Mechanisms of Hepatic Very Low Density Lipoprotein Overproduction in Insulin Resistance

EVIDENCE FOR ENHANCED LIPOPROTEIN ASSEMBLY, REDUCED INTRACELLULAR ApoB DEGRADATION, AND INCREASED MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN IN A FRUCTOSE-FED HAMSTER MODEL.

(Received for publication, June 23, 1999, and in revised form, January 10, 2000)

Changiz Taghibiglou‡, André Carpentier§, Stephen C. Van Iderstine‡, Biao Chen‡, Debbie Rudy‡, Andrea Aiton‡, Gary F. Lewis§, and Khosrow Adeli‡

From the ‡Department of Laboratory Medicine and Pathobiology, Hospital for Sick Children, University of Toronto, and the §Department of Medicine, Division of Endocrinology and Metabolism, The Toronto Hospital, University of Toronto, Toronto, Ontario M5G 1X8,Canada

A novel animal model of insulin resistance, the fructose-fed Syrian golden hamster, was employed to investigate the mechanisms mediating the overproduction of very low density lipoprotein (VLDL) in the insulin resistant state. Fructose feeding for a 2-week period induced significant hypertriglyceridemia and hyperinsulinemia, and the development of whole body insulin resistance was documented using the euglycemic-hyperinsulinemic clamp technique. In vivo Triton WR-1339 studies showed evidence of VLDL-apoB overproduction in the fructose-fed hamster. Fructose feeding induced a significant increase in cellular synthesis and secretion of total triglyceride (TG) as well as VLDL-TG by primary hamster hepatocytes. Increased TG secretion was accompanied by a 4.6-fold increase in VLDL-apoB secretion. Enhanced stability of nascent apoB in fructose-fed hepatocytes was evident in intact cells as well as in a permeabilized cell system. Analysis of newly formed lipoprotein particles in hepatic microsomes revealed significant differences in the pattern and density of lipoproteins, with hepatocytes derived from fructose-fed hamsters having higher levels of luminal lipoproteins at a density of VLDL versus controls. Immunoblot analysis of the intracellular mass of microsomal triglyceride transfer protein, a key enzyme involved in VLDL assembly, showed a striking 2.1-fold elevation in hepatocytes derived from fructose-fed versus control hamsters. Direct incubation of hamster hepatocytes with various concentrations of fructose failed to show any direct stimulation of its intracellular stability or extracellular secretion, further supporting the notion that the apoB overproduction in the fructose-fed hamster may be related to the fructose-induced insulin resistance in this animal model. In summary, hepatic VLDL-apoB overproduction in fructose-fed hamsters appears to result from increased intracellular stability of nascent apoB and an enhanced expression of MTP, which act to facilitate the assembly and secretion of apoB-containing lipoprotein particles.

Insulin resistance is an extremely common pathophysiological trait that is implicated in the development of a number of important human diseases including Type 2 diabetes, atherosclerosis, hypertension, and dyslipidemia (1, 2). Many studies have suggested that insulin resistance may be a factor in causing dyslipidemia (3–7). The insulin resistant state is commonly associated with lipoprotein abnormalities that are risk factors for coronary heart disease, including hypertriglyceridemia, high levels of VLDL (1), low levels of high density lipoprotein cholesterol (8), and small, dense LDL (9). These metabolic abnormalities together with hypertension and Type 2 diabetes may cluster in the same individual, constituting a syndrome referred to as the metabolic Syndrome X (2). It has been suggested that the most fundamental defect in these patients is resistance to insulin-stimulated glucose uptake, which leads to compensatory hyperinsulinemia, enhanced VLDL secretion by the liver, and hypertriglyceridemia (10). Hypertriglyceridemia is the most common lipid abnormality in subjects with insulin resistance. Early kinetic studies suggested that the hypertriglyceridemia associated with insulin resistance is due to an increase in VLDL-TG production (11–14), but the cellular mechanisms of this process have not been clearly determined.

Insulin has been shown to acutely inhibit the hepatic production of VLDL-TG in both in vitro and in vivo studies (reviewed in Refs. 15 and 16). Short-term hyperinsulinemia also inhibits hepatic secretion of apolipoprotein B (apoB) by perfused rat liver (17), primary rat hepatocytes (18–20), human hepatocytes (21), as well as in human subjects in vivo (22, 23) (reviewed in Refs. 15 and 16). Interestingly however, obese, chronically hyperinsulinemic and insulin-resistant human subjects were resistant to the acute inhibitory effects of insulin on VLDL apoB (22). Furthermore, stable isotope studies in humans have shown that VLDL1 production is acutely inhibited by insulin in normal subjects but not in insulin-resistant patients with Type 2 diabetes (24–26). Primary rat hepatocytes, incubated in vitro with high concentrations of insulin for 3 days, no longer respond to insulin suppression of VLDL apoB secretion, and secrete higher basal levels of VLDL-apoB (27). A similar resistance to the acute suppressive effects of insulin has been observed in HepG2 cells (28). Secretion of VLDL by hepatocytes from hypertriglyceridemic and hyperinsulinemic Zucker fatty rats (fa/fa), is also resistant to the inhibitory effect

© 2000 by The American Society for Biochemistry and Molecular Biology, Inc.
Mechanisms of Hepatic VLDL Overproduction in Insulin Resistance

8417

Insulin regulates hepatic synthesis and secretion of apoB, either directly or indirectly, by its effects on lipid availability (15). Acute insulin exposure reduces the synthesis of apoB in cultured hepatocytes (20) and increases the rate of apoB degradation (20). Studies in our laboratory, using cell-free translation systems, have shown that insulin attenuates the rate of apoB mRNA translation (30, 31). It has also been suggested that apoB availability may become a limiting factor in VLDL assembly and secretion in insulin-treated hepatocytes (32). Recent studies by Sparks and co-workers (33, 34) have suggested that insulin inhibits apoB secretion through activation of phosphoinositide 3-kinase. Phosphoinositide 3-kinase activity which phosphorylates phosphoinositol in the 3′-position of the inositol ring (35) appears to be necessary for insulin-dependent inhibition of apoB secretion by rat hepatocytes (33, 34). Insulin acts by causing the activation and localization of phosphoinositide 3-kinase to the site of apoB synthesis (34).

In the present study, we have employed a new animal model of chronic hyperinsulinemia and insulin resistance, namely, the fructose-fed Syrian golden hamster. The Syrian golden hamster has been used with increasing frequency in recent years to study hepatic lipid metabolism (36–38). Hamsters develop hypertriglyceridemia, hypercholesterolemia, and atherosclerosis in response to a modest increase in dietary cholesterol and saturated fat (39, 40). The hamster has attracted increasing attention as a model for lipoprotein research since its lipoprotein metabolism appears to closely resemble that in humans. The main plasma cholesterol carrier in the hamster is LDL (40, 41). Furthermore, hamster liver produces VLDL containing only apoB-100 with a density close to that of human VLDL (42, 43), unlike the rat, which has been used extensively for studies of VLDL metabolism and the effects of insulin resistance, and whose liver secretes both apoB-48 and apoB-100. Carbohydrate induced insulin resistance in rodents has been previously well documented. Reaven and colleagues (44–47) were among the first groups to use sucrose or fructose feeding to induce insulin resistance in rats. Hamsters can also be made obese, hyperinsulinemic, hypertriglyceridemic, and insulin-resistant by fructose feeding (48). Fructose feeding appears to interfere with glucose utilization in vivo, inducing an insulin resistant state (48). It thus appears feasible to induce insulin resistance in the hamster and use the insulin-resistant hamster model to study the mechanisms controlling hepatic VLDL-apoB secretion.

MATERIALS AND METHODS

Male Syrian golden hamsters (Mesocricetus auratus) were purchased from Charles River (Montreal, PQ). Fetal bovine serum (certified grade), liver perfusion medium, hepatocyte wash medium, liver digest medium, and hepatocyte attachment medium were obtained from Life Technologies (Grand Island, NY). Rabbit anti-hamster apoB antisera was prepared commercially by Lampire Biological Laboratories (Pipersville, PA) using hamster LDL prepared in our laboratory. Specificity of this commercial preparation of anti-apoB polyclonal antibody and lack of any cross-reactivity to other hamster apolipoproteins (apoA-I or apoE) was confirmed by immunoblotting analysis of purified plasma lipoprotein fractions. Anti-bovine MTP antibody was generously provided by Dr. S. P. Tam, Queen’s University.

Animal Protocols—Male Syrian golden hamsters were obtained from Charles River (Montreal, PQ). Fetal bovine serum, 1.0 μg/ml insulin, 1× penicillin-streptomycin, and penicillin-streptomycin (15× 106 cells/35-mm plate). After 4 h or overnight incubation at 37 °C, 5% CO2, attached cells were used to carry out the experiments.

Determination of the Synthesis and Secretion of Cellular and Secreted Lipids—Primary hepatocytes were pulsed for 3 or 18 h with 5 μCi/ml
Fructose feeding caused a significant elevation of plasma free fatty acids (p = 0.0045) and an elevation of plasma cholesterol level that approached statistical significance (p = 0.0550), following a 2-week period of fructose feeding. Two-way ANOVA was used to compare the glucose, insulin, and Ginf curves of the fructose-fed and control groups during the last 30 min of the clamp. S_j is expressed in arbitrary units (litter−kg−1 min−1). Two-way ANOVA was used to compare the glucose, insulin, and Ginf curves of the fructose-fed and control groups during the last 30 min of the clamp. A two-tailed paired t test was used to compare the mean baseline versus mean clamp S_j values.

RESULTS

Metabolic Effects of Fructose Feeding in Syrian Golden Hamsters—Fig. 1 shows the physiological changes observed in control and fructose-fed hamsters after a 2-week feeding period. Fructose-fed hamsters gained body weight at approximately the same rate as that for control hamsters over the 2-week feeding period (data not shown). Fructose-fed hamsters showed a significant elevation of plasma TG (p = 0.0309) and an elevation of plasma cholesterol level that approached statistical significance (p = 0.0550), following a 2-week period on a fructose-rich diet (Fig. 1, A and B). There was also a significant elevation (p = 0.0110) of plasma insulin level (Fig. 1C). In addition, fructose feeding caused a significant elevation of plasma free fatty acids (p = 0.0045) as shown in Fig. 1D.
However, plasma glucose levels did not differ significantly (p = 0.9452) between control and fructose-fed hamsters (Fig. 1E). Overall, fructose feeding induced significant elevation in plasma levels of TG, insulin, and free fatty acids.

Evidence for Development of Insulin Resistance in Fructose-fed Hamsters: Euglycemic Hyperinsulinemic Clamp Studies—Fig. 2 shows the results of the euglycemic hyperinsulinemic clamp studies, which were performed in 9 fructose-fed hamsters and 10 control hamsters. The plasma glucose levels (Fig. 2A) did not change from baseline and were significantly higher in the fructose-fed versus control animals during the last 30 min of the clamp (5.0 ± 0.4 vs 3.9 ± 0.3 mmol/liter, p < 0.01). Although the insulin levels (Fig. 2B) tended to be higher in the fructose-fed versus control group during the last 30 min of the clamp (2394 ± 441 vs 2002 ± 272 pmol/liter), this difference was not significant. However, the Ginf (Fig. 2C) during the last 30 min of the clamp was significantly lower in fructose-fed versus control animals (26.0 ± 6.5 μmol kg⁻¹ min⁻¹ vs 39.5 ± 9.4 μmol kg⁻¹ min⁻¹, p < 0.01). This difference in Ginf, especially in the face of higher levels of both glucose and insulin during the clamp in fructose-fed versus control hamsters, confirms that the former are more insulin resistant than the latter. This is shown by the calculation of S, (Fig. 2D) which was significantly lower in fructose-fed versus control hamsters (2.7 ± 0.8 × 10² vs 4.8 ± 0.4 × 10² liter² kg⁻¹ min⁻¹, p = 0.03).

In Vivo Evidence of VLDL-ApoB Overproduction in Fructose-fed Hamsters—Fig. 3A shows the increase of VLDL-TG over 90 min following the intravenous administration of Triton WR-1339. The increase in both fructose-fed and control hamsters was linear (mean R squared 0.98 ± 0.01 and 0.91 ± 0.05 for the fructose-fed and control group, respectively). VLDL-TG increase over time tended to be higher in fructose-fed hamsters but this difference was not statistically significant (0.051 ± 0.010 versus 0.034 ± 0.006 μmol/ml/min in the fructose fed versus control group, respectively, p = 0.13). Similarly, the VLDL-TG secretion rate was 30% higher in the fructose-fed than in the control group (0.23 ± 0.03 versus 0.16 ± 0.03 μmol/min, respectively) although this difference did not reach statistical significance (p = 0.14) (inset of Fig. 3A).

VLDL-apoB increased linearly in both groups (mean R squared 0.96 ± 0.01 and 0.97 ± 0.01 for the fructose-fed and control group, respectively), as shown in Fig. 3B. The slope of the increase in VLDL-apoB over time was significantly steeper in the fructose-fed versus control group (2.27 ± 0.04 versus 1.55 ± 0.14 μg/ml/min, respectively, p < 0.001). Consequently, the VLDL-apoB secretion rate was 31% higher in the fructose-fed versus control group (10.26 ± 0.47 versus 7.13 ± 0.73 μg/min, respectively, p < 0.005) (inset of Fig. 3B).

Evidence that Direct Incubation with Fructose Does Not Directly Affect Hepatic ApoB Secretion by Primary Hamster Hepatocytes—It was important to determine if fructose can directly induce the hepatic synthesis and secretion of apoB-100 in hamster hepatocytes since such a direct effect would complicate the interpretation of our data relating apoB overproduction to the development of fructose-induced insulin resistance. Freshly isolated hamster hepatocytes from control, chow-fed hamsters were incubated with different concentrations of fructose for a 24-h period and synthesis and secretion of apoB were monitored by pulse labeling with [³⁵S]methionine. Fig. 4A shows a dose-response study of the effect of fructose on hepatic apoB. Cellular accumulation and extracellular secretion of apoB were unaffected in the presence of increasing concentrations of fructose in the culture media. Even at the highest concentration of 3 mM, there was no significant effect on the synthesis or secretion of apoB in primary hamster hepatocytes. To further confirm a lack of direct effect of fructose on hamster apoB biogenesis, we incubated cultured hepatocytes for a period of up to 3 days with exogenous fructose at the highest concentration (3 mM). Cells incubated for 1, 2, or 3 days were then subjected to pulse-chase labeling (45 min pulse, 1–2 h chase) to determine the extent of hamster apoB secretion and its intracellular stability in hamster hepatocytes. Fig. 4, B-G, show the effects of fructose incubation for periods of 1–3 days. Panels B, D, and F...

![Fig. 2. Euglycemic hyperinsulinemic clamp studies in fructose-fed hamsters (white bars) versus normal (or control) chow-fed animals (black bars). Mean glucose levels (A) were slightly but significantly higher in fructose-fed versus control animals during the last 30 min of the clamp period (p < 0.01). Mean insulin levels (B) were slightly but not significantly higher in the fructose-fed versus control hamsters during the clamp period. The glucose infusion rate (Ginf) (C) during the clamp period was significantly lower in fructose-fed versus control animals (p < 0.01). Although the insulin levels (Fig. 2B) were slightly but not significantly higher in the fructose-fed versus control hamsters during the clamp period. The glucose infusion rate (Ginf) (C) during the clamp period was significantly lower in fructose-fed versus control animals (p < 0.01). The calculated insulin sensitivity index (SI, see "Materials and Methods") (D) was also significantly lower in the fructose-fed versus control hamsters (p = 0.03). Fructose-fed (n = 9), control hamsters (n = 10).]
primary hamster hepatocytes also revealed no significant change in cholesteryl ester secretion (Fig. 5B). Interestingly, however, the secretion of TG was significantly elevated in fructose-fed hamsters (Fig. 5B). Conversely, hepatocytes from fructose-fed hamsters secreted significantly lower levels of free cholesterol (Fig. 5B). The decline in free cholesterol secretion was accompanied by an increase in its intracellular levels, suggesting that fructose feeding of hamsters has an inhibitory effect on the release of free cholesterol from hepatocytes. In the case of TG, both the cellular and secreted levels were elevated, suggesting that fructose feeding enhanced the synthesis of TG and its secretion from the cell.

We also analyzed the secreted levels of core lipids associated with VLDL particles secreted by primary hepatocytes. Following radiolabeling of hamster hepatocytes, the cultured media was subjected to ultracentrifugation to isolate the VLDL fraction. The radiolabeled lipids associated with media VLDL were then analyzed by thin layer chromatography. Secretion of VLDL-TG was also significantly induced in fructose-fed hamsters whereas VLDL-cholesteryl ester secretion was unaffected by fructose feeding (data not shown). The observed increase in VLDL-TG secretion compared well with the increase in the intracellular and secreted levels of total TG reported in Fig. 5, A and B.

Overproduction of VLDL-ApoB in Hepatocytes from Fructose-fed Hamsters—Primary hepatocytes isolated from hamster liver secrete apoB at a density of VLDL (Fig. 6 and Ref. 42). To determine the effect of fructose feeding on VLDL-apoB secretion, we performed in vitro steady state labeling experiments in which hepatocytes from control and fructose-fed hamsters were radiolabeled for a 2-h period. Culture media containing secreted lipoprotein particles was then collected and subjected to ultracentrifugation to isolate VLDL. Radiolabeled apoB associated with VLDL particles was immunoprecipitated and analyzed by SDS-PAGE and fluorography. Fig. 6 shows the immunoprecipitable VLDL-apoB secreted by control and fructose-fed hepatocytes. There was a highly significant (4.6-fold) elevation in the amount of VLDL-apoB secreted into the media in fructose-fed hepatocytes. Increased VLDL-apoB levels suggest the secretion of a considerably higher number of VLDL particles by fructose-fed hepatocytes compared with control hepatocytes.

Turnover Rate of ApoB in Control and Fructose-fed Hepatocytes—We employed pulse-chase labeling experiments to assess the stability and secretion of apoB in hepatocytes isolated from control and fructose-fed hamsters. Isolated hepatocytes were pulsed for 45 min and then chased for 1 and 2 h. Cellular and media apoB was immunoprecipitated and analyzed by SDS-PAGE and fluorography. Fig. 7 shows the intracellular turnover and extracellular secretion of apoB in control and fructose-fed hepatocytes. A large percentage of newly synthesized, radiolabeled apoB disappeared from control cells over the 2-h chase with a small percentage appearing in the media (Fig. 7, A and B). The disappearance rate of apoB in fructose-fed hepatocytes was considerably slower, with only about 25% of apoB having been lost during the 2-h chase (Fig. 7A). The increased stability of apoB in fructose-fed hepatocytes was accompanied by a dramatic increase in the secreted level of newly-synthesized apoB. As shown in Fig. 7B, fructose-fed hepatocytes secreted about 20% of newly synthesized apoB compared with only about 5% in control cells.

Stability of ApoB in Permeabilized Primary Hamster Hepatocytes—Permeabilized cells have been used previously to investigate post-translational degradation of apoB, allowing for detection of specific degradation intermediates, including a 70-kDa fragment (61). We have recently applied the permeabilization protocol to primary hamster hepatocytes and have

![Fig. 3. In vivo production of VLDL-apoB and VLDL-TG in control and fructose-fed hamsters. A, VLDL-TG (VLDL-TG) concentration over time after intravenous administration of Triton WR-1339 in fructose-fed (n = 6, closed circles) versus control hamsters (n = 7, open circles). The slope of the curve tended to be higher in the former group (p = 0.13). The VLDL-TG secretion rate is shown in the inset in the fructose-fed animals (black bars) compared with the controls (white bars) (p = 0.14). B, VLDL-apoB concentration over time during the same experiments as in A. The slope of the curve was significantly higher in the fructose-fed animals (closed circles) compared with the controls (open circles) (p < 0.001). The VLDL-apoB secretion rate is shown in the inset in the fructose-fed (closed bars) compared with the control hamsters (open bars) (*, p < 0.005).](http://www.jbc.org/)

...show hamster apoB secretion, while panels C, E, and G show the stability of apoB as assessed by the total apoB remaining in cells and media over a 2-h chase. There was no detectable stimulation of apoB secretion or stability with fructose treatment for up to 3 days. There was actually some inhibition of apoB secretion observed at day 2, but overall the entire experiment revealed no specific effect.

Effect of Fructose Feeding on Hepatic Synthesis and Secretion of Lipids—Primary hamster hepatocytes isolated from normal chow-fed and fructose-fed hamster livers were used to determine the effect of fructose feeding on the hepatic synthesis and secretion of total lipids. There was a small decrease in cellular levels of cholesteryl ester, although this change was not statistically significant (Fig. 5A). However, the intracellular levels of TG and free cholesterol were both significantly increased in hepatocytes from fructose-fed hamsters (Fig. 5A). Analysis of radiolabeled lipids in culture media...
investigated hamster apoB stability and turnover in this cell model system. Utilizing the permeabilized cell model system, we attempted to determine the effect of fructose feeding on the turnover of apoB. Control and fructose-fed hepatocytes were pulse-labeled, permeabilized, and then chased for a 2–3-h period. Fig. 8 shows the turnover of full-length hamster apoB-100 in permeabilized control and fructose-fed hepatocytes. Hamster apoB-100 was significantly more stable in fructose-fed hepatocytes as judged from the considerably higher intracellular level of apoB remaining in permeabilized cells after a 3-h chase. There was approximately a 2-fold higher level of apoB-100 remaining in fructose-fed hepatocytes following completion of the chase period (Fig. 8).

**Effect of Fructose Feeding on Intracellular Assembly of ApoB-containing Lipoproteins**—To directly investigate the formation of apoB-containing lipoprotein particles in hamster hepatocytes, cells were pulse-labeled, chased for 0 and 1 h, and then subjected to subcellular fractionation. Nascent lipoproteins accumulated in the microsomal lumen were fractionated by sucrose gradient centrifugation and immunoprecipitated with anti-hamster apoB antibody. Fig. 9 illustrates the pattern of nascent lipoproteins accumulated in the lumen of control hepatocytes compared with that of lipoproteins detected in fructose-fed hepatocytes. Luminal apoB-containing lipoproteins in both control and fructose-fed hepatocytes were predominantly recov-

---

2 C. Taghibiglou, D. Rudy, S. Van Iderstine, A. Aiton, D. Cavallo, and K. Adeli, *J. Lipid Res.* in press.
Control and fructose-fed hepatocytes. Primary hepatocytes immediately following attachment to culture plates were pulsed for 18 h with ester (CE, TG), and triglyceride (TG); bovine serum albumin. A, monitored by labeling cells for 3–5 h with cholesterol, and cholesteryl ester. TG synthesis and secretion were facilitated assembly of apoB-containing lipoproteins in control and fructose-fed hepatocytes. Control cells had a significantly higher level of total lumenal apoB after a 1-h chase. This observation suggests that a small pool of nascent lipoproteins exhibiting a density typical of LDL (Fig. 9B). Also intriguing was the detection of high density lipoprotein-size lipoproteins in the lumen of control hepatocytes but not that of fructose-fed hepatocytes. This observation suggests that a small pool of nascent hamster lipoproteins may form a dense, secretion-incompetent pool in normal hamster hepatocytes as previously reported in HepG2 cells (62, 66). The absence of high density lipoprotein-like apoB-containing lipoproteins in microsomes of fructose-fed hepatocytes may in turn suggest a higher efficiency of lipoprotein assembly under this metabolic condition. Finally, when the radiolabeled apoB in all fractions of the gradient were collected, density adjusted to 1.006 g/ml, and adjusted media was subjected to ultracentrifugation for 18 h at 55,000 rpm in SW55 rotor to float the VLDL fraction. The VLDL fraction was then collected and immunoprecipitated with an specific anti-hamster apoB antibody. The immunoprecipitates were analyzed by SDS-PAGE and fluorography. Quantitation of apoB was performed by scintillation counting of the apoB-100 band (mean ± S.D., n = 4). *, significantly different from control (p < 0.05).

**DISCUSSION**

Although overproduction of VLDL-TG and VLDL-apoB has been well demonstrated in the insulin-resistant state in both humans and animal models, few data are available on the underlying cellular mechanisms involved, particularly those directly affecting the apoB protein itself. The majority of studies have focused on the acute effects of insulin, while the role of chronic hyperinsulinemia and insulin resistance in VLDL overproduction have been understudied. In the present study, we have simultaneously examined the specific impact of inducing an insulin-resistant condition on the rate of apoB expression at the potential regulatory steps of synthesis, intracellular degradation, and lipoprotein assembly. We employed a fructose-fed hamster model to investigate the above mechanisms in the state of insulin resistance. This model offers advantages over the more commonly used fructose-fed rat model, in that the metabolism of its apoB-containing lipoproteins is more similar to that of humans.
It has been well documented that fructose feeding in rodents including hamsters (48), results in chronic hyperinsulinemia, an insulin resistance state, and hyperlipidemia. A previous study (48) clearly demonstrated the feasibility of inducing insulin resistance and chronic hyperinsulinemia in fructose-fed hamsters. The fructose protocol employed in the current study was also similar to those previously shown to induce insulin resistance in the rat (67). The data from in vivo hyperinsulinemic-euglycemic clamp studies presented in this article also support the induction of an insulin-resistant condition in the fructose-fed hamster model. The in vivo clamp study suggests whole body resistance to insulin action and reduced rate of in vivo glucose uptake.

Our in vivo Triton 1339 studies suggested the hepatic overproduction of VLDL-apoB in fructose-fed hamsters. The fructose protocol employed in the current study was also similar to those previously shown to induce insulin resistance in the rat (67). The data from in vivo hyperinsulinemic-euglycemic clamp studies presented in this article also support the induction of an insulin-resistant condition in the fructose-fed hamster model. The in vivo clamp study suggests whole body resistance to insulin action and reduced rate of in vivo glucose uptake.

Mechanisms of Hepatic VLDL Overproduction in Insulin Resistance

FIG. 7. Intracellular stability of hamster apoB in control and fructose-fed hepatocytes. Primary hamster hepatocytes were pulsed for 45 min with 100 μCi/ml [35S]methionine, and the radioactivity was chased for 1 and 2 h in the presence of 5 mM excess cold methionine. Media and cells were collected and apoB was immunoprecipitated with a specific anti-hamster apoB antibody followed by SDS-PAGE and fluorography. A, apoB stability expressed as percent apoB remaining in cells + media (total apoB) in control and fructose-fed hepatocytes at 0 time (beginning of the chase), 1 h chase, and 2 h chase. B, distribution of immunoprecipitable apoB in cells and media expressed as a percentage of radiolabeled apoB in cells at 0 time (mean ± S.D., n = 3). *, significantly different from control (p < 0.05).

FIG. 8. Post-translational stability of hamster apoB in permeabilized primary hepatocytes. Primary hamster hepatocytes were pulsed for 45 min with 100 μCi/ml [35S]methionine. Cells were then permeabilized with digitonin (50 μg/ml), and the permeabilized cells were incubated in a CSK buffer for 2 and 3 h prior to immunoprecipitation with an anti-hamster apoB antibody. A, hamster apoB-100 radioactivity was quantified by cutting and scintillation counting of the bands and apoB degradation was assessed by calculating the percent apoB remaining in cells under various conditions. *, significantly different from control (p < 0.05).

FIG. 9. Intracellular distribution of nascent apoB-containing lipoproteins in microsomal lumen of control and fructose-fed hepatocytes. Cultured primary hamster hepatocytes were pulsed for 45 min with [35S]methionine and the radioactivity was chased for 0 or 1 h. Labeled cells were then subjected to homogenization and fractionation of microsomes. Luminal lipoproteins were extracted from microsomes by carbonate treatment and separated from the membrane fraction by centrifugation followed by fractionation on a sucrose gradient. After centrifugation, gradient fractions were collected and immunoprecipitated with an anti-hamster apoB antibody. Immunoprecipitates were analyzed by SDS-PAGE and fluorography and apoB radioactivity was quantitated by cutting and scintillation counting of the apoB-100 band. A, luminal lipoproteins in control hepatocytes at 0 and 1 h chase; B, luminal lipoproteins in fructose-fed hepatocytes at 0 and 1-h chase.
observation which limits the usefulness of these animals as a model of human pathophysiology (70). Although fructose feeding has been shown to induce an increase in VLDL-TG production in vivo in rats (50, 51) these previous studies did not investigate the VLDL-apoB production rate.

The insulin-resistant, fructose-fed hamster model thus provided an excellent system to investigate the intracellular mechanisms that may mediate the considerable VLDL-apoB overproduction observed. A number of important observations were made which appear to explain the VLDL-apoB overproduction in this model. First, there was a significant enhancement of intracellular stability of newly synthesized apoB with only a minor fraction being sorted to intracellular degradation. The increased intracellular stability of apoB in fructose-fed hepatocytes was evident both in intact cells as well as in permeabilized cells. Turnover of nascent apoB was slowed in intact fructose-fed hepatocytes compared with control cells. This observation may or may not be related to an enhanced rate of apoB translocation across the endoplasmic reticulum membrane. Whether stimulated translocation of apoB across the endoplasmic reticulum membrane is responsible for the enhanced intracellular stability is currently unknown and awaits further analysis of apoB translocation status in normal and fructose-fed hepatocytes. We are currently investigating this question by analyzing translocation status of apoB in both isolated microsomes as well as in permeabilized cells.

Further analysis of lipoprotein formation in hepatocytes derived from fructose-fed animals revealed a considerable stimulation of VLDL assembly under this metabolic condition. This was evident from reduced formation of LDL-like apoB-containing lipoproteins and increased accumulation of VLDL particles in fructose-fed hepatocytes. These observations argue for enhanced efficiency of VLDL assembly in the microsomal lumen of fructose-fed hepatocytes. Facilitated assembly of hamster VLDL may be related to an increased availability of core lipids, an increased availability of freshly translated apoB, and/or increased activity of MTP. Analysis of intracellular lipid biosynthesis revealed a significant increase in intracellular TG levels, which may in turn contribute to increased assembly of VLDL. In addition, intracellular stability of nascent apoB was also increased, making a higher pool of nascent apoB molecules available for VLDL assembly. Most interesting, however, was an increased mass of MTP detected in fructose-fed hepatocytes. MTP catalyzes the transfer of lipids to the apoB molecule and is an important factor involved in the assembly of apoB-containing lipoproteins (71, 72). Inhibition of the activity of MTP blocks the assembly and secretion of apoB-containing lipoprotein particles (73). Thus it is reasonable to conclude that an increased intracellular mass of MTP can enhance the VLDL assembly process, leading to formation and secretion of an increased number of mature particles. Furthermore, the combination of an increased abundance of MTP, in the presence of both higher availability of TG as well as apoB, strongly favors the formation of VLDL particles and their secretion from the cell. Insulin is known to acutely diminish both the MTP mRNA level as well as the mass of MTP protein (74). The insulin effect was shown to be dose- and time-dependent and mediated through the insulin receptor (75). Despite acute inhibition of MTP mRNA levels, short-term insulin treatment (24 h) did not change MTP activity levels due to the slow turnover rate of MTP, $t_{1/2} = 4.4$ days. These observations suggested that sustained changes in MTP mRNA levels would be required to affect MTP protein levels (75). The 5° ends of both human and hamster MTP genes contain a negative insulin response element whose activity is negatively regulated by insulin (76). Very recent studies in Otsuka Long-Evans Tokushima Fatty rat, an animal model of Type 2 diabetes, characterized by visceral obesity and hyperlipidemia, has shown enhanced expression of acyl-coenzyme A synthetase, and MTP mRNA in the absence of insulin resistance (77). These investigators suggested that the enhanced expression of both acyl-coenzyme A synthetase and MTP genes associated with visceral fat accumulation, prior to the development of insulin resistance, may be involved in the pathogenesis of hyperlipidemia in obese animal models with Type 2 diabetes (77). In contrast to these findings, MTP protein levels were found to be unaltered in the streptozotocin diabetic rat and 10-day-old suckling rats, animal models in which VLDL-TG secretion is markedly reduced (78). Thus, whether increased MTP causes the increased stability and assembly of VLDL in insulin resistance or is merely secondary to the increase in intracellular lipid synthesis is currently unknown.

Hepatic overproduction of VLDL in the state of insulin resistance may result from direct hepatic effects of insulin as well as indirect metabolic effects, such as increased availability of free fatty acids (FFA) for TG secretion (23). In the present study, we found significantly elevated plasma levels of free fatty acids in fructose-fed hamsters, suggesting that increased flux of FFA into the liver may contribute to VLDL overproduction. However, we did not measure in vivo FFA flux in fructose-fed hamsters and cannot confirm the impact of plasma FFA elevation on in vivo VLDL production rates. It is also important to note that the rate of VLDL-apoB secretion from primary hamster hepatocytes was measured under identical concentrations of free fatty acids in the culture media, for both control and fructose-fed hepatocytes. Elevated FFAs in the presence of hyperinsulinemia may have induced hepatic enzymes responsible for channeling FFAs into secretory rather than oxidative pathways, which could have had lasting effects in the cultured hepatocytes.

In conclusion, the fructose-fed hamster model has allowed us to address a number of important questions regarding the intracellular mechanisms that modulate hepatic VLDL assembly and secretion. The evidence obtained in this model suggests that the hepatic overproduction of apoB observed in insulin
Mechanisms of Hepatic VLDL Overproduction in Insulin Resistance

resistance may be caused by the combined effect of an increased expression of MTP, increased hepatocyte neutral lipid availability, and reduced degradation of apoB, which can in turn facilitate the assembly and secretion of apoB-containing lipoprotein particles. Precisely which of these factors occurs directly as a result of hepatic hyperinsulinemia or insulin resistance and which are secondary to the extrahepatic effects of insulin is not currently known. The hepatic effects may be due to a direct action of insulin or may be secondary to an increased lipid availability. The mechanisms for enhanced intracellular stability of apoB and increased extracellular secretion are currently unknown and will require further investigation particularly on whether fructose feeding affects apoB translocation. Enhanced activity of MTP may contribute to intracellular stability of apoB, but whether it is sufficient by itself to explain the VLDL overproduction is unknown. Further studies are required to fully investigate the mechanisms by which insulin resistance can influence either the expression or intracellular stability of MTP and thus exert a stimulatory effect on VLDL assembly and secretion. Of particular interest is the interaction of MTP abundance/activity, intracellular apoB stability, and core lipid availability in determining the efficiency of the VLDL assembly process.

REFERENCES

1. Moller, D. E., and Flier, J. S. (1993) N. Engl. J. Med. 325, 938–948
2. Reaven, G. M. (1990) Diabetes 37, 1595–1607
3. Kissebah, A. H., Alfarsi, S., and Adams, P. W. (1981) J. Biol. Chem. 256, 1128–1136
4. Reaven, G. M., and Chen, Y. D. (1988) Diabetes Care 11, 390–393
5. Lin, M. C., Gordon, D. A., Sharp, D., Mullaney, D., Yao, Z., Gregg, R. E., and Wetterau, J. R. (1995) Am. J. Clin. Nutr. 61, 892–895
6. Reaven, G. M., Chen, Y. D., Culliy, M. D., and Reaven, G. M. (1980) Metabolism 29, 303–305
7. Tobey, T. A., Mondon, C. E., Zavaroni, I., and Reaven, G. M. (1982) Metabolism 31, 608–612
8. Kazumi, T., Vranic, M., and Steiner, G. (1985) J. Clin. Invest. 76, 265–275
9. Miller, L. L. (1973) in Isolated Liver Perfusion and Its Applications (Miller, L. L., ed) pp. 11–52, Raven Press, New York
10. Adeli, K. (1994) J. Biol. Chem. 269, 9166–9175
11. Macri, J., and Adeli, K. (1997) J. Biol. Chem. 272, 7328–7337
12. Boren, J., Wettesten, M., Björk, A., Thorlin, T., Bondjers, G., Wiklund, O., and Olofsson, S. O. (1990) J. Biol. Chem. 265, 10550–10564
13. Adeli, K., Wettesten, M., Asp, L., Mohammad, A., Macri, J., and Olofsson, S. O. (1997) J. Biol. Chem. 272, 5031–5039
14. Laemmli, U. K. (1970) Nature 227, 680–685
15. Bergman, R. N., Finegood, D. T., and Ader, M. (1985) Endocrinology 117, 1145–1150
16. Kazumi, T., Vranic, M., and Steiner, G. (1986) Am. J. Physiol. 250, E235–E330
17. Yoshino, G., Hirano, T., Maeda, E., Murata, Y., Naka, Y., Nagata, K., Kazumi, T., and Urayama, T. (1995) Atherosclerosis 118, 133–139
18. Patsch, W., Haidar, A., and Schonfeld, G. (1983) J. Lab. Clin. Med. 95, 1773–1782
19. Sletten, J. A., and Chakravarthy, S. (1981) J. Lipid Res. 22, 1274–1283
20. Dashti, N., Williams, D. L., and Alaupovic, P. (1989) J. Clin. Invest. 84, 136–150
21. Salhanick, A. I., Schwartz, S. I., and Amatruda, J. M. (1991) J. Biol. Chem. 266, 1277–1288
22. Wetterau, J. R., Aggerbeck, L. P., Bouma, M. E., Eisenberg, C., Munck, A., and Wetterau, J. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7328–7332
23. Arbeeny, C. M., Meyers, D. S., Bergquist, K., and Gregg, R. E. (1992) J. Lipid Res. 33, 843–851
24. Malmstrom, R., Packard, C. J., Caslake, M., Bedford, D., Stewart, P., and Redinger, R. N. (1991) Comp. Biochem. Physiol. A 99, 223–228
25. Wright, D. W., Hansen, R. I., Mondon, C. E., and Reaven, G. M. (1993) Am. J. Clin. Nutr. 58, 879–885
26. Siedentop, S. K., Chen, Y. D., Culliy, M. D., and Reaven, G. M. (1980) Metabolism 29, 303–305
27. Tobey, T. A., Mondon, C. E., Zavaroni, I., and Reaven, G. M. (1982) Metabolism 31, 608–612
28. Kazumi, T., Vranic, M., and Steiner, G. (1985) J. Clin. Invest. 76, 1128–1136
29. Sparks, J. D., and Sparks, C. E. (1994) Biochem. Biophys. Res. Commun. 205, 417–422
30. Dallal, N., Williams, D. L., and Alaupovic, P. (1989) J. Lipid Res. 30, 1365–1373
31. Sparks, J. D., and Sparks, C. E. (1994) Biochem. Biophys. Res. Commun. 205, 417–422
32. Wetterau, J. R., Agerbeck, L. P., Bouma, M. E., Eisenberg, C., Munck, A., and Wetterau, J. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7328–7332
33. Wetterau, J. R., Agerbeck, L. P., Bouma, M. E., Eisenberg, C., Munck, A., and Wetterau, J. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7328–7332
34. Phung, T. L., Roncone, A., de Mesy Jensen, K. L., Sparks, C. E., and Sparks, J. D. (1997) J. Biol. Chem. 272, 30693–30702
35. Whitman, M., Downes, C. P., Keeler, M., Keller, T., and Cantley, L. (1988) J. Biol. Chem. 263, 644–646
36. Jackson, B., Gee, A. N., Martinez-Cayuela, M., and Suckling, K. E. (1990) Biochim. Biophys. Acta 1045, 21–28
37. Inoue, A., Cheng, Y. Q., and Yamamoto, M. (1990) J. Lipid Res. 31, 1839–1992
38. Kissebah, A. H., Alfarsi, S., and Adams, P. W. (1981) J. Biol. Chem. 256, 1128–1136
39. Lin, M. C., Gordon, D., and Wetterau, J. R. (1995) J. Lipid Res. 36, 1073–1081
40. Kissebah, A. H., Yamashita, H., Shimomura, I., Funahashi, T., Ishigami, M., Aragane, K., Miyako, K., Nakamura, T., Takemura, K., Man, Z., Toide, K., Nakayama, N., Fukuda, Y., Lin, M. C., Wetterau, J. R., and Matsuzawa, Y. (1998) Hepatology 27, 557–562
41. Brett, D. J., Pease, R. J., Scott, J., and Gibbons, G. F. (1995) Biochem. J. 310, 11–14

Downloaded from http://www.jbc.org/ by guest on July 26, 2018
Mechanisms of Hepatic Very Low Density Lipoprotein Overproduction in Insulin Resistance: EVIDENCE FOR ENHANCED LIPOPROTEIN ASSEMBLY, REDUCED INTRACELLULAR ApoB DEGRADATION, AND INCREASED MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN IN A FRUCTOSE-FED HAMSTER MODEL

Changiz Taghibiglou, André Carpentier, Stephen C. Van Iderstine, Biao Chen, Debbie Rudy, Andrea Aiton, Gary F. Lewis and Khosrow Adeli

J. Biol. Chem. 2000, 275:8416-8425.
doi: 10.1074/jbc.275.12.8416

Access the most updated version of this article at http://www.jbc.org/content/275/12/8416

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 77 references, 32 of which can be accessed free at http://www.jbc.org/content/275/12/8416.full.html#ref-list-1