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# Intestinal assembly and secretion of highly dense/lipid-poor apolipoprotein B48–containing lipoprotein particles in the fasting state: Evidence for induction by insulin resistance and exogenous fatty acids☆

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**Abstract**

Emerging evidence suggests that overproduction of intestinally derived apolipoprotein (apo) B48–containing lipoprotein particles may be an important contributor to both fasting and postprandial dyslipidemia in insulin-resistant states. Mechanisms regulating the assembly and secretion of apoB48-containing lipoproteins are not fully understood particularly in the diabetic/insulin-resistant intestine. In the present study, we have investigated the density profile of apoB48 lipoproteins assembled in primary hamster enterocytes. Both intracellular and secreted apoB48 particles were examined in intestinal enterocytes isolated from normal or insulin-resistant fructose-fed hamsters, as well as in enterocytes treated with exogenous oleic acid. Microsomal luminal contents and culture media were analyzed by discontinuous and sequential ultracentrifugation on sucrose and KBr gradients, respectively. ApoB48 was mostly secreted on VLDL-, LDL-, and denser HDL-sized particles in the fasting state. In pulse-chase labeling experiments, nascent apoB48-containing particles initially accumulated in the microsomal lumen as HDL-sized particles, with subsequent formation of apoB48-VLDL particles, with only a minute amount of chylomicrons observed. Treatment with 720 $\mu$mol/L of oleic acid, increased microsomal apoB48 HDL synthesis, and induced a marked shift toward lighter more buoyant particles. A marked enhancement in assembly of apoB48-containing lipoproteins was also observed in the microsomal lumen of fructose-fed hamster enterocytes, suggesting facilitated assembly and secretion of dense intestinal lipoprotein particles in insulin-resistant states. Overall, these observations suggest that a major proportion of apoB48-containing lipoprotein particles is assembled and secreted as highly dense, HDL-sized particles. The production of these small, dense, and potentially atherogenic apoB48 particles can be stimulated by increased free fatty acid flux as well as in insulin-resistant diabetes.


## 1. Introduction

Chylomicrons are light particles ($d$ < 1.000 g/mL) which are heterogeneous in size (diameters 80-1000 nm) and consist of >90% neutral lipid, predominantly triacylglycerol (TAG) with some cholesteryl ester, stabilized by a shell of amphipathic lipids (phospholipids, cholesterol), and a structural protein, apolipoprotein (apo) B48 [1]. ApoB48 is the major apolipoprotein produced by both human and hamster intestine and is essential for the assembly and secretion of intestinal chylomicrons [1,2]. Intestinal cells secrete apoB48-containing very low density lipoprotein (VLDL) and chylomicrons for the transport of biliary and dietary lipids (reviewed in Ref. [3]). The major function of chylomicrons is thought to be the transport of dietary fat, whereas constitutively synthesized intestinal VLDL-sized particles appear to provide a mechanism for the reabsorption of endogenous lipids [3].

Mechanisms regulating intestinal chylomicron assembly and secretion have received increasing attention in recent years. As with hepatocytes, it has been shown that assembly of apoB48-containing lipoproteins is a 2-step process in intestinal cells [4]. The first step produces dense apoB48 phospholipid-rich particles, whereas in the second step, these dense particles rapidly acquire the bulk of the TAG

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and phospholipids, forming large chylomicron-sized particles [4]. Interestingly, there is published evidence that in the fasting state, the intestine is capable of synthesizing and secreting VLDL-like particles from endogenously synthesized substrates [5,6]. There is evidence that the intestine maintains a basal rate of apoB48 secretion and that this rate is increased in insulin resistance [7-9]. There is also evidence for the production of small dense apoB48-containing lipoprotein particles by intestinal enterocytes. In primary enterocytes from rabbits fed a low-fat diet, newly synthesized and immunodetectable apoB48 was only found in a density subfraction similar to high-density lipoprotein [4]. A large proportion of newly synthesized apoB was also found to be secreted as VLDL as well as LDL-sized particles in differentiated CaCo-2 cells [10]. There is also evidence that hamster enterocytes secrete both higher density apoB48 particles (HDL, LDL) and more buoyant apoB48 particles (chylomicrons, VLDL) [11]. The significance of these differences in density distribution of apoB48 lipoproteins, factors regulating the degree of lipidation, as well as potential alterations in pathological conditions such as diabetes, is poorly understood.

Our understanding of the mechanisms regulating intestinal lipoprotein assembly has lagged behind our understanding of the assembly and secretion of hepatic VLDL. This is mainly caused by the lack of an appropriate cell model. Data are currently available from only 2 intestinal models, differentiated CaCo-2 cells and isolated primary rabbit enterocytes [4,12]. More recently, our laboratory has used the Syrian golden hamster to investigate intestinal lipoprotein production in both normal chow-fed animals, as well as fructose-fed (FF) insulin-resistant animals. The Syrian golden hamster is an attractive model to investigate regulation of lipoprotein metabolism as its lipoprotein metabolism closely resembles that of human beings and has a distinct advantage of tissue-specific expression of apoB100 and apoB48 by the liver and intestine, respectively [13-16]. Our recently published studies identified an interesting link between the development of an insulin-resistant state and deregulation of intestinal lipoprotein metabolism [11]. Chronic fructose feeding stimulated intestinal apoB48 secretion with a concomitant increase in apoB48 stability in fasted animals. The overproduction of apoB48 was accompanied by enhanced intestinal lipid synthesis in the form of free cholesterol, cholesterol ester, and triglyceride, as well as increases in both mass and activity of microsomal triglyceride transfer protein (MTP). These results suggest that in insulin-resistant or diabetic animals, there may be a mechanism causing enhanced intestinal secretion of lipoproteins in the fasting state. Fructose feeding may enhance this basal level of lipoprotein secretion through increased de novo lipogenesis and increased MTP availability. Comparison of plasma lipoproteins from FF animals showed a marked shift toward secretion of larger, less dense, chylomicrons in the insulin-resistant animals. Interestingly, fatty acid synthesis appeared to be stimulated upon fructose feeding. The data suggest that in addition to hepatic lipoproteins, intestinal lipoproteins may be oversecreted, and this may contribute significantly to the overall dyslipidemia associated with the insulin-resistant state. These abnormalities in intestinal lipoprotein metabolism may also explain the profound postprandial dyslipidemia observed in insulin-resistant states.

In the present study, we have used primary intestinal enterocytes from chow-fed and FF hamsters to investigate the process of assembly and secretion of apoB48-containing lipoproteins. Our observations in this report suggest that dense HDL- and LDL-sized apoB48 particles represent a major fraction of intestinally derived lipoproteins, and production of these potentially atherogenic particles can be enhanced with higher intestinal free fatty flux as well as in the insulin-resistant state.

## 2. Experimental procedures

### 2.1. Materials

Oleic acid (OA) and all fine chemicals were obtained from Sigma Aldrich (St Louis, Mo). Tissue culture medium and fetal bovine serum were from Life Technologies (Burlington, Ontario, Canada). Matrisperse was from Collaborative Biomedical Products (BD Pharmingen, Mississauga, Ontario, Canada). [$^{35}$S] methionine was obtained from Perkin-Elmer Life Sciences (Woodbridge, Ontario, Canada). Rabbit antihamster apoB antibody was commercially prepared for our laboratory. Zysorbin was from Zymed Laboratories (Inter Medico, Markham, Ontario, Canada).

### 2.2. Animal protocols

Male Syrian golden hamsters (*Mesocricetus auratus*, Charles River, Montreal, Quebec, 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 2 to 3 weeks, and hamster weight was monitored every 2 days. Animals were fasted 16 hours before isolation of intestinal enterocytes.

### 2.3. Isolation of primary hamster enterocytes

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 [17]. 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 inhalation (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 culture dish containing 7 mL of ice-cold Matrisperse and incubated at 4°C for 1 hour without agitation. The dish was then gently shaken to separate the villi, and the villi suspension was twice washed in ice-cold phosphate-buffered saline and centrifuged at 180 *g* for 5 minutes. After the final spin, the villi were resuspended in Dulbecco modified Eagle medium (DMEM) supplemented with 1% fetal bovine serum and placed in an incubator (37°C, 5% $CO_2$, 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 greater than 90% cell viability at 4 hours, based on total protein synthesis activity as well as the synthesis of apoB48. The incorporation of [$^{35}$S] methionine into trichloroacetic acid–precipitable protein indicated a high degree of viability.

### 2.4. Metabolic labeling of intact primary hamster enterocytes

Primary hamster enterocytes were preincubated in methionine-free DMEM at 37°C for 45 minutes and pulse-labeled with 150 μCi/mL of [$^{35}$S] methionine for 60 to 120 minutes. For OA experiments, pulse was performed in the presence or absence of 720 μmol/L OA conjugated to bovine serum albumin in a 1:8 molar ratio. For pulse-chase experiments, the cells were washed twice and chased in DMEM supplemented with 40 mmol/L methionine. After 1- and 2-hour chase, cells and media were harvested.

### 2.5. Isolation of microsomes and density ultracentrifugation of luminal contents

Cells were washed once with 3 to 5 mL of 250 mmol/L sucrose + 3 mmol/L imidazole, pH 7.4, and once with 3 to 5 mL of 50 mmol/L sucrose + 3 mmol/L imidazole, pH 7.4, centrifuged (1500 rpm, 4°C, 3 minutes), and harvested into 1 mL of 50 mmol/L sucrose + 3 mmol/L imidazole, pH 7.4, containing protease inhibitors (5 mmol/L EDTA, 1 mmol/L ethyleneglycotetraacetic acid, 2 mmol/L phenylmethylsulfonylfluoride [PMSF], 100 kallikrien-inactivating units [KIU]/mL aprotinin). Cells were kept on ice and homogenized with 20 strokes of a Dounce homogenizer, 200 μL of 49% sucrose solution was added, and cells were homogenized with 5 additional strokes. Cell homogenates were transferred into a microfuge tube and centrifuged at 2200 *g* for 10 minutes at 4°C to remove heavy nuclei and nonhomogenized cells. The supernatant was centrifuged using a SW55Ti swinging bucket rotor (Beckman Coulter Instruments, Fullerton, Calif) at 100 000 *g* for 1 hour at 4°C. The pellet containing the microsomal fraction was fractionated into luminal and membrane-enriched fractions by incubation of the microsomal fraction in 1 mL of 100 mmol/L sodium carbonate, pH 11.5, + 0.025% deoxycholate + 1.2 M potassium chloride + protease inhibitors for 30 minutes on ice with occasional gentle shaking [18]. After carbonate extraction of luminal contents, bovine serum albumin was added to a final concentration of 0.5%, and the suspension was centrifuged at 100 000 *g* for 1 hour at 4°C. Luminal contents were supplemented with additional protease inhibitors and subjected to ultracentrifugation either on a discontinuous sucrose gradient or on sequential KBr gradient. For sucrose gradients, microsomes were layered as follows: 1.5 mL of 49% sucrose + 3 mmol/L imidazole, 3 mL of 25% sucrose + 3 mmol/L imidazole, 2 mL of 20% sucrose + 3 mmol/L imidazole, 3.2 mL microsome sample, 1.9 mL of 5% sucrose + 3 mmol/L imidazole, and 0.9 mL of 0% sucrose + 3 mmol/L imidazole, and centrifuged at 207 000 *g* for 65 hours at 4 °C. Gradients were fractionated into 1- to 1.5-mL fractions, and each fraction was subjected to immunoprecipitation for apoB48. For KBr gradients, microsome sample was layered underneath KBr $d = 1.006$. For chylomicrons and large VLDL, the top 1 mL was collected after centrifugation at 105 000 *g* at 4°C for 32 minutes and 2 hours, respectively. The gradient was then centrifuged at 150 000 *g* at 4°C for 17 hours, the gradient was fractionated into 10 additional fractions, and each fraction was subjected to immunoprecipitation for apoB48.

### 2.6. Immunoprecipitation and SDS-PAGE

Immunoprecipitation was performed as described previously [19]. Briefly, 500 μL of solubilizing buffer and 5 μL of rabbit antihamster apoB antibody were added to each sample. Samples were incubated at 4°C for 18 hours with gentle shaking. After incubation with primary antibody, 100 μL of a 50% Zysorbin slurry (protein A) was added, and samples were mixed at room temperature for 1 hour. The samples were centrifuged at 13 000 rpm for 3 minutes, and the supernatant was removed. The pellet was washed 3 times with 1 mL of cold immunoprecipitation wash buffer (10 mmol/L Tris-HCl, pH 7.4, 2 mmol/L EDTA, 0.1% sodium dodecyl sulfate [SDS], 1% Triton X-100) resuspending the pellet for 10 minutes between each wash. Samples were prepared for electrophoresis by resuspending in 100 μL SDS–polyacrylamide gel electrophoresis (PAGE) sample buffer and boiling for 5 minutes. ApoB was resolved by 6% SDS-PAGE at 70 V, for 16 hours. The gels were exposed to Kodak Hyperfilm (Eastman Kodak, Rochester, NY) for 24 to 96 hours at −80 °C. The radioactivity in the apoB48 band was quantified by digestion of the gel slices by addition of 200 μL 60% perchloric acid and 400 μL hydrogen peroxide and 18-hour incubation at 60°C. Radioactivity was quantified by a scintillation counting. Alternately, quantitative analysis of apoB48 was performed using imaging densitometry.

## 3. Results

### 3.1. Primary hamster enterocytes assemble highly dense lipoprotein particles in the fasting state

To determine the effect of fructose feeding on apoB48 particle formation, we carried out density fractionation of

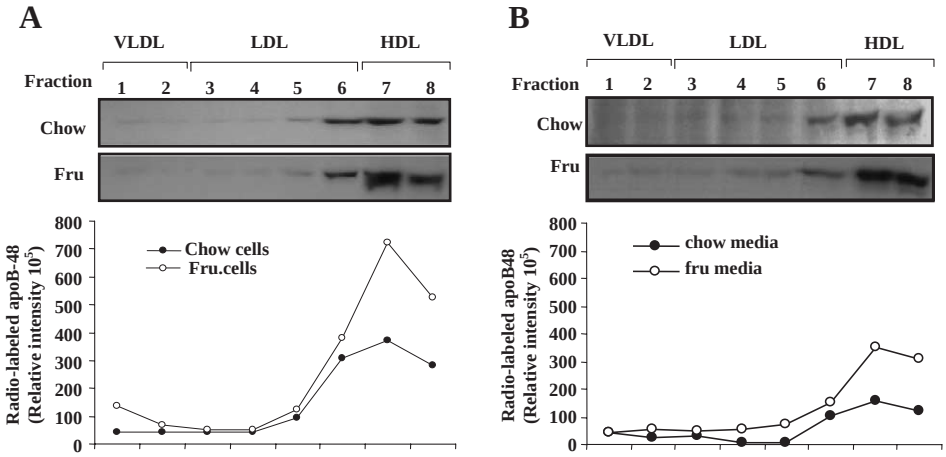


Fig. 1. Assembly and secretion of apoB48-containing lipoproteins in chow-fed and FF hamster enterocytes. Analysis of density profile by sucrose gradient fractionation. Enterocytes were isolated from hamsters fed a chow or fructose diet for 2 to 3 weeks. Cells were labeled ex vivo with [$^{35}$S] methionine for 2 hours and homogenized, and the total microsomal fraction was recovered by ultracentrifugation. The luminal content of the microsomes was extracted with 0.1 mol/L sodium carbonate, 0.025% deoxycholate, and 1.2 mol/L potassium chloride. The extracts were fractionated on discontinuous sucrose gradients, and apoB48 lipoproteins from each fraction were recovered by immunoprecipitation using antihamster apoB antibody. ApoB protein was resolved using 6% SDS-PAGE and visualized by autoradiography. ApoB48 was quantified by densitometry. A, Density distribution of radiolabeled apoB48-containing lipoproteins from ER luminal contents of chow-fed or FF hamster enterocytes; (B) density distribution of radiolabeled apoB48-containing lipoproteins secreted into the medium ex vivo by chow-fed or FF hamster enterocytes. Data show a representative experiment (7 independent experiments).

apoB48-containing lipoproteins of villus enterocytes from hamsters fed either chow or a high-fructose diet. After an overnight fast, enterocytes were isolated and were steady state–labeled. ApoB48 was found to be distributed across various lipoprotein fractions (Figs. 1A and B). Interestingly, there appeared to be a considerable amount of apoB48 present in high- and low-density lipoprotein fractions in media obtained from both chow-fed and FF hamsters (Fig. 1B). FF hamsters had higher media apoB48 in almost all lipoprotein fractions in a range of different densities including large chylomicrons. There was a switch from the secretion of small particles ($d > 1.006$ g/mL) toward larger particles ($d < 1.006$ g/mL) in enterocytes from FF hamsters. The secretion of total apoB48-containing lipoproteins by FF hamster enterocytes was higher by approximately 3-fold when compared with chow-fed hamster, although the cellular increase in large chylomicron-apoB48 was more pronounced at almost 6-fold (Fig. 1A). The increased chylomicron-apoB48 levels suggest the secretion of a considerably higher number of large chylomicron particles by FF hamster

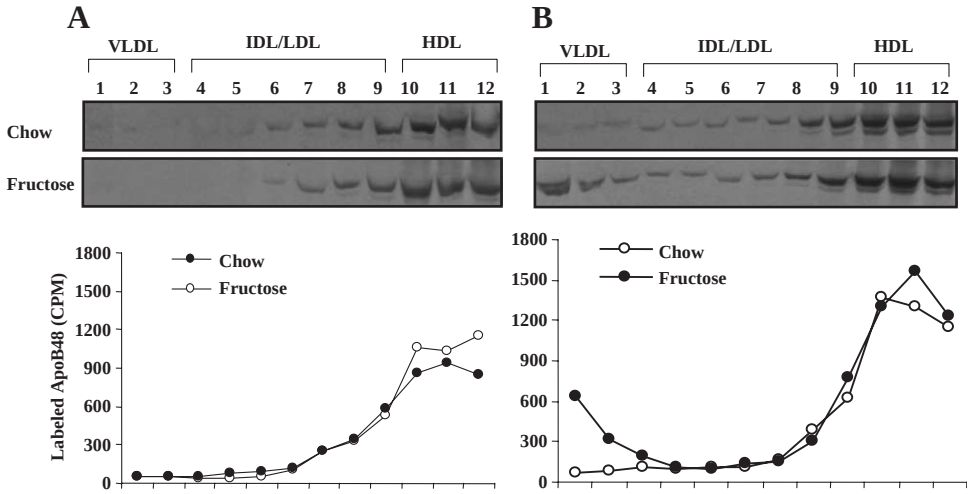


Fig. 2. Assembly and secretion of apoB48-containing lipoproteins in chow-fed and FF hamster enterocytes. Analysis of density profile by KBr gradient fractionation. Microsomal luminal contents were extracted from [$^{35}$S]-labeled hamster enterocytes as described in Fig 1. The extracts were fractionated using a sequential KBr gradient as described under Experimental procedures. ApoB48 lipoproteins from each fraction were recovered by immunoprecipitation using antihamster apoB antibody. ApoB protein was resolved using 6% SDS-PAGE and visualized by autoradiography. ApoB radioactivity was quantified by cutting out the bands corresponding to apoB48. A, Density distribution of radiolabeled apoB48-containing lipoproteins from ER luminal contents of chow-fed or FF hamster enterocytes; (B) density distribution of radiolabeled apoB48-containing lipoproteins secreted into the medium ex vivo by chow-fed or FF hamster enterocytes. Data show a representative experiment (4 independent experiments).

enterocytes. This indicates that fructose feeding markedly increased both the size and number of lipoprotein particles secreted into the media.

These studies were also repeated using sequential ultracentrifugation on KBr gradients (Fig. 2) to confirm the results obtained with sucrose gradient fractionation studies (above). Under different dietary conditions (chow and fructose-enriched diets), we analyzed lipoprotein precursors in the luminal contents of primary hamster enterocytes isolated from animals fed either a chow diet or a high-fructose diet for 2 to 3 weeks. Hamster enterocytes were pulsed with [$^{35}$S] methionine (to radiolabel newly synthesized apoB48). Microsomal luminal contents were prepared and fractionated on KBr salt gradients to separate newly assembled lipoprotein precursor particles. Luminal contents of enterocytes isolated from chow-fed hamster enterocytes contained immunodetectable apoB48 and newly synthesized radiolabeled apoB48 in both HDL- and small LDL-sized fraction with a density of 1.02 to 1.21 g/mL, similar to that of plasma LDL/HDL. In the luminal contents of FF hamster enterocytes, newly synthesized apoB48 was also present predominantly in the LDL/HDL range, although there was a slight shift toward the larger sized particles in the FF state. Analysis of the secreted apoB48 lipoproteins in the media of primary enterocytes showed that enterocytes from chow-fed hamsters secreted

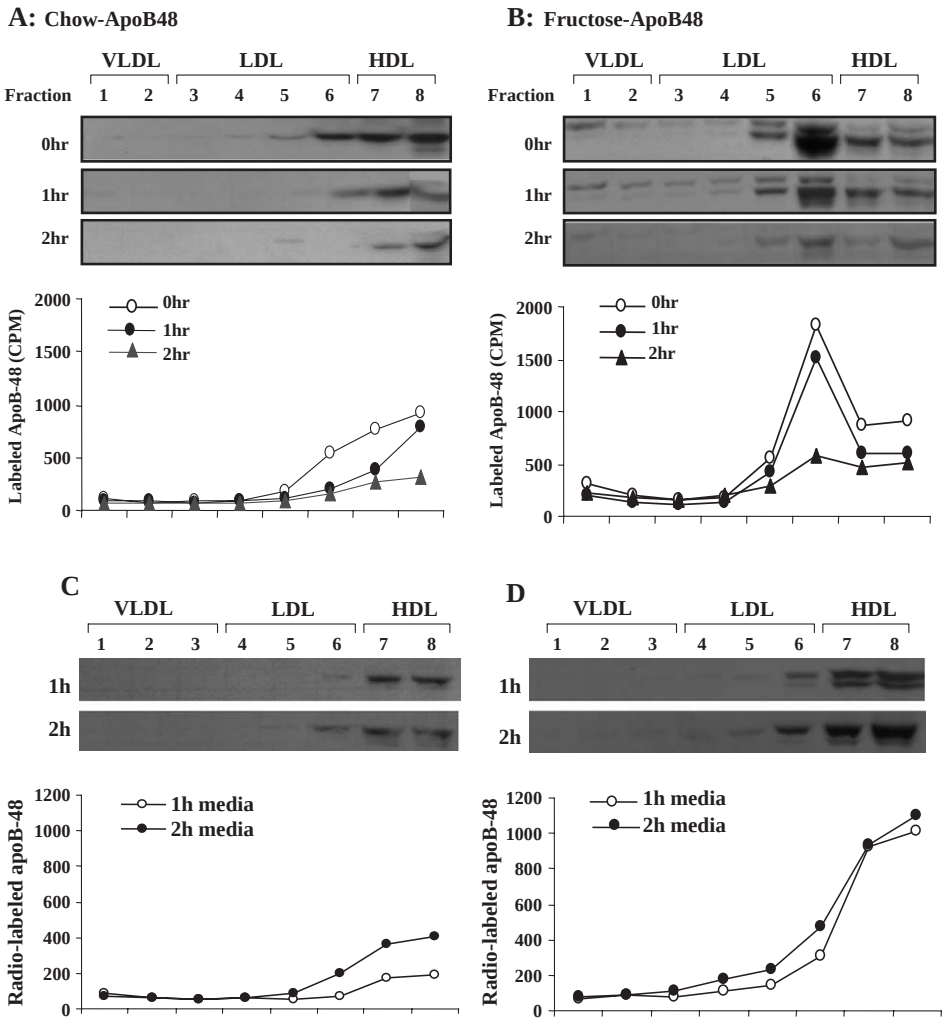


Fig. 3. Rate of intracellular assembly and secretion of intestinal apoB48-containing lipoproteins. Cultured primary hamster enterocytes were pulsed for 45 minutes with [$^{35}$S] methionine, and the radioactivity was chased for 0, 1, or 2 hours. Labeled cells were then subjected to homogenization and 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 as described in Fig. 1. After centrifugation, gradient fractions were collected and immunoprecipitated with an antihamster apoB antibody. Immunoprecipitates were resolved by 6% SDS-PAGE and visualized by fluorography. ApoB radioactivity was quantified by cutting out the bands corresponding to apoB48. A, Luminal lipoproteins in control enterocytes at 0-, 1-, and 2-hour chase; (B) luminal lipoproteins in FF enterocytes at 0-, 1-, and 2-hour chase; (C) medium lipoproteins in control enterocytes at 1- and 2-hour chase; (D) medium lipoproteins in FF enterocytes at 1- and 2-hour chase. Nascent lipoproteins accumulated in the microsomal lumen were fractionated by sucrose gradient centrifugation and immunoprecipitated with antihamster apoB antibody. ApoB protein was resolved using 6% SDS-PAGE and visualized by autoradiography. Data show a representative experiment (4 independent experiments).

the newly assembled lipoproteins at the LDL/HDL density as well as some larger apoB48-containing particles with densities of larger LDL/VLDL, suggesting further lipidation of some of the dense particles before secretion. Analysis of media lipoproteins from FF hamster enterocytes also showed secretion of dense LDL/HDL particles; however, a greater proportion of particles were present in the media having densities in the range of VLDL and chylomicrons. These data clearly suggest that the insulin-resistant state induced by fructose feeding increases both the size and number of particles produced by the hamster intestine.

### *3.2. Time course of the intracellular assembly and secretion of intestinal apoB48-containing lipoproteins*

To monitor the formation of apoB-containing lipoprotein particles in the secretory lumen of hamster enterocytes, cells were pulse-labeled using [$^{35}$S] methionine, radioactivity was chased for 0, 1, and 2 hours, and cells were subjected to subcellular fractionation. Fig. 3 illustrates the pattern of nascent lipoproteins accumulated in the media or microsomal lumen of control enterocytes from chow-fed hamsters compared with that of lipoproteins detected in enterocytes from FF hamsters. Luminal apoB-containing lipoproteins corresponding to VLDL were recovered from the top of the gradient (fraction 1), whereas fractions 5 to 8 correspond to LDL and HDL. At time 0 hour, FF hamster enterocytes had a higher level of LDL-like lipoproteins with a small pool fractionating at lower VLDL density (Fig. 2B). At 1- and 2-hour chase, there was a gradual loss of radiolabeled apoB48 lipoprotein particles present in the lumen presumably caused by extracellular secretion into the culture medium. In contrast to FF enterocytes, most of the apoB-containing lipoproteins isolated from the microsomal luminal of chow-fed hamster enterocytes at time 0 hour did not have a VLDL-like density with only a minor fraction of the total pool of nascent lipoproteins exhibiting a density typical of LDL (Fig. 2A). The majority of apoB48 lipoproteins was present at the bottom of the gradient corresponding to highly dense HDL-sized lipoproteins. Over the 2-hour chase, most of the luminal lipoproteins disappeared from the secretory lumen with a predominant loss of LDL-sized particles. At 2-hour chase, a small amount of HDL-sized particles was still present in the microsomal lumen of chow-fed hamster enterocytes. The data appear to suggest that under basal and fasting conditions, most of the newly synthesized apoB48 is incorporated into dense HDL- and LDL-sized lipoprotein particles which are rapidly lost from the secretory compartment because of secretion and possibly intracellular degradation. ApoB48-containing LDL was preferentially secreted at 1- and 2-hour chase times compared with HDL-sized particles, which appeared to move out of the microsomal lumen at a slower rate. The appearance of LDL-like apoB-containing lipoproteins as well as a small amount of VLDL-apoB48 in microsomes from FF hamster enterocytes appears to suggest a higher efficiency of lipoprotein assembly in these cells.

Analysis of secreted lipoproteins in the media of primary hamster enterocytes showed the presence of a considerable number of HDL- and LDL-sized apoB48-containing lipoproteins. Primary enterocytes from FF hamsters secreted a considerably higher number of particles at both 1- and 2-hour chase. Most of the lipoproteins appeared in the culture medium within the first hour of chase with only a

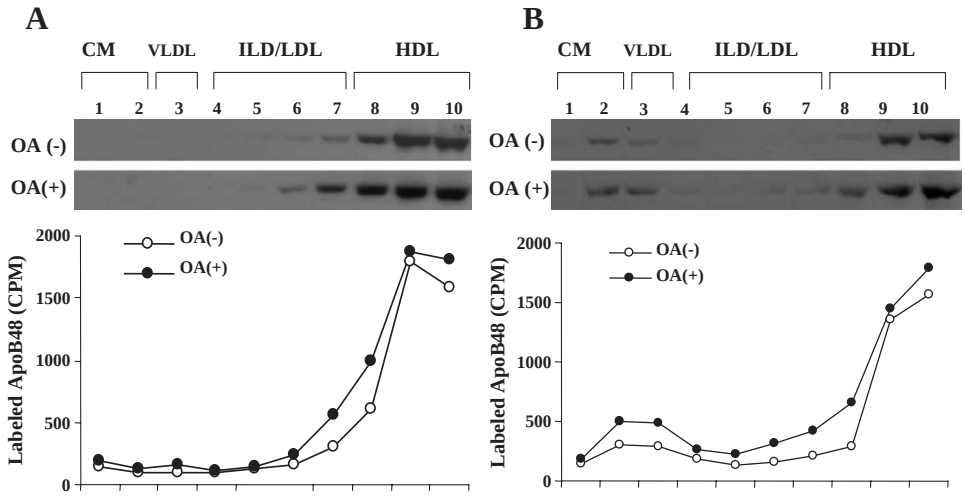


Fig. 4. Effect of exogenous OA on the intracellular assembly and secretion of intestinal apoB48-containing lipoproteins. Primary enterocytes were labeled with [$^{35}$S] methionine in the presence and absence of 720 $\mu$mol/L OA for 120 minutes, the microsomal fraction and the culture medium were recovered, and the microsomes were extracted with deoxycholate/carbonate as described under Experimental procedures. Luminal extracts (A) and medium (B) were subjected to discontinuous sucrose gradient ultracentrifugation, and apoB48 was recovered by immunoprecipitation using an antihamster apoB antibody. ApoB protein was resolved using 6% SDS-PAGE and visualized by autoradiography. The bands were cut out of the dried gel and digested, and the radioactivity was counted. Data show a representative experiment (3 independent experiments).

small increase in the second hour suggesting a rapid release of dense apoB48 containing lipoproteins after their formation in the secretory pathway of enterocytes. The density profile of lipoproteins secreted under the 2 conditions described (chow-fed vs FF) was not markedly different.

### *3.3. Effect of exogenous OA on the intracellular assembly and secretion of intestinal apoB48-containing lipoproteins*

We next examined the effect of exogenous fatty acid supplementation on the density profile of apoB48-containing lipoproteins secreted by hamster enterocytes. Freshly isolated enterocytes from chow-fed hamsters were treated with and without OA for 2 hours and then subjected to metabolic labeling. Microsomal and secreted lipoproteins were then fractionated by ultracentifugation. Intracellularly, OA treatment resulted in the formation of a considerably greater number of lipoproteins in both the HDL and LDL size range (Fig. 4A). The higher assembly of these lipoprotein subclasses appeared to result in a considerably higher number of particles secreted into the culture medium (Fig. 4B). OA treatment resulted in higher levels of secreted lipoproteins with density in the range of HDL, LDL, and VLDL/chylomicrons. The presence of VLDL/chylomicrons in the OA-treated condition suggests that OA treatment induced the conversion of a higher number of dense apoB48-containing particles to larger, more buoyant lipoprotein particles (Fig. 4).

## 4. Discussion

Recent data in our laboratory have shown that fructose feeding in hamsters induced an insulin-resistant state, which was accompanied by a metabolic dyslipidemic state [20]. In the present study, we used the FF hamster model to explore potential alterations in intestinal production of chylomicrons. Ex vivo experiments using cultured primary enterocytes isolated in the fasting state demonstrated that chronic fructose feeding caused a considerable increase in intestinal production of lipid-poor apoB48 lipoproteins with a less marked trend toward higher lipid in VLDL. The hypersecretion of apoB48-containing lipoproteins into the culture medium also confirmed these findings. There was a considerably greater secretion of both large and small intestinal particles in enterocytes isolated from FF versus chow-fed hamsters based on KBr gradient ultracentrifugation. These findings imply that there is increased lipoprotein production upon prolonged fructose feeding and induction of an insulin-resistant state. This phenomenon may occur because the efficiency of apoB48 incorporation into small lipid-poor apoB48-containing lipoproteins, even in the fasting state, is dependent on lipid availability in the intestinal cell. An increased number of apoB48 lipoprotein particles may be recovered because of more efficient intestinal fat absorption in the FF hamster and may be contributed to by any of the 3 essential factors required for lipoprotein assembly: de novo lipogenesis, apo48 stability, and MTP availability. The model used in this study may be limited by animal to animal variations in MTP activity and rates of lipogenesis; however, it has been reported that overall, both these parameters are increased in numerous models of insulin resistance and type 2 diabetes [11,21]. Our laboratory has previously observed an increase in both MTP mass and de novo lipogenesis with fructose feeding [11]. Increased MTP mass and lipogenesis coupled with the increased stability of apoB seen with fructose feeding may be important factors in the noticeable overall shift toward larger, less dense chylomicronlike particles seen in our current, as well as previous studies [11].

It is generally assumed that chylomicrons transport predominantly exogenously ingested TAG derived from dietary sources, whereas VLDL particles transport endogenous TAG from liver synthesis. There is, however, growing evidence that in the fasting state, the intestine synthesizes VLDL-like particles constitutively [6,22]. Some observations suggest a significant role of intestinal VLDL-like TAG secretion in the endogenous hypertriglyceridemia in diabetic rats. Previous investigations revealed that during absorption, a substantial fraction (>50%) of total mesenteric lymphatic TAG is derived from endogenously synthesized sources [6]. Furthermore, Gangl and Ockner [5] have shown that during lipid infusion in rats, incorporation of labeled plasma fatty acid into intestinal lymph TAG increases 6-fold when compared with the fasting state. Our findings of increased apoB48 lipoprotein secretion after treatment of enterocytes with a fatty acid 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 TAG may be under hormonal and nutritional control and can potentially be modulated in insulin-resistant states [23].

Studies in McA-RH7777 cells have shown that the first apoB48-containing particle seen in the luminal content of the microsomal fraction is a dense HDL-like particle suggesting that apoB48 VLDL is formed in 2 steps [18]. The first step is the formation of a precursor HDL-like particle. This HDL particle is then converted to apoB48 VLDL after further rapid lipidation in a second step. Cartwright and Higgins [4] also found direct evidence for a 2-step assembly of apoB48-containing lipoproteins in the lumen of the smooth endoplasmic reticulum (ER) of rabbit enterocytes. In this model, small dense HDL-sized lipoproteins were composed of phospholipids derived from a preexisting pool. Larger VLDL-sized lipoproteins were formed upon rapid addition of TAG to the preexisting particles. These results are supported by studies in CaCo-2 cells showing that apoB48 acquires preexisting phospholipids, perhaps from the ER membrane during synthesis to form HDL-sized lipoprotein particles [24]. In enterocytes from chow-fed rabbits, the rate-limiting step appears to be the transfer of apoB48 and TAG from the membrane to the lumen of the ER. The synthesis of larger VLDL-sized particles is dependent on availability of TAG for a second assembly step [4]. It was also shown that both TAG

synthesis and MTP activity were required for the second step of assembly.

The comparison of apoB48 degradation in primary hamster enterocytes isolated from FF and chow-fed hamsters revealed a marked enhancement of intracellular stability of newly synthesized apoB48 with only a minor fraction being sorted to intracellular degradation in FF hamsters [11]. An increased secretion of apoB48-containing lipoproteins accompanied the increased intracellular stability of apoB48 in FF hamster. Similar results were obtained by Zoltowska et al [25] in the *Psammomys obesus* model of obesity and insulin resistance with marked increases in de novo triglyceride synthesis and lipoprotein assembly that resulted in intestinal oversecretion of apoB48-containing lipoproteins. Importantly, treatment with an insulin-sensitizer drug, rosiglitazone, could normalize intestinal overproduction of apoB48-containing lipoproteins in an insulin-resistant hamster model fed a high-fat diet or infused with exogenous fatty acid [26,27]. In the present study, we observed that the assembly of apoB48 VLDL was delayed compared with the formation of the apoB48-containing HDL-like particle in FF hamster and failed to detect nascent polypeptides in fractions with a density lower than LDL based on pulse-chase experiments in chow-fed hamster. This supports the idea that apoB48-containing VLDL particles are formed in 2 steps, the first being the formation of the HDL-like particle, which in the second step is converted into the VLDL particle. 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 HDL-sized particles and increased secretion of chylomicron and VLDL-sized lipoprotein particles in FF hamster enterocytes. This finding further suggests the enhanced efficiency of apoB48 particle assembly in FF hamster enterocytes.

In summary, our studies in chow-fed and FF hamsters suggest that dense HDL- and LDL-sized apoB48 particles represent a major fraction of intestinally derived lipoproteins in a primary enterocyte culture system, and production of these potentially atherogenic particles can be enhanced with higher intestinal free fatty flux as well as in the insulin-resistant state. Further studies are currently underway to investigate the cellular factors modulating the formation of these dense lipoprotein particles in the intestine and dysregulation in the insulin-resistant state.

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