Metabolic effects of dietary cholesterol in an animal model of insulin resistance and hepatic steatosis

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Basciano H, Miller AE, Naples M, Baker C, Kohen R, Xu E, Su Q, Allister EM, Wheeler MB, Adeli K. Metabolic effects of dietary cholesterol in an animal model of insulin resistance and hepatic steatosis. Am J Physiol Endocrinol Metab 297: E462–E473, 2009.—Although the atherogenic role of dietary cholesterol has been well established, its diabetogenic potential and associated metabolic disturbances have not been reported. Diet-induced hamster models of insulin resistance and dyslipidemia were employed to determine lipogenic and diabetogenic effects of dietary cholesterol. Metabolic studies were conducted in hamsters fed diets rich in fructose (40%), fat (30%), and cholesterol (0.05–0.25%) (FFC) and other test diets. Short-term feeding of the FFC diet induced insulin resistance, glucose intolerance, hypertriglyceridemia, and hypercholesterolemia. Prolonged feeding (6–22 wk) of the FFC diet led to severe hepatic steatosis, glucose intolerance, and mild increases in fasting blood glucose, suggesting progression toward type 2 diabetes, but did not induce ß-cell dysfunction. Metabolic changes induced by the diet, including dyslipidemia and insulin resistance, were cholesterol concentration dependent and were only markedly induced on a high-fructose and high-fat dietary background. There were significant increases in hepatic and plasma triglyceride with FFC feeding, likely due to a 10- to 15-fold induction of hepatic stearoyl-CoA desaturase compared with chow levels (P < 0.03). Hepatic insulin resistance was evident based on reduced tyrosine phosphorylation of the insulin receptor-ß, IRS-1, and IRS-2 as well as increased protein mass of receptor-evident based on reduced tyrosine phosphorylation of the insulin receptor-evident based on reduced tyrosine phosphorylation of the insulin receptor.

Although the atherogenic role of dietary cholesterol has been well established, its diabetogenic potential and associated metabolic disturbances have not been reported. Diet-induced hamster models of insulin resistance and dyslipidemia were employed to determine lipogenic and diabetogenic effects of dietary cholesterol. Metabolic studies were conducted in hamsters fed diets rich in fructose (40%), fat (30%), and cholesterol (0.05–0.25%) (FFC) and other test diets. Short-term feeding of the FFC diet induced insulin resistance, glucose intolerance, hypertriglyceridemia, and hypercholesterolemia. Prolonged feeding (6–22 wk) of the FFC diet led to severe hepatic steatosis, glucose intolerance, and mild increases in fasting blood glucose, suggesting progression toward type 2 diabetes, but did not induce ß-cell dysfunction. Metabolic changes induced by the diet, including dyslipidemia and insulin resistance, were cholesterol concentration dependent and were only markedly induced on a high-fructose and high-fat dietary background. There were significant increases in hepatic and plasma triglyceride with FFC feeding, likely due to a 10- to 15-fold induction of hepatic stearoyl-CoA desaturase compared with chow levels (P < 0.03). Hepatic insulin resistance was evident based on reduced tyrosine phosphorylation of the insulin receptor-ß, IRS-1, and IRS-2 as well as increased protein mass of protein tyrosine phosphatase 1B. Interestingly, nuclear liver X receptor (LXR) target genes such as ABCA1 were upregulated on the FFC diet, and dietary supplementation with an LXR agonist (instead of dietary cholesterol) worsened dyslipidemia, glucose intolerance, and upregulation of target mRNA and proteins similar to that of dietary cholesterol. In summary, these data clearly implicate dietary cholesterol, synergistically acting with dietary fat and fructose, as a major determinant of the severity of metabolic disturbances in the hamster model. Dietary cholesterol appears to induce hepatic cholesterol ester and triglyceride accumulation, and diet-induced LXR activation (via cholesterol-derived oxysterols) may possibly be one key underlying mechanism.

Liver X receptor; Syrian golden hamster; apolipoprotein B

Insulin resistance and many facets of the metabolic syndrome are often linked to the macronutrient content of the diet, and there is evidence that excessive consumption of macronutrients such as carbohydrates, fats, and even protein may eventually lead to the development of insulin resistance (34, 37). Diets high in saturated fats and cholesterol have been demonstrated to induce weight gain, insulin resistance, and hyperlipidemia in humans and animals (11, 16). Several rodent models of diet-induced insulin resistance have been developed to enhance our understanding of the underlying mechanisms associated with complications due to dyslipidemia (reviewed in Refs. 7, 19, 21, and 33). In addition, high dietary cholesterol has been shown to cause accumulation of lipids via enhanced scavenger receptor activity, increased non-HDL cholesterol, increased inflammatory markers, and decreased expression of enzymes involved in bile acid synthesis and cholesterol excretion in humans, rabbits, and the Zucker diabetic fatty rat (17, 18, 25).

High dietary fat intake has been shown to induce perturbations in insulin signaling and rates of lipid synthesis via increased hepatic free fatty acid (FFA) flux and triglyceride (TG) accumulation (8). Insulin resistance can also be induced by consumption of refined carbohydrates, and excessive intake of these may be particularly deleterious with respect to increasing the risks of insulin resistance (24). Specifically, diets high in fructose have been shown to contribute to weight gain, hyperlipidemia, and metabolic disturbances (26, 29).

Several rodent models of diet-induced insulin resistance have been employed to investigate potential underlying mechanisms. Among these is the “sand rat” (Psammomys obesus), a gerbil that develops obesity and insulin resistance when its habitual diet of desert plants is replaced by standard rodent chow. These animals exhibit signs of hyperglycemia, insulin resistance, dyslipidemia, obesity, and hyperphagia (2, 38). There is also evidence of insulin receptor (IR)-signaling pathway dysfunction, hepatic insulin resistance, and insensitivity to the satiety factor leptin (32, 39).

The fructose-fed Syrian golden hamster model has also been very informative in studies of diet-induced hepatic and intestinal insulin resistance. Fructose feeding for a 2-wk period induces hepatic and whole body insulin resistance accompanied by increases in plasma TG, cholesterol, and FFA but does not cause obesity or type 2 diabetes (35). Fructose-induced insulin resistance is associated with a considerable rise in the production of hepatic VLDL-apolipoprotein (apo)B and -TG (35). Interestingly, intestinal overproduction of apoB-48 lipoproteins has also been demonstrated in this model (14, 15). Thus the fructose-fed hamster is a model of relatively mild insulin resistance, hypertriglyceridemia, and increased hepatic and intestinal apoB lipoprotein overproduction.

In the present study, we characterize a novel dietary model that combines fat, fructose, and cholesterol (the FFC diet) in a manner that realistically represents macronutrient consumption in the Western diet, illustrating the progression of a chronic dietary induction of metabolic syndrome. We present new and intriguing data clearly implicating dietary cholesterol as a major determinant

* These authors contributed equally to the work described in this article.

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of the degree of diet-induced hypertriglyceridemia and insulin resistance. Supplementation of a high-fructose/high-fat diet with cholesterol led to more severe dyslipidemia, aggravation of insulin resistance, and progression toward hyperglycemia/type 2 diabetes. These effects were cholesterol concentration dependent. Overall, our data in the hamster suggest that dietary cholesterol acts synergistically with dietary fructose and fat to induce a severe insulin-resistant state with severe metabolic complications.

MATERIALS AND METHODS

Animal protocols. Male Syrian golden hamsters (Mesocricetus auratus, 80–110 g) were obtained from Charles River (Montreal, QC, Canada) and housed individually on an alternating 12:12-h light-dark cycle with free access to food and water. After 1 wk of acclimatization, hamsters were fasted for 5 h prior to baseline blood collection and then placed on either the control diet (chow; Dyets, Bethlehem, PA) or one of three test diets enriched in fat and fructose (FF) or FFC. Table 1 shows detailed compositional analysis of the FFC test diets. Body weight and food consumption were monitored at regular intervals throughout the study period. Upon termination of the study, liver tissue sections were analyzed for TG and cholesterol concentrations following collection at a rate of 0.5 ml/min.

Intraperitoneal glucose tolerance test. Baseline blood glucose was measured following a 5-h fast, as described above. Hamsters were then given an intraperitoneal injection of 2 g glucose/kg body wt (as a solution of 30% glucose in water). Glucose was measured from saphenous vein blood samples at subsequent intervals of 15, 30, 60, and 120 min.

Lipid extraction and TG and cholesterol mass measurement in tissue. Lipid extraction and analysis were performed as described previously (31).

In vivo TG production. To determine whether FFC feeding promotes increased in vivo release of TG from the liver, a catheter was placed in the jugular vein of Syrian golden hamsters that were fed each of the test diets for 2 wk. The day after cannulation the hamsters were fasted for 12 h, and blood samples were collected at 0, 30, 60, and 90 min (300 µl/time point) following an iv bolus (600 mg/kg) of 20% (wt/vol) Triton-WR1339 (Sigma) in saline. Blood samples were centrifuged at 6,000 rpm at 4°C for 10 min to separate plasma. Plasma TG was measured using Trigs kit (Randox), and expression of apoB and apoA-I were assessed by immunoblotting. Since Triton-WR1339 effectively blocks the activity of lipoprotein lipase in vivo and therefore blocks VLDL particle clearance, the total amount of VLDL-apoB and VLDL-TG is proportional to the amount of newly synthesized VLDL. This method has been used previously in our hamster model (23).

Immunoblot analysis. Frozen tissues were homogenized in solubilizing buffer (3 mmol/l imidazole, 250 mmol/l sucrose at pH 7.5; 1 mmol/l PMSF, 100 kallikrein-inactivating units/ml aprotinin) using a mechanical homogenizer. For subcellular isolation, homogenates were centrifuged (2,200 × g) to separate the nuclear pellet and the cytosol. Nuclear pellets were then lysed using buffer A (PBS containing 1% NP-40, 24 mmol/l sodium deoxycholate, 5 mmol/l EDTA, 1 mmol/l EGTA, 2 mmol/l PMSF, 100 kallikrein-inactivating units/ml aprotinin, pH 7.4). Protein concentrations of tissue lysates were determined using the Bradford method (DC Protein assay kit; Bio-Rad, Hercules, CA). Equal amounts of protein from all samples were resolved by SDS-PAGE and detected by immunoblotting using antibodies obtained from the following sources: sterol regulatory element-binding protein-1c (SREBP-1c) from Santa Cruz Biotechnology (Santa Cruz, CA), fatty acid synthase (FAS) from Novus Biologicals (Littleton, CO), peroxisome proliferator-activated receptor (PPAR)α from Research Diagnostics (Concord, MA), and protein tyrosine phosphatase 1B (PTP1B) from Upstate Cell Signaling (Lake Placid, NY). Determination of tyrosine phosphorylation of IR and IR substrate-1 and -2 in primary hamster hepatocytes. Hamsters were treated in vivo with 5 U/kg of insulin for 2 min via the inferior vena cava. Livers were harvested, homogenized in the presence of phosphatase inhibitors, and analyzed for IR, IR substrate (IRS)-1, and IRS-2 as described (36).

Real-time quantitative PCR analysis. Messenger RNA levels of a number of genes (see Table 2) were assessed by real-time quantitative RT-PCR. PCR reactions were carried out using SYBR Green PCR Master Mix (Applied Biosystems, Streetsville, ON, Canada). Relative quantities of mRNA were calculated from threshold cycle (CT) values by the comparative CT method, using 18S rRNA as an internal reference.

Pancreatic islet morphology. Pancreata were extracted from the hamsters and snap-frozen in optimum cutting temperature compound (Sakura Finetek, Torrance, CA) using liquid N2 and then stored at −80°C. Sections (5–10 µm) were prepared from each pancreas at three levels separated by 100 µm (n = 3 chow fed and n = 3 FFC fed for 20 wk). Pancreatic sections were mounted onto slides and stained for insulin or with Oil Red O and counterstained with hematoxylin. Slides were digitized on a brightfield scanner at ×200 magnification. Analysis of staining area was performed with ImageScope software (Aperio Technologies, Vista, CA) using the positive pixel count algorithm.

Statistical analyses. Statistical significance was evaluated using one-way ANOVA. Posttest analysis was performed to determine the significance between groups, using unpaired two-way Student’s t-tests. Parameters were deemed to be significantly different if P < 0.05.

RESULTS

The severity of metabolic disturbances in the FFC model is cholesterol concentration dependent. Hamster feeding studies were performed using four test diets: normal rodent chow (control diet), a high-fructose/high-fat diet (FF), FF supple-

Table 1. Detailed composition of the hamster diets

<table>
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<th>Chow</th>
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<td>41.18</td>
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Dietary analysis, %mass

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<tr>
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<td>0.01</td>
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FF, fat and fructose; FFC, fat, fructose, and cholesterol.

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Table 2. Genes assessed by real-time quantitative RT-PCR

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Continued...
mented with low cholesterol (FFC 0.05%), and FF containing a higher cholesterol content (FFC 0.25%). Hamsters on all of the test diets showed a trend toward increased body weight gain compared with chow (Fig. 1A); however, this did not reach statistical significance after 6 wk of feeding (n = 6). In addition, all of the treatment groups showed a trend toward increased epididymal fat pad weight, but again this was non-significant (Fig. 1B). No significant differences in food consumption (Fig. 1C) or fasting blood glucose (Fig. 1D) were observed during the 6-wk study period (n = 6). However, among the treatment groups, slight increases in plasma insulin were observed at 2 and 4 wk of feeding, and by 6 wk the FFC (0.25%) group had significantly higher insulin levels compared with chow-fed controls (0.73 and 0.16 ng/ml, respectively, P < 0.05).

Fig. 1. Fat, fructose, and cholesterol (FFC)-induced metabolic disturbances. Effects of various test diets on weight gain (A), epididymal fat content (B), daily food consumption (C), blood glucose (D), and plasma insulin, cholesterol, and triglyceride (TG) (E–G). Hamsters were maintained for 6 wk on one of the following test diets: chow, fat and fructose (FF; 40% fructose/30% fat), or FFC (FF diet supplemented with either 0.05 or 0.25% cholesterol). Blood was collected following a 5-h fasting period. Values are means ± SE (n = 6). *P < 0.05 vs. chow; #P < 0.05 vs. FF dietary background. Lipoproteins from plasma were analyzed by fast-performance liquid chromatography (FPLC) following 4 wk of feeding. FPLC profiles for cholesterol (H) and TG (I) are representative of plasma pools from 3 animals.

Table 2.—Continued

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<thead>
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| SREBP-1 and -2, sterol regulatory element-binding protein-1 and -2, respectively; FAS, fatty acid synthase; apoB, -A-I, -E, and C-III, apolipoproteins B, A-I, E, and C-III, respectively; MTP, microsomal triglyceride transfer protein; SCD-1, stearoyl-CoA desaturase-1; LXRα, liver X receptor-α; PPARα, peroxisome proliferator-activated receptor-α; FXR, farnesyl X receptor; DGAT2, diacylglycerol acyltransferase-2; CPT Iα, carnitine palmitoyltransferase Iα; ACADVL, acyl-CoA dehydrogenase, very long chain; LCAT, lecithin-cholesterol acyltransferase; PGC-1α, PPARγ coactivator-1α; CETP, cholesteryl ester transfer protein; LDLR, LDL receptor; ABCA1, ATP-binding cassette transporter A1; SR-BI, scavenger receptor class B type I; PEPCK1, phosphoenolpyruvate carboxykinase 1; G6PD, glucose-6-phosphate dehydrogenase. Primer pairs for PCR were designed using Primer3 software and sequence information obtained from Genbank.

*No hamster sequence available. In these cases primers were designed to highly conserved regions determined by multiple sequence alignments performed on closely related species.
All diets induced significant increases in plasma cholesterol compared with chow, although the increase was greatest with FFC (0.25%) feeding (Fig. 1F). The FFC (0.25%) diet also caused a significant increase in plasma TG (Fig. 1G) compared with both chow and FF-fed animals. Initially, the metabolic effects of dietary cholesterol were tested on several dietary backgrounds, including chow, fat alone, and fructose alone. Interestingly, cholesterol supplementation of the chow diet did not induce dyslipidemia or other metabolic changes (data not shown). These effects were observed only when cholesterol was introduced on the high-fructose and high-fat (FF) background.

Since plasma lipid and insulin levels appear to reach their maximum levels at the 2- to 4-wk mark, the following studies were performed in this time period. Plasma samples from the four different diet groups were pooled and subjected to FPLC fractionation following 4 wk of feeding (Fig. 1, H and I). VLDL-TG was increased three- and fourfold in the 0.05% FFC and 0.25% FFC diet groups, respectively. VLDL cholesterol was also increased two- and five-fold in the 0.05% FFC and 0.25% FFC groups, respectively. In addition, there was a 0.5- and a 2.5-fold increase in the HDL cholesterol fraction of 0.05% FFC and 0.25% FFC groups, respectively.

Cholesterol content of the FFC diet determines the degree of insulin resistance and the extent of hepatic steatosis. Glucose tolerance tests were performed on hamsters from all three dietary groups at 2 and 4 wk. FF and FFC (0.25%) fed animals showed evidence of glucose intolerance and insulin insensitivity (Fig. 2A). At 2 wk both FFC (0.25%) and FF showed decreased insulin sensitivity compared with chow, and by 4 wk this effect was exacerbated. In addition, by 4 wk the FFC (0.25%)-fed group showed significantly greater insulin resistance compared with the FF group as well. Hamsters fed FFC (0.05%) also showed significant insulin resistance compared with chow at 4 wk. This effect was slightly more severe than that seen with the FF diet; however, 0.25% FFC-fed hamsters were significantly more insulin resistant than 0.05% FFC-fed hamsters (data not shown). This indicates that the addition of cholesterol to the FF diet has a dose-dependent effect on the severity of insulin resistance.

Figure 2, B and C, shows hepatic TG and total cholesterol content by mass. The increases in both hepatic TG mass and total cholesterol appear to be dependent on the cholesterol concentration of the diet, with highest levels observed with the FFC (0.25%) diet. The liver morphology (Fig. 2D) between the different diet-fed groups was even more striking. There was a modest increase in liver size after 20 wk on the FFC (0.05%) diet; however, FFC (0.25%)-fed animals showed a remarkable increase in liver size. In addition, gross pathology of the FFC (0.25%)-fed liver showed evidence of considerable lipid deposition and steatosis.

FFC feeding induces increased TG production. In vivo TG production was monitored following an intravenous dose of Triton-WR1339 (which blocks lipoprotein lipolysis and clearance; Fig. 3). Hamsters fed one of the four test diets for 2 wk were cannulated and fasted, and Triton-WR1339 was administered to block lipoprotein lipase-mediated clearance of TG-rich lipoproteins. Blood samples were collected at various time points.

Fig. 2. Cholesterol content of the FFC diet determines the degree of insulin resistance and the extent of hepatic steatosis. A: intraperitoneal (ip) glucose tolerance at 2 (top) and 4 wk (bottom) (n = 5). B and C: hepatic tissue TG and total cholesterol content, respectively (n = 6–8). D: hamster livers at 20 wk. *P < 0.05 vs. chow. IPGTT, intraperitoneal glucose tolerance test.
points to measure plasma TG, apoA-I, and apoB. Triton infusion resulted in a time-dependent increase in TG accumulation among all diet groups. FFC (0.25%)-fed hamsters had consistently higher TG levels compared with all other diet groups; however, when the slope of each curve was calculated, it was observed that chow-fed hamsters had the greatest rate of plasma TG increase. Although fasting TG levels are higher in FFC (0.25%)-fed animals, the rate of hepatic TG production is higher in chow-fed animals, indicating that the increase in TG levels might be due to decreased catabolism of TG rather than increased TG production. Plasma levels of apoA-I and apoB were also assessed by immunoblotting at each time point, but no significant differences were observed between any of the diets tested (data not shown).

Long-term FFC feeding: effects on blood glucose, plasma lipids, and pancreatic β-cell function. We next evaluated the effect of long-term FFC feeding. To produce this more severe model of insulin resistance and dyslipidemia, we extended FFC feeding to 22 wk. The FFC diet caused a significant increase in fasting blood glucose compared with chow-fed controls at 8 wk ($P < 0.05$; Fig. 4A), which was normalized by 12 wk on the diet. Increased fasting blood glucose concentrations were accompanied by increased circulating plasma insulin levels in the FFC animals, which was statistically significant at 2 and 8 wk (Fig. 4B). Insulin levels normalized by 20 wk, suggesting possible induction of a compensatory mechanism that improved insulin sensitivity and/or reduced insulin secretion.

Plasma cholesterol and TG concentrations, significantly elevated by FFC feeding as early as 2 wk, remained elevated throughout the course of the study (Fig. 4, C and D, respectively, $P < 0.05$). Cholesterol feeding did not induce neutral lipid accumulation in pancreatic islets or β-cell dysfunction. After 20 wk of FFC, pancreata were stained with insulin and counterstained with hemotoxylin. The islets of the hamsters did not show any gross changes in islet architecture; however, there was a small but significant increase in the average islet size in the FFC hamsters ($P < 0.05$; Fig. 4E). In addition, Oil Red O staining of pancreatic sections showed that there was no neutral lipid accumulation in islets after 20 wk on either the chow or FFC diet (data not shown). No significant changes were observed in hepatic mitochondrial area/number (based on staining of liver tissue sections for the mitochondrial enzyme succinic acid dehydrogenase; data not shown).

**FFC-induced alteration in mRNA levels of key metabolic genes.** Hepatic mRNA abundance for a number of key lipogenic and metabolic genes was measured by real-time PCR using hepatic tissue from 2- and 20-wk chow- and FFC-fed hamsters (Fig. 5A). After 2 wk of FFC, hepatic mRNA for SREBP-1c, a key regulator of lipogenesis, was increased 2.4-fold ($P = 0.023$) compared with chow-fed controls. This
FFC feeding alters key genes involved in lipid metabolism. We next attempted to further elucidate the mechanism underlying the effects of cholesterol feeding on lipid metabolism; therefore, another study was conducted to determine the effects of prolonged FFC feeding on the expression of several metabolic genes (Fig. 6). FFC feeding for 6 wk caused a moderate, but significant, increase in expression of PPARγ coactivator-1α (PGC-1α; 1.8-fold, n = 6, P < 0.0001), a transcriptional coactivator of PPARγ that promotes mitochondrial biogenesis and increases the transcription of genes involved in oxidative metabolism. Expression of two mitochondrial enzymes, ACADVL (acyl-CoA dehydrogenase, very-long-chain; involved in the oxidative metabolism of very-long-chain fatty acids) and carnitine palmitoyltransferase I (CPT I), which mediate the transport of long-chain fatty acids across the outer mitochondrial membrane, were both increased with FFC feeding (1.4-fold for both, n = 6, P < 0.005). These results suggest
that FFC induces increases in activity of specific mitochondrial enzymes, possibly in an attempt to deal with the oversupply of lipid substrate originating from consumption of the FFC diet. In addition, supplemental dietary cholesterol resulted in significantly decreased LDL receptor (LDLR) expression in a dose-dependent manner. LDLR is key to the uptake and catabolism of LDL particles. As such, this suggests that FFC diet may reduce lipoprotein uptake, which could contribute to the major increase in plasma TG observed with FFC feeding.

LXR activation mimics the dyslipidemic and glucose-intolerant effects of dietary cholesterol. To better understand the mechanism behind the cholesterol effect observed here, we compared the effect of high-cholesterol feeding together with activation of the nuclear receptor liver X receptor (LXR). One of the primary metabolic pathways of cholesterol involves its conversion to oxysterol intermediates, which act as natural activators of LXR (4). Thus it appears likely that dietary cholesterol may exert some of its metabolic effects via LXR activation. LXR agonist supplementation was compared directly with cholesterol supplementation on the same dietary background (FF) over 2 wk. After 2 wk of dosing with the LXR agonist TO901317 (20 mg kg\(^{-1}\)day\(^{-1}\) in 1% carboxymethylcellulose by oral gavage), both the cholesterol-supplemented and the LXR-activated groups exhibited large increases in plasma TG (7.5- and 5.3-fold, respectively; Fig. 7A). This increase appeared to be mediated in part by the FF diet, since LXR activation in chow-fed animals resulted in only a fourfold increase in plasma TG (data not shown). Both dietary cholesterol and LXR activation also increased plasma cholesterol (Fig. 7A), albeit to a more modest degree (100 and 40% above baseline, respectively). Furthermore, the FF and TO901317 (FFLXR) and FFC groups demonstrated increased hepatic lipid accumulation, with both groups showing significantly higher TG in the liver compared with chow animals (\(P < 0.05\); Fig. 7B). However, the FFC group was the only one that showed a significant increase in hepatic cholesterol storage (\(P < 0.05\)). These lipid increases were accompanied by altered plasma lipoprotein profiles; both the LXR agonist and supplemental dietary cholesterol caused plasma TG and cholesterol to shift significantly toward the VLDL fraction (Fig. 7, C and D).

To gain insight into the integrity of the insulin response in the treated hamsters, the glucose disposal of the animals in each group was measured by an intraperitoneal glucose tolerance test. Regular monitoring of blood glucose for 2 h following glucose injection revealed that both the FFC and FFLXR groups exhibited impaired glucose tolerance compared with the chow-fed group (Fig. 8A). These results indicate that the effects of dietary cholesterol may be at least partially mediated through activation of LXR.

Effect of chronic LXR activation on hepatic gene expression. Changes in expression of select target genes were assessed to delineate some of the molecular pathways that may be responsible for the dyslipidemia observed following LXR activation (Fig. 8B). Since LXR is a transcription factor, one would expect activation to increase transcription of its downstream target genes, including SCD and FAS. After 16 days, SCD mRNA levels were significantly altered by the combination of the FF diet and the LXR agonist, achieving a level 2.6 times that of chow-fed animals (\(P = 0.033\)). FFC-fed animals showed a nearly significant twofold increase in FAS expression relative to chow (\(P = 0.06\)).

Other transcription factors, including SREBP-1c and carbohydrate response element-binding protein (ChREBP), are
known to be controlled directly by LXR (9, 28). The FFC and FFLXR groups elicited roughly similar fourfold increases in SREBP-1c mRNA relative to chow-fed animals (P < 0.007). The addition of LXR agonist combined with FF stimulated a 4.3-fold increase (P < 0.03) in ChREBP mRNA. However, Chrebp’s expression was not potently stimulated with the FFC diet, which achieved only a 2.8-fold increase in expression relative to chow (P = 0.052).

The cholesterol transporter ATP-binding cassette transporter A1 (ABCA1) is a direct LXR target. Its level was measured to verify experimental LXR activation. Both treatment groups showed an increase in ABCA1 mRNA following 16 days of treatment compared with the chow-fed group (P < 0.0001). Abca1 regulation was equivalent for both groups, with FFLXR and FFC groups able to induce an approximately ninefold increase in expression.

![Fig. 7](image-url) Treatment with an LXR agonist (TO901317) mimics the effects of dietary cholesterol on plasma and hepatic lipids and lipoproteins. Hamsters were fed chow, FFC (0.25%), or FF and TO901317 (FFLXR; 20 mg·kg⁻¹·day⁻¹) for 16 days. A: plasma lipids are expressed as % increase over baseline levels (n = 6). Hepatic lipids (B) and plasma lipoproteins (C and D) were analyzed following the 16-day treatment period (n = 6). *P < 0.05 vs. chow-fed controls.

![Fig. 8](image-url) Effect of chronic LXR agonist treatment on whole body glucose tolerance and hepatic mRNA levels. A: ip glucose tolerance and area under the curve (AUC) values following 16-day feeding with chow, FFC (0.25%), or FFLXR (n = 6). B: hepatic mRNA levels were measured by real-time quantitative RT-PCR. Means ± SE are expressed relative to chow-fed controls (n = 6). *P < 0.05 vs. chow.
DISCUSSION

A new nutritionally induced insulin-resistant hamster model was employed in this study, clearly highlighting the importance of nutrient-nutrient interaction, specifically the interaction of fructose with dietary fat and cholesterol, all of which are present in significant amounts in a typical Western diet. This is a novel animal model that combines the advantages of the well-characterized fructose-fed hamster model with the use of a diet that more realistically represents macronutrient consumption in the current Western diet. Our data appear to implicate dietary cholesterol as a major determinant of the severity of insulin resistance and dyslipidemia, at least in this hamster model. Hamsters fed the FFC diet showed very rapid progression toward insulin resistance and dyslipidemia (1–2 wk, with dyslipidemia evident as early as 5 days; data not shown). Prolonged feeding (6–22 wk) led to elevations in fasting blood glucose levels (suggesting development toward overt type 2 diabetes) and hepatic steatosis (due largely to liver accumulation of cholesterol ester). The development of these metabolic disturbances was cholesterol concentration dependent. Cholesterol supplementation of chow diet did not induce these metabolic defects; however, our data suggest that cholesterol acts synergistically with dietary fructose and fat to induce a state of severe dyslipidemia, hepatic steatosis, and insulin resistance. It is noteworthy that data published in the early 1990s by Fungwe and colleagues (12, 13) also showed that dietary cholesterol can induce profound hypertriglyceridemia and increased VLDL secretion in rats. They suggested that the lipogenic effects of dietary cholesterol are secondary to stimulation of de novo lipogenesis and reduced fatty acid oxidation. More recently, Deng et al. (9) demonstrated that combining fructose and cholesterol in the rat diet can induce cardiac insulin resistance and dyslipidemia. In addition, cholesterol feeding in rabbits has been shown to induce fatty liver (20). These studies demonstrate that cholesterol feeding can induce aspects of the metabolic syndrome in a variety of rodent models and dietary backgrounds.

Previous work in our laboratory utilizing fructose-fed hamsters indicates that fructose feeding alone can induce increases in hepatic TG secretion and promote insulin resistance; these observations were exacerbated in our current study with the addition of dietary fat and cholesterol. The additive effect of fat and cholesterol together with fructose resulted in a much more severe phenotype and exacerbated increases in plasma insulin and TG levels (specifically in the VLDL fraction). In addition, fructose feeding by itself did not increase fasting plasma glucose or cholesterol levels (35), contrary to what we observed here with the FFC diet. Hepatic lipid accumulation and liver steatosis were also much more pronounced with FFC feeding. This effect is similar to what has been reported in rats, where fructose feeding causes increases in plasma TG and insulin levels. However, with FFC feeding in hamsters, these conditions are exacerbated by cholesterol resulting in a three- to fourfold increase in these parameters compared with a merely twofold increase in the fructose-fed rat (3). Our results demonstrate clearly the ability of dietary cholesterol to promote dyslipidemia and insulin resistance when coupled with the fat/fructose diet and establish the FFC-fed hamster as a model of insulin resistance, dyslipidemia, and hepatic steatosis more severe than other previously used models.

It was shown recently that a cellular cholesterol transporter, ABCA1, may be responsible for maintaining cholesterol homeostasis and normal insulin secretion in pancreatic β-cells (5), suggesting that cholesterol accumulation may be a contributing factor in β-cell dysfunction. In the present FFC-fed hamster model, there was no evidence of neutral lipid accumulation in the pancreatic islets of FFC hamsters and no significant β-cell defect. However, the hamsters (especially those on the high-cholesterol diet) did appear to develop peripheral insulin resistance between 4 and 12 wk, as evidenced by the marked increase in plasma insulin levels accompanied by unchanged fasting glucose concentrations. Further evidence to support this comes from the drastic shift in the intraperitoneal glucose tolerance test seen among hamsters in the high-cholesterol group. Although the other test diets display similar trends, the effect is most pronounced in the high-cholesterol group, indicating the potential role of cholesterol in exacerbating the effects of dietary fructose and fat.

Our results also demonstrate that FFC (0.25%) feeding increases mRNA for PGC-1α (which promotes mitochondrial biogenesis and increases the transcription of genes involved in oxidative metabolism), ACADVL (which is involved in oxidative metabolism of very-long-chain fatty acids), and CPT Iα (which mediates transport of these fatty acids across the mitochondrial membrane). These findings suggest that hepatic mitochondrial function may be enhanced to compensate for the large amount of lipid substrate available in FFC-fed hamster hepatocytes.

Lipid and lipoprotein data in the FFC model show evidence of a severe dyslipidemic state, suggesting that dietary cholesterol supplementation may act synergistically with dietary fructose and fat to induce profound alterations in lipid/lipoprotein metabolism through novel mechanisms. High-cholesterol feeding induced increased fasting TG plasma levels, and there was a profound increase in hepatic cholesterol mass. Increases in plasma TG were not a result of increased hepatic TG secretion, as indicated by the Triton experiments. This would indicate that the most likely mechanism of hyperlipidemia would be decreased clearance. This is supported by the observation that FFC feeding resulted in decreased LDLR expression. LDLR is integral in uptake and degradation of LDL particles, and its decline is most likely the principal cause of the observed hyperlipidemia in the FFC-fed animals. Cholesterol feeding may alter the composition of lipoprotein particles, and this may induce changes in lipolysis, uptake, and clearance.

Dietary cholesterol is also known to play an important role in the regulation of hepatic lipid/lipoprotein output at the transcriptional level. The nuclear transcription factor LXR is a cholesterol sensor and can potentially be activated by metabolites derived from dietary cholesterol. In the FFC model, we observed marked increases in SCD-1 mRNA and protein levels, suggesting increased conversion of fatty acids to TG. These data suggest selective activation of SCD-1-mediated fatty acid esterification in this model, which may be in response to the need to desaturate and esterify the large amount of dietary fatty acids presented to the liver from the FFC diet. SCD is thought to be upregulated mainly via SREBP-1-dependent signaling, although studies have shown that cholesterol and lipids can regulate SCD gene expression through a mechanism independent of SREBP-1 maturation, controlling both...
SCD’s transcription and mRNA stability (28). Results from this study showing steady or even declining levels of SREBP-1 after 20 wk suggest that upregulation of SCD likely occurred independently of SREBP-1 pathways. SCD activity has also been shown to be rapidly increased upon the feeding of high-carbohydrate diets (40). Carbohydrate control of SCD regulation independent of SREBP-1 signaling was found in a study involving long-term fructose feeding in SREBP-1c−/− mice (27). In this study, SCD was induced even in the absence of SREBP-1c maturation. Finally, a recent study by Chu et al. (6) also showed that SCD has an LXR response element and can be activated independently of SREBP-1. Increased SCD-1 expression may be explained by oxysterol-mediated activation of LXR, a known transcriptional activator of SCD-1 (30). Together, past data combined with the current results suggest SCD as a point of convergence for the dietary components of fat, fructose, and cholesterol. These data also indicate that the extensive hepatic steatosis that was observed with FFC feeding could be a result of LXR activation of SCD-1, since LXR activation has been shown to lead to increased hepatic TG accumulation in mice (30). Since cholesterol metabolites are potential activators of LXR, this could explain the increased steatosis seen with the increases in dietary cholesterol. Interestingly, we also found that activation of LXR induces effects remarkably similar to those seen with cholesterol feeding. Direct comparison of cholesterol supplementation with LXR activation on the FF diet background showed that both treatments caused reduced glucose tolerance, increased plasma lipids, and dysregulated lipoprotein profiles. The similarity in the effects of cholesterol and the LXR agonist on the expression of key target genes such as SREBP-1c, ABCA1, and ChREBP appears to underlie the similar metabolic changes observed. Since cholesterol molecules are naturally converted to oxysterols after consumption, and since oxysterols are natural LXR activators, we postulate that chronic dietary cholesterol feeding may induce metabolic abnormalities via chronic induction of LXR and its downstream metabolic pathways. It is also likely that there is increased de novo TG synthesis from fructose. Increased lipogenesis, the lack of greater liver TG secretion, and a possible reduction in fatty acid oxidation may all contribute to fatty liver.

Evidence of reduced hepatic insulin signaling in the FFC diet-fed hamster model was observed after merely 2 wk of feeding, as shown by reductions in the tyrosine phosphorylation of the IRβ subunit and decreases in the mass of both IRS-1 and IRS-2. A likely contributor to the tyrosine dephosphorylation of the IRβ in the FFC diet-fed hamsters is the elevation of the hepatic protein level of PTP1B, a tyrosine phosphatase that is responsible for the dephosphorylation and inactivation of the IR and previously shown to be increased with fructose feeding (36). Insulin signal transduction is generally impaired by chronic high-fat feeding. In both rats and hamsters, it has been shown that hepatic insulin-stimulated receptor autophosphorylation is significantly reduced upon high-fructose feeding (36). Both the protein and mRNA levels of IRS-1 and IRS-2 were reduced in rats fed high-fat diets (1), likely associated with increased hepatic FFA flux (22).

In conclusion, data presented in this article indicate that a more severe dyslipidemia and progression toward overt type 2 diabetes can be induced when high-fructose feeding is combined with high-fat and cholesterol diets. The marked effects of dietary cholesterol on the metabolic phenotype in this model were particularly intriguing and suggest a potentially important role of dietary cholesterol (and nutrient-nutrient interactions) in the rising rates of obesity and type 2 diabetes in humans. New clinical nutritional trials are needed to test this hypothesis in humans.

REFERENCES

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