The genes for apolipoprotein B and microsomal triglyceride transfer protein are expressed in mouse and human heart tissues. Why the heart would express these “lipoprotein assembly” genes has been unclear. Here we demonstrate that the beating mouse heart actually secretes spherical lipoproteins. Moreover, increased cardiac production of lipoproteins (e.g., in mice that express a human apolipoprotein B transgene) was associated with increased triglyceride secretion from the heart and decreased stores of triglycerides within the heart. Increased cardiac production of lipoproteins also reduced the pathological accumulation of triglycerides that occurs in the hearts of mice lacking long-chain acyl coenzyme A dehydrogenase. In contrast, blocking heart lipoprotein secretion (e.g., in heart-specific microsomal triglyceride transfer protein knockout mice) increased cardiac triglyceride stores. Thus, heart lipoprotein secretion helps regulate cardiac triglyceride stores and may protect the heart from the detrimental effects of surplus lipids.

Apolipoprotein (apo)B and microsomal triglyceride transfer protein (MTP) play critical roles in the assembly and secretion of lipoproteins in hepatocytes and intestinal enterocytes (1–3). The concept that the function of apoB and MTP is to export lipids was remarkable because the heart has always been viewed as a prodigious importer of lipids from the plasma lipoproteins produced in the liver and intestine (4). We further hypothesized that lipoprotein secretion by cardiac myocytes might function to unload surplus lipids from cells. A mechanism for exporting surplus lipids would make teleological sense, given that elevated levels of fatty acids and fatty acid intermediates can have deleterious effects on cardiac function (11–13) and that increased amounts of triglyceride storage can compromise contractile function and cause cell death (14, 15).

In the current study, we sought to determine whether the beating heart actually secretes spherical lipoproteins containing a core of neutral lipids and, if so, to assess the effect of lipoprotein secretion on myocardial lipid homeostasis. To address the possibility that lipoproteins are synthesized by the heart, we used a Langendorff apparatus to perfuse mouse hearts and then examined the perfusion medium for secreted lipoproteins and lipids. To assess the impact of lipoprotein secretion on cardiac lipid homeostasis in vivo, we measured cardiac triglyceride stores in genetically modified mice that had increased levels of cardiac lipoprotein production and in mice with a blockade of cardiac lipoprotein production.

**EXPERIMENTAL PROCEDURES**

Mouse Models—Hemizygous human apoB transgenic mice (HuBTg1/o) generated with an 80-kb human genomic clone (p158) have been described (16). The HuBTg1/o mice, which express human apoB in the liver and heart but not in the intestine (9, 16, 17), were backcrossed to C57BL/6 mice more than 14 times. Long-chain acyl coenzyme A dehydrogenase-deficient (encoded Acadl−/−) mice have been described (18). Transgenic mice expressing Cre under the control of an α-myosin heavy chain promoter (αMHC-Cre; Ref. 19) were obtained from Dr. Michael Schneider (Baylor College of Medicine, Houston, TX).
Mice homozygous for a conditional allele of the microsomal triglyceride transfer protein gene (Mttp<sup>floxed</sup>) were described previously (5). All except the HuBTg<sup>Cre</sup>+/o mice had a mixed genetic background. The mice were housed in a pathogen-free barrier facility with a 12-h light/ dark cycle and fed rodent chow containing 4.5% fat (Ralston Purina, St. Louis, MO). Mttp<sup>Cre</sup>+/o mice were assessed by Southern blot analysis or by polymerase chain reaction (PCR).

Heart-specific Mttp knockout mice (Mttp<sup>floxed</sup>oMHC-Cre<sup>Cre</sup>+/o) were generated by breeding Mttp<sup>floxed</sup>oMHC-Cre<sup>Cre</sup>+/o mice with oMHC-Cre transgenic mice. Recombination within Mttp was assessed by Southern blot analysis of NaCl-precipitated genomic DNA (5). Mttp mRNA levels in the hearts of Mttp<sup>Cre</sup>+/o mice were assessed by reverse transcriptase (RT)-PCR. In brief, 25 ng of total heart RNA was subjected to retrograde perfusions in a Langendorff apparatus at 80 cm of H<sub>2</sub>O pressure with continuously circulating Krebs-Henseleit bicarbonate (KHB) medium (120 mM NaCl, 25 mM NaH<sub>2</sub>CO<sub>3</sub>, 5 mM KCl, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 2.5 mM CaCl<sub>2</sub>, 0.1 mM EDTA, and 11 mM glucose). All noncardiac tissues (lung, thymus, trachea, and external fat deposits) were dissected away from the heart, and the aorta was cannulated with an 18-gauge plastic cannula. The hearts were subjected to retrograde perfusions in a Langendorff apparatus at 80 cm of H<sub>2</sub>O pressure with continuously oxygenated (95% O<sub>2</sub> and 5% CO<sub>2</sub>) KHB medium. A modified KHB medium containing 1.2 mM oleate bound to 3% albumin was used as described previously (20). The heart rate (n = 30 hearts) was 303 ± 47 beats/min (mean ± S.D.), and the coronary flow was 5.1 ± 1.6 ml/min. In 10 experiments, an MTP inhibitor (BMS-197636; Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ) was added to the perfusion medium at a final concentration of 2 μM.

Examination of Lipoprotein Particles by Electron Microscopy—Fresh plasma samples (0.5 ml) and heart perfusion medium samples (9 ml) were adjusted to d 1.10 g/ml with KBr and overlaid with 3 ml of d 1.063 g/ml KBr solution. The samples were then ultracentrifuged in an SW40 Ti rotor (Beckman Instruments, Palo Alto, CA) at 40,000 rpm for 18 h at 12 °C. The top 0.5 ml from the ultracentrifugation tubes was removed and adjusted to d 1.10 g/ml and a volume of 3 ml. The samples were further centrifuged for 30 min at a final concentration of 1.063 g/ml and ultracentrifugation in an SW55 Ti rotor (Beckman Instruments) for 18 h at 12 °C. A carbon grid was placed on the liquid surface and then examined for lipoproteins by negative-staining electron microscopy (21, 22).

Triglyceride Secretion from Isolated Mouse Hearts—To assess cardiac triglyceride secretion, hearts were perfused with continuously circulating KHB medium containing 1.2 mM [3H]oleate (Amersham Pharmacia Biotech) bound to 3% albumin (specific activity, 5 mCi/liter). Samples of the perfusate were collected at baseline and at 15, 30, and 60 min. After lyophilization, lipids were extracted with 2:1 chloroform/methanol (v/v) and separated by thin layer chromatography (TLC). The fatty acid and triglyceride radioactivities were quantified in a scintillation counter. The sample activity of [3H]oleate was the latter to compensate for differences in recovery between samples. The d 1.063 g/ml fractions of perfusion medium samples obtained at 60 min were prepared as described above for the electron microscopy studies. At the end of the second ultracentrifugation, lipids within the top 0.5 ml of the density gradient were extracted with 3:2 hexane/isopropanol (v/v) and separated by TLC, and the triglyceride radioactivity was quantified in a scintillation counter.

Mouse Cardiac Myocyte Cultures—Cardiac myocytes were prepared from 1–5-day-old neonatal HuBTg<sup>+/o</sup> and nontransgenic hearts by serial trypsinization of pieces of heart tissue as described (23). The cells were plated for 90 min at 37 °C in minimum essential medium with Hank’s basic salt solution, 50 units/ml penicillin (Sigma), vitamin B12 (Sigma), and 5% bovine calf serum (HyClone Laboratories, Logan, UT).

RESULTS

Lipoprotein Secretion by the Heart—Hearts from HuBTg<sup>+/o</sup> and nontransgenic littermate controls were perfused in a Langendorff apparatus, and the perfusate was examined for lipoproteins by electron microscopy. Each mouse heart was
first perfused exhaustively in a noncirculating fashion with KHB perfusion medium (retrograde coronary perfusion of 5 ml/min for 60 min) to remove any plasma lipoproteins. The hearts were then perfused with KHB perfusion medium containing 1.2 mM oleate, and the buffer was circulated. After 5 min, virtually no lipoproteins were detectable in the perfusion medium (Fig. 1, A and B), indicating that most plasma lipoproteins had been washed away. After 60 min, the perfusion medium from both HuBTg+/o and nontransgenic mice contained spherical lipoproteins (Fig. 1, C and D). Most of these lipoproteins in the perfusates from HuBTg+/o mouse hearts were in the low density lipoprotein size range (18.3 ± 5.7 nm in diameter (mean ± S.D.); n = 200 particles). The same was true for the lipoproteins in the perfusates of the nontransgenic hearts (18.5 ± 6.9 nm; n = 200). The lipoproteins in the plasma of the nontransgenic mice were larger and more variable in size (22.9 ± 17 nm; range, 12–106 nm; n = 200; Fig. 1E). The lipoproteins in the plasma of the HuBTg+/o mice were skewed toward smaller particles (17.5 ± 4.3 nm; n = 200), reflecting the larger numbers of low density lipoprotein particles in the plasma of those mice (Ref. 16 and Fig. 1F).

The heart perfusion experiments suggested that the beating heart secretes lipoproteins. The fact that the lipoproteins were spherical suggested that the heart must secrete core lipids (e.g., triglycerides). To address the issue of triglyceride secretion by the heart, we perfused mouse hearts for 60 min with continuously circulating medium containing 1.2 mM [3H]oleate. [3H]triglycerides were detected in the perfusates of nontransgenic hearts, and the amounts increased with time (Fig. 2A). Significantly more [3H]triglycerides were present in the perfusates of the hearts of the HuBTg+/o mice at 60 min (Fig. 2A). Adding an MTP inhibitor to the medium reduced the amount of [3H]-triglycerides secreted from the HuBTg+/o mouse hearts (Fig. 2A).

The difference in the amounts of triglycerides in the perfusates of HuBTg+/o and nontransgenic hearts reflected, in large part, the different amounts of [3H]triglycerides in the d < 1.063-g/ml fraction. At 60 min, the amount of [3H]-triglycerides in the d < 1.063-g/ml fraction was significantly greater for the HuBTg+/o mice than for the nontransgenic mice (Fig. 2B).

We suspected that lipoproteins are produced by cardiac myocytes. This conclusion was bolstered by experiments with cardiac myocytes from newborn HuBTg+/o mice. HuBTg+/o myocytes expressed the apoB gene, as judged by RT-PCR (Fig. 3A). After incubating the myocytes with [14C]oleate, HuBTg+/o myocytes secreted ~2-fold more 14C-triglycerides than the nontransgenic cardiac myocytes (2.9-, 1.6-, 1.3-, 2.0-, and 2.1-fold more in five experiments). An example of one such experiment is shown in Fig. 3B.

Heart Lipoprotein Secretion and Cardiac Triglyceride Stores—Gene defects that block lipoprotein secretion are associated with increased intracellular lipid stores in the liver and intestine (2, 6, 24). We hypothesized that interfering with lipid and lipoprotein secretion in the heart might also increase triglyceride stores in the heart. We compared heart triglyceride stores in heart-specific MTP knockout mice (Mttp+/floxed mice carrying an MHC-Cre transgene) and littermate controls (nontransgenic Mttp+/floxed mice; n = 20 in each group). Heart-specific recombination occurred as predicted in mice carrying the αMHC-Cre transgene (Fig. 4A), and Mttp mRNA levels were reduced by ~90% (Fig. 4B). Although there was no difference in liver triglyceride stores in the two groups of mice (Fig. 4C; p = 0.77), the triglyceride stores in hearts of the Mttp+/floxed/αMHC-Cre mice were almost 2-fold higher than in controls (p < 0.0005; Fig. 4C). The increase in cardiac triglyceride stores was almost certainly attributable to the Mttp gene excision and not simply to the expression of the αMHC-Cre transgene. In a control experiment, there was no difference in cardiac triglyceride stores in αMHC-Cre transgenic mice and littermate control mice that were homozygous for a wild-type Mttp allele (2.40 ± 0.40 μmol/g in αMHC-Cre transgenic mice (n = 4) versus 2.75 ± 0.80 μmol/g in the nontransgenic mice (n = 5); p = 0.45).

To investigate whether increased heart lipoprotein production reduces cardiac triglyceride stores, we assessed heart triglyceride stores in C57BL/6 HuBTg+/o mice (n = 30 females) and nontransgenic littermate controls (n = 29 females). The plasma triglycerides and cholesterol levels were higher in the HuBTg+/o mice than in nontransgenic controls (Fig. 5A), but there were no differences in plasma glucose or free fatty acid levels (Fig. 5B). Cardiac triglyceride stores were reduced by ~75% in the HuBTg+/o mice (p < 0.0001; Fig. 5C). There was no difference in liver triglyceride stores (Fig. 5C).

Long-chain acyl coenzyme A dehydrogenase (Acadl) is required for the oxidation of long-chain fatty acids within mitochondria. Homozygous Acadl−/− mice have elevated plasma fatty acid levels and accumulate pathological levels of triglycerides in both heart and liver in response to fasting (18). To determine whether increased heart lipoprotein production from the human apoB transgene affects heart triglyceride stores in Acadl−/− mice, we measured heart triglyceride stores in Acadl-deficient mice that carried the human apoB transgene (Acadl−−/−HuBTg+/o) and in nontransgenic Acadl−/− littermate control mice. As before, the plasma cholesterol and triglyceride levels were higher in the Acadl−/−/−/HuBTg+/o than in the Acadl−/−/− mice (Fig. 6A), but there were no differences in glucose or fatty acid levels (Fig. 6B). Heart triglyceride stores were significantly lower in Acadl−−/−HuBTg+/o mice than in the Acadl−/−/− controls (p < 0.01; Fig. 6C). Once again, however, the human apoB transgene did not affect triglyceride stores in the liver (Fig. 6C).

We suspected that the increased triglyceride stores within the hearts of Acadl-deficient mice might be associated with the...
Acadl، Bituric acid-reactive substances were lower in nontransgenic hearts, and HuBTg/o hearts perfused in the presence of the MTP inhibitor BMS-197636 (n = 10 in each group). Hearts were perfused with circulating Krebs-Henseleit bicarbonate solution containing 1.2 mM [3H]oleate (5 mCi/ml) bound to 3% bovine serum albumin. Coronary flow samples (9 ml) were drawn at baseline and after 15, 30, and 60 min of perfusion. The incorporation of the oleate into triglycerides was assessed by TLC followed by scintillation counting. +, p < 0.05 versus HuBTg/o hearts. In each group, the fold-increase in [3H]-triglycerides from baseline to 60 min was significant (p < 0.05). B, [3H]-Triglycerides in the d < 1.063-g/ml fraction of the 60-min coronary flow samples. +, p < 0.05 versus HuBTg/o hearts.

FIG. 2. Triglyceride secretion from hearts perfused in a Langendorff apparatus. A, accumulation of [3H]-triglycerides in the perfusate of HuBTg/o hearts, nontransgenic hearts, and HuBTg/o hearts perfused in the presence of the MTP inhibitor BMS-197636 (n = 10 in each group). Hearts were perfused with circulating Krebs-Henseleit bicarbonate solution containing 1.2 mM [3H]oleate (5 mCi/ml) bound to 3% bovine serum albumin. Coronary flow samples (9 ml) were drawn at baseline and after 15, 30, and 60 min of perfusion. The incorporation of the oleate into triglycerides was assessed by TLC followed by scintillation counting. +, p < 0.05 versus HuBTg/o hearts. In each group, the fold-increase in [3H]-triglycerides from baseline to 60 min was significant (p < 0.05). B, [3H]-Triglycerides in the d < 1.063-g/ml fraction of the 60-min coronary flow samples. +, p < 0.05 versus HuBTg/o hearts.

FIG. 3. Apolipoprotein B and triglyceride secretion from cardiac myocyte cultures. A, RT-PCR analysis of human apoB gene expression in the hearts and cardiac myocytes from HuBTg/o mice. B, autoradiograph of a TLC plate showing cellular and secreted lipids from cardiac myocytes of HuBTg/o mice and nontransgenic controls. The myocytes were grown in medium containing 10 mM [14C]oleate for 24 h. Lipids in the cells and in the medium were extracted and separated by TLC. In control experiments, labeled HuBTg/o myocytes were incubated with [35S]Promix. A small amount of 35S-labeled human apoB100 could be detected in the medium from HuBTg/o myocytes labeled with [35S]methionine (data not shown), in line with previously published results (7).

secretion of more buoyant lipoproteins from the heart. However, this did not appear to be the case. The buoyant density of the apoB100-containing lipoproteins produced by minced-up pieces of hearts from Acadl–/– HuBTg/o mice was the same as that of lipoproteins produced by pieces of HuBTg/o hearts (Fig. 6D).

The lower cardiac levels of lipids in Acadl–/– HuBTg/o mice than in Acadl+/+ mice raised the possibility that the hearts of those two groups of mice might contain different amounts of lipoperoxides. Interestingly, the cardiac levels of triacontan acid-reactive substances were lower in Acadl–/– HuBTg/o mice than in Acadl+/+ mice (p < 0.001; Fig. 6E), suggesting that increased levels of lipoprotein production in Acadl+/+ HuBTg/o mice also reduce lipid peroxide levels.

**DISCUSSION**

This study shows that the beating mouse heart actually synthesizes and secretes spherical lipoproteins and also secretes triglycerides. The fact that the secretion of spherical lipoproteins would be accompanied by triglyceride secretion was not particularly surprising, given that the principal function of apoB in other tissues is to package neutral lipids, particularly triglycerides, for secretion. The amount of triglyceride secretion from the heart increased with increased levels of lipoprotein synthesis and secretion (e.g. in HuBTg/o mice) and decreased with reduced levels of lipoprotein secretion (e.g. in the setting of an MTP inhibitor). Importantly, our studies suggest that the expression of lipoprotein assembly genes influences cardiac lipid homeostasis. Blocking lipoprotein secretion by knocking out Mtp in the heart significantly increased cardiac triglyceride stores within the heart after an 18-h fast. Increasing lipoprotein secretion with a human apoB transgene also affected cardiac triglyceride stores. Cardiac triglyceride stores in HuBTg/o mice were ~75% lower than in nontransgenic littermates. The human apoB transgene also reduced the accumulation of triglycerides and triacontan acid-reactive substances in the hearts of mice lacking Acadl.

Why would the heart, a prodigious importer and consumer of lipids, secrete lipoproteins? One obvious possibility is that lipoprotein secretion is involved in reverse triglyceride transport, exporting surplus and potentially toxic lipids away from the heart. That cardiac triglyceride stores were reduced in the HuBTg/o mice and increased in the heart-specific Mtp knock-out mouse is consistent with this possibility. Lipoprotein secretion from the heart could be particularly relevant during a prolonged fast or during exercise, when both the uptake and the oxidation of fatty acids increase (28). Interestingly, an increased rate of triglyceride depletion from working rat hearts cannot be fully explained by increases in β-oxidation, prompting speculation that triglycerides can be removed by other mechanisms (29, 30). Our studies raise the possibility that some of this unexplained triglyceride turnover could be accounted for by lipoprotein secretion by the heart.

The fact that perturbations in cardiac lipoprotein production were associated with reciprocal changes in cardiac triglyceride stores supports a role for lipoprotein secretion in exporting lipids away from the heart and into the general circulation. However, lipoprotein secretion might very well play a role in
redistribution of lipids between myocytes, just as apoE secretion by astrocytes may play a role in redistributing cholesterol within the central nervous system (31).

Inherited defects in the mitochondrial β-oxidation pathway can cause a striking accumulation of triglycerides in the heart and are associated with heart failure as well as sudden cardiac death (13). In mice lacking Acadl, triglycerides accumulate during fasting (18). We found that the human apoB transgene significantly reduced cardiac triglyceride stores in HuBTg mice but not to the low levels that we observed in HuBTg mice with wild-type levels of Acadl expression. We suspect that the capacity of heart lipoprotein secretion to export lipids is relatively limited and fixed. Two pieces of evidence tend to support this concept. First, the absolute magnitude of the transgene-associated reduction in triglyceride stores was quite similar in the presence and absence of Acadl deficiency. Second, even though the Acadl-deficient mice had increased triglyceride stores in the heart, the lipoproteins secreted by the hearts of HuBTg/Acadl−/− mice had similar buoyant densities.

Hepatic triglyceride stores were not affected by the human apoB transgene, either in the presence or in the absence of Acadl deficiency, despite high-level transgene expression in the liver. Why would the effects of the transgene on triglyceride stores be different in the heart and the liver? One potential explanation is that the liver serves a unique role as a clearinghouse for triglycerides and that any transgene-induced increase in triglyceride secretion in the heart is simply balanced by an increase in lipid uptake, thereby preventing net changes in triglyceride stores. Alternatively, transgenic overexpression of apoB in the liver may not be associated with increased triglyceride secretion. In the liver, lipoprotein particles containing very small amounts of triglycerides and cholesterol esters are thought to be assembled in the rough endoplasmic reticulum as apoB is translated (32). However, the bulk of triglycerides in nascent very low density lipoprotein particles are thought to be added later in a specialized compartment of the smooth endoplasmic reticulum (32–34). In this so-called second step of lipoprotein assembly, small “apoB particles” are thought to fuse with large apoB-free triglyceride particles, generating nascent very low density lipoprotein for secretion. Because most lipids in the liver are added independently of apoB production, overexpression of apoB might have a negligible impact on triglyceride secretion rates (and thus little impact on triglyceride stores). In line with this view, hepatic triglyceride secretion rates in HuBTg mice and controls are similar.

In contrast to the liver, the heart seemingly lacks the capacity for this second step (we found that most of the lipoproteins in the perfusates of mouse hearts were small, much smaller.
than very low density lipoprotein in normal mouse plasma; Ref. 35). Thus, unlike the situation in the liver, the amount of apoB synthesis and secretion in the heart may regulate triglyceride secretion rates. Of note, certain hepatic cancer cell lines have lost the capacity for the second step of lipoprotein assembly and thus secrete low density lipoprotein-sized particles (similar to those that we observed in the heart perfusates). In those cell lines, apoB overexpression increases lipid secretion (36, 37).

The heart-specific *Mtp* knockout mice had increased cardiac triglyceride stores. Whether this finding is relevant to the human lipoprotein deficiency syndrome abetalipoproteinemia is not clear. There have been occasional cases of cardiomyopathy in abetalipoproteinemia (38), but as far as we are aware, no one has assessed cardiac lipid stores in this condition. Even if such measurements were feasible, they might be difficult to interpret. Patients with abetalipoproteinemia have extremely low plasma triglyceride levels (typically \(-5 \text{ mg/dl};\) Ref. 2), which might protect them from increased triglyceride storage in the heart. Our results might, however, be relevant to the treatment of human hyperlipidemic patients with MTP inhibitors (39, 40). Those drugs might influence cardiac triglyceride stores, although further studies under some conditions are needed.

Our studies support the notion that cardiac lipoprotein secretion is involved in reverse triglyceride transport. High levels of triglycerides in the heart cause diminished contractile function, hypertrophy, and myocyte death (14, 15, 41). Therefore, lipoprotein secretion might be physiologically important, serving to ward off the deleterious effects of surplus lipids. We would not be surprised if future studies uncovered roles for lipoprotein secretion in other tissues. Recently, we were intrigued to learn that both apoB and MTP are expressed in the \(\beta\) cells of pancreatic islets,\(^3\) where the accumulation of lipids is thought to be toxic (42).

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