Assembly of very low density lipoproteins in mouse liver: evidence of heterogeneity of particle density in the Golgi apparatus

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Abstract **The assembly of very low density lipoproteins (VLDL) by hepatocytes is believed to occur via a two-step process. The first step is the formation of a dense phospholipid and protein-rich particle that is believed to be converted to VLDL by the addition of bulk triglyceride in a second step. Previous studies in our laboratory led us to hypothesize a third assembly step that occurs in route to or in the Golgi apparatus. To investigate this hypothesis, nascent lipoproteins were recovered from Golgi apparatus-rich fractions isolated from mouse liver. The Golgi fractions were enriched 125-fold in galactosyltransferase and contained lipoprotein particles averaging approximately 35 nm in diameter. These lipoproteins were separated by ultracentrifugation into two fractions: d < 1.006 g/ml and d1.006–1.210 g/ml. The d < 1.006 g/ml fraction contained apolipoprotein B-100 (apoB-100), apoB-48, and apoE, while the d1.006–1.210 g/ml fraction contained these three apoproteins as well as apoA-I and apoA-IV. Both fractions contained a 21-kDa protein that was isolated and sequenced and identified as major urinary protein. Approximately 50% of the apoB was recovered with the denser fraction. To determine if these small, dense lipoproteins were secreted without further addition of lipid, mice were injected with Triton WR1339 and [^{3}H]leucine, and the secretion of apoB-100 and apoB-48 into serum VLDL (d < 1.006 g/ml) and d1.006–1.210 g/ml fractions was monitored over a 2-h period. More than 80% of the newly synthesized apoB-48 and nearly 100% of the apoB-100 were secreted with VLDL. These studies provide the first characterization of nascent lipoproteins recovered from the Golgi apparatus of mouse liver. We conclude that these nascent hepatic Golgi lipoproteins represent a heterogeneous population of particles including VLDL as well as a population of small, dense lipoproteins. The finding of the latter particles, coupled with the demonstration that the primary secretory product of mouse liver is VLDL, suggests that lipid may be added to nascent lipoproteins within the Golgi apparatus.**—Swift, L. L., K. Valyi-Nagy, C. Rowland, and C. Harris. **Assembly of very low density lipoproteins in mouse liver: evidence of heterogeneity of particle density in the Golgi apparatus.** ***J. Lipid Res.*** **2001.** 42: **218–224.**

Supplementary key words VLDL • apoB-100 • apoB-48 • hepatic lipoprotein assembly

The assembly of very low density lipoproteins (VLDL) by the liver is a complex process, which is poorly understood [for review, see refs. (1, 2)]. Biochemical and morphologic evidence has been presented in support of a model in which assembly is accomplished via a two-step process (3, 4). The first step occurs in the rough endoplasmic reticulum (ER) during the translation of apolipoprotein B (apoB) and results in a small, dense, high density lipoprotein (HDL)-like particle. In the second step, additional core material, primarily triglyceride, is added to the primordial HDL-like particle to form a VLDL. This step is believed to occur in the smooth-surfaced ends of the rough ER. It is unclear whether assembly is completed in the ER or whether the Golgi apparatus plays an active role in the assembly process (5–9). On the basis of the size and the lipid-to-apoB ratios of nascent lipoproteins recovered from the rough ER and Golgi apparatus of rat livers, Rusinol, Verkade, and Vance (5) concluded that assembly of VLDL is completed in the rough ER. However, a number of studies have suggested a role for the Golgi apparatus in the addition of lipid to the nascent particle (6–9). For example, Bamberger and Lane (6) concluded that the assembly of triglyceride with apoproteins occurs in the Golgi. Likewise, studies in rat liver suggested that the *trans*-Golgi region appeared to be the major intracellular site of assembly of apoB with triglyceride (7, 8). Studies in our laboratory suggest addition of lipid after the nascent particles leave the rough ER (10). By comparing nascent VLDL (d < 1.006 g/ml) recovered from the rough ER with that recovered from Golgi apparatus-rich fractions, we concluded that an additional assembly step occurs while the particles are in route to the Golgi apparatus or within the Golgi apparatus. In fact, we estimated that up to 50% of the total VLDL triglyceride and 30–40% of the phospholipid is added after the particle leaves the rough ER.

Abbreviations: apoB, apolipoprotein B; ER, endoplasmic reticulum; HDL, high density lipoproteins; MTP, microsomal triglyceride transfer protein; MUP, major urinary protein; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; VLDL, very low density lipoproteins.

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With increasing interest in lipoprotein metabolism in mice and the availability of unique transgenic models such as apoB-48- and apoB-100-only mice, we have expanded our studies to the mouse model. In this article we report on the isolation of Golgi apparatus-rich fractions from mouse liver and the recovery of nascent lipoproteins from these fractions. The finding of a population of small, dense, apoB-containing particles within the Golgi apparatus, coupled with the demonstration that most of the newly synthesized hepatic apoB is secreted with VLDL, suggests a role for the Golgi apparatus in the addition of lipid to forming lipoproteins.

MATERIALS AND METHODS

Animals

Male ICR mice (approximately 30 g) were purchased from Harlan Industries (Indianapolis, IN). The animals were maintained in the Animal Care facility and received food (Wayne Lab Blox, Allied Mills, Inc., Chicago, IL) and water ad libitum for at least 5 days but no longer than 10 days prior to the experiments.

Isolation of cell fractions

Animals were anesthetized with a ketamine-xylazine cocktail, and killed by cutting the terminal abdominal aorta. The liver was quickly removed, rinsed in cold water, trimmed of excess fat and connective tissue, and weighed. The procedure for isolating Golgi apparatus was based on previously published procedures developed for use with rat liver (11). Livers (usually three, with a total mass of 5–6 g) were minced finely with scalpels, placed in homogenizing buffer (1.5 ml/g) consisting of 0.1 M phosphate buffer (pH 7.3), 0.25 M sucrose, 1% dextran, and 0.01 M $MgCl_2$, and homogenized at a setting of 45 for 20 s, using a Virtishear (VirTis, Gardiner, NY). The homogenate from three livers was placed in one tube and centrifuged in an SW-40 rotor (Beckman, Palo Alto, CA) at 2,600 rpm for 10 min, at which point the speed was increased to 6,900 rpm and the samples spun for an additional 30 min. The supernatant was discarded except when used for isolation of microsomes (see below). Homogenizing buffer (1.7 ml) was added to the pellet. The top one third of the pellet was gently dislodged from the remainder of the pellet, resuspended, and layered on 8 ml of 1.2 M sucrose. The samples were centrifuged in the SW-40 rotor at 5,100 rpm for 10 min, 10,200 rpm for 10 min, and 25,600 rpm for 45 min. The material banding on the 1.2 M sucrose was carefully removed, diluted with ice-cold distilled water, and pelleted with the SW-40 rotor (6,900 rpm for 30 min). A small piece of the final pellet was fixed in glutaraldehyde for analysis by electron microscopy. The remainder of the pellet was suspended in 15 mM Tris, 0.154 M NaCl (pH 7.4). A drop of the suspension was used for negative staining, and an aliquot was taken for protein assay. The remainder was quick frozen and stored at −70°C.

Microsomes were isolated by centrifuging the first supernatant, using an SW-41 rotor (Beckman), at 30,000 rpm for 60 min. The pellet was washed by resuspending it in 15 mM Tris, 0.154 M NaCl (pH 7.4), and pelleting under the same conditions. The final microsomal pellet was resuspended in 15 mM Tris, 0.154 M NaCl (pH 7.4), and quick frozen.

Iodination experiments

Low density lipoprotein (LDL) was isolated from human serum by ultracentrifugation between the densities 1.019 and 1.055 g/ml, using a 50 Ti rotor (Beckman). The fraction was washed once at the upper density; dialyzed against 0.154 M NaCl, 0.01% ethylenediaminetetraacetic acid (EDTA), pH 7.4; and iodinated with $Na^{125}I$ (100 mCi/ml; Amersham Pharmacia Biotech, Piscataway, NJ) according to a modification of the McFarlane ICl method (12, 13). The final specific activity of the iodinated LDL ranged between 100 and 200 dpm/ng protein. Greater than 99% of the radioactivity was trichloroacetic acid precipitable, and less than 2% of the radioactivity was extractable with chloroform-methanol. By sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography, greater than 99% of the radioactivity was associated with apoB-100. The iodinated LDL (2 μCi) was injected into the mice under ketamine-xylazine anesthesia via the retro-orbital plexus, and the animals were killed 15 min later. Golgi apparatus-rich fractions were isolated as described above. Radioactivity was determined in the homogenate and the final Golgi samples using a Beckman model 5500 γ counter.

Isolation of nascent Golgi and serum lipoproteins

Golgi fractions were incubated on ice with an equal volume of 0.2 M sodium carbonate (pH 11.0) for 60 min. The membranes were pelleted with an Optima TLX tabletop ultracentrifuge (Beckman) with a TLA 120.2 rotor (120,000 rpm for 30 min). The supernatant was dialyzed against 0.154 M NaCl, 0.01% EDTA (pH 7.4) and a d < 1.006 g/ml fraction was isolated with the TLA 120.2 rotor. The samples were spun for 3 h at 120,000 rpm, and the top 250–300 μl was removed after tube slicing. The density of the infranatant was raised to 1.210 g/ml with solid KBr, and the d1.006–1.210 g/ml fraction was floated by spinning for 4.5 h at 120,000 rpm in the TLA 120.2 rotor. The top 250–300 μl was removed after tube slicing, and both the d < 1.006 and d1.006–1.210 g/ml fractions were dialyzed against 0.01% EDTA (pH 7.4) with a final change against distilled water prior to lyophilization.

Blood (usually 0.4–0.8 ml) was collected from the inferior vena cava with a 1-ml syringe and 25-gauge needle. Serum lipoprotein fractions (d < 1.006 and d1.006–1.210 g/ml) were isolated as described above for Golgi lipoproteins.

To validate the conditions used for isolating nascent Golgi lipoproteins, VLDL was isolated from rat plasma by conventional ultracentrifugation (Beckman 40 rotor, 38,000 rpm, 18 h), and an aliquot of this VLDL was refloated in the TLA 120.2 rotor under conditions identical to those used for isolation of the d < 1.006 g/ml fraction from Golgi contents. The average recovery of triglyceride in the d < 1.006 g/ml fraction was 93% (n = 7) and the average recovery of total apoB in the d < 1.006 g/ml fraction as determined by SDS-PAGE and densitometry was 95%. In addition, a d < 1.210 g/ml fraction was isolated from mouse serum, using the TLA 120.2 rotor under conditions identical to those used to isolate the d1.006–1.210 g/ml fraction from the Golgi. Greater than 98% of the total cholesterol was recovered in this fraction.

Analytical and enzymatic assays

Protein was determined by the bicinchoninic acid method (Pierce, Rockford, IL), modified to eliminate interference by lipid (14) and using bovine serum albumin as standard. Galactosyltransferase was assayed by the method of Morré, Merlin, and Keenen (15) modified to include ATP at an initial concentration of 1 mM to reduce nonspecific hydrolysis of the UDP galactose. NADPH-cytochrome-c_2 reductase was assayed by the method of Masters, Williams, and Kamin (16) as modified by Wannagat, Adler, and Ockner (17).

SDS-PAGE and immunoblot analyses

Proteins were separated with a Novex system (Novex, San Diego, CA) with NuPAGE Bis-Tris gels (4–12% gradients). The samples

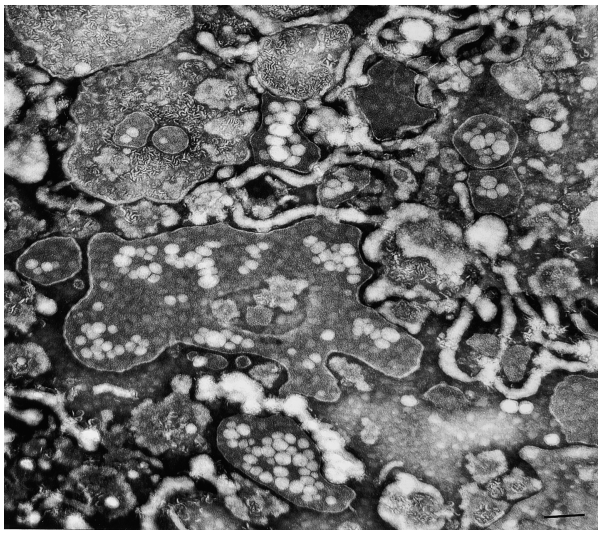

Fig. 1. Negative-stain electron micrograph of mouse hepatic Golgi apparatus-rich fraction. Golgi fractions were stained with 2% phosphotungstic acid, pH 6.5, on carbon-coated grids. Present are large central plates, tubular network, and secretory vesicles. Nascent lipoproteins are visible within the plates and vesicles. Bar = 100 nm.

were solubilized in NuPAGE lithium dodecyl sulfate sample buffer, and gels were run with the morpholinepropanesulfonic acid SDS running buffer. Gels were stained with Coomassie blue and destained. The relative amounts of protein present were estimated by densitometry, using a Bio-Rad (Hercules, CA) GS700 imaging densitometer with Molecular Analyst software (18).

For immunoblotting, the proteins were transferred to a nitrocellulose membrane, using the Novex system with NuPAGE transfer buffer containing 10% methanol. The membrane was blocked in 5% nonfat milk, incubated with primary antibody, washed extensively, and incubated with alkaline phosphatase-conjugated secondary antibody. Antibody conjugates were detected with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate.

Radioisotope incorporation studies

To study the secretion of newly synthesized lipoproteins, mice were anesthetized with ketamine-xylazine and injected via the tail vein with Triton WR-1339 (800 mg/kg) dissolved in normal saline (0.25 g/ml) and 50 μCi of L-[4,5-^{3}H]leucine (141 Ci/mmol; Amersham Pharmacia Biotech) in normal saline (pH 7.4). At 30, 60, and 120 min after injection blood was collected from the inferior vena cava. Aliquots of serum (50 and 100 μl) were taken for isolation of lipoprotein fractions as described above. The apoproteins from the fractions were separated by SDS-PAGE, stained with Coomassie blue, and destained. The gels were dried, and apoB-100 and apoB-48 were cut from the gel and prepared for scintillation counting, using NCS tissue solubilizer (Amersham Pharmacia Biotech). Samples were counted in a Beckman LS 6800 scintillation counter. Radioactivity in the apoproteins was determined, and expressed as disintegrations per minute per 100 μl of serum.

Electron microscopy

Samples obtained from the final Golgi pellet were fixed in 2% glutaraldehyde in phosphate buffer (pH 7.2) overnight. The samples were rinsed in 7.5% phosphate-buffered sucrose and postfixed in Millonig's osmium tetroxide. They were stained en bloc with aqueous uranyl acetate, dehydrated, and embedded in Araldite. Thin sections were doubly stained with uranyl acetate and lead citrate. Negatively stained grids of the Golgi fraction were prepared with carbon-stabilized Formvar-coated grids (Ted Pella, Redding, CA) and aqueous 2% phosphotungstic acid (pH 6.5). The grids were viewed in a Philips 300 electron microscope (Philips Electronic Instruments, Mahwah, NJ), and various fields were selected and photographed. The diameters of lipoproteins within vesicles and plates were measured using Canvas after scanning photographs with Molecular Analyst software.

RESULTS

The purity of the Golgi fractions was evaluated by morphologic and biochemical approaches. By negative-stain electron microscopy, the fractions exhibited morphologic characteristics consistent with the known features of the Golgi apparatus (**Fig. 1**). The fractions contained large platelike structures with and without associated tubular network. In some places, the plates were ruptured, permitting the negative stain to penetrate the structures and revealing electron-translucent lipoprotein particles. The tubular network appeared to be filled with electron-

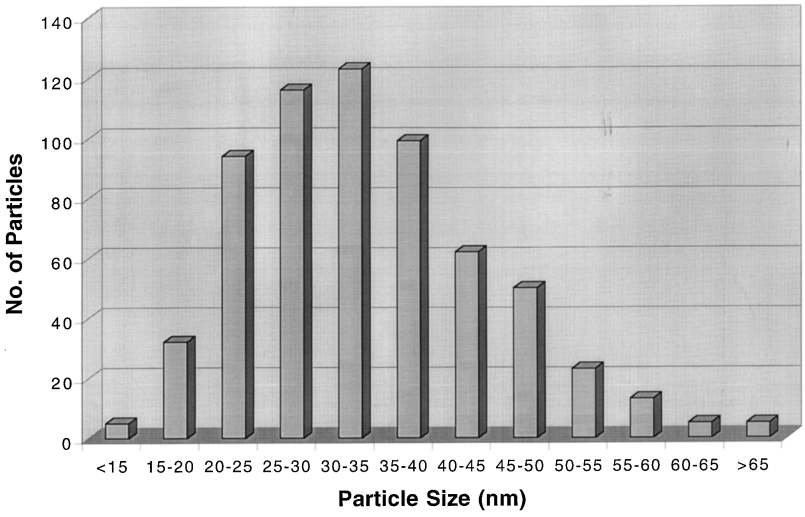

Fig. 2. Histogram displaying the size distribution of lipoprotein particles within Golgi plates and vesicles. The average diameter of the particles was 35 nm, and the median diameter was 32.5 nm (n = 634 particles).

translucent material, and the appearances of bulges in the tubules were consistent with this material representing nascent lipoprotein particles. Lipoprotein-filled vesicles were also observed. Intravesicular particles as well as particles inside plates ranged in size from 15 to 65 nm, with an average diameter of 35 nm and a median diameter of 32.5 nm (**Fig. 2**). By comparison, serum VLDL measured 42.9 ± 12.5 nm. By thin-section electron microscopy we observed cisternae in cross-section, sometimes appearing in parallel stacks, as well as tubules and vesicles that contained VLDL-sized particles (**Fig. 3**). Structures with characteristics of multivesicular bodies were not observed in our preparations, although morphologic analysis cannot exclude the possibility of minor contamination by the endosomal compartment.

The specific activity of galactosyltransferase, an enzyme widely accepted as a marker for Golgi apparatus, was enriched approximately 125-fold compared with the liver homogenate (**Table 1**). The specific activities of different preparations ranged from 500 to 1,300 nmol transferred per milligram of protein per hour. NADPH-cytochrome-c_2 reductase was not elevated above homogenate levels in the Golgi fractions, but was elevated approximately 4.2-fold in the microsomal fraction.

To monitor endosomal contamination mice were injected with ^{125}I-labeled LDL 15 min prior to being killed. Approximately 15% of the injected LDL was recovered in the liver homogenate after 15 min. The Golgi fractions contained less than 0.2% (0.14 ± 0.02, n = 3) of the total liver radioactivity.

The apoproteins of Golgi d < 1.006 and d1.006–1.210 g/ml fractions were separated by SDS-PAGE (**Fig. 4**). The d < 1.006 g/ml fraction contained apoB-100, apoB-48, apoE, and an unidentified protein at a molecular mass of slightly less than 21 kDa. The d1.006–1.210 g/ml fraction contained the same proteins, but also contained a protein that migrated with apoA-I and an additional protein with a molecular mass of 46 kDa. The latter protein was shown by immunoblotting to be apoA-IV (Fig. 4B). ApoB-48 was more prominent than apoB-100 in both fractions, with the apoB-100/apoB-48 ratio being 0.127 ± 0.057 in the d < 1.006 g/ml fraction and 0.084 ± 0.026 in the d1.006–1.210 g/ml fraction. The ratio of apoE to apoB-48 was similar in the two fractions (1.061 ± 0.114, d < 1.006 g/ml vs. 0.811 ± 0.142, d1.006–1.210 g/ml). Approximately 50% of the apoB was recovered with the d < 1.006 g/ml fraction.

TABLE 1. Marker enzyme analysis of mouse Golgi fractions

Fraction	Galactosyl Transferase	NADPH-cytochrome c_2 Reductase
	nmol galactose transferred/ mg protein/h	*μmol reduced/ mg protein/min*
Homogenate	5.87 ± 1.34 (5)	0.169 + 0.027 (5)
Golgi	727.2 ± 321.6 (5)	0.186 + 0.021 (4)
Microsomes	n.d.	0.713 + 0.058 (5)

Data represent means ± SD. Numbers in parentheses represent number of experiments. n.d., Not determined.

The 21-kDa protein was identified by N-terminal sequencing as major urinary protein (MUP), a member of a family of proteins synthesized in large amounts in mouse liver and subsequently excreted in urine (19–22). Confirmatory evidence of the identity of this protein as MUP was obtained by Western blot analysis (data not shown).

To determine the serum lipoprotein fractions into which apoB-containing lipoproteins were secreted in the mouse, the animals were injected with Triton WR-1339 and [^{3}H]leucine, and the appearance of labeled apoB in the serum d < 1.006 and d1.006–1.210 g/ml fractions was monitored. The results are shown in **Fig. 5**. Newly synthesized apoB-48 appeared in the d1.006–1.210 g/ml fraction at the 30-min time point, before any newly synthesized apoB-48 or apoB-100 appeared in the serum VLDL fraction. At 60 and 120 min after isotope injection the majority of newly synthesized apoB-48 was recovered in the VLDL fraction. Nearly all (>90%) of the newly synthesized apoB-100 in the serum was recovered with the VLDL fraction.

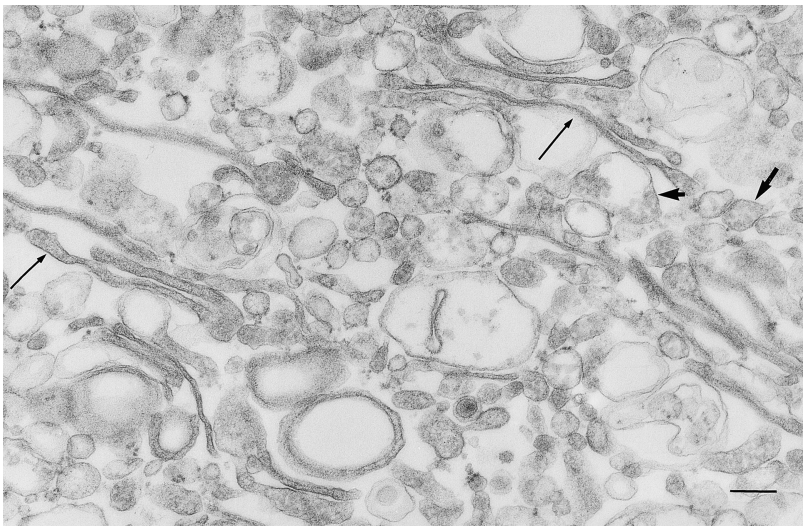

Fig. 3. Thin-section electron micrograph of mouse hepatic Golgi apparatus-rich fractions. Cisternae (arrows) are prominent along with vesicles (arrowheads) containing nascent lipoproteins. Bar = 250 nm.

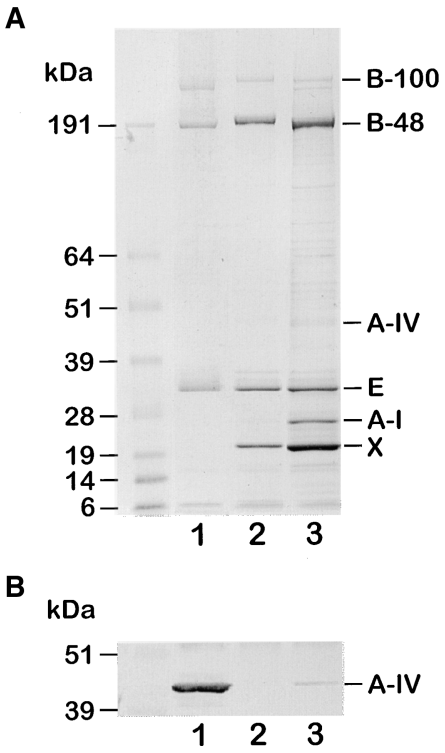

Fig. 4. SDS-polyacrylamide gel and Western blot of serum lipoproteins and nascent lipoproteins recovered from mouse hepatic Golgi apparatus-rich fractions. Gels (4–12% acrylamide gradient) were run as described in Materials and Methods and stained with Coomassie blue (A) or transferred to nitrocellulose paper and probed with antibody to mouse apoA-IV (B). A: Lane 1, serum d < 1.006 g/ml; lane 2, Golgi d < 1.006 g/ml; lane 3, Golgi d1.006–1.210 g/ml. B: Lane 1, serum d1.006–1.210 g/ml; lane 2, Golgi d < 1.006 g/ml; lane 3, Golgi d1.006–1.210 g/ml.

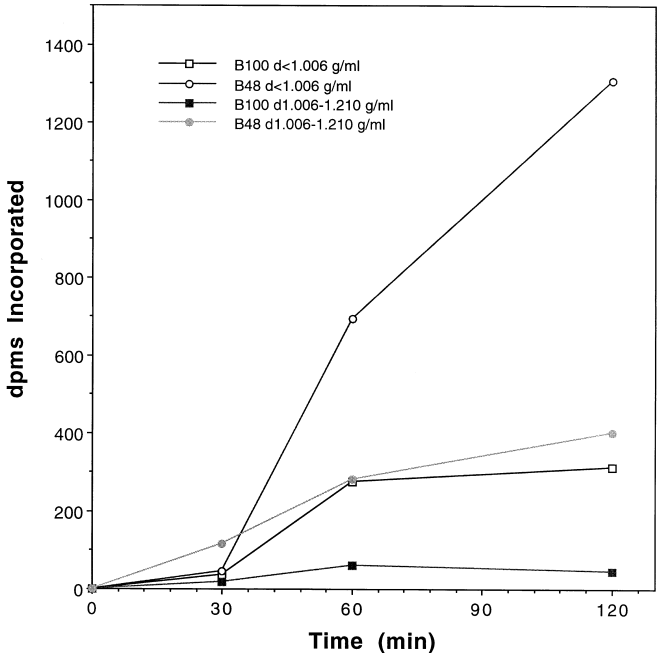

Fig. 5. Secretion of newly synthesized apoB in mouse. Mice were anesthetized with ketamine-xylazine, injected with Triton WR1339 (800 mg/kg) and [^{3}H]leucine (50 μCi/mouse), and killed by exsanguination at 30, 60, and 120 min postinjection. Two serum lipoprotein fractions were isolated by ultracentrifugation: d < 1.006 g/ml and d1.006–1.210 g/ml. The lipoprotein apoproteins were separated by SDS-PAGE, stained with Coomassie blue, and destained. ApoB-100 and apoB-48 were cut from the gel and radioactivity was determined by scintillation counting. Radioactivity is expressed as dpm/100 μl of serum. Each point represents the average of two experiments.

DISCUSSION

Previous studies in our laboratory on hepatic lipoprotein assembly have utilized the rat as an animal model. With increasing interest in lipoprotein metabolism in the mouse and the availability of unique transgenic mouse models we have expanded our studies to the mouse. In this article we report the isolation of highly purified Golgi apparatus-rich fractions from mouse liver and the recovery and characterization of nascent lipoproteins from the vesicles, tubules, and plates of the Golgi apparatus. Our studies provide evidence not only of VLDL in the Golgi apparatus, but also of a population of small, dense, apoB-containing lipoproteins. Through radioisotope incorporation studies we conclude that a fraction of these small lipoproteins is secreted without further addition of lipid while the remainder is secreted as VLDL, suggesting that additional lipid is added within the Golgi compartment.

A concern in the study is the possible contamination of the Golgi fractions by elements of the endosomal compartment and associated remnant lipoproteins cleared by receptor-mediated processes (23). We believe endosomal contamination in our preparations was minimal, on the basis of the following evidence. First, structures characteristic of multivesicular bodies and the endocytic system as described by others (24–27) were not observed by negative-stain or thin-section electron microscopy. The fractions did exhibit, however, the characteristic morphologic features associated with the Golgi apparatus (Figs. 1 and 3). Second, the Golgi fractions were enriched 125-fold in galactosyltransferase with some preparations exceeding 200-fold enrichment (Table 1). This enrichment of Golgi marker enzyme is greater than that reported previously for mouse liver Golgi apparatus (28), and exceeds even that of rat liver Golgi fractions that are thought to have minimal endosomal contamination (23). Third, injection of ^{125}I-labeled LDL to mark the endocytic compartment revealed little or no contamination. The total radioactivity recovered in the Golgi fractions was much less than 1% (0.14%) of the total liver homogenate and much less than that reported by Hornick et al. (26) in rat liver Golgi preparations. The recovery of iodine in the Golgi was similar to what we have previously reported for rat liver Golgi preparations (29, 30). This small amount of ^{125}I in the Golgi fractions does not necessarily imply contamination by endosomes or multivesicular bodies, as ^{125}I-labeled LDL could be nonspecifically trapped in Golgi fractions during the isolation procedure. Taken together, these results provide strong evidence that endosomal contamination in the mouse liver Golgi fractions was minimal and that the

lipoproteins recovered from the Golgi fractions represent newly assembled lipoproteins.

Another concern is possible contamination by the ER. NADPH-cytochrome-c_2 reductase activity was not elevated although it was measurable in our Golgi fractions. Given the enrichment of the fractions in galactosyltransferase, it is unlikely that this low level of ER contamination contributes to the nascent lipoproteins recovered from the Golgi. It has been reported that the amount of apoB and associated lipids in the lumen of the Golgi is 7- to 10-fold higher than in the ER (5). Others have also reported the low content of lipoproteins in microsomes as compared with the Golgi (31). However, to investigate this further we recovered content lipoproteins from equivalent protein aliquots of microsomes and Golgi apparatus and separated the fractions on SDS gels. We were unable to detect by Coomassie staining apoB-100, apoB-48, or apoE in the microsome content fractions while these proteins were visible in the Golgi content fractions. We conclude that the presence of small amounts of ER in our Golgi fractions would not contribute significantly to the nascent lipoproteins recovered from the Golgi fractions.

The size of some of the nascent lipoproteins within the plates and vesicles of the Golgi fractions was small (Fig. 2), prompting us to isolate not only the VLDL fraction ($d < 1.006$ g/ml) but also a higher density fraction (d1.006–1.210 g/ml). The apoprotein composition of these two fractions was similar, the only major difference being the relative amounts of apoA-I and apoA-IV in the two fractions. ApoA-IV was found only in the d1.006–1.210 g/ml fraction, and apoA-I was predominantly in the d1.006–1.210 g/ml fraction, although it was detected in the $d < 1.006$ g/ml fraction by immunoblot analysis (data not shown). The segregation of these two apoproteins primarily with the denser lipoprotein fraction is an interesting finding that underscores the unique nature of the lipoproteins isolated at d1.006–1.210 g/ml compared with those isolated at $d < 1.006$ g/ml. Consistent with our measurements of intravesicular lipoproteins was the finding that approximately 50% of the total apoB was recovered in the denser lipoprotein fraction. To eliminate the possibility that Golgi VLDL particles were not completely recovered by our ultracentrifugal procedures, the methodology for isolating nascent VLDL was validated in experiments with plasma VLDL. The finding of apoB-containing lipoproteins in the d1.006–1.210 g/ml fraction from mouse Golgi suggested either that mouse liver secretes small apoB-containing lipoproteins or that lipoprotein assembly continues in the Golgi apparatus, with the small lipoproteins being converted to VLDL-sized particles prior to secretion.

We investigated the possibility that mouse liver secretes small apoB-containing lipoproteins. For these studies mice were injected via the tail vein with Triton WR-1339 to block not only the removal of lipoproteins from the plasma compartment but also the intravascular conversions that occur as a result of lipase-mediated events. Labeled apoB-48 appeared in the d1.006–1.210 g/ml fraction of the plasma compartment within 30 min of Triton-isotope injection (Fig. 5), prior to the appearance of any newly synthesized apoB-48 or apoB-100 in the VLDL fraction. Secretion of these particles in the d1.006–1.210 g/ml fraction composed a small percentage of the total apoB-48 secreted, with the majority appearing in the VLDL fraction. The results suggest that there is a pool of apoB-48 that does not undergo bulk lipid transfer in the ER or Golgi apparatus and is rapidly secreted as a lipid-poor "high density lipoprotein-like" particle. A number of studies have reported that the liver of the rat secretes small, dense, apoB-containing high density lipoproteins (32, 33). In addition, Borén, Rustaeus, and Olofsson (4) and Rustaeus et al. (34) demonstrated that apoB-48-containing HDL are formed in McArdle RH 7777 cells. It may not be unexpected, then, to find that mouse liver secretes apoB-48 in the higher density fractions. However, it is interesting to note that we find little evidence for the secretion of any apoB-100 in the higher density fraction. The important observation from our studies is that the bulk of newly synthesized apoB (both apoB-100 and apoB-48) secreted by the liver is recovered in the VLDL fraction. Therefore, while approximately 50% of the apoB recovered with the nascent Golgi lipoproteins is associated with dense particles (d1.006–1.210 g/ml) (Fig. 4), most of the newly synthesized apoB is secreted with the VLDL particles ($d < 1.006$ g/ml) (Fig. 5). This suggests that some of the small, dense, apoB-48-containing lipoproteins recovered in the d1.006–1.210 g/ml fraction obtains additional lipid prior to secretion.

In summary, these studies provide the first characterization of nascent lipoproteins recovered from Golgi apparatus-rich fractions of mouse liver. These lipoproteins include a population of small, dense, apoB-containing lipoproteins as well as VLDL. Our data demonstrate that a fraction of the small, dense particles is secreted, apparently without further addition of lipid, but that the majority of the newly synthesized apoB is secreted as VLDL. This suggests that additional lipid may be added to these particles within the Golgi apparatus. Other investigators have suggested that lipid is added to the maturing lipoprotein particles within the Golgi apparatus (6–9). The source of the lipid and the mechanism by which the lipid is added to the particles are unknown. Studies by Hebbachi and Gibbons (35) have shown that inhibition of microsomal triglyceride transfer protein (MTP) results in a decreased removal of labeled triglyceride from the membranes of the *trans*-Golgi in rat hepatocytes. It is possible that this membrane triglyceride serves as a pool for addition to lipoproteins within the Golgi apparatus. In this regard preliminary studies in our laboratory have shown a rapid incorporation and turnover of labeled triglyceride in the Golgi membranes of mice after injection of [^{3}H]glycerol. Furthermore, we have identified MTP within the Golgi content fraction, and in vitro lipid transfer assays have demonstrated triglyceride transfer activity in this fraction. We speculate that Golgi membrane triglyceride may be utilized by MTP for lipidation of nascent Golgi lipoproteins. Further work is necessary to delineate the steps in VLDL assembly and to define precisely the role of the Golgi apparatus in the assembly process.

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