *et al*, using the PPAR gamma agonist rosiglitazone have shown that insulin sensitization can significantly ameliorate VLDL secretion in the fructose-fed hamster model, both *in vivo* and *ex vivo* (135). In addition to normalizing plasma glucose levels of insulin resistant animals, rosiglitazone treatment improved the defect in insulin-induced tyrosine phosphorylation of the insulin receptor, IRS-1, and IRS-2 with a concomitant decrease in IRS-1 and IRS-2 mass. Rosiglitazone treatment also decreased the PTP-1B levels that were initially increased by fructose feeding. Finally, there was a decrease in the MTP mass that had also been initially increased by fructose feeding. These observations suggest that normalization of insulin and glucose metabolism may attenuate several mechanisms that stabilize apoB in the insulin resistant state.

2.8. Potential link between alterations in ER proteases in insulin resistance and overproduction of hepatic VLDL

There is increasingly strong evidence that apoB degradation may also occur within the ER as well as further along the secretory pathway. We have used a permeabilized cell system to directly demonstrate the existence of a non-proteasomal degradative pathway that is responsible for specific fragmentation of apoB that consistently results in the generation of a 70 kDa fragment (137,138). Changes in the insulin signaling pathway coincided with drastic suppression of ER-60 that was accompanied by an increase in the synthesis and secretion of apoB. Data from other laboratories also support the involvement of multiple proteolytic pathways in apoB degradation. A proteomic analysis approach has yielded 99 novel and unique proteins from different subcellular compartments that potentially bind to apoB (139). More recently, Gillian-Daniel *et al* showed that expression of recombinant forms of the LDL receptor that were retained within the ER caused apoB degradation within this compartment (140). Work in our own laboratory has shown that cellular apoB and more recently ER lumenal apoB-containing lipoproteins (unpublished observations) are associated with ER-60, an ER-localized cysteine protease, in HepG2 cells. We had previously shown that ER-60 is associated with apoB based on direct cross-linking of ER-60 with apoB in HepG2 cells (141). Adenovirus-mediated overexpression of ER-60 resulted in a decrease in apoB secretion that was not affected by proteasomal inhibitors (unpublished observations). An important observation in the insulin resistant, fructose-fed hamster model was that livers of fructose-fed hamsters expressed a lower level of ER-60, compared to chow-fed control animals. Interestingly, we have found that treatment of fructose-fed hamsters with rosiglitazone, an insulin sensitizer, results in normalization of the ER-60 protein in the liver. This suggests that ER-60

protein levels are chronically responsive to hepatic insulin signaling. We have analyzed the 5' promoter of the ER-60 gene and have found that it contains putative IREs, SRE, SRE3, and NF-Y motifs that may mediate insulin and/or sterol regulation of ER-60. Downregulation of ER-60 protease may thus contribute to the enhanced stability of apoB in livers of fructose-fed hamsters and result in higher assembly and secretion of VLDL (Figure 2).

2.9. Role of intestinal lipoproteins in metabolic dyslipidemia

Numerous studies have shown that there is an increase in postprandial triglyceride-rich lipoproteins in subjects with insulin resistance and type 2 diabetes (142-146). In the fasting state, increased fasting remnant lipoproteins, such as large VLDL and chylomicron remnants have been observed in insulin resistant subjects. Postprandially, a strong correlation exists between plasma triglycerides and plasma insulin and the TG response to a fat meal, as well as the postprandial levels of large VLDL and chylomicron remnants (147,148). Currently it is not known whether the accumulation of these potentially atherogenic remnant lipoproteins occurs as a result of increased intestinal secretion of apoB48-containing chylomicrons, of diminished clearance from the circulation, or both (reviewed in 149). There is a noticeable lack of literature regarding the biogenesis and secretion of apoB48-containing lipoproteins from the intestine of insulin resistant and type 2 diabetic patients. Early studies showed that in the fasting state the intestine is capable of synthesizing and secreting VLDL-like particles from endogenously synthesized substrate (150,151). Based on studies in rats (152) and dogs (153), it has been estimated that the intestinal contribution to fasting total body TG production is between 10% to 40% of total plasma TG. It has been suggested that the intestine maintains a basal rate of apoB48 secretion in the fasting state, and that this is increased in the diabetic intestine (144,148,154). The contribution of the intestine in fasting hypertriglyceridemia is also markedly increased in diabetic rats (145). Studies in human subjects with coronary artery disease (144), diabetic patients (142), and diabetic rats (145), have all pointed to the important role of the intestine in increased plasma chylomicron remnants.

In humans, apoB48-containing chylomicrons are exclusively synthesized within enterocytes, and this synthesis is believed to be largely driven by dietary fat consumption. There is some evidence, however, suggesting that *de novo* synthesized lipid and plasma FFA can also act as substrates for the assembly and secretion of apoB48-containing lipoproteins.

Experiments in our own laboratory using the fructose-fed hamster have shown that chronic fructose feeding stimulates intestinal apoB48 secretion in fasted animals (155). There was a concomitant increase in apoB48 stability accompanying this. This overproduction of apoB48 was accompanied by enhanced intestinal lipid synthesis in the form of free cholesterol, cholesteryl ester, and TG, as well as an increase in both MTP mass and activity. These results suggest that in insulin resistant or diabetic animals, there may be a mechanism causing enhanced intestinal secretion of lipoproteins in the fasting state. Chronic fructose feeding may enhance this basal level of lipoprotein secretion through increased *de novo* lipogenesis and increased MTP availability. The same effect was not observed upon acute, two days fructose feeding or *in vitro* incubation of hamster enterocytes with fructose for one hour. In addition, the direct incorporation of fructose into intestinal lipoproteins was not apparent suggesting that it is a poor substrate for *de novo* lipogenesis in enterocytes. Comparison of plasma lipoproteins from fructose-fed animals showed a significant shift toward secretion of larger, less dense, chylomicrons in the insulin resistant animals. Interestingly, fatty acid synthesis appeared to be stimulated upon fructose feeding. Using the fatty acid synthase inhibitor cerulenin, which inhibited the synthesis of both fatty acid and TG (156,157), we found that *de novo* lipogenesis was required for the secretion of apoB48-containing lipoproteins from enterocytes. There may be a constitutive rate of lipoprotein synthesis and secretion that occurs in the intestine. Collectively, these results suggest that facilitated lipoprotein secretion occurs in the intestine due to increased *de novo* lipogenesis and MTP availability. Intestinal lipoproteins that are oversecreted may, therefore, contribute significantly to overall dyslipidemia suggesting a potential new target for the treatment of metabolic dyslipidemia associated with insulin resistance and diabetes.

3. CONCLUSION AND PERSPECTIVES

It is only relatively recently, that we have begun to understand the molecular mechanisms involved in lipoprotein assembly and secretion in normal and insulin resistant states. Hepatic lipoprotein overproduction of VLDL is now widely accepted as a key abnormality underlying the development of metabolic dyslipidemia. Emerging evidence appears to support the notion that hepatic VLDL overproduction requires an interaction between enhanced lipid substrate availability and hepatic insulin resistance (Figure 2). High FFA flux as commonly observed in insulin resistant states is critical to increasing intracellular substrate availability for augmented assembly and secretion of hepatic apoB-containing lipoproteins. This alone appears to be insufficient however to enhance VLDL overproduction in the context of an insulin sensitive liver. Development of hepatic insulin resistance is likely to result in increased intracellular synthesis and stability of apoB and together with high availability of core lipids, contribute to a stimulated state of VLDL assembly and secretion. The contribution of intestinal lipoprotein secretion into the plasma compartment may also have to be taken into account as recent results suggest that intestinal lipoproteins that are oversecreted may contribute significantly to the fasting and postprandial dyslipidemia associated with the insulin resistant state. Recent studies have identified a number of key molecules that may play important roles in development of insulin resistance (e.g. PTP-1B) and hepatic and intestinal lipoprotein overproduction (e.g. MTP, ER-60). Further research is now underway to more clearly define the critical pathways that link defects in insulin signaling transduction and processes responsible for

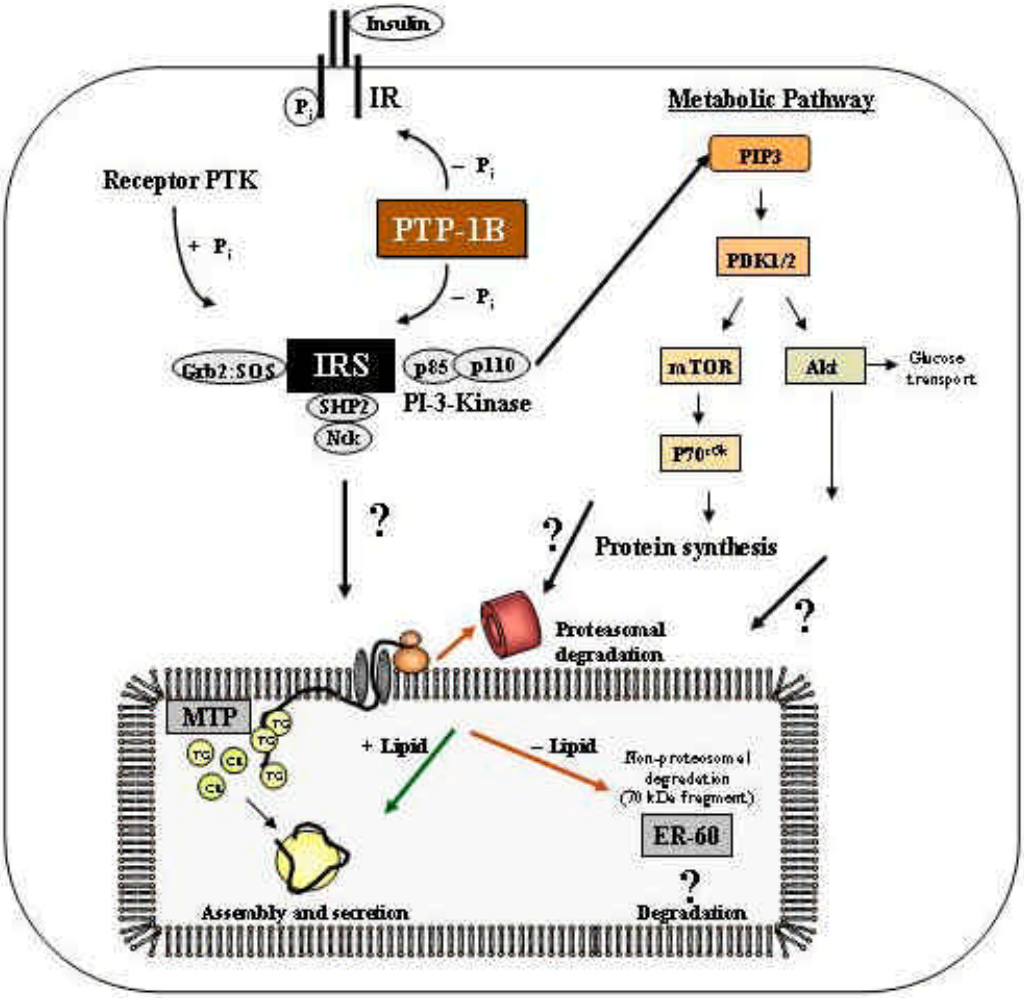

Figure 2. Postulated Links between Hepatic Insulin Signaling and VLDL-apoB Secretion in Insulin-Resistant States. Insulin induces signal transduction via two major signaling pathways: the mitogenic, MAP-kinase-mediated pathway and the metabolic, PI 3-kinase-mediated pathway. Insulin acutely reduces apoB secretion, however in insulin resistance there is reduced sensitivity to inhibitory action of insulin on apoB. Enhanced expression of PTP-1B, a key negative regulator of insulin signaling, may be a key initiating factor in inducing hepatic insulin resistance and consequently increased synthesis and stability of apoB. However, stimulation of VLDL-apoB secretion also requires high availability of core lipoprotein lipid which can be supplied from the high FFA flux commonly observed in insulin resistant states. Reduced expression of ER-60, a putative protease involved in intra-luminal apoB degradation, may be an additional important factor in enhanced stability of apoB.

lipoprotein assembly and secretion. Recent development of specific transgenic and knockout animal models (such as the PTP-1B knockout mice, and adenovirus-mediated PTP-1B or ER-60 overexpression in the hamster) will undoubtedly be invaluable to our further understanding of these factors and their role in linking insulin resistance with disorders of lipoprotein metabolism.

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Abbreviations: ACAT, acyl-CoA cholesterol acyl transferase; ALLN, N-acetyl-leucyl-leucyl-norleucinal; apoB, apolipoprotein B; CE, cholesteryl ester; ER, endoplasmic reticulum; FAS, fatty acid synthase; FFA, free fatty acid; HDL, high density lipoproteins; HMG coA, 3-hydroxy-3-methylglutaryl coenzyme A; IRE, insulin response element; IRS, insulin receptor substrate; LDL, low density lipoproteins; Lp, lipoprotein; MEM, minimum essential medium; MTP, microsomal triglyceride transfer protein; PPAR, peroxisome-proliferator activated receptor; PTPase, phosphotyrosyl-protein phosphatase; PTP-1B, protein tyrosine phosphatase 1B; SRE, sterol response element; SREBP, sterol response element binding protein; STZ, streptozotocin; TG, triglyceride; VLDL, very low density lipoprotein

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