

Fasting and Postprandial Overproduction of Intestinally Derived Lipoproteins in an Animal Model of Insulin Resistance

EVIDENCE THAT CHRONIC FRUCTOSE FEEDING IN THE HAMSTER IS ACCOMPANIED BY ENHANCED INTESTINAL *DE NOVO* LIPOGENESIS AND ApoB48-CONTAINING LIPOPROTEIN OVERPRODUCTION*

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Insulin-resistant states are characterized by hypertriglyceridemia, predominantly because of overproduction of hepatic very low density lipoprotein particles. The additional contribution of intestinal lipoprotein overproduction to the dyslipidemia of insulin-resistant states has not been previously appreciated. Here, we have investigated intestinal lipoprotein production in a fructose-fed hamster model of insulin resistance previously documented to have whole body and hepatic insulin resistance, and hepatic very low density lipoprotein overproduction. Chronic fructose feeding for 3 weeks induced significant oversecretion of apolipoprotein B48 (apoB48)-containing lipoproteins in the fasting state and during steady state fat feeding, based on (a) *in vivo* Triton WR1339 studies of apoB48 production as well as (b) *ex vivo* pulse-chase labeling of intestinal enterocytes from fasted and fed hamsters. ApoB48 particle overproduction was accompanied by increased intracellular apoB48 stability, enhanced lipid synthesis, higher abundance of microsomal triglyceride transfer protein mass, and a significant shift toward the secretion of larger chylomicron-like particles. ApoB48 particle overproduction was not observed with short-term fructose feeding or *in vitro* incubation of enterocytes with fructose. Secretion of intestinal apoB48 and triglyceride was closely linked to intestinal enterocyte *de novo* lipogenesis, which was up-regulated in fructose-fed hamsters. Inhibition of fatty acid synthesis by cerulenin, a fatty acid synthase inhibitor, resulted in a dose-dependent decrease in intestinal apoB48 secretion. Overall, these findings further suggest that intestinal overproduction of apoB48 lipoproteins should also be considered as a major contributor to the fasting and postprandial dyslipidemia observed in response to chronic fructose feeding and development of an insulin-resistant state.

The metabolic syndrome, which is characterized by fasting hypertriglyceridemia, insulin resistance, glucose intolerance, hypertension, and obesity (1), appears also to include impaired postprandial lipoprotein metabolism (2, 3). Postprandial triglyceride-rich lipoproteins and especially chylomicron remnants have been implicated as risk factors for atherosclerosis and progression of coronary artery disease, based on both experimental work (4, 5) and cross-sectional studies (6–8). Emerging evidence suggest that intestinal lipoproteins may be particularly atherogenic in diabetes (9). We (10, 11) and others (12–15) have shown previously that there is an elevation of postprandial triglyceride (TG)¹-rich lipoproteins in subjects with insulin resistance and type 2 diabetes and that fasting hypertriglyceridemia predicts this abnormal postprandial response to a fat load. A strong correlation also exists between plasma insulin and the postprandial TG response to a fat meal, and the postprandial levels of large VLDLs and chylomicron remnants (3, 16). In the fasting state, plasma insulin, a marker of insulin resistance, is also related to fasting plasma levels of large VLDL and CM remnants (16) and increased fasting remnant lipoproteins have been observed in insulin-resistant subjects (3, 17–19).

It is not known whether the accumulation of these potentially atherogenic chylomicron remnant lipoproteins occurs as a result primarily of increased intestinal secretion of apoB48-containing chylomicron particles, diminished clearance from the circulation or both. Perhaps because intestinal fat absorption is highly efficient and because the intestine is felt primarily to be an absorptive rather than a secretory organ, the majority of investigations have focused on the retarded plasma clearance of alimentary lipoproteins as an underlying mechanism for postprandial hypertriglyceridemia (9). Mechanistic information regarding the biogenesis of apoB48-containing lipoproteins in the intestine of insulin-resistant and type 2 diabetic patients is strikingly absent from the literature. Early studies showed that in the fasting state the intestine is capable of VLDL-like particle secretion from endogenously synthesized substrate (20). The intestinal contribution to the fasting total body TG production was estimated to range from 10 to 40% of total plasma TG based on studies in rats (20–24) and mongrel dogs (25). It was later suggested that the intestine maintains a basal rate of apoB48 secretion in the fasting state, which is

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¹ The abbreviations used are: TG, triglyceride; apoB48, apolipoprotein B-48; CE, cholesteryl ester; FC, free cholesterol; FF, fructose-fed; MTP, microsomal triglyceride transfer protein; VLDL, very low density lipoprotein; BSA, bovine serum albumin; TRL, triglyceride-rich lipoprotein; *S*_ρ, flotation density.

increased in the diabetic intestine (13). The contribution of the intestine to fasting endogenous hypertriglyceridemia is also markedly increased in diabetic rats (22, 26). In addition, evidence from studies of healthy men (27), women with CAD (18), diabetic patients (13), and diabetic rats (26) have all pointed to the important role of the intestine in causing increased plasma chylomicron remnant lipoproteins.

The mechanisms of intestinal chylomicron assembly and secretion in normal enterocytes are not fully understood (reviewed by Hussain (28)) and there has been a tendency to extrapolate findings from studies of hepatic cells to enterocytes. There has, however, been some progress recently using differentiated CaCo2 cells and primary rabbit enterocytes (29–31), and two models have been proposed for the intracellular assembly of chylomicrons (28). The assembly of chylomicrons is a unique characteristic of enterocytes (32), which is largely driven by dietary fat consumption, however, there is evidence suggesting that *de novo* synthesized lipid and plasma fatty acids can also act as substrates for the assembly and secretion of apoB48-containing lipoproteins (33, 34).

The Syrian golden hamster has attracted increasing attention as a model for studies of lipoprotein metabolism, because it appears to more closely resemble that in humans (35, 36). The tissue-specific expression of apoB100 (only in the liver) (37, 38) and apoB48 (only in the intestine) is a distinct advantage of the hamster model. Previously, we reported that high fructose feeding for a 3-week period induced significant hypertriglyceridemia and hyperinsulinemia and the development of whole body insulin resistance in the Syrian golden hamster (39). Hepatic VLDL apoB overproduction was also directly associated with attenuated hepatic insulin signaling and insulin resistance (40). In the present study, we present both *in vivo* and *ex vivo* evidence that development of a nutritionally induced insulin-resistant state in the hamster may also be accompanied by overproduction of intestinal apoB48-containing lipoproteins in both the fasting and postprandial states.

EXPERIMENTAL PROCEDURES

Animal Protocols—Male Syrian golden hamsters (*Mesocricetus auratus*, Charles River, Montreal, QC, Canada) were housed in pairs and were given free access to food and water. After blood collection, animals were placed on either a control diet (normal chow) or fructose-enriched diet (hamster diet with 60% fructose, pelleted; Dyets Inc., Bethlehem, PA). The diet was continued for 3 weeks and hamster weight was monitored every 2 days. Animals were fasted 16 h before isolation of intestinal enterocytes.

Isolation of Primary Hamster Enterocytes and Hepatocytes—To investigate the molecular mechanisms of apoB48 biogenesis and chylomicron assembly we have developed a method for the isolation of adult viable villi from Syrian golden hamster small intestine. The protocol developed for isolation of epithelial cells from hamster small intestine was based on that described by Perreault and Beaulieu (41). In this protocol, dissociation of the intestinal epithelial from the mesenchyme is achieved by using Matrisperse, a dissociating solution initially designed to recover epithelial cells grown on extracellular matrix. Specimens of small intestine from hamsters weighing between 88 and 110 g were obtained after anesthesia by isoflurane (4 in 50% oxygen and 50% nitrous oxide). The small intestine was opened longitudinally, washed in phosphate-buffered saline, and cut into 5 × 5-mm fragments. The fragments obtained were transferred to a plastic culture dish containing 7 ml of ice-cold Matrisperse (Collaborative Biomedical Products, BD PharMingen, Mississauga, Ontario, Canada) and incubated at 4 °C for 4 h without agitation. The dish was then gently shaken to separate the villi, and the villi suspension was washed twice in ice-cold phosphate-buffered saline (180 × g, 5 min). After the final spin, the villi were resuspended in Dulbecco's modified Eagle's medium supplemented with 1% fetal bovine serum and placed in an incubator (37 °C, 5% CO₂, 95% air, 100% humidity).

The viability and functional specificity of primary enterocytes were examined by trypan blue exclusion assay, protein synthesis rate, and secretion of a specific intestinal protein, apoB48. We consistently obtained more than 90% cell viability, for 4 h, based on total protein

synthesis activity as well as the synthesis of apoB48. The incorporation of [³⁵S]methionine into trichloroacetic acid-precipitable protein indicated a high degree of viability (103,406 ± 21,343 cpm/mg of protein/h; *n* = 8).

Metabolic Labeling of Intact Primary Hamster Enterocytes—Primary hamster enterocytes were preincubated in methionine-free Dulbecco's modified Eagle's medium at 37 °C for 30 min and pulsed with 30–50 μCi/ml of [³⁵S]methionine for 20–25 min. After the pulse, the cells were washed twice and chased in Dulbecco's modified Eagle's medium supplemented with 40 mM methionine. At various chase times, triplicate dishes were harvested, and cells were lysed in solubilization buffer (42). The lysates were used for immunoprecipitation as described (42).

In Vivo Determination of Intestinal Particle Production Rates—Male Syrian golden hamsters, weighing 88–110 g, were studied after 3 weeks of fructose feeding or chow diet as described above. At the end of the 3-week feeding period, femoral venous and arterial catheters were inserted under isoflurane anesthesia as previously described (39), for blood sampling and Triton administration, respectively. The animals were fasted overnight for 12 h prior to insertion of the catheters in the morning, followed by either fasting studies or the fat feeding studies performed that afternoon. They were allowed to awaken from anesthesia and were unrestrained in their cages for the duration of fasting or fat feeding study. The fat feeding study was conducted by manually administering 400 μl of lard orally by gavage at 0 h and then every 20 min over a 1½ time period. Preliminary experiments (*n* = 4) demonstrated that this method of feeding resulted in constant *S*_r > 400 (*i.e.* large TG-rich lipoprotein (TRL)) and *S*_r 100–400 (small TRL), TG and apoB48 concentrations between 60 and 80 min (*i.e.* the time period of measurement of intestinal particle production rates following administration of Triton). For the fat feeding experiment, 1 h after starting feeding (at 60 min) an intravenous bolus of Triton WR-1339 (Sigma) was administered. Blood samples were drawn through the arterial catheter at 60 and 80 min (total blood volume withdrawn was 1.2 ml). *S*_r > 400 and *S*_r 100–400 particles were isolated by ultracentrifugation. TG and apoB48 was quantified as previously described (43, 44).

The fact that the hamster liver makes a negligible amount of apoB48 (37, 38) makes the measurement of apoB48 a good indicator of intestinally derived lipoprotein. Large and small apoB48 and TG secretion rates were performed by multiplying the slope of the concentration increase of apoB48 (in μg/ml/min) and TG (in μmol/ml/min), respectively, over time by the intravascular distribution volume estimated as 3.8 ml/100 g body weight, as previously described (39). A two-tailed unpaired *t* test was used to compare the large and small TRL TG and apoB48 secretion rates between groups.

SDS-PAGE and Fluorography—SDS-PAGE and fluorography were performed essentially as described (45). To quantify the radioactivity associated with apoB48, the bands corresponding to these proteins were visualized by fluorography, excised from the gel, digested, and subjected to scintillation counting. Chemiluminescent immunoblotting was carried out as described (42).

Lipid and Lipoprotein Analysis—Primary enterocytes were pulsed for 3 h with 10 μCi/ml [³H]acetate to assess the rate of synthesis and secretion of cholesterol and CE. TG synthesis and secretion were monitored by labeling cells for 3 h with 5 μCi/ml [³H]oleate bound to bovine serum albumin. Thin layer chromatography of lipid extracts were performed as described (42).

Density gradient ultracentrifugation was performed based on a method previously optimized to isolate large chylomicron, small chylomicron, and VLDL from cell culture media by sequential ultracentrifugation (31). To separate the *S*_r 20–60 lipoproteins (VLDL), centrifugation was continued for 17 h and the top 1 ml collected. The rest of the gradient was fractionated into seven additional 1.5-ml fractions. Fractions 2–4 and 5–7 were considered intermediate density lipoprotein/low density lipoprotein (*d* = 1.02–1.063 g/ml) and high density lipoprotein (1.063–1.1 g/ml), respectively.

RESULTS

Primary Hamster Enterocytes Synthesize and Secrete ApoB48-containing Lipoproteins—Pulse-chase experiments were performed to analyze the stability and secretion of apoB48 in intestinal cells isolated from chow-fed hamsters. Villus enterocytes from hamsters synthesized and secreted exclusively apoB48 and there was no evidence of apoB100 synthesis or secretion (Fig. 1). Intracellular apoB48 was not quantitatively secreted and there was evidence of intracellular degradation of apoB48 (Fig. 1). The

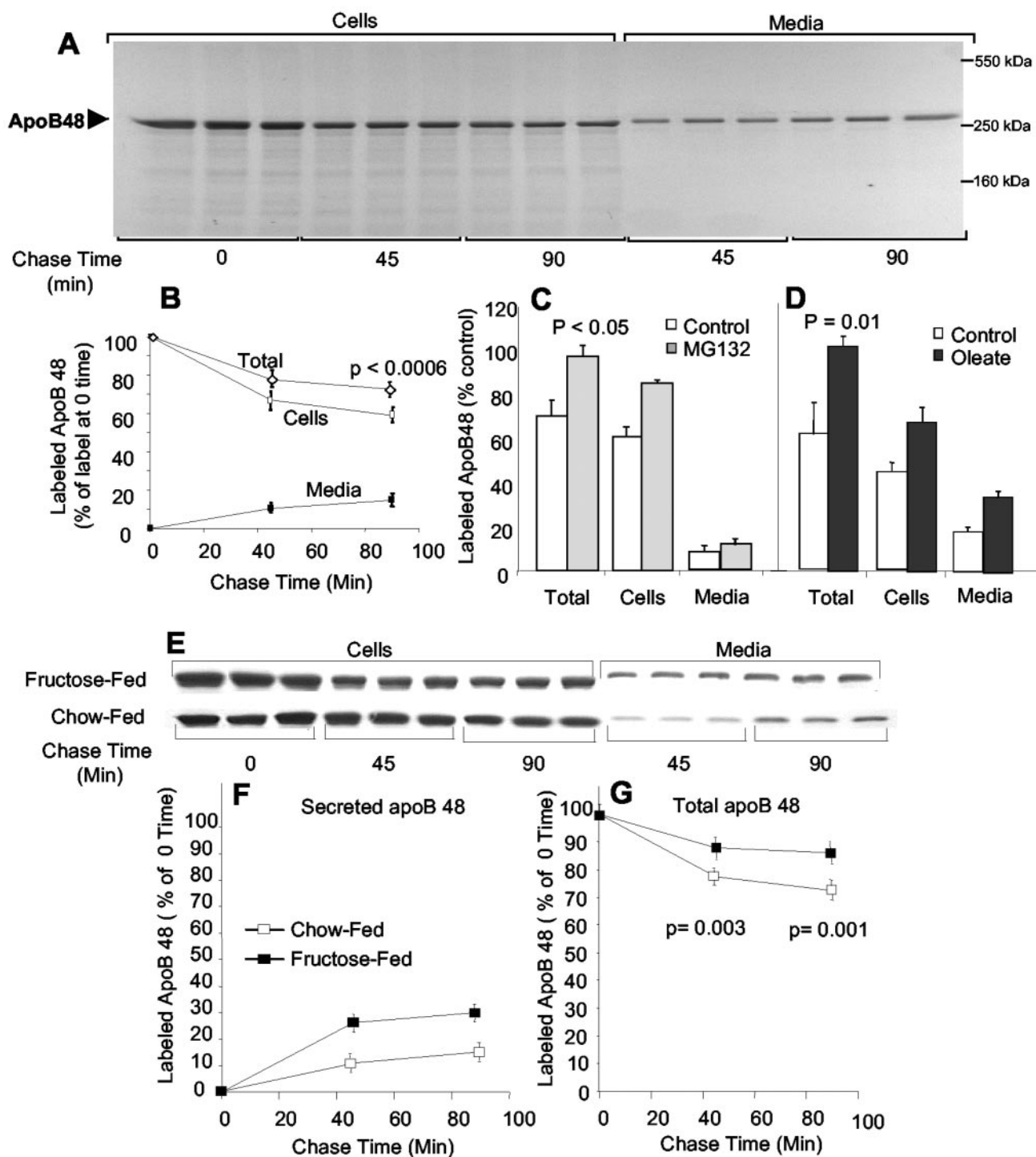


FIG. 1. Ex vivo secretion and regulation of apoB48 by primary hamster enterocytes and stimulation in FF hamsters. Primary enterocytes, isolated from fasting hamsters, were pulsed with [³⁵S]methionine and chased for 0, 45, and 90 min. The media samples and cell lysates collected at each chase time point were subjected to immunoprecipitation and then analyzed by SDS-PAGE and fluorography. *Panel A* shows a representative experiment in a chow-fed hamster (four separate pulse-chase labeling experiments were performed with similar results). Note that hamster enterocytes exclusively secreted apoB48 and there was no evidence of apoB100. *Panel B* shows the distribution of apoB48 secreted into the medium (filled squares) as well as cellular and total apoB48 (open squares and open diamonds). In *panels C* and *D*, similar experiments ($n = 3$) were performed in the presence of MG132 (25 μ M) and oleic acid (0.72 mM, complexed to albumin; oleic acid/bovine serum albumin ratio, 8:1). Shown are the total labeled cellular and secreted apoB48 recovered from control and MG132-treated enterocytes (*panel C*) or from control and oleic acid-treated enterocytes (*panel D*). In *panels E-G*, pulse-chase labeling experiments were performed in primary enterocytes isolated from hamsters fed either a chow or fructose-enriched diet (following a 16-h fast). *Panel E* shows a representative experiment in chow-fed ($n = 3$) and FF ($n = 3$) hamsters. *Panel F* and *G* show the distribution of immunoprecipitable apoB48 in media (*panel F*), and cell + media (total) (*panel G*), respectively.

percentage of newly synthesized apoB48 degraded after a 90-min chase was estimated at $31 \pm 4\%$, $p < 0.0006$ (Fig. 1B). *In vitro* treatment of enterocytes with a proteasome inhibitor (N-carbobenzoxy-L-leucyl-L-leucyl-L-norleucinal, MG132)

(25 μ M) significantly reduced apoB48 degradation (Fig. 1C), suggesting the involvement of the ubiquitin-proteasome system in intestinal apoB48 secretion. Similarly, incubation with oleic acid (0.72 mM bound to bovine serum albumin at a 8:1 ratio) enhanced

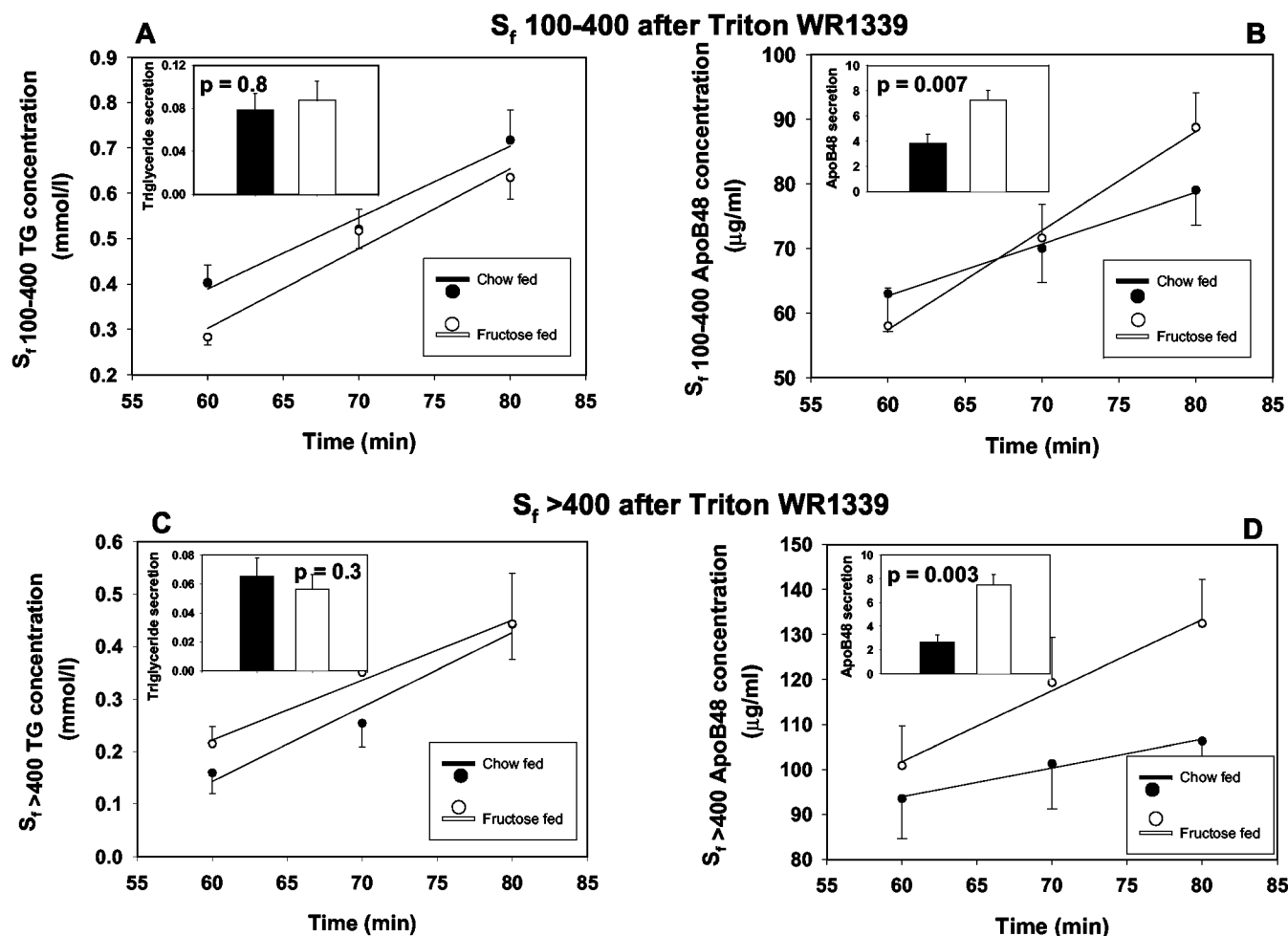


FIG. 2. *In vivo* production of TG and apoB48 in control and FF hamsters in the fasting state. A, S_f 100–400 TG concentration over time after intravenous administration of Triton WR-1339 in FF ($n = 7$, open circles) versus control hamsters ($n = 7$, closed circles). The TG secretion rate (in μ mol/min) is shown in the inset in the FF animals (open bars) compared with the controls (closed bars) ($p = 0.8$). B, S_f 100–400 apoB48 concentration over time during the same experiments as in A. The apoB48 secretion rate (in μ g/min) is shown in the inset in the FF (open bars) compared with the control hamsters (closed bars) ($p = 0.007$). C, S_f > 400 TG concentration over time during the same experiments as in A. D, S_f > 400 apoB48 concentration over time during the same experiments as in A. The apoB48 secretion rate (in μ g/min) is shown in the inset in the FF (open bars) compared with the control hamsters (closed bars) ($p = 0.003$).

intestinal apoB48 stability and increased its extracellular secretion (Fig. 1D).

Chronic Fructose Feeding Stimulates Intestinal ApoB48 Secretion in Fasted Hamsters—Chronic fructose feeding for a period of 3 weeks was previously shown to induce hepatic VLDL-apoB overproduction concomitant with attenuated hepatic insulin signaling and whole body insulin resistance (39, 40). Here we investigated intestinal lipoprotein secretion in the fructose-fed (FF) hamster model. First, we employed pulse-chase labeling experiments to assess the stability and secretion of apoB48 in villus enterocytes isolated from chow-fed and FF hamsters (Fig. 1E). Fig. 1, F and G, shows the extracellular secretion and the intracellular turnover of apoB48 in chow-fed and FF hamster enterocytes. Enterocytes from FF hamsters secreted about 30% of the newly synthesized apoB48 over a 90-min chase compared with about 15% in chow-fed controls. There was also evidence of enhanced apoB48 stability with only 7% of apoB48 having been lost during the 90-min chase in FF hamster enterocytes compared with ~30% in chow-fed hamster enterocytes. It should be noted that in Fig. 1, F and G, apoB48 synthesis and secretion were normalized for both total cellular protein mass and the incorporation of [35 S]methionine into total trichloroacetic acid-insoluble proteins. Therefore, the stimulation of apoB48 synthesis and secretion was not a

sequence of global effects of fructose feeding on protein synthesis and secretion.

In Vivo Evidence of Fasting Intestinal Lipoprotein Overproduction in FF Hamsters—The *in vivo* production of apoB48 and TG were measured in fasted hamsters in two fractions, S_f 100–400 and S_f > 400 fractions, based on Triton WR-1339 experiments. In the S_f 100–400 fraction, the TG secretion rate was similar in both groups (0.09 ± 0.01 versus 0.10 ± 0.03 μ mol/min, respectively, $p = 0.8$) (Fig. 2A). There was a 2-fold increase in apoB48 secretion rate in the S_f 100–400 fraction in the FF hamsters compared with control hamsters (7.26 ± 0.75 and 3.85 ± 0.72 μ g/min for the FF and control group, respectively, $p = 0.007$) (Fig. 2B).

In the S_f > 400 fraction there was an ~3-fold increase in the apoB48 secretion rate in the FF hamsters compared with control hamsters (7.46 ± 0.75 and 2.65 ± 0.60 μ g/min for the FF and control groups, respectively, $p = 0.003$) (Fig. 2D). As in the S_f 100–400 fraction, there was no difference in the S_f > 400 TG secretion rate between FF and control hamsters (0.064 ± 0.012 versus 0.163 ± 0.069 μ mol/min, respectively, $p = 0.3$) (Fig. 2C).

In Vivo Evidence of Postprandial Intestinal Lipoprotein Overproduction in FF Hamsters—To further examine intestinal lipoprotein production postprandially in FF hamsters, a series of *in vivo* Triton WR-1339 studies were performed during

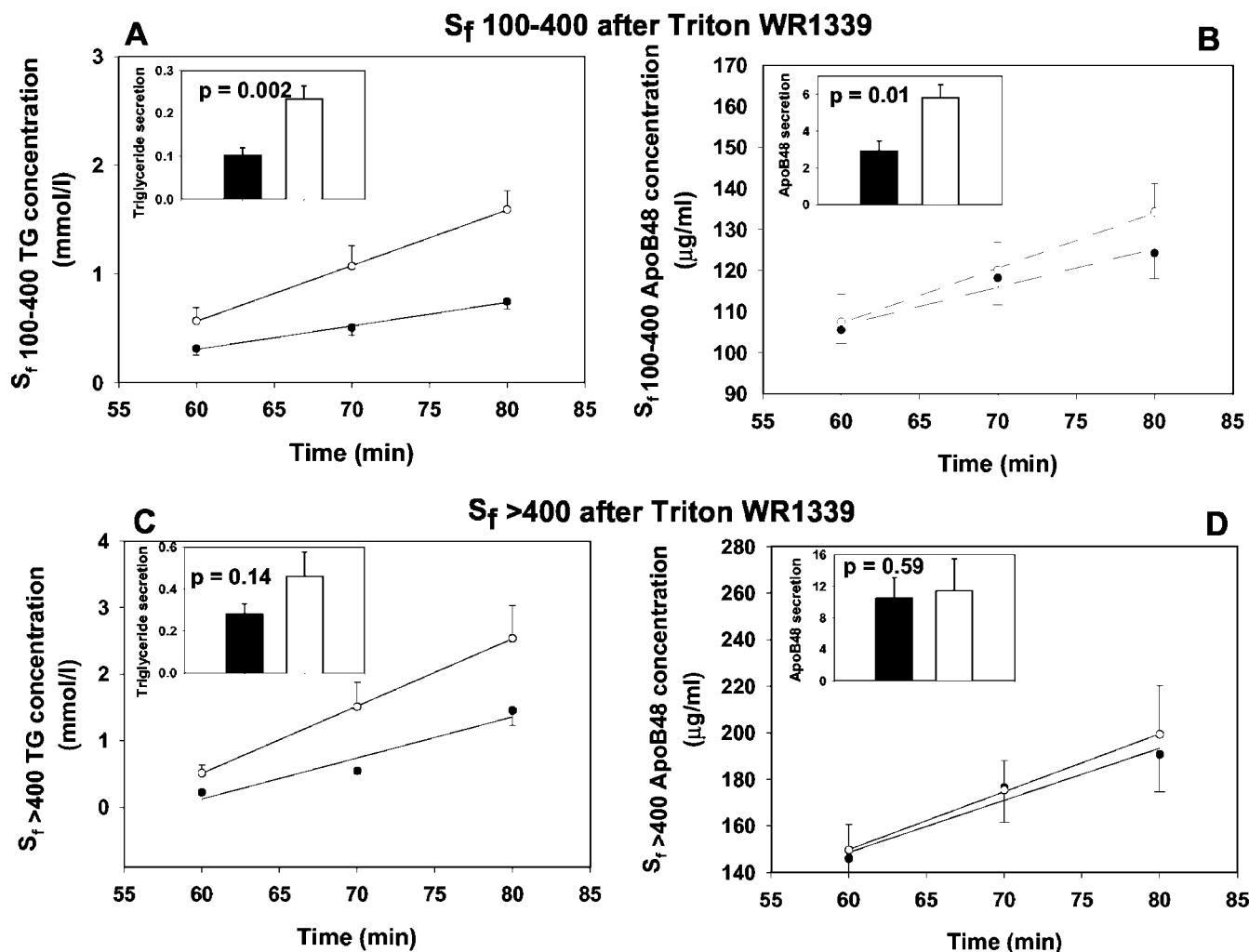


FIG. 3. *In vivo* production of TG and apoB48 in control and FF hamsters postprandially during constant fat feeding. Fasted (16 h) hamsters were manually administered 400 μ l of lard orally by gavage at 0 h and then every 20 min over a 1½-h time period. Animals were used for *in vivo* Triton WR-1339 experiments as described under "Experimental Procedures." Panel A shows S_f 100–400 TG concentration over time after intravenous administration of Triton WR-1339 in FF ($n = 9$, open circles) versus control hamsters ($n = 9$, closed circles). The slope of the line was significantly higher in the former group ($p = 0.002$). The TG secretion rate is shown in the inset in the FF animals (open bars) compared with the controls (closed bars) ($p = 0.002$). B, S_f 100–400 apoB48 concentration over time during the same experiments as in A. The slope of the line was significantly higher in the FF animals (open circles) compared with the controls (closed circles) ($p = 0.01$). The apoB48 secretion rate is shown in the inset (open bars) compared with the control hamsters (closed bars) ($p = 0.01$). C and D show the $S_f > 400$ TG and apoB48 increments after Triton, respectively, in FF (open circles) and chow-fed (closed circles) and production rates (inset). There were no significant differences between chylomicron production rates in FF versus chow-fed hamsters.

steady state fat feeding. Fasted (16 h) hamsters were manually administered 400 μ l of lard orally by gavage at 0 h and then every 20 min over a 1½-h time period. Animals were then used for *in vivo* Triton WR-1339 experiments and the production rate of apoB48 was determined in chow-fed versus FF hamsters, in two density fractions, S_f 100–400 and $S_f > 400$. In the S_f 100–400 fraction, the TG secretion rate was more than 2-fold higher in the FF versus control group (0.23 ± 0.03 versus 0.10 ± 0.02 μ mol/min, respectively, $p = 0.002$) (Fig. 3A). Similarly, the apoB48 secretion rate was significantly higher in the FF hamsters (5.81 ± 0.71 and 2.93 ± 0.53 μ g/min for the FF and control group, respectively, $p = 0.01$) (Fig. 3B).

The large TRL ($S_f > 400$) TG secretion rate was not statistically different between the FF and control groups (0.46 ± 0.12 versus 0.28 ± 0.05 μ mol/min, respectively, $p = 0.14$) (Fig. 3C). In addition, the apoB48 secretion rate was similar in the FF and control hamsters (11.46 ± 4.04 versus 10.47 ± 2.63 μ g/min, respectively, $p = 0.59$) (Fig. 3D).

Overproduction of ApoB48 in FF Hamster Is Accompanied by Enhanced Intestinal Lipid Synthesis and Secretion, and In-

creased MTP Mass and Activity—Primary hamster enterocytes isolated from chow-fed and FF hamsters were used to determine the synthesis and secretion of free cholesterol, CE, and TG. Fig. 4, A–C, shows the effect of fructose feeding on the intestinal synthesis and secretion of total lipids. The intracellular levels of FC, CE, and TG were significantly increased in enterocytes isolated from FF hamster. This increase was particularly dramatic for FC (~6-fold). Evaluation of radiolabeled lipids in culture media of primary hamster enterocytes also revealed significantly elevated secretion of FC, CE, and TG levels in FF hamster (Fig. 4, A–C).

Facilitated secretion of apoB48 and core lipoprotein lipids in FF hamster enterocytes could be related to an increased mass and/or activity of MTP, the key factor involved in the lipoprotein assembly process. To test this hypothesis equal quantities of cell lysate (20 μ g) were analyzed by immunoblotting. As shown in Fig. 4D, there was a significant increase in protein mass of MTP in FF hamster enterocytes compared with chow-fed controls.

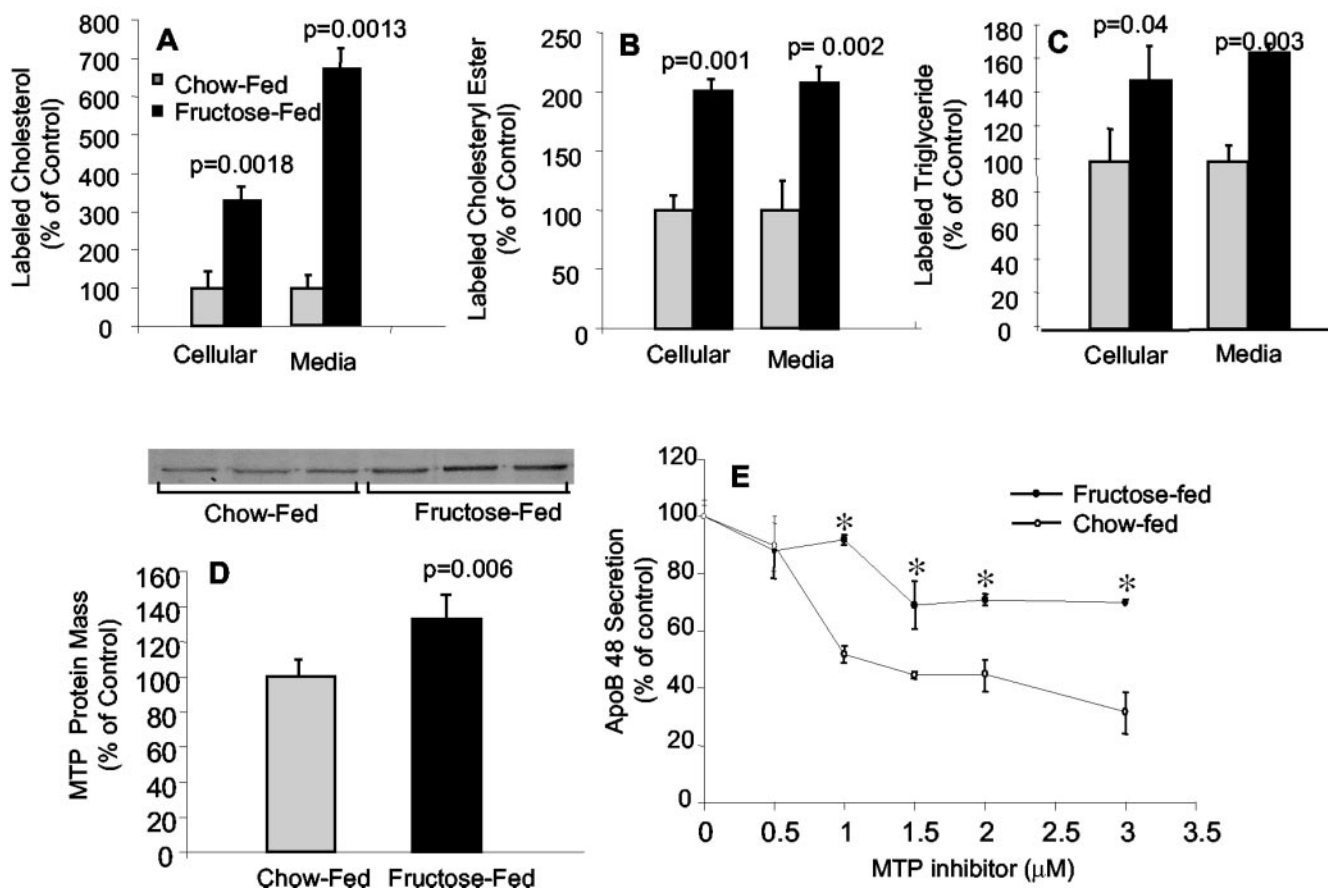


FIG. 4. **Effect of fructose feeding on intestinal lipid production and the mass and activity of MTP.** *Panels A–C*, primary enterocytes isolated from chow-fed ($n = 3$) and FF ($n = 3$) hamsters following a 16-h fast were pulsed with [^3H]acetate or [^3H]oleate/bovine serum albumin. *Panel A*, cellular and secreted level of FC. *Panel B*, cellular and secreted levels of CE. *Panel C*, cellular and secreted levels of TG. *Panels D and E* shows a comparison of the mass and activity of MTP in enterocytes from control and FF hamsters. *Panel D*, enterocytes from chow-fed ($n = 3$) and FF ($n = 3$) hamsters (following a 16-h fast) were solubilized and equal amounts of cell protein ($20 \mu\text{g}$) were subjected to SDS-PAGE, and proteins were transferred onto polyvinylidene difluoride membrane. Immunoblotting was performed to detect the 97-kDa MTP subunit with a rabbit anti-hamster MTP antiserum. Shown is a representative immunoblot (*upper panel*) and densitometric quantitation (*lower panel*) from three experiments expressed as a percentage of the MTP mass detected in chow-fed hamster intestine. *Panel E*, differential sensitivity of apoB48 secretion to MTP inhibitor, BMS-197636, in enterocytes isolated from chow-fed ($n = 3$) and FF ($n = 3$) hamsters. Enterocytes were pretreated with different concentrations of the MTP inhibitor and then pulsed for 25 min with [^{35}S]methionine and chased for 1 h. The results are expressed as labeled apoB48 secreted as a percent of that in control, Me_2SO -treated cells.

We also assessed the sensitivity of intestinal apoB48 secretion to MTP inhibition in enterocytes from chow-fed and FF hamsters, by performing titration experiments with an MTP inhibitor, BMS-197636. As shown in Fig. 4E, apoB48 secretion by enterocytes from chow-fed and FF hamsters exhibited significantly different sensitivities toward the MTP inhibitor. This is an indirect indication of a higher MTP activity in enterocytes from FF hamsters.

Effect of Fructose Feeding on the Distribution of Secreted Lipoproteins from Villus Enterocytes and Plasma Profile of ApoB48 Lipoproteins—To determine the effect of fructose feeding on apoB48 particle formation, we analyzed the density profile of secreted lipoproteins following steady state labeling of cultured enterocytes isolated from fasted hamsters. Fig. 5, A–F, shows the immunoprecipitable apoB48 particles secreted by control and FF hamster enterocytes. Control hamster enterocytes secreted apoB48 particles in a range of different densities including large chylomicrons ($S_f > 400$) (Fig. 5A). There was a switch from the secretion of small-sized particles ($d > 1.006 \text{ g/ml}$) toward larger sized particles ($d < 1.006 \text{ g/ml}$) in FF hamster enterocytes (Fig. 5B). The secretion of total apoB48-containing lipoproteins by FF hamster was higher by ~3-fold when compared with chow-fed hamster, although the increase in large chylomicron-apoB48 ($S_f > 400$) was more pronounced at almost 6-fold (Fig. 5C). The increased chylomicron-apoB48 lev-

els suggest the secretion of a considerably higher number of large chylomicron particles by FF hamster enterocytes. This indicates that fructose feeding markedly increased both the size and number of lipoprotein particles secreted into the media.

Experiments were also conducted to assess *ex vivo* secretion of apoB48 in a postprandial (fat-fed) state. The fat loading was performed as described under “Experimental Procedures.” One hour after the start of fat feeding, the animals were sacrificed and isolated villus enterocytes were subjected to pulse-chase labeling experiments to assess the secretion of apoB48 particles. As shown in Fig. 5D, in a fat-fed/postprandial state the secretion of chylomicron ($S_f > 400$) and total apoB48 particles were significantly higher in enterocytes isolated from FF hamsters.

We also carried out density fractionation of apoB48-containing lipoproteins from plasma of both chow-fed and FF hamsters and compared the profile with that observed in cultured cells. Fig. 5E shows the fasting plasma profile of apoB48-containing particles as determined by density gradient centrifugation of plasma followed by immunoblotting of all fractions with an anti-hamster apoB antibody. ApoB48 was found to be distributed across various lipoprotein fractions. Interestingly, there appeared to be a considerable amount of apoB48 present in high- and low-density lipopro-

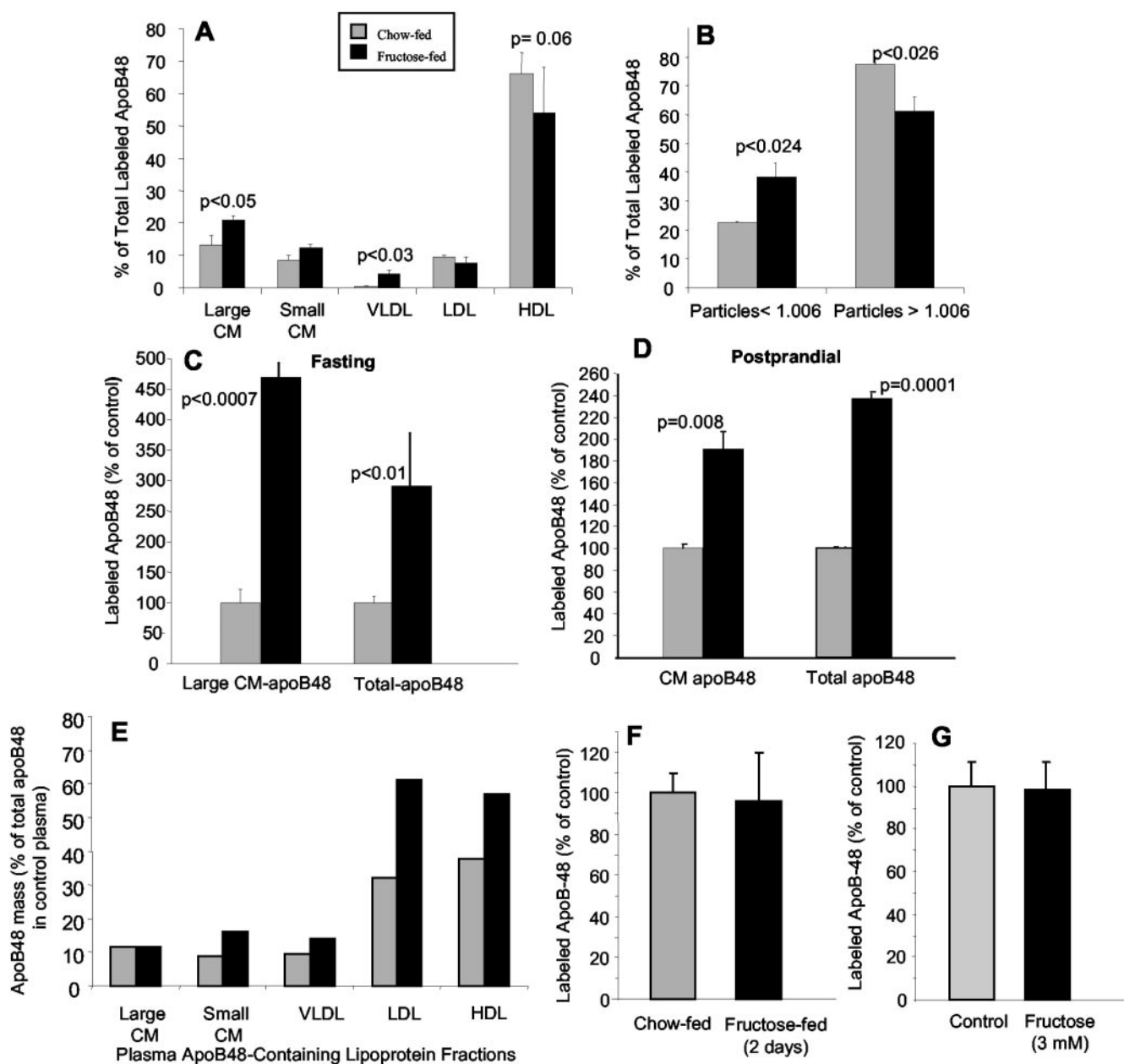


FIG. 5. Evidence for altered density distribution of intestinally derived apoB48-containing lipoproteins in FF hamsters in the fasting state and postprandially. Panels A–C, density profile of secreted lipoproteins in enterocytes isolated from chow-fed and FF hamsters (following a 16-h fast) after a 1-h pulse with [³⁵S]methionine and a 2-h chase. Panel A shows the distribution of apoB48-containing lipoproteins in media collected from chow-fed and FF hamster enterocytes. Panel B shows a comparison of the distribution of apoB48-lipoproteins of various densities. Panel C shows the comparison of total apoB48 and chylomicron-apoB48 secretion in chow-fed and FF hamster. In panel D, similar experiments were performed as above but in enterocytes isolated from chow-fed ($n = 3$) and FF ($n = 3$) hamsters postprandially (following 16 h fast and 1 h fat feeding). Panel E shows the density profile of apoB48-containing lipoproteins in plasma obtained from both chow-fed and FF hamsters. Plasma from fasting hamsters was subjected to density gradient centrifugation followed by immunoblotting of all fractions with an anti-hamster apoB antibody. Control experiments were also carried out to determine intestinal apoB48 lipoprotein secretion in response to short-term (2 day) fructose feeding of hamsters as well as the *ex vivo* exposure to exogenous fructose. Panel F, enterocytes from fasting chow-fed ($n = 3$) or FF (2 day) hamsters ($n = 3$) were pulse chased and the secreted lipoproteins ($S_f > 400$) were assessed by ultracentrifugation. Panel G, primary hamster enterocytes (from 16-h fasted animals, $n = 3$) were incubated with 3 mM fructose for 1 h, pulsed for 1 h with [³⁵S]methionine, and chased for 2 h, and secreted apoB48 was determined in the chylomicron ($S_f > 400$) fraction.

tein fractions in plasma obtained from both chow-fed and fructose-fed hamsters. Fructose-fed hamsters had higher plasma apoB48 in almost all lipoprotein fractions with a small shift toward lighter particles.

ApoB48 Overproduction Was Not Observed with Acute Fructose Feeding or in Vitro Incubation of Hamster Enterocytes with Fructose—We also performed two sets of control experiments. First, to investigate the short-term effect of fructose feeding on apoB48 lipoprotein formation, *ex vivo* experiments were per-

formed on intestinal enterocytes isolated from hamsters fed a fructose-enriched diet for 2 days. As shown in Fig. 5E, there was no significant difference in the secreted level of intestinal chylomicron-apoB48 between chow-fed and 2-day FF hamsters. Second, primary enterocytes from chow-fed hamsters were incubated *in vitro* in the presence of fructose (3 mM, 1 h) and the secretion of chylomicron apoB48 was monitored. There was no significant change in the secreted level of radiolabeled apoB48-containing chylomicron particles following incubation with

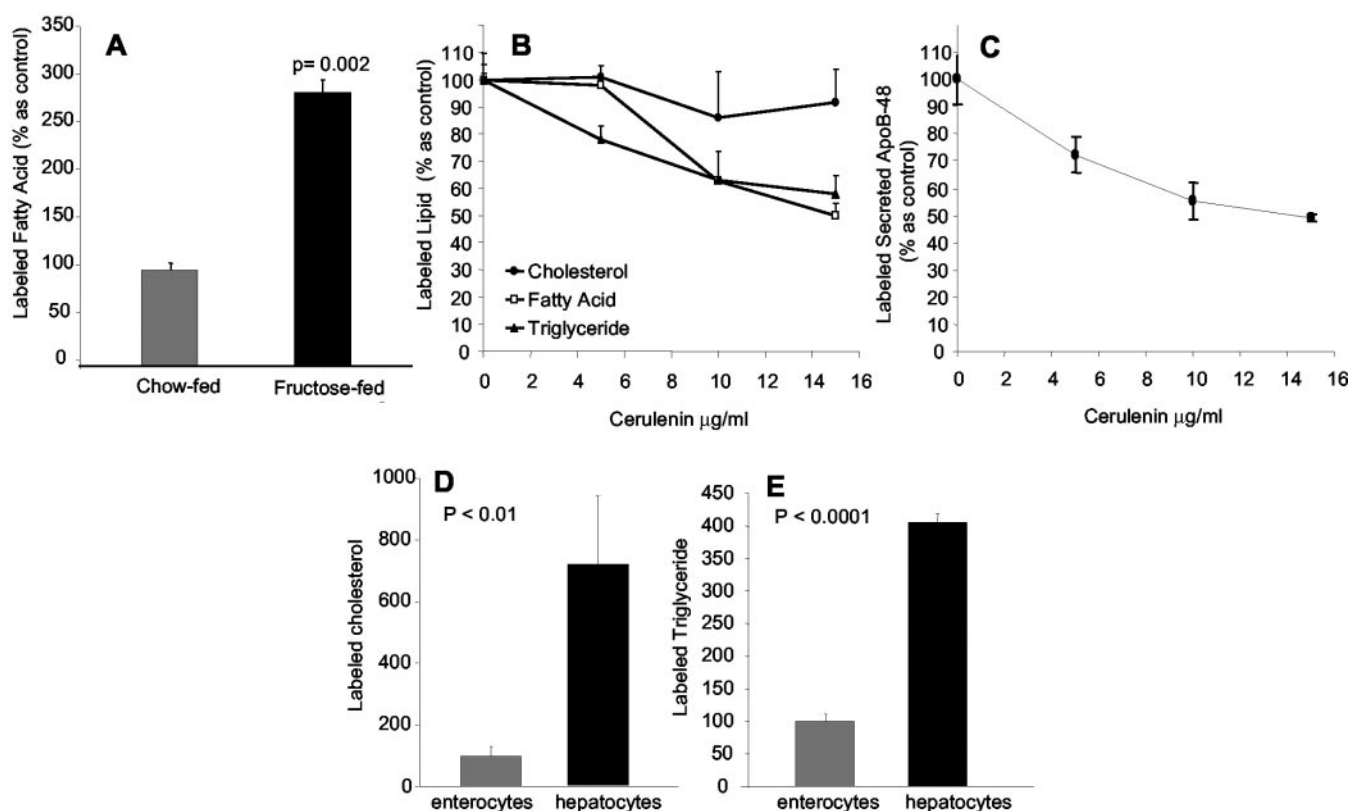


FIG. 6. Fatty acid synthesis is stimulated with fructose feeding and fasting apoB48 secretion is dependent on *de novo* lipogenesis. Panel A, primary hamster enterocytes isolated from chow-fed and FF hamster, after a 16-h fast, were pulsed with [³H]acetate and fatty acid synthesis was assessed by thin layer chromatography. Panel B, dose-dependent effect of different concentrations (0, 5, 10, and 15 µg/ml) of cerulenin, a fatty acid synthase inhibitor, on incorporation of [³H]acetate in fatty acid, TG, and cholesterol in primary enterocytes isolated from chow-fed hamsters ($n = 3$). Panel C, effect of cerulenin treatment (0, 5, 10, and 15 µg/ml) on secretion of apoB48 lipoprotein secretion in primary hamster enterocytes. Enterocyte cultures were treated with cerulenin for 1 h and pulsed with [³⁵S]methionine for 25 min. After a 60-min chase period, the media were collected and apoB48 was immunoprecipitated. In control experiments, enterocytes and hepatocytes were assessed for their ability to incorporate [¹⁴C]fructose (1 µCi/ml, 3 h) into secreted cholesterol (panel E) and TG (panel F). Data were normalized for total protein mass.

high fructose levels (Fig. 5F), ruling out acute stimulation of intestinal chylomicron secretion by exogenous fructose.

Fatty Acid Synthesis Was Stimulated with Fructose Feeding and Fasting ApoB48 Secretion Is Dependent on *de Novo* Lipogenesis—Primary hamster enterocytes from chow-fed and FF hamsters were labeled with [³H]acetate to determine the effect of fructose feeding on intestinal fatty acid synthesis (Fig. 6A). There was a marked increase in labeled fatty acid synthesis in FF hamster enterocytes. To examine the role of *de novo* synthesis of fatty acid and TG on secretion of apoB48-containing lipoprotein, cerulenin, a natural fatty acid synthase inhibitor was employed. Fig. 6B shows a dose-response effect of cerulenin on incorporation of [³H]acetate into cholesterol, fatty acid, and TG. Cerulenin treatment inhibited synthesis of both fatty acid and TG, whereas synthesis of cholesterol was unchanged. Similar dose-response experiments were performed to also measure the effect of inhibition of fatty acid synthesis by cerulenin on intestinal apoB48 secretion. Fig. 6C shows that treatment of enterocytes with cerulenin, during a pulse-chase experiment, led to inhibition of apoB48 particle secretion in a dose-dependent manner.

Dietary Fructose Was Not an Efficient Substrate for Intestinal Lipid Secretion—Finally, we also incubated hamster enterocytes with [¹⁴C]fructose to determine whether fructose can act as a substrate for intestinal lipoprotein lipid secretion. In comparison with hamster hepatocytes, enterocytes only minimally incorporated [¹⁴C]fructose into media lipids over a 3-h period. (Fig. 6, D and E), suggesting that fructose is a poor substrate for *de novo* lipogenesis in the enterocyte.

DISCUSSION

In the present study we have demonstrated overproduction of intestinal apoB48-containing lipoproteins in the FF Syrian golden hamster, a model of dietary-induced insulin resistance and hypertriglyceridemia. Hypersecretion of apoB48 lipoproteins was demonstrated not only in response to fat feeding but also in the fasted state. We showed that chronic but not acute fructose feeding is associated with greater stability of intracellular apoB48, enhanced intestinal enterocyte *de novo* lipogenesis, and up-regulation of the key enzyme involved in intestinal lipoprotein assembly, MTP. The mechanism of intestinal overproduction of apoB48-containing lipoprotein particles in this insulin-resistant animal model therefore has a number of similarities to hepatic overproduction of apoB100-containing lipoprotein, as we have previously demonstrated (39). These findings suggest that intestinal overproduction of apoB48-containing lipoproteins in insulin-resistant states may be an important contributor to the elevation of circulating TG-rich lipoproteins, both in the fasting and fed states, and potentially could be an important contributor to atherosclerosis in this condition.

It is generally assumed that chylomicrons transport predominantly exogenously ingested TG derived from dietary sources, whereas VLDL particles transport endogenous TG from liver synthesis. There is, however, growing evidence that in the fasting state the intestine synthesizes VLDL-like particles constitutively (20, 46). As far back as 1969, Ockner *et al.* (20) found intestinally derived VLDL-size particles in fasting lymph of rats and suggested this to be the major lipoprotein secreted in

the fasting state, responsible for the transport of endogenous lipids. Furthermore, Risser *et al.* (22) compared the intestinal contribution to endogenous VLDL-TG production in control and insulin-deficient streptozotocin-induced diabetic rats. Using the Triton WR-1339 procedure in the fasting state, the authors found a large increase (more than 2-fold) in intestinal secretion of VLDL-TG in diabetic rats. Using a mesenteric lymph fistula rat model, Popper *et al.* (26) also found that during fasting, diabetic rats have a greater than 2-fold increase in TG output from intestine. The TG was carried mainly by VLDL-like particles *in vivo*. These observations suggest a significant role of intestinal VLDL-like TG secretion in the endogenous hypertriglyceridemia in diabetic rats. Previous investigations (33) revealed that during absorption a substantial fraction (>50%) of total mesenteric lymphatic TG is derived from endogenously synthesized sources. Furthermore, Gangl and Ockner (34) have shown that during a luminal lipid infusion in rats, incorporation of labeled plasma fatty acid into intestinal lymph TG increases 6-fold when compared with the fasting state. Our findings of increased *de novo* synthesis of fatty acid after fructose feeding and decreased apoB48 lipoprotein secretion after treatment of enterocytes with a fatty acid synthase inhibitor suggest that endogenous production of fatty acids, in the fasting state, plays an important role in apoB48 lipoprotein secretion by the hamster intestine. This endogenous source of TG may be under hormonal and nutritional control and can potentially be modulated in insulin-resistant states (47).

Recent data in our laboratory has shown that fructose feeding in hamsters induced an insulin-resistant state, which was accompanied by hepatic VLDL-apoB overproduction (40). In the present study, we employed the FF hamster model to explore potential alterations in intestinal chylomicron production. First, *in vivo* Triton WR-1339 studies following a fat load demonstrated that chronic fructose feeding caused a significant increase in intestinal production of both TG and apoB48 in small TRL with a nonsignificant trend toward higher levels in large TRL. This overproduction of apoB48 lipoproteins occurred in the face of an equivalent level of infused fat load. *In vivo* experiments performed in the fasting state confirmed hypersecretion of apoB48-containing lipoproteins. In contrast to the postprandial studies, however, the fasting studies showed significantly greater secretion of both large ($S_f > 400$) and small (S_f 100–400) intestinal particles in FF *versus* chow fed hamster. We speculate that the higher basal fasting secretion rate of large particles is not apparent in the postprandial state because postprandially absorption of ingested fat becomes the major determinant of chylomicron ($S_f > 400$) secretion rate, thereby eliminating any potential difference between insulin-resistant (FF) and control animals. The lack of a significant difference between FF and the control hamster TG secretion rate in both large and small fractions in the fasting *in vivo* studies may relate to the lower sensitivity of the TG assay than the apoB48 assay at these extremely low levels of TG in the fasting experiments. Alternatively, these findings could imply that there is increased production of small, lipid-poor apoB48-containing lipoproteins in the fasting state with fructose feeding.

The *in vivo* findings were confirmed by the *ex vivo* experiments employing cultured primary enterocytes. Because there is one apoB48 molecule per particle and because the efficiency of apoB48 incorporation into the particle is highly dependent on lipid availability in the intestinal cell, the increased number of lipoprotein particles may be because of more efficient intestinal fat absorption in the FF hamster, or other factors. The former possibility is less likely because fat absorption is known

to be highly efficient and is rarely the rate-limiting factor in determining the postprandial TG excursion (48, 49). A series of *ex vivo* experiments were also conducted in enterocytes from fasted hamsters that allowed us to address the mechanism, independent of dietary fat consumption. Three essential factors for assembly of intestinally derived lipoproteins including *de novo* lipogenesis, stability of apoB48 particles, and MTP were examined. Analysis of lipid biosynthesis revealed a significant increase in intracellular and secreted TG, FC, and CE, accompanied by an increase in intracellular fatty acid synthesis in enterocytes isolated from FF hamsters. This increase was more pronounced for both intracellular and secreted FC. The small intestine is second only to the liver in the rate of *de novo* cholesterol synthesis and in some animal models, including hamster, the intestine actually contributes more cholesterol to total body stores than does liver (50). Previous studies have shown that cholesterol synthesis in the small intestine is increased in animal models of diabetes (51, 52) and type 2 diabetic patients (15). Increased intestinal cholesterol esterification has also been reported in diabetes (53, 54).

The comparison of apoB48 degradation in primary hamster enterocytes isolated from FF and chow-fed hamsters revealed a significant enhancement of intracellular stability of newly synthesized apoB48 with only a minor fraction being sorted to intracellular degradation in FF hamster. An increased secretion of apoB48-containing lipoproteins accompanied the increased intracellular stability of apoB48 in FF hamster. Furthermore, Western blotting experiments demonstrated an increased mass of MTP in FF relative to chow-fed hamster. These observations were confirmed by the detection of a lower degree of sensitivity of apoB48 secretion to MTP inhibition in FF hamster enterocytes. Gleeson *et al.* (55) also reported an increased MTP mRNA level in intestine of streptozotocin-induced diabetic rats. In the present study, overproduction of apoB48-containing particles in enterocytes isolated from fasted FF hamsters may be attributable to the combination of an increased abundance of MTP, and the presence of both higher availability of core lipoprotein lipids, TG, and cholesterol, as well as apoB48. The analysis of lipoprotein formation in enterocytes derived from FF hamsters revealed a considerable stimulation of chylomicron assembly under this metabolic condition. This was evident from the reduced formation of small-sized particles and increased secretion of large-sized lipoprotein particles in FF hamster enterocytes. This finding further suggested the enhanced efficiency of apoB48 particle assembly in FF hamster enterocytes. Control studies with short-term (2 day) fructose feeding, and *in vitro* incubation of hamster enterocytes with high fructose or [^{14}C]fructose appear to rule out the possibility that fructose can directly stimulate intestinal lipid and lipoprotein synthesis and secretion, or act as a substrate for *de novo* lipogenesis in the intestine. This finding is in agreement with previous reports that fructokinase activity is minimal in the intestine (56).

In conclusion, the evidence obtained in the FF hamster model suggests that intestinal overproduction of apoB48 containing particles occurs in response to chronic fructose feeding and may result from an interaction between induction of *de novo* lipogenesis, a higher availability of core lipids, higher intracellular stability of apoB48, and an increased abundance of MTP, leading to facilitated lipoprotein assembly and secretion. The finding that intestine may secrete apoB48-containing lipoproteins in the fasting state from *de novo* lipid synthesis is particularly intriguing. We postulate that there is ongoing production of intestinally derived lipoprotein particles in the postabsorptive state. Chronic fructose feeding and the development of an insulin-resistant state may increase this basal

(fasting) rate of endogenous VLDL-like particle production through induction of *de novo* lipogenesis and enhanced abundance of MTP. This alteration in basal synthesis of lipoproteins might in turn hypersensitize the intestine to dietary fat consumption such that insulin-resistant animals would exhibit an exaggerated response to the same dose of dietary fat because of the availability of a higher number of primordial VLDL-size particles. It is important to note that the evidence for a link between insulin resistance and overproduction of intestinal apoB48 lipoproteins is thus far indirect and it is possible that these occur by independent mechanisms. Further studies are needed to more directly examine the link between impaired insulin signaling in the enterocytes and intestinal lipoprotein overproduction. Overall, our results support the notion that the small intestine is not merely an absorptive organ but rather plays an active role in lipid homeostasis in both the fed and post-absorptive states.

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