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FoxO1 integrates insulin signaling to VLDL production

Adama Kamagate and **H. Henry Dong***
Rangos Research Center; Division of Immunogenetics; Department of Pediatrics; Children's Hospital of Pittsburgh; University of Pittsburgh School of Medicine; Pittsburgh, Pennsylvania USA

Abstract

Very low-density lipoproteins (VLDL) are triglyceride-rich particles. VLDL is synthesized in hepatocytes and secreted from the liver in a pathway that is tightly regulated by insulin. Hepatic VLDL production is stimulated in response to reduced insulin action, resulting in increased release of VLDL into the blood under fasting conditions. Circulating VLDL serves as a vehicle for transporting lipids to peripheral tissues for energy homeostasis. Conversely, hepatic VLDL production is suppressed in response to increased insulin release after meals. This effect is critical for preventing prolonged excursion of postprandial plasma lipid profiles in normal individuals. In subjects with obesity and type 2 diabetes, the ability of insulin to regulate VLDL production becomes impaired due to insulin resistance in the liver, resulting in excessive VLDL secretion and accumulation of triglyceride-rich particles in the blood. Such abnormality in lipid metabolism characterizes the pathogenesis of hypertriglyceridemia and accounts for increased risk of coronary artery disease in obesity and type 2 diabetes. Nevertheless, the molecular basis that links insulin resistance to VLDL overproduction remains poorly understood. Our recent studies illustrate that the forkhead transcription factor FoxO1 acts in the liver to integrate hepatic insulin action to VLDL production. Augmented FoxO1 activity in insulin resistant livers promotes hepatic VLDL overproduction and predisposes to the development of hypertriglyceridemia. These new findings raise an important question: Is FoxO1 a therapeutic target for ameliorating hypertriglyceridemia? Here we discuss this question in the context of recent advances toward our understanding of the pathophysiology of hypertriglyceridemia.

Keywords

hypertriglyceridemia; FoxO1; MTP; ApoB; VLDL

Etiology of Hypertriglyceridemia

Hypertriglyceridemia is a hallmark of metabolic syndrome and is characterized by a triad plasma lipid profile, i.e., increased triglyceride (TG) and low-density lipoprotein (LDL) levels, and decreased high-density lipoprotein (HDL) levels.[1,2] Due to its pro-atherogenic potential, hypertriglyceridemia is considered an independent risk factor for coronary artery disease.[3–12] Hypertriglyceridemia increases the incidence of cardiovascular disease by 32% in men and 76% in women, independent of plasma HDL-C levels.[13–15] To date, the pathophysiology of hypertriglyceridemia is incompletely understood. Its close association with adiposity and type 2 diabetes implicates insulin resistance as a causative factor in the development of hypertriglyceridemia.[10,16–18] As a result of insulin resistance, adipose tissue undergoes

*Correspondence to: H. Henry Dong; Rangos Research Center; Children's Hospital of Pittsburgh; 3460 5th Avenue; Rm 5140; Pittsburgh, Pennsylvania 15213 USA; Tel.: +1.412.692.6324; Fax: +1.412.692.5809; Email: dongh@pitt.edu.

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unrestrained fat mobilization, resulting in elevated plasma free fatty acid (FFA) levels. An increased FFA flux into the liver stimulates hepatic lipogenesis and promotes VLDL-TG overproduction, contributing to the pathogenesis of hypertriglyceridemia (Fig. 1).[16,19,20]

Treatment of Hypertriglyceridemia

Hypertriglyceridemia is often associated with visceral obesity, insulin resistance, hyperinsulinemia, hypertension or diabetes, a cluster of conditions that are collectively termed "metabolic syndrome". As a result, patients with hypertriglyceridemia are primarily recommended with exercise or dietary supplements of omega-3 fatty acid-enriched food or congestion of fish oil. If these non-pharmacological interventions fail to correct atherogenic lipid profiles, pharmacological therapies are instituted with fibrates or niacin. These two commonly prescribed triglyceride-lowering agents act to reduce plasma triglyceride levels via distinct mechanisms, but each with its own limitations. Fibrates are fibric acid derivatives that act as agonists of peroxisome proliferator-activated receptor alpha (PPAR-α) to enhance fatty acid oxidation in peripheral tissues and promote triglyceride VLDL-TG clearance from plasma.[21–23] Niacin appears to target G protein-coupled receptors GPR109A and GPR109B (also known as HM74A and HM74, respectively) in adipose tissues to limit fat mobilization and reduce plasma levels of FFA, the substrate for hepatic VLDL production.[24–27] More detailed discussions on pharmacological intervention of hypertriglyceridemia have been reported elsewhere.[28–31] Here we focus our review on the molecular basis that governs insulin-dependent regulation of VLDL production, while bearing in mind the question of how hepatic VLDL production becomes unrestrained in insulin resistant subjects with metabolic syndrome.

Insulin Regulation of VLDL Production

VLDL is assembled and produced in liver, which depends on substrate availability and is tightly regulated by insulin.[32,33] Under fasting conditions, hepatic VLDL production is induced, resulting in increased VLDL secretion into the blood. In response to postprandial insulin release, hepatic VLDL production is suppressed to limit plasma triglyceride excursion.[34–37] Such an acute inhibitory mechanism of insulin action on VLDL production is critical for rapid adaptation by the liver to metabolic shift between fasting and refeeding for maintaining plasma lipids within the physiological range.

VLDL assembly in hepatocytes is conducted by microsomal triglyceride transfer protein (MTP), an endoplasmic reticulum resident protein. MTP (MW, 88 kDa) is regarded as a molecular chaperone. When heterodimerized with its small subunit protein disulphide isomerase (PDI, 58 kDa) in the endoplasmic reticulum (ER), MTP catalyzes the transfer of lipid to nascent apolipoprotein B (apoB), a rate-limiting step in hepatic VLDL production.[38–40] MTP is also produced in the intestine and is responsible for lipidation of apoB48 for the production of chylomicrons.[40–45] In humans, a lack of MTP activity, resulting from genetic lesions in its gene, causes abetalipoproteinemia or Bassen-Kornzweig syndrome, a rare autosomal recessive disorder that is characterized by defects in the assembly and secretion of triglyceride-rich lipoproteins. Patients with abetalipoproteinemia manifest severe lipid disorders and multiple vitamin deficiencies, due to the impairment in dietary fat absorption secondary to defects in intestinal chylomicron secretion.[46–50] This clinical condition is recapitulated in mice with genetic MTP depletion, as $MTP^{-/-}$ homozygous mice are associated with the inability to manufacture VLDL and die at the E10.5 stage during embryonic development.[50–52] MTP haploinsufficiency is associated with increased hepatic fat deposition due to markedly reduced VLDL secretion in $MTP^{+/-}$ heterozygous mice.[49] In contrast, hepatic MTP overproduction results in excessive VLDL-TG secretion and significantly elevated plasma TG levels.[38] Pharmacological inhibition of MTP activity is shown to reduce VLDL production and decrease plasma cholesterol levels in subjects with

familial hypercholesterolemia.[53] DNA polymorphism at -493G/T in the human MTP promoter is linked to altered triglyceride metabolism and increased risk of coronary heart disease.[54–58] Furthermore, there is emerging evidence that diminished MTP activity is a compounding factor for advanced alcoholic liver disease.[59,60] Together these data highlight the critical role of MTP in VLDL assembly and secretion in health and disease. This has spurred intensive investigations to understand hepatic regulation of MTP gene expression in order to gain insights into the pathophysiology of hypertriglyceridemia associated with obesity and diabetes.

Hepatic Regulation of MTP Production

Consistent with its importance in triglyceride metabolism, hepatic MTP production is regulated in response to physiological cues. Hagan et al.,[61] first report that MTP gene expression is negatively regulated by insulin in cultured HepG2 cells. This observation is corroborated by Lin et al.,[62] who show in HepG2 cells that insulin inhibits MTP expression in a dose- and time-dependent manner. In parallel with these findings, hepatic MTP mRNA levels are significantly upregulated, correlating with augmented VLDL-TG secretion in a number of animal models with insulin resistance and aberrant triglyceride metabolism, including non-diabetic obese Zucker rats,[63,64] high fat-induced obese mice,[65,66] and high fructose-induced hypertriglyceridemic hamsters.[67–70] Pharmacological intervention, which improves insulin resistance and ameliorates metabolic dyslipidemia, is associated with reduced MTP expression and diminished VLDL-TG output in hypertriglyceridemic models.[67,69,71,72] These data are consistent with the idea that insulin exerts an inhibitory effect on MTP gene expression in the liver. Loss of insulin inhibition is thought to be a contributor for unrestrained MTP expression and VLDL overproduction in insulin resistant subjects.

To account for the underlying mechanism, Au et al.,[73] show in HepG2 cells that insulin regulates MTP gene expression via the activation of mitogen-activated protein kinase (MAPK). However, a caveat of this study is the use of a relatively shorter version of the human MTP promoter (−250/+86 nt) in their luciferase reporter system. Furthermore, targeted blockage of MAPK activity only results in partial inhibition of insulin-mediated reduction in VLDL-apoB production,[74] suggesting that other mechanisms are involved in insulin-dependent inhibition of MTP and VLDL-TG production in the liver.

Wolfrum and Stoffel[75] show that the forkhead box A2 (Foxa2) contributes to hepatic regulation of MTP expression. Foxa2 in complex with its co-activator PGC-1β stimulates hepatic MTP mRNA expression, contributing to increased VLDL secretion from the liver.[76] In response to insulin action, Foxa2 is phosphorylated and dissociated from PGC-1β, contributing to the reduction in hepatic MTP and VLDL production.[76] However, Foxa2 is predominantly localized in the cytoplasm in response to hyperinsulinemia,[76] arguing against its direct action in promoting MTP gene expression in insulin resistant states. This has led to the postulation that the absence of Foxa2 in the nucleus in the liver is compensated by other factors such as hepatocyte nuclear factor 4 (HNF4) and peroxisome proliferator-activated receptor alpha (PPAR-α) under hyperinsulinemic conditions.[77] Indeed, a recent study by Sheena et al.,[72] demonstrates that hepatocyte nuclear factor 4-alpha (HNF-4α) targets the human MTP promoter for trans-activation in cultured HepG2 cells. This effect is complemented by hepatocyte nuclear factor 1-alpha (HNF-1α) and counteracted by β,β-tetramethyl-hexadecanedioic acid acting as an HNF-4α antagonist. Nevertheless, the significance of this finding in the pathogenesis of hypertriglyceridemia remains to be determined.

Peroxisome proliferator-activated receptor alpha (PPAR-α) is shown to stimulate hepatic MTP mRNA expression in primary cultures of mouse and rat hepatocytes.[78] However, this finding seems paradoxical, as targeted activation of PPAR-α with anti-hypertriglyceridemia therapy

such as fibrates helps attenuate hepatic MTP activity and curb VLDL-TG overproduction in animal models with diet-induced dyslipidemia.[67,69,71,79] PPAR-α is known to bind specifically to a highly conserved DNA sequence that comprises 2-hexamer repeats with one nucleotide in between (AGGTCAXAGGTCA) in target promoters. Such a consensus PPAR-α binding site is lacking within a 5-kb DNA region (−5000/+1 nt) of human and mouse MTP promoters. Further studies are needed to reconcile the stimulatory action of PPAR-α on hepatic MTP production with the ameliorating effect of PPAR-α agonists on hypertriglyceridemia.

Although MTP plays an obligatory role in the lipidation of apoB for VLDL assembly and secretion, there is evidence that hepatic VLDL production is upregulated without significantly altering hepatic MTP mRNA levels in genetically modified mice that over-express human apoB but lack brown adipose tissue (Batless).[80] In keeping with this observation, two independent groups show that the late addition of core lipids to apoB molecules, a critical step for VLDL maturation in the lumen of ER, is independent of MTP, as selective inhibition of MTP activity does not seem to affect apoB100 secretion during the later stages of lipoprotein assembly.[81,82] While MTP is required for transferring lipids to nascent apoB polypeptides in coupling with translation during the initial phase of VLDL assembly, these data argue against the requirement of MTP in the late stage of VLDL assembly, in which bulk core lipids are incorporated into poorly lapidated apoB for the production of mature VLDL particles.[81,83] Thus, it remains an unsettled question of whether MTP is absolutely necessary for the late stage of VLDL assembly. Studies are needed to further delineate the VLDL secretion pathway for better understanding of the molecular basis of hypertriglyceridemia.

Hepatic Regulation of apoB Expression

ApoB is a structural component of triglyceride-rich lipoproteins. There are two forms of apoB, namely apoB100 and apoB48 that are differentially expressed in humans.[84] ApoB100 is expressed in the liver and is responsible for VLDL-TG production in the post-absorptive phase, whereas apoB48 is produced in the intestine and required for postprandial chylomicron secretion. In mice and rats, apoB48 is also produced in the liver.[85] It is noteworthy that apoB48 is translated from its distinct mRNA that is derived from apoB100 mRNA editing, a posttranscriptional process by which a C is converted to a U at nucleotide 6666. This single nucleotide change results in the conversion of CAA (Gln-2153) to a stop codon UAA. As a result, apoB48 comprises the N-terminal 48% of apoB100.[86,87]

Unlike MTP that is an ER-resident chaperone, apoB is a secretory glycoprotein whose lipidation by MTP is essential for VLDL assembly and secretion. As depicted in Figure 2, this dynamic process of VLDL assembly depends on substrate availability, which is counteracted by insulin.[33,88–91] Consistent with this model is the evidence that the synthesis of apoB is closely coordinated with MTP protein activity.[92,93] In the presence of lipids, nascent apoB undergoes rapid lipidation that is facilitated by MTP. This process is kinetically coupled with translation and translocation of apoB polypeptides into the ER lumen for VLDL assembly and secretion. In the absence of lipids, nascent apoB molecules are unable to engage in lipidation and are destined for proteasome-mediated degradation.[83,94] This fine-tuning mechanism serves as a quality control process for allowing physiological secretion of VLDL particles in the access of lipids.

Given its importance in lipoprotein metabolism, the molecular mechanism underlying insulin- and/or substrate-dependent regulation of apoB secretion has received intensive investigations. One potential mechanism suggests that insulin inhibits apoB secretion by stimulating apoB degradation. Consistent with this notion is the observation that insulin suppresses apoB expression and promotes proteasome-mediated apoB degradation in cultured HepG2 cells, primary rat hepatocytes, and perfused rat livers.[91,95–100] Alternatively, insulin inhibits

hepatic apoB secretion by limiting its substrate FFA availability.[91,101,102] Indeed, insulin has been shown to restrain FFA mobilization from adipose tissue by inhibiting the hormone-sensitive lipase.[103] It is thought that such an inhibitory action of insulin along with substrate availability is critical for the liver to adjust the rate of hepatic VLDL-TG secretion in response to changes in metabolic states. In insulin resistant states, an increased FFA flux into the liver, resulting from unrestrained fat mobilization in adipose tissue, augments apoB secretion, contributing to hepatic VLDL-TG overproduction and the development of dyslipidemia. Indeed, hepatic apoB production is markedly elevated, accompanied by increased VLDL-TG secretion in animal models with whole-body insulin resistance and altered triglyceride metabolism.[67,70,94,104–110] There is clinical evidence that elevated plasma apoB levels, which reflect the number of small, dense LDL particles in plasma, are a significant predictor of cardiovascular risk in subjects with metabolic syndrome.[111–116]

As discussed above, hepatic apoB production is tightly regulated at the posttranslational level. This process has been viewed as a safeguarding mechanism for protecting against the development of steatosis by enhancing VLDL secretion in the response to lipid overload into the liver. This view raises an important question: why the liver cannot rid of excessive lipids and avoid steatosis by accelerating VLDL secretion in the face of lipid excess such as in obesity? A significant clue to this question derives from the study by Ota et al.,[107] who show that hepatic apoB production is subject to regulation by ER stress, an adaptive response that is elicited by the accumulation of unfolded or misfolded proteins in the ER lumen. They show that an increased lipid infiltration into the liver induces ER stress and compromises the secretory pathway. This effect inhibits hepatic apoB secretion and instigates lipid accumulation, contributing to the development of steatosis.[107] This lipid-induced hepatic ER stress along with concomitant steatosis is detectable in both genetic and dietary models of obese mice.[107,117,118] Likewise, we and others show that high fructose-induced hypertriglyceridemic hamsters also exhibit hepatic ER stress, accompanied by excessive fat deposition in the liver.[67,70,108,119] Together these data elucidate that lipid-induced ER stress links aberrant apoB secretion to the development of hepatic steatosis associated with obesity.

FoxO1 Integrates Insulin Signaling to Hepatic VLDL Production

FoxO1 is a nuclear transcription factor that belongs to a protein family characterized by a highly conserved DNA binding motif, termed “forkhead” domain, including FoxO1 (FKHR), FoxO3a (FKHRL1), FoxO4 (AFX) and FoxO6 in mammals.[120–122] These fork-head proteins are substrates of the Akt/PKB and SGK kinases and play important roles in insulin action.[120, 121,123–128] Insulin exerts its inhibitory effect on gene expression via a highly conserved insulin response element (IRE) with its core nucleotide sequence (TG/ATTTT/G) in the promoter. In the absence of insulin, FoxO1 resides in the nucleus (Fig. 3A) and binds as a trans-activator to IRE, enhancing promoter activity. In response to insulin, FoxO1 is phosphorylated through the PI3K-dependent pathway, resulting in its nuclear exclusion (Fig. 3B) and inhibition of target gene expression.[123,129–133] Failure in phosphorylation of FoxO1 results in its constitutive nuclear localization and trans-activation of gene expression.[130,134]

As shown in Figure 4, FoxO1 comprises two structural domains, the amino forkhead domain that is necessary for DNA binding and the carboxyl trans-activation domain that is responsible for stimulating promoter activity.[131] These two domains are functionally separable, as the carboxyl domain, when fused with a heterologous Gal4-DNA binding domain, is capable of stimulating Gal4 promoter activity.[135] In contrast, a truncated version of FoxO1 containing the forkhead domain is able to bind IRE DNA, but its binding does not result in trans-activation of promoter activity.[136] This unique property accounts for its dominant-negative phenotype in suppressing target gene expression.[129,136]

Recently, we demonstrate that FoxO1 mediates insulin-dependent regulation of MTP expression in modulating hepatic VLDL secretion.[66] We show in cultured HepG2 cells that hepatic MTP production is stimulated by FoxO1 and inhibited by insulin. This effect correlates with the ability of FoxO1 to bind at its target site in the MTP promoter, resulting in trans-activation of MTP promoter activity. Deletion or mutation of the FoxO1 binding site disables FoxO1 binding to the MTP promoter and abrogates insulin-dependent regulation of hepatic MTP production. FoxO1 gain-of-function, resulting from either FoxO1 transgenic expression or adenovirus-mediated FoxO1 production in the liver, augments hepatic MTP expression and promotes apoB secretion, contributing to a significant induction in both the number and size of VLDL-TG particles. Both VLDL-TG production and apoB secretion are increased in response to elevated FoxO1 production in HepG2 cells and mice. Conversely, FoxO1 loss-of-function, caused by RNAi-mediated FoxO1 knockdown in the liver, suppresses hepatic MTP expression and reduces hepatic VLDL-TG output in mice.

These data shed light on the mechanism that the liver has evolved to adjust hepatic VLDL-TG assembly and secretion to maintain lipid homeostasis and energy balance in different physiological states. It follows that in response to postprandial insulin release, FoxO1 is phosphorylated and excluded from the nucleus, resulting in the inhibition of hepatic MTP expression. This effect acts to abate VLDL-TG production and limit postprandial lipid excursion in the blood. As a result of insulin resistance, FoxO1 is preferentially localized in the nucleus due to the inability of FoxO1 to undergo insulin-dependent phosphorylation and unclear exclusion. This effect augments FoxO1 transcriptional activity in promoting hepatic MTP and VLDL-TG overproduction. These data suggest that unleashed FoxO1 activity in insulin resistant livers plays an important role in linking impaired insulin action to excessive VLDL-TG secretion, contributing to the development of hypertriglyceridemia in obesity and type 2 diabetes.[66,137] Consistent with this model, we show that hepatic MTP abundance and hepatic VLDL-TG production are markedly upregulated, correlating with augmented hepatic FoxO1 activity in multiple models of mice with altered triglyceride metabolism, including high fat-induced obese mice, diabetic db/db mice, FoxO1-transgenic mice, and high fructose-induced hypertriglyceridemic hamsters.[66,67,108,129,138,139]

These data are consistent with the idea that FoxO1 dysregulation is associated with aberrant hepatic metabolism.[139–141] Further support of this notion is provided by Valenti et al.,[142] who report that an enhanced FoxO1 activity is associated with nonalcoholic steatohepatitis in humans. This raises an important hypothesis that selective inhibition of FoxO1 activity in insulin resistant livers would curb hepatic VLDL-TG overproduction and ameliorate hypertriglyceridemia. As a proof of the concept, Samuel et al.,[143] show that targeted inhibition of FoxO1 by an anti-sense oligonucleotide approach results in significant improvement in peripheral insulin sensitivity, glucose and lipid metabolism in high fat-induced obese mice. Likewise, we demonstrate that functional inhibition of FoxO1 by adenovirus-mediated production of FoxO1 dominant-negative mutant in the liver improves whole-body insulin sensitivity and reduces hyperinsulinemia, contributing to improved carbohydrate metabolism in diabetic *db/db* mice.[129] Furthermore, FoxO1 haplo-insufficiency protects from high fat-induced insulin resistance and lipid disorders in insulin receptor-deficient diabetic mice.[144] In keeping with observation, Dong et al., show that liver-specific depletion of FoxO1 is sufficient to restore the metabolic abnormality in diabetic mice with genetic deletion of both insulin receptor substrate 1 (IRS1) and 2 (IRS2) genes.[145]

Our recent data of using RNAi-mediated FoxO1 knockdown approach to curb hepatic VLDL-TG overproduction have validated the concept that FoxO1 deregulation contributes to aberrant hepatic metabolism and selective inhibition of FoxO1 in insulin resistant livers contributes to improved glucose and lipid metabolism.[139,140] It also prompts an urgent call for the

development of an antagonist compound for targeted inhibition of FoxO1 activity in vivo for testing its therapeutic value in preclinical models of diabetic dyslipidemia.

Conclusion

FoxO1 has emerged as an important player in integrating insulin signaling to downstream target gene expression in carbohydrate metabolism. FoxO1 mediates the inhibitory effect of insulin on the expression of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), two key enzymes in hepatic gluco-neogenesis.[120,121,139] Under fasting conditions, FoxO1 expression along with its nuclear distribution is increased, accounting for its augmented transcriptional activity to promote hepatic gluconeogenesis. Under fed conditions, FoxO1 is phosphorylated and translocated to the cytoplasm, resulting in inhibition of gluconeogenesis in the liver. These two reciprocal mechanisms play a critical role in maintaining blood glucose levels within a narrow physiological range in different metabolic states.[120,121]

Our recent data suggest that a similar mechanism is exploited by the liver to regulate hepatic VLDL-TG secretion for maintaining normal triglyceride metabolism.[66] This is achieved via FoxO1-mediated regulation of MTP and apoB production in response to insulin action in the liver. As illustrated in Figure 5, hepatic insulin signaling bifurcates at FoxO1 to target different sets of genes in glucose and lipid metabolism. Such a FoxO1-accentuated regulatory mechanism is evolved for synchronizing insulin-dependent regulation of hepatic glucose production and VLDL-TG secretion for priming the liver to respond to metabolic shift between fasting and refeeding states. However, an impaired ability of insulin to check FoxO1 activity consequently triggers concomitant perturbations in glucose and lipid metabolism, accounting for concurrent manifestations of both hyperglycemia and hypertriglyceridemia in insulin resistant subjects with obesity and/or type 2 diabetes.

It is of note that FoxO1 is a nuclear transcriptional factor that is ubiquitously expressed. However, a comprehensive survey of FoxO1 function in other insulin sensitive tissues, including the brain,[146,147] skeletal muscle,[148,149] pancreas[150,151] and adipose tissue,[152,153] is beyond the scope of this article. Due to space limitation in this article, we have centered our review on recent advances made toward our understanding of the underlying mechanism of VLDL overproduction in the liver, a prominent pathological feature of hypertriglyceridemia. In addition, there is emerging evidence that intestinal overproduction of apoB48-containing lipoproteins is a compounding factor for the pathogenesis of postprandial lipaemia and diabetic dyslipidemia in insulin resistant subjects.[64,154–156] In accordance with these findings, intestinal MTP and apoB48 expression levels are markedly elevated in enterocytes isolated from hyperlipidemic animals.[157–159] Nevertheless, the molecular pathway from insulin resistance to aberrant production of apoB48-containing lipoprotein particles remains largely undefined. It is noteworthy that FoxO1 is also expressed in the intestine,[138] but its role in regulating intestinal MTP and apoB48 expression in response to insulin action remains obscure. Further studies are needed to decipher the molecular basis underlying intestinal overproduction of apoB48-containing lipoproteins for better understanding the pathophysiology of postprandial lipaemia that are closely associated with obesity and type 2 diabetes.

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Abbreviations

FoxO1 forkhead box O1

MTP microsomal triglyceride transfer protein

ApoB apolipoprotein B

IRS insulin receptor substrate

PEPCK phosphoenolpyruvate carboxykinase

G6Pase glucose-6-phosphatase

PPARα peroxisome proliferator activated receptor alpha

PGC1β PPARgamma coactivator-1beta

Foxa2 forkhead box a2

HNF4α hepatocyte nuclear factor 4alpha

HNF1α hepatocyte nuclear factor 1alpha

VLDL very low-density lipoprotein

LDL low density lipoprotein

HDL high density lipoprotein

TG triglyceride

FFA free fatty acid

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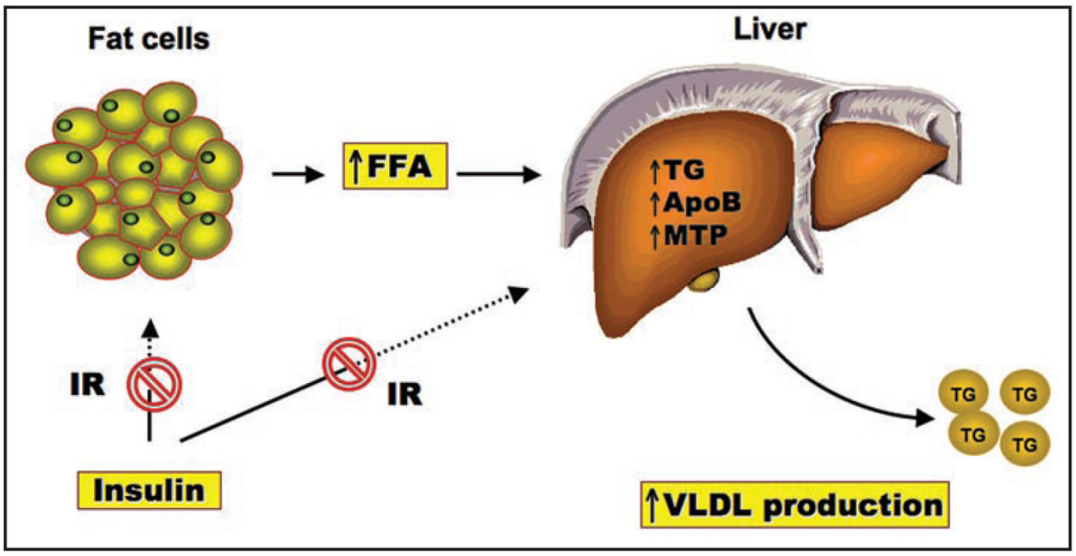

Figure 1.
Hepatic VLDL overproduction. Unrestrained fat mobilization, resulting from peripheral insulin resistance (IR), leads to increased FFA flux into the liver. This effect stimulates hepatic lipogenesis and promotes VLDL overproduction, contributing to hypertriglyceridemia in insulin resistant subjects with obesity and type 2 diabetes.

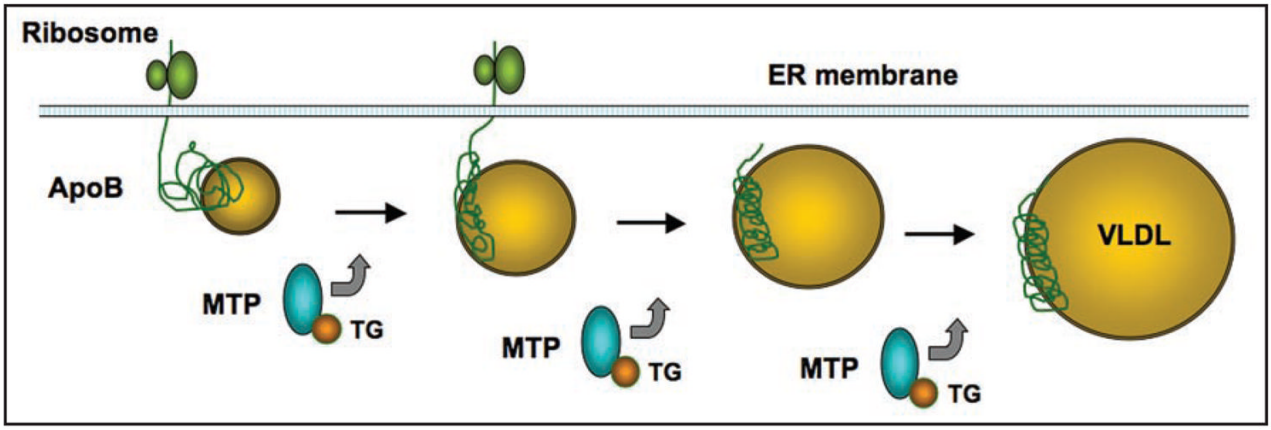

Figure 2.
Hepatic VLDL assembly. The assembly of VLDL initiates with the lipidation of nascent apoB polypeptides in a process that is mechanistically coupled with apoB translation and translocation into the ER lumen. MTP acts as a molecular chaperone for transporting lipids to nascent apoB molecules, resulting in the production of triglyceride (TG)-rich VLDL particles. It remains a debatable issue of whether MTP is required for the late stage of VLDL assembly, in which bulk core lipids are incorporated into poorly lapidated apoB for the maturation of VLDL particles.

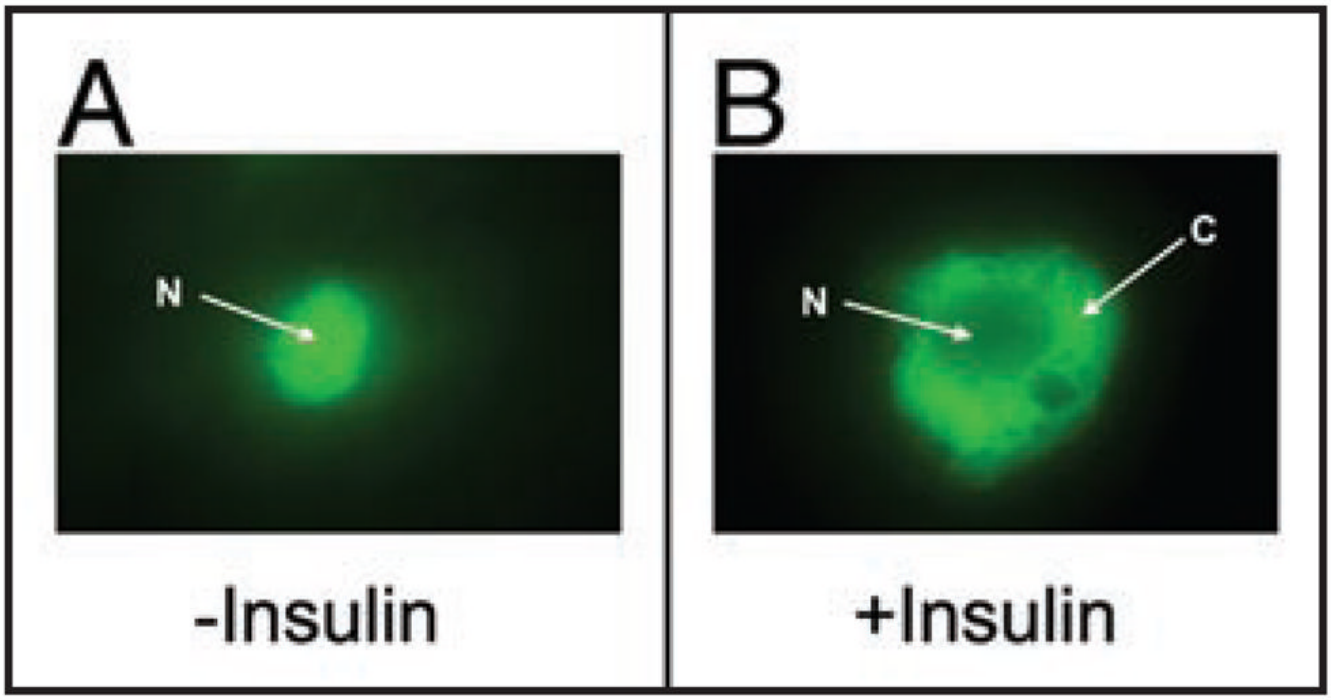

Figure 3.
Insulin-mediated FoxO1 protein trafficking in HepG2 cells. (A) FoxO1 is localized to the nucleus (N) in the absence of insulin. (B) In response to insulin, FoxO1 is translocated to the cytoplasm (C). HepG2 cells pre-transfected with a plasmid encoding FoxO1-GFP fusion protein are exposed to insulin (100 nM) for 15 min, followed by immunofluorescent microscopy.

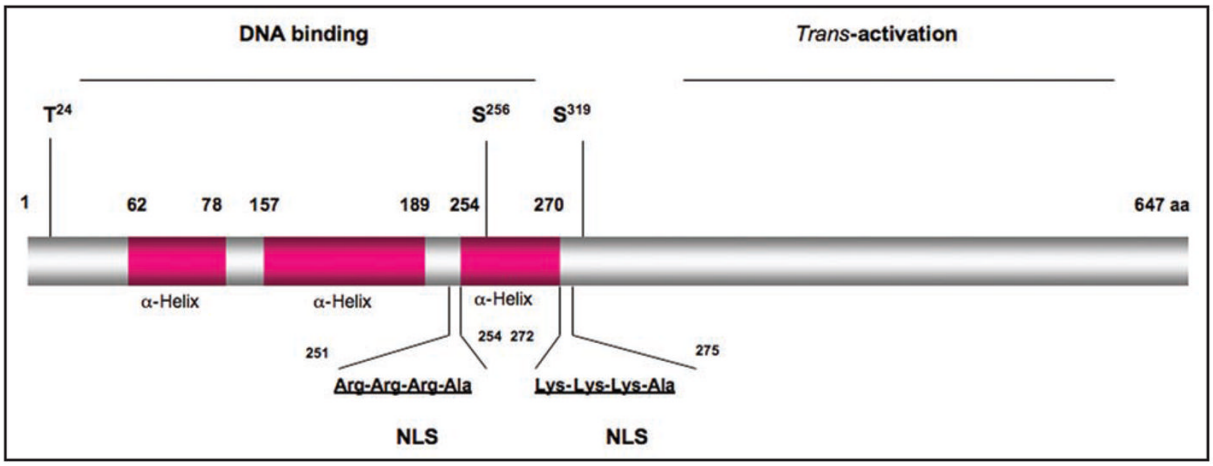

Figure 4.

Schematic depiction of FoxO1 protein. FoxO1 comprises the amino DNA binding domain and carboxyl trans-activation domain. The DNA binding domain is formed by three α-helix structural motifs. Within the DNA binding domain are two consensus nuclear localization signals (NLS) and three highly conserved phosphorylation sties (T24, S256 and S319). Phosphorylation of FoxO1 in response to insulin promotes FoxO1 translocation from the nucleus to cytoplasm. This effect results in inactivation of FoxO1 transcriptional activity and inhibition of target gene expression, as FoxO1 is removed from its active nuclear location.

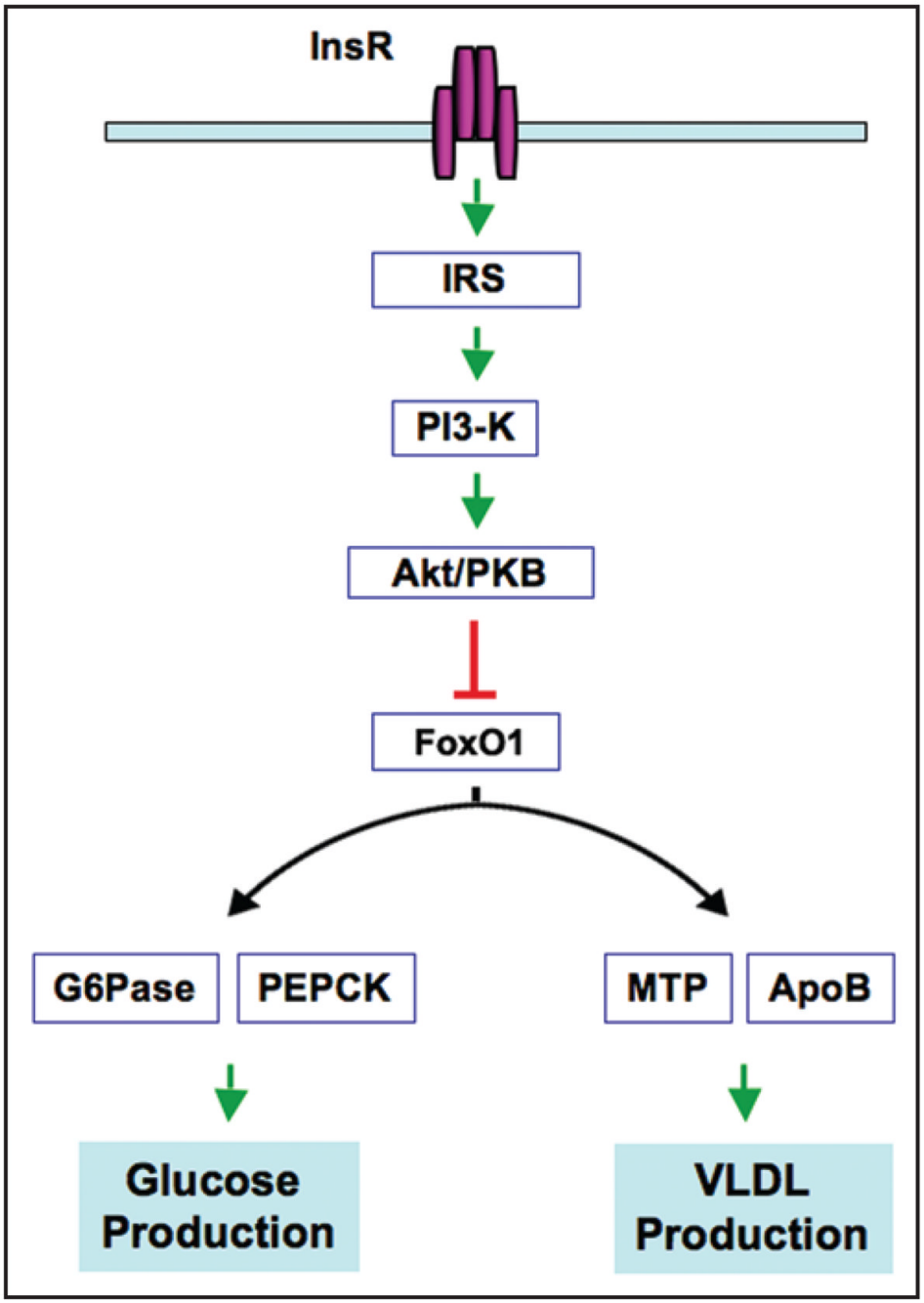

Figure 5.
FoxO1 integrates insulin signaling to hepatic glucose and VLDL production. Insulin binds to its receptors (Insr) at cell surface, resulting in the activation of a cascade of events including insulin receptor substrate (IRS), PI3-kinase, and Akt (known as protein kinase B, PKB). Insulin inhibits FoxO1 activity via Akt/PKB-dependent phosphorylation and nuclear exclusion. This effect is instrumental for liver to curb hepatic glucose and VLDL-TG production and limit postprandial glucose and lipid excursion. Loss of insulin inhibition of FoxO1 activity in insulin resistant livers results in excessive production of both glucose and VLDL-TG, contributing to the dual pathogenesis of hyperglycemia and hypertriglyceridemia in diabetes.