Lipid accumulation is associated with cardiac dysfunction in diabetes and obesity. Transgenic mice expressing non-transferable lipoprotein lipase (LpL) with a glycosylated phosphatidyl-inositol (GPI) anchor in cardiomyocytes have dilated cardiomyopathy. However, the mechanisms responsible for lipid accumulation and cardiomyopathy are not clear. Hearts from 3-month-old mice expressing GPI-anchored human LpL (hLpLGPI) had increased fatty acid oxidation and heart failure genes and decreased glucose transporter genes. 6-month-old mice had increased mRNA expression and activation of the apoptosis marker caspase-3. Moreover, hLpLGPI hearts had significant cytochrome c release from mitochondria to cytosol. Low density lipoprotein uptake was greater in hLpLGPI hearts, and this was associated with more intracellular apolipoprotein B (apoB).

To test whether lipid accumulation in the hLpLGPI heart is reduced by cardiac expression of apoB, hLpLGPI mice were bred with transgenic human apoB (HuB)-expressing mice. Hearts of HuB/hLpLGPI mice had less triglyceride (38%) and free fatty acids (19%), secreted more apoB, and expressed less atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP) and more glucose transporter 4 (GLUT4). The increased mortality of the mice was abrogated by the transgenic expression of apoB. Therefore, we hypothesize that cardiac apoB expression improves cardiomyopathy by increasing lipid resecretion from the heart.

Lipid accumulation in tissues is associated with defective function and apoptosis. This process, termed lipotoxicity (1), occurs in a number of tissues and is thought to be the etiology of non-alcoholic steato-hepatitis and type 2 diabetes due to lipid-induced insulin resistance (2). Moreover, some forms of genetic cardiomyopathy, such as carnitine palmitoyltransferase 1 (CPT-1) deficiency (3), are due to defects in lipid metabolism. Diabetic and obesity cardiomyopathy also involve some degree of lipotoxicity.

Lipotoxic cardiomyopathy has been found in experimental animals. Zucker fatty rats that have a defect in leptin actions have lipid infiltration within the heart and a reduction in cardiac contractility (4). Accumulation of lipids due to alterations in intracellular pathways of lipid metabolism in mice with cardiac overexpression of fatty acyl CoA synthetase (5) and the transcription factor PPARα (6) leads to cardiomyopathy. Our laboratory surprisingly discovered that transgenic expression of a cardiomyocyte-anchored form of lipoprotein lipase (hLpLGPI) also caused dilated cardiomyopathy (7). Lipoprotein lipase (LpL) is the primary enzyme responsible for conversion of lipoprotein triglyceride (TG) into free fatty acids (FFA). Although hLpLGPI hearts assimilate more very low density lipoproteins (VLDL) lipids from the circulation, the downstream steps leading to muscle dysfunction are not clear.

Hearts acquire lipids from three sources: FFA associated with albumin, lipolysis of lipoprotein TG by LpL, and internalization of whole lipoproteins. These processes supply the immense metabolic demands of cardiac muscle. However, there may also be mechanisms to prevent overaccumulation of lipids that lead to cardiac muscle dysfunction. Elimination of lipids occurs by release of FFA, lipid oxidation, or lipoprotein secretion. Lipoprotein secretion has generally been thought to be limited to liver and gut. However, Young’s laboratory (8) noted that mice expressing human apolipoprotein B (apoB) had robust apoB expression in the heart; apoB mRNA expression was 3% of that of the liver; and microsomal triglyceride transfer protein, the protein required to add lipid to apoB, was also found in the heart. Hearts synthesized and secreted lipoproteins (8). Cardiac apoB expression led to reduced heart lipids after induction of diabetes in mice (9) and in the presence of long chain acyl CoA dehydrogenase deficiency (10). This supported the hypothesis that heart apoB enables the heart to secrete excess lipid in lipoproteins (8). To test whether apoB would affect cardiomyopathy, we studied whether transgenic apoB expression reduces heart lipids and affects heart failure markers and premature death in hLpLGPI mice.

MATERIALS AND METHODS

Mice and Diets—hLpLGPI transgenic mice (7) and human apoB transgenic mice (HuB) (10, 11) have been described. The hLpLGPI mice were crossbred with HuB mice, originally created by Callow et al. (12), to obtain HuB/hLpLGPI mice. Two diets were used: normal chow diet that contained 4.5% (w/w) fat with 0.02% (w/w) cholesterol and Western-type diet (WD), 21% (w/w) fat (polyunsaturated/saturated = 0.07) with free fatty acids; LDL, low density lipoproteins; VLDL, very low density lipoproteins; PPARα, peroxisome proliferator-activated receptor-α; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid; TC, tyramine cellulose; AMPK, AMP-activated protein kinase.
0.15% cholesterol and 19.5% casein (Harlan Teklad, Madison, WI) (13). Diabetes was induced by intraperitoneal injection of streptozotocin (STZ, Sigma; 50 mg/kg of body weight) for 5 consecutive days (14). Mice with blood glucose of greater than 250 mg/dl were considered diabetic. For some studies, 1-month-old mice were fed WD. All mice were housed in a temperature-controlled (25°C) facility with a 12-h light/dark cycle and given free access to food. Blood from fasted (24 h) mice was collected from the retro-orbital plexus into tubes containing EDTA. Plasma TG, total and free cholesterol, FFA, and glucose were determined enzymatically by kits (Sigma) in duplicate (15).

**Analysis of RNA Microarray Data**—Total RNA was isolated from hearts of 2-month-old wild type and hLpLGPI mice. Reverse transcription used oligo(dT)24 with T7 promoter sequences attached followed by strand cDNA synthesis. cRNA was then amplified and biotinylated using T7 RNA polymerase (Enzo Diagnostics, Inc., Farmingdale, NY) prior to hybridization to the Affymetrix genechip mouse U74aV2 using the Affymetrix-recommended protocol (16). For comparison between experiments, expression data were globally scaled to an average intensity of 1000 using the Affymetrix Microarray Suite™ software. Significant changes were calculated by the two-tailed Student’s t test at p < 0.05.

**Northern Blot Analysis**—Total RNA (10 µg) was isolated from hearts using TRIzol reagent (Invitrogen), subjected to electrophoresis in 1% agarose gel containing formamide, and transferred to nylon filters (Hybond N, Amersham Biosciences). Northern blot analyses were performed using the radiolabeled cDNA probes for caspase-3, atrial natriuretic factor (ANF), brain natriuretic peptide (BNP), glucose transporter 4 (GLUT4), CPT-1, and acyl-CoA oxidase (ACO). The data were normalized to the expression of glyceraldehyde-3-phosphate dehydrogenase.

**Caspase-3 Activity Assay**—Hearts were homogenized with a glass-Teflon homogenizer in an extract buffer, which contained 25 mM HEPES buffer (pH 7.4), 5 mM EDTA, 2 mM dithiothreitol, 0.1% CHAPS, and 1.0 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged at 14,000 rpm for 30 min. The supernatant was diluted with an assay buffer (50 mM HEPES buffer (pH 7.4), 1 mM EDTA, 10 mM DDT, 100 mM NaCl, 0.1% CHAPS, and 10% glycerol (pH 7.4)) and incubated at 37°C with 200 mM caspase-3 substrate (N-acetyl-Asp-Glu-Val-Asp-pNA; CalBiochem). Cleavage of the substrate was monitored at 405 nm using a microplate reader and recorded in 10-min intervals for 2 h. The specific activity was expressed in picomoles per minute per mg of protein.

**Western Blot Analysis**—Hearts from transgenic mice and matched control littermates were snap-frozen in liquid nitrogen. Tissues were disrupted by 60 strokes in a glass-Teflon homogenizer in buffer containing 20 mM Tris (pH 7.4), 1 mM EDTA, 255 mM sucrose, 1 mM phenylmethylsulfonyl fluoride, and the protease inhibitor mixture (Amersham Biosciences). Proteins were quantified by Lowry (Sigma) and 20 µg of protein separated by 5% SDS-PAGE and analyzed by Western blot using a mouse antibody directed against human apoB (Amersham Biosciences). For analysis of mitochondrial cytochrome c release, homogenized supernatants were pelleted by centrifugation at 60,000 rpm and resuspended in 1% SDS, 150 mM NaCl, 50 mM Tris (pH 7.4), 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and the protease inhibitor mixture. 20 µg of mitochondrial fraction protein and 30 µg of soluble fraction protein were separated by 10% SDS-PAGE and analyzed by Western blot using a mouse antibody directed against cytochrome c (PharMingen 6598A 1: 500 dilution; PharMingen). Detection was per-
formed using a horseradish peroxidase-coupled anti-mouse antibody (Amersham Biosciences) and chemiluminescence reagents (Amersham Biosciences). Lipoprotein Uptake—LDL were isolated from hHb mice (n = 20) by sequential ultracentrifugation at 1.025 < d < 1.063 g/ml for 16 h. Immediately after isolation, LDL were labeled with non-hydrolyzable 1,3,4,6-tetrachloro-3-[125I]-labeled tyramine cellobiose (TC) (17). 125I-TC was prepared using a horseradish peroxidase-coupled anti-mouse antibody (Amersham Biosciences) and chemiluminescence reagents (Amersham Biosciences) and used within 1 week. 125I-diphenylglycouril (Iodogen), and linked to lipoproteins were dialyzed extensively against saline containing 1 mg/ml EDTA, sterilized by filtration through an Acrodisc (0.45 μm), stored at 4 °C, and used within 1 week. An aliquot of the lipid-containing medium was added to an Eppendorf tube. The tube was placed in a heating block and mixed gently every 30 min. After a total 20-h study. At the end of the experiment, mice were anesthetized, exsanguinated, and then perfused with 10 ml of phosphate-buffered saline containing 1 mg/ml EDTA. Isolated organs were quickly rinsed with ice-cold phosphate-buffered saline and weighed, and radiiodine was quantified by automatic gamma counter (WALLAC, 1470 wizard, PerkinElmer Life Sciences).

Heart Lipid Analysis—Hearts were rapidly removed and homogenized in ice-cold 1 N NaCl buffer containing protease inhibitors to prevent TG hydrolysis. Lipids were extracted from hearts (50 mg) according to methods modified from that of Folch et al. (19). Briefly, snap-frozen heart tissues were homogenized and extracted twice with chloroform/methanol (2:1 v/v) solution. The organic phase was dried under nitrogen gas and resolubilized in chloroform. An aliquot of the Folch extraction was resuspended in an aqueous solution containing 2% Triton X-100 (13). Total cholesterol, free cholesterol, TG, and FFA were determined by enzymatic kit as described (15).

Metabolic Labeling of Hearts—apoB secretion from the hearts of control mice and mice with human apoB transgene expression was assessed as described (8). Hearts were dissected free from the thoracic cavity, opened longitudinally, and flushed with ice-cold incubation medium (methionine- and cysteine-free Dulbecco’s modified Eagle’s medium, Sigma) supplemented with 7% fetal calf serum, 1.6 mM glucose, and 1.6 mM sodium pyruvate. The whole heart (~100 mg) was minced with a razor blade. After the tissue was washed twice with 1.0 ml of incubation buffer, a total of 1.0 ml of labeling medium (incubation medium containing 1.0 Ci of [35S]methionine (Amersham Biosciences)) was added to an Eppendorf tube. The tube was placed in a 37 °C heating block and mixed gently every 30 min. After a total

### TABLE I

Comparison of gene expression in LPL GPI and wild type mice: affymetrix U34A array analysis

| Lipid metabolism | Ratio | p value | Accession no. |
|------------------|-------|---------|---------------|
| CD36             | 1.1   | NS      | L23108        |
| PGC-1            | 1.1   | NS      | AF049330      |
| Acyl-CoA oxidase | 1.9   | < 0.01  | AJ38894       |
| CPT-1            | 1.7   | < 0.05  | X59583        |
| PPAR-α           | 1.2   | NS      | X57638        |
| PPAR-γ           | 1.0   | NS      | U10374        |
| B-FABP           | 0.8   | NS      | U10482        |
| FATP             | 0.7   | NS      | U19397        |
| Fatty acid synthase | 1.6 | NS      | X13135        |
| Fatty acid binding protein | 0.9 | NS      | X23461        |
| UCP-2            | 1.3   | NS      | U69135        |
| UCP-3            | 3.0   | NS      | AB101742      |
| Acyl-CoA-cholesterol acyltransferase-2 | 1.3 | NS | AF078751 |
| Lecithin cholesterol acyltransferase | 1.5 | NS | J051754 |
| GPAT             | 0.8   | NS      | U11630        |
| DGAT             | 0.9   | NS      | AF078752      |
| SRBI             | 1.4   | < 0.05  | U37799        |
| ABC1             | 0.8   | NS      | U60020        |
| Steroyl-CoA desaturase 1 | 5.3 | < 0.05 | M26270 |
| Steroyl-CoA desaturase 2 | 1.7 | NS | M21265 |
| Fatty acid synthase | 2.0  | < 0.05  | X13135        |
| Lipoproteins     |       |         |               |
| apoa-I           | 1.5   | NS      | U79573        |
| apoa-II          | 1.0   | NS      | X26277        |
| apoC-I           | 1.5   | NS      | Z22961        |
| apoC-II          | 0.8   | NS      | Z22216        |
| apoE             | 1.0   | NS      | D00466        |
| LpL              | 1.1   | NS      | M63335        |
| LDL receptor     | 1.3   | NS      | L34317        |
| LDL receptor     | 1.1   | NS      | Z19521        |
| Glucose metabolism |     |         |               |
| GLUT1            | 0.4   | < 0.05  | M22998        |
| GLUT4            | 0.8   | < 0.05  | M32338        |
| AMP-activated protein kinase | 0.6 | < 0.05 | AF036535 |
| IRS-1            | 2.4   | NS      | X69722        |
| IRS-2            | 0.6   | NS      | AF097037      |
| Vesicle transport protein (munc-18c) | 1.7 | < 0.05 | U19521 |
| PDK-1            | 0.7   | NS      | AF079535      |
| MEK2B            | 1.0   | NS      | D50311        |
| MEK2C            | 0.5   | NS      | L13171        |
| Heart failure marker |   |         |               |
| SERCA2a          | 0.7   | NS      | AF029982      |
| cTnT             | 1.3   | NS      | U43428        |
| eNOS             | 0.5   | < 0.05  | U53142        |
| Troponin T       | 1.6   | NS      | AJ13711       |
| Myosin light chain, cardiac | 3.6 | < 0.05 | M19436 |
| ANF              | 2.7   | < 0.05  | K02761        |
| BNP              | 1.6   | < 0.05  | D28873        |
| Na+/Ca2+ exchanger | 1.5 | < 0.05 | AF046466 |
| Cardiotrophin-1  | 0.9   | NS      | U18366        |
| Cardiac calnexin | 1.5 | < 0.05 | AF088244 |
| Calmodulin       | 1.3   | NS      | M25784        |
| Caspase-3        | 1.7   | NS      | U54803        |
| CK               | 1.0   | NS      | X03233        |

Fig. 2. Evidence for apoptosis in hLpL GPI mice. A. cytochrome c (cyt c). Cardiac tissues from 5–6-month-old hLpL GPI mice and non-transgenic control littermates were homogenized and separated by differential density centrifugation to yield a membrane fraction (mitochondria) and soluble fraction (cytosol). Membrane protein (20 μg) and soluble protein (30 μg) were separated by SDS-PAGE and analyzed with Western blotting using an anti-cytochrome c antibody. B, Northern blot analysis. 10 μg of total RNA was isolated from heart and subjected to Northern blot analysis for caspase-3. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is shown as a control for loading. C, caspase activity. Activation of caspase-3 was measured by enzymatic assay for its activity. *, p < 0.05 versus wild type mice.
apoB antibody. Molecular weight is indicated on the SDS-PAGE and analyzed by Western blotting using an anti-human wild type background. Shown in Fig. 1, cardiomyopathy, and mortality were similarly altered on the half-normal LpL. We then tested whether the gene changes, allowed expression of the anchored hLpL in animals that had crossed onto the LpL heterozygous background (7). This alleviated for 2 h. The [35S]methionine-labeled immunoprecipitated proteins added to protein-A agarose (Sigma) for immunoprecipitation and incubated with a protease inhibitor mixture. After overnight incubation, the medium was recycled for four mice of each genotype were produced; this allowed calculation of a standard deviation of mRNA expression and significance testing. Fatty acid oxidation genes, ACO and CPT-1, were increased as noted previously (7). Scavenger receptor type B-class I (SRBI) was increased. Two SREBP-regulated genes, stearoyl-CoA desaturase 1 (SCD-1) and fatty acid synthase, were also increased; however, genes involved in cholesterol metabolism including HMG-CoA reductase, LDL receptor, and long chain fatty acyl CoA synthase were unchanged (data not shown). Genes for apoproteins (AI, AII, Cc, E, and B) and receptors (LDL and VLDL) were unchanged. GLUT1, GLUT4, and AMP-activated protein kinase (AMPK) were decreased, and heart failure markers (ANF, BNP, and myosin light chain) were increased.

**Evidence of Apoptosis**—Previous studies failed to show evidence of apoptosis in 2-month-old mice (7). To test whether apoptosis only was manifest in older mice, we examined heart tissue for evidence of apoptosis through the release of cytochrome c from mitochondria and the expression of caspase-3 (Fig. 2A) and increased expression of caspase-3 (Fig. 2B). 6-month-old hLpLGPI mice showed a 2.73-fold increase in cardiac caspase-3 activity (Fig. 2C). However, young mice showed no mitochondrial release of cytochrome c, and the expression and activity of caspase-3 was not different from wild type (data not shown). Therefore, apoptosis was only manifest in older animals.

**ApoB in Hearts**—Although apoB expression was not altered as assessed in the microarray study, we tested whether cardiac tissue from hLpLGPI transgenic mice had increased amounts of apoB by Western blot analysis; regulation of apoB depends on posttranscriptional mechanisms (21). As shown in Fig. 3A, hLpLGPI hearts had more apoB. Increased tissue apoB might have been an adaptive response. It is also possible that the increase in apoB represented greater amounts of lipoprotein uptake into these hearts.

**Results**

**Effects of hLpLGPI Transgene on the Wild Type Background**—Previously, we studied hLpLGPI-expressing mice crossed onto the LpL heterozygous background (7). This allowed expression of the anchored hLpL in animals that had half-normal LpL. We then tested whether the gene changes, cardiomyopathy, and mortality were similarly altered on the wild type background. Shown in Fig. 1, A and B, are the mortality curves of breeding male and female mice. Both genders had reduced survival when the transgene was expressed; at 36 weeks, only 61.5% of male and 63.6% of female mice survived. We then compared breeding and non-breeding female mice. After 36 weeks, mortality of non-breeding females was not significantly different from wild type (data not shown). Therefore, the hLpLGPI transgene leads to increased mortality that is similar in males and breeding females.

**Effects of Diabetes and WD in hLpLGPI Transgene**—Two interventions that had increased mortality in another model of lipotoxicity were studied (20), high fat diets and STZ-induced diabetes. After 36 weeks, hLpLGPI mice fed a WD had increased plasma TG (78.9 ± 14.2 on chow and 104.2 ± 10.5 mg/dl on WD) and plasma glucose (81.5 ± 17.5 mg/dl on chow and 150 ± 45.5 mg/dl on WD). STZ-treated hLpLGPI had increased plasma TG and glucose, but the levels did not differ from similarly treated wild type mice; TG was 178.0 ± 74.8 mg/dl in wild mice and 185.7 ± 55.2 mg/dl in hLpLGPI mice, and glucose was 409.3 ± 109.2 mg/dl in wild mice and 419.6 ± 125.9 mg dl in hLpLGPI STZ-treated mice. Neither WD (Fig. 1, C and D) nor STZ (Fig. 1, E and F) affected the mortality of the mice.

**Gene Changes in hLpLGPI Mice**—Using gene arrays, we specifically evaluated four clusters of genes: lipid oxidation enzymes, glucose metabolic genes, heart failure markers, and lipoproteins (Table I). We utilized younger mice (2 months old) to assess metabolic changes that were not confounded by the presence of heart failure, which occurs at a later age. Microarrays for four mice of each genotype were produced; this allowed calculation of a standard deviation of mRNA expression and significance testing. Fatty acid oxidation genes, ACO and CPT-1, were increased as noted previously (7). Scavenger receptor type B-class I (SRBI) was increased. Two SREBP-regulated genes, stearoyl-CoA desaturase 1 (SCD-1) and fatty acid synthase, were also increased; however, genes involved in cholesterol metabolism including HMG-CoA reductase, LDL receptor, and long chain fatty acyl CoA synthase were unchanged (data not shown). Genes for apoproteins (AI, AII, Cc, E, and B) and receptors (LDL and VLDL) were unchanged. GLUT1, GLUT4, and AMP-activated protein kinase (AMPK) were decreased, and heart failure markers (ANF, BNP, and myosin light chain) were increased.

**Table II**

|                | Cholesterol | TG   |
|----------------|-------------|------|
| Wild           | 69.5 ± 5.5  | 78.3 ± 21.1 |
| hLpLGPI        | 67.2 ± 2.6  | 72.5 ± 24.0 |
| HuB            | 152.2 ± 12.4 | 186.9 ± 23.6 |
| Hub/hLpLGPI    | 142.1 ± 5.0  | 183.3 ± 30.0 |

* p < 0.05 versus wild type mice.

**p < 0.05 versus hLpLGPI mice.**
We then determined whether the cardiac apoB content was altered by the HuB transgene. Both HuB and HuB/hLpL$^{GPI}$ had much greater amounts of intracellular apoB (Fig. 3B) than did hearts not expressing HuB. However, the greatest concentration of cardiac apoB was in the double transgenic line. This increase could have resulted from either increased uptake of plasma apoB, expected in mice expressing hLpL$^{GPI}$ (7), or greater cardiac apoB synthesis. To test this, we performed lipoprotein kinetic studies using LDL obtained from HuB transgenic mice that was labeled with the trapped ligand, TC. Plasma turnover of this LDL was similar in all four genotypes (Fig. 4, A and B). However, hearts of mice expressing the hLpL$^{GPI}$ transgene trapped nearly twice as much label as did HuB, but the uptake did not differ from hLpL$^{GPI}$ hearts (Fig.

### TABLE III

Heart lipids in hLpL$^{GPI}$ and HuB/hLpL$^{GPI}$ mice

|                   | Wild    | HuB     | hLpL$^{GPI}$ | HuB/hLpL$^{GPI}$ |
|-------------------|---------|---------|-------------|------------------|
| Total cholesterol | 2.33 ± 0.01 | 2.61 ± 0.09 | 3.60 ± 0.24$^a$ | 3.01 ± 0.07$^b$  |
| Free cholesterol  | 0.71 ± 0.03 | 0.71 ± 0.15 | 0.89 ± 0.07$^a$ | 0.75 ± 0.06$^b$  |
| Triglyceride      | 4.50 ± 0.44 | 5.24 ± 0.38 | 6.25 ± 1.41    | 3.90 ± 0.20$^a$  |
| Free fatty acid   | 84.01 ± 7.7  | 88.79 ± 3.06 | 104.49 ± 3.35$^a$ | 85.01 ± 1.66$^b$  |

$^a$ p < 0.05 versus wild type mice.

$^b$ p < 0.05 versus hLpL$^{GPI}$ mice.

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**Fig. 4.** LDL kinetic studies in HuB and HuB/hLpL$^{GPI}$ mice. A, plasma clearance of $^{125}$I-TC-LDL in wild type and hLpL$^{GPI}$ mice. $^{125}$I-TC-LDL obtained from HuB mice was used in LDL kinetic studies. $^{125}$I-TC-LDL was injected into wild type (open circles line, n = 3) and hLpL$^{GPI}$ (closed circles line, n = 5). B, plasma clearance of $^{125}$I-TC-LDL in HuB and HuB/hLpL$^{GPI}$ mice. $^{125}$I-TC-LDL was injected into HuB (open triangles line, n = 5) and HuB/hLpL$^{GPI}$ (closed triangles line, n = 4). At the indicated times, blood was collected. C, heart TC-LDL uptake. 20 h after injection, the mice were perfused with phosphate-buffered saline containing 1 mg/ml EDTA, and the indicated hearts were taken out, washed, and counted. The data are shown as percentage of wild type mice (cpm/g of tissue). Values are expressed as mean ± S.D. ($^*$, p < 0.05; **, p < 0.01 versus wild type mice).
**ApoB Reduces Cardiomyopathy**

4209

Fig. 5. ApoB secretion from hearts of wild type, hLpL

ApoB100

4C). Therefore, the increase in intracellular apoB was due, at least in part, to endogenous apoB synthesis.

Increased apoB should not lead to reduced cardiac lipids; in fact, the opposite should have occurred. Therefore, we tested whether cardiac secretion of apoB was altered in the HuB/hLpL mice. In hearts without transgenic expression of apoB, apoB secretion was not detected (Fig. 5). ApoB production by wild type hearts has been reported by others (8); therefore, the sensitivity of our assay might have been lower due to the use of a different antibody. Heart apoB100 secretion was evident in HuB expression mice. ApoB100 secretion was increased 31% in the double transgenic mice as compared with HuB mice. A second band, the size of apoB48, immunoprecipitated from the medium. A similar size protein that immunoprecipitated with anti-apoB antiserum from hearts expressing HuB was reported by others (8); however, the lack of apoB activity in the heart (8) suggests that this is either a breakdown product or a non-specific band. Therefore, the reduction in heart lipid likely results from increased heart lipid secretion.

**Effects of HuB Expression on Gene Expression and Mortality—**Heart failure markers, ANF and BNP, were reduced in HuB/hLpL mice as compared with hLpL mice (Fig. 6A). Moreover, GLUT4 was increased in HuB/hLpL mice as compared with hLpL mice. There was no obvious change in CPT-1 and ACO mRNA.

Mice were followed for 6 months, and survival and echocardiographic parameters were assessed. HuB/hLpL mice had on average a 19.1% improvement in cardiac fractional shortening, from 0.27 to 0.32% (Fig. 6B). Most remarkably, HuB/hLpL mice had no increased mortality as compared with wild type mice and much greater survival than did hLpL mice (Fig. 6, C and D). Thus, expression of apoB in the heart and reduction of heart lipids prevented cardiomyopathic death.

**DISCUSSION**

Dilated cardiomyopathy occurs in patients with diabetes, severe obesity, and inborn errors of lipid metabolism. Transgenic expression of genes that increase lipid accumulation and oxidation in the mouse heart also causes dilated cardiomyopathy and is thought to reproduce these disorders (3–6). Previously, we noted that expression of the hLpL transgene on the heterozygous LpD-deficient background did not alter plasma lipoprotein levels but led to the development of a dilated cardiomyopathy. In the current report, we show the following. 1) hLpL expression on the wild type background leads to cardiomyopathy. 2) This is associated with increased expression of genes mediating fatty acid oxidation and a decrease in glucose transporters and AMPK. 3) Heart failure genes and genes indicating apoptosis are elevated in these hearts. 4) Transgenic expression of apoB reduced cardiac lipid and the expression of heart failure markers. Moreover, this completely corrected premature death in hLpL mice.

Lipotoxic cardiomyopathy is associated with alterations in cellular lipid metabolism. This occurs due to an accumulation of lipids associated with defective fat oxidation with leptin deficiency (4), increased oxidation and increased expression of fatty acid transporters due to PPARα overexpression (6), and accumulation of more fatty acids due to expression of long chain fatty acyl CoA synthase (5). In contrast to these other models, the hLpL transgene leads to increased lipid uptake from circulating lipoproteins (7).

These mice develop cardiomyopathy at 4–6 months of age. This is associated with premature demise of both male and female mice. Females that are not breeding are resistant to this, suggesting that the hormonal and lipid changes or the physiologic demands of pregnancy and birth accelerate the cardiac dysfunction.

We were surprised that neither diabetes nor high fat diets exacerbated the cardiomyopathy. In contrast, similar interventions led to greater mortality in PPARα transgenic mice (6). Perhaps lipid uptake pathways mediated by the LpL transgene were already operating at maximal capacity, and lipid uptake could not be further increased.

The gene changes associated with lipotoxicity in this model were explored. Although heart failure markers are elevated in young mice, only older animals had evidence of apoptosis (release of cytochrome c from mitochondria, caspase-3 expression and activity). Similarly, PPARα and fatty acyl CoA transgenes appear to differ in the development of apoptosis; the latter, but not former, mice have evidence of apoptosis by 5–6 months.

Lipotoxicity has been postulated to result from excess lipid oxidation (22) or the accumulation of a toxic lipid intermediate. Recently, cellular studies have shown that palmitate, but not oleate, leads to apoptosis (20). Perhaps this saturated fatty acid, its metabolic product, or cellular processes affected by palmitate lead to cellular dysfunction or death.

Information from our studies comparing hLpL and HuB/hLpL mice shed some light on the mechanism of lipid-induced cardiac dysfunction. Transgenic expression of apoB reduced heart lipids and both ANF and BNP. Because HuB/hLpL hearts secrete more apoB, it is likely that this transgene increased lipid removal from the heart. This conclusion is supported by studies that showed a reduction in heart lipid due to apoB expression in long chain acyl CoA dehydrogenase knockout mice (10). In addition, these investigators found that cardi-specific loss of microsomal triglyceride transfer protein, leading to an inability to produce lipoproteins in the heart, increased cardiac triglyceride stores. HuB/hLpL hearts did not have less lipid uptake or more lipid oxidation. Lipoprotein uptake was assessed using TC-labeled LDL. TC is a trapped intracellular ligand that is internalized and not released (17, 18). Therefore, 125I-TC-LDL accurately reflects heart LDL uptake; the label is not lost after lipoprotein degradation. LDL accumulation was markedly increased in hearts expressing hLpL, and the increased uptake was not reduced by the HuB transgene. We assessed expression of genes involved in FFA oxidation. CPT-1 is the rate-limiting enzyme for long chain fatty acid transfer into the mitochondria, and ACO mediates FFA oxidation in the peroxisome. mRNA levels for neither of these genes were significantly changed. Thus, it is unlikely that HuB expression altered oxidation pathways.

Removal of accumulated lipid by apoB, we hypothesized, would reduce cardiomyopathy and premature death. Indeed, we found that this was true; along with the reduction in cardiac failure genes, the excess mortality of older animals was totally eliminated by the HuB transgene. Thus, loss of accumulating lipids,
but not changes in mitochondrial lipid oxidation, appears to be important. A recent study in another line of mice that overexpress LpL both in heart and in muscle found that crossing this line with PPARα knockouts led to cardiac toxicity (23). Thus, in this model, lipids, but not lipid oxidation that should be decreased with PPARα deficiency, leads to lipotoxicity.

Of interest, GLUT4 mRNA was decreased in hLpLGPI hearts (Table I) and was then up-regulated along with the reduction in cardiac lipids accompanying HuB expression. Thus, it appears that either the excess lipid or the development of heart failure modulated this gene. Conversely, the levels of GLUT4 could have affected heart function by allowing the hearts to utilize more glucose. Others have noted that transgenic expression of cardiac-specific GLUT1 is protective for ischemia-induced cardiac dysfunction (24). Our studies demonstrate a connection between GLUT4 gene expression and lipids and demonstrate that hLpLGPI hearts have reduced expression that is improved along with the reduction of cardiac lipids due to transgenic expression of apoB.

In summary, cardiac expression of hLpLGPI is a model for dilated cardiomyopathy due to increased lipid uptake. Such a process may occur with diabetes, in which peripheral hydroly-
sis of lipoprotein TG is reduced, whereas cardiac uptake of fatty acids is increased (25). Cardiac dysfunction in hLpL1(2/2) hearts is reduced, whereas cardiac uptake of fatty lipid oxidation.

A protective mechanism that operates in the setting of reduced cardiac lipid secretion of apoB-containing lipoproteins may be a pro-

in hearts of patients with coronary artery disease (26). Thus, heart secretion of apoB-containing lipoproteins may be a pro-

strates the previously postulated beneficial role of apoB to relieve hearts of excess lipid (9). ApoB expression is increased

by reduction of cardiac lipids suggests that lipid oxidation,

mechanisms of cardiac lipotoxicity are unknown. Its alleviation

is associated with apoptosis. Most importantly, the toxicity is

reduced by expression of apoB, which reduces cardiac lipid. The

is increased (25). Cardiac dysfunction in hLpLGPI

of lipoprotein TG is reduced, whereas cardiac uptake of fatty

diac lipid oxidation.

per se, does not lead to cardiomyopathy. Our study also demonstra-

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