Overexpression of Apolipoprotein B in the Heart Impedes Cardiac Triglyceride Accumulation and Development of Cardiac Dysfunction in Diabetic Mice

The heart secretes apolipoprotein B (apoB) containing lipoproteins. Herein, we examined whether the overexpression of a human apoB transgene in the heart affects triglyceride accumulation and development of cardiac dysfunction in streptozotocin-treated diabetic mice. Blood glucose, plasma free fatty acids, and plasma triglycerides were similarly affected in diabetic wild type mice and diabetic apoB transgenic mice as compared with non-diabetic mice of the same genotype. After 12 weeks, heart triglycerides were increased by 48% in diabetic wild type mice. These mice displayed an increased expression of brain natriuretic peptide and deterioration of heart function on echocardiography. In diabetic apoB transgenic mice, heart triglyceride levels were identical to those in non-diabetic wild type and apoB transgenic mice, and brain natriuretic peptide expression as well as echocardiographic indexes of heart function were only marginally affected or unaffected. The findings suggest that triglyceride accumulation in the heart is important for development of diabetic cardiomyopathy in mice, and that lipoprotein formation by cardiomyocytes plays an integrated role in cardiac lipid metabolism.

Liver and intestinal cells secrete triglyceride-rich lipoproteins. This ability is dependent on the expression of the apoB and microsomal triglyceride transfer protein (MTP) genes (1, 2). MTP transfers triglycerides onto the apoB polypeptide chain during its translation into the endoplasmic reticulum. ApoB serves as the principal structural protein in the resulting lipoprotein particles that are secreted from the cells. Studies of mice, which overexpress a human apoB transgene, revealed that cardiac myocytes in addition to hepatocytes and absorptive enterocytes also express the apoB and MTP genes (3) and secrete apoB containing lipoproteins (4). The apoB mRNA is not edited in cardiac myocytes (5). Consequently, the heart secretes lipoproteins containing the full-length apoB100 protein rather than the truncated apoB48 protein (4). Because the formation of apoB-containing lipoproteins serves as an effective means of secreting large amounts of triglycerides from liver and intestinal cells, we hypothesized previously that the physiological role of lipoprotein formation in the heart could be the removal of triglycerides from myocytes that are not used as fuel, i.e. a reverse triglyceride transport pathway (6, 7).

Recently, the idea has been put forward that triglyceride accumulation in cardiac muscle cells adversely affects cardiac function (8). Diabetes is associated with aberrations in cardiac fuel metabolism and a ~2-fold increase in cardiac triglyceride content (9, 10). In diabetic rats, this triglyceride accumulation occurs in parallel with compromised cardiac performance (11, 12). Echocardiographic studies have also revealed abnormal cardiac function in young diabetic individuals without coronary heart disease (13). It is unknown as to what extent triglyceride accumulation in cardiac myocytes affects the development of diabetes-induced cardiac dysfunction.

In this study, we examined whether the overexpression of a human apolipoprotein B transgene in the heart might attenuate cardiac lipid accumulation and consequently modulate signs of cardiac dysfunction in streptozotocin-treated diabetic mice.

EXPERIMENTAL PROCEDURES

Animals—5–7-week-old wild type mice (C57BL/6 mice, 20 female and 20 male mice) and human apoB transgenic mice (C57BL/6N-TgTGm1Cgg, 20 female and 20 male mice) were obtained from M&B (Ry, Denmark) housed at the Panum Institute, University of Copenhagen (Copenhagen, Denmark) and fed standard laboratory chow (Altromin number 1314, Rutgers, Denmark). The apoB transgenic mice were generated with a ~87-kilobase pair human genomic fragment from a genomic P1 bacteriophage clone, p158. This transgene directs human apoB overexpression in the liver and in the heart but not in the intestine (7, 14). The transgenic mice used in this study had been backcrossed to a C57BL6 background ~14 times. The study was approved by the Danish Government body supervising animal experiments (Dyreforsøgsstilsynet).

Diabetes—Streptozotocin (STZ) (40 μg/g/day) (Sigma) was injected intraperitoneally once a day for five consecutive days followed by a two-day recovery and injections for an additional 2 days. STZ (4 μg/μl) was dissolved in citrate buffer (pH 4.5, 4 °C), kept on ice, and injected within 20 min. Control mice received the citrate buffer vehicle (200 μl/mouse/day) following the same treatment regimen.

Tissue and Blood Samples—Blood samples for plasma insulin and lipid analyses were drawn into tubes containing Na2-EDTA and centrifuged at ~4000 × g for 10 min at 4 °C. Plasma was stored at ~80 °C until analyses. After echocardiographic examination, the heart and liver were removed and briefly rinsed in 0.9% NaCl (4 °C). The heart was carefully cleaned of pericardial fat. Cross-sectional slices of the ventricular portion of heart including right and left ventricular tissue and liver biopsies were snap-frozen in liquid nitrogen and stored at ~141 °C for subsequent lipid and RNA extraction. A ~3-mm slice from the apex of the heart was fixed in 3% paraformaldehyde.
TABLE I
Basic characteristics

|                | Wild type | STZ | Control | STZ | Control |
|----------------|-----------|-----|---------|-----|---------|
| **Male mice**  |           |     |         |     |         |
| Body weight (g)| 23.8 ± 0.6* | 25.4 ± 0.5 | 24.7 ± 0.8* | 26.8 ± 0.3 |
| Blood glucose, fed | 15.5 ± 2.2* | 7.2 ± 0.3 | 16.5 ± 1.9* | 7.8 ± 0.4 |
| Blood glucose, fasted | 11.6 ± 1.4* | 5.4 ± 0.3 | 12.7 ± 0.9* | 7.4 ± 0.2 |
| Insulin (µmol/liter) | 406 ± 75' | 750 ± 139 | 364 ± 32' | 1021 ± 176 |
| Free fatty acids | 0.37 ± 0.02 | 0.31 ± 0.02 | 0.38 ± 0.03 | 0.35 ± 0.02 |
| Triglycerides | 0.77 ± 0.10 | 0.57 ± 0.06 | 1.14 ± 0.04 | 1.13 ± 0.06 |
| Free glycerol | 0.86 ± 0.03' | 0.77 ± 0.02 | 0.74 ± 0.02 | 0.80 ± 0.03 |
| Total cholesterol | 2.16 ± 0.14 | 1.91 ± 0.06 | 2.50 ± 0.15 | 2.48 ± 0.09 |
| HDL-cholesterol | 1.67 ± 0.12 | 1.70 ± 0.06 | 1.48 ± 0.06 | 1.61 ± 0.05 |
| LDL+VLDL-cholesterol | 0.49 ± 0.19 | 0.11 ± 0.02 | 1.32 ± 0.15' | 0.87 ± 0.10 |
| **Female mice** |           |     |         |     |         |
| Body weight (g) | 21.7 ± 0.5 | 215 ± 0.4 | 21.0 ± 0.7 | 22.8 ± 0.3 |
| Blood glucose, fed | 6.9 ± 0.2 | 6.0 ± 0.3 | 10.0 ± 1.0c | 7.1 ± 0.2 |
| Blood glucose, fasted | 7.1 ± 0.2c | 5.4 ± 0.2 | 9.0 ± 1.3 | 7.0 ± 0.3 |
| Insulin (µmol/liter) | 254 ± 44 | 228 ± 29 | 205 ± 28 | 326 ± 65 |
| Free fatty acids | 0.36 ± 0.05 | 0.30 ± 0.03 | 0.42 ± 0.03c | 0.33 ± 0.03 |
| Triglycerides | 0.43 ± 0.02 | 0.44 ± 0.04 | 0.97 ± 0.06 | 0.89 ± 0.03 |
| Free glycerol | 0.68 ± 0.03 | 0.68 ± 0.02 | 0.67 ± 0.01 | 0.67 ± 0.02 |
| Total cholesterol | 1.59 ± 0.06 | 1.77 ± 0.11 | 2.67 ± 0.11 | 2.59 ± 0.11 |
| HDL-cholesterol | 1.45 ± 0.05 | 1.51 ± 0.06 | 1.36 ± 0.02 | 1.36 ± 0.05 |
| VLDL+LDL-cholesterol | 0.15 ± 0.05 | 0.28 ± 0.09 | 1.32 ± 0.12 | 1.23 ± 0.09 |

*a p < 0.0005 compared with controls.
*b p < 0.005.
*c p < 0.05.

**Echocardiography**—The mice were anesthetized with a subcutaneous injection of 1:1 hypnorm (Fentanyl, Fluanisone (10 mg/ml), Janssen)/diazepam (6 ml/kg) before transthoracic echocardiography, and Doppler flow analyses were performed with the Vivid Five instrument (GE Ultrasound, Copenhagen, Denmark) and a 10-MHz transducer head (15). Echocardiographic investigations were repeated in each mouse after an intraperitoneal injection of dobutamine (1.0–1.5 µg/g body weight) (16). Echocardiography and data analyses were performed by E. Bollano without any knowledge of treatment and mouse genotype.

**Plasma Analyses**—The glucose concentration in tail blood was determined with a Humacheck Plus glucose meter (Human, Wiesbaden, Germany). Plasma insulin was measured with ELISA by Dr. B. Rolin (Novo Nordisk A/S). Plasma lipid concentrations were determined with enzymatic kits: free fatty acids (Wako NEFA C kit, TriChem Aps, Frederiksvend, Denmark), triglycerides (GPO-TRINDER, Sigma; this kit also measures free glycerol), total cholesterol (CHOD-PAP, Roche Molecular Biochemicals), and high density lipoprotein (HDL) cholesterol (HDL-C, Roche Molecular Biochemicals).

**Tissue Lipid Analyses**—Lipids were extracted from heart (~40 mg) and liver (~140 mg) biopsies with chloroform/methanol. For enzymatic quantification of triglycerides, glycerol, and cholesterol with the same kits as for plasma analyses, the tissue extracts were redissolved in isopropl alcohol with 1% Triton X-100 (2 µl/mg wet weight of tissue, Sigma). For thin layer chromatography (TLC) (17), the extracts were redissolved in toluene (1 µl/mg wet weight of tissue). TLC plates (20 × 20 cm, DC-Fertigplatten SIL G-25, Macherey-Nagel, Düren, Germany) were impregnated with Na2EDTA (1 mm, pH 5.5) by ascending development, dried, and washed in chloriform:methanol:water (60:40:10). After the activation of the TLC plates at 110 °C for 30 min, 1 µl of tissue extract and standards was applied. The plates were developed in a six-step procedure using chloriform:methanol:water (60:40:10); chloriform:methanol:water (65:40:5); chloriform, methanol, ethyl acetate, 2-propanol, ethanol, 25% KCL (22:15:35:20:9); ethanol:toluene:diethyl ether (3:60:40); diethyl ether:heptane (8:94); and pure n-heptane. After development, the plates were placed in 10% cupric sulfate (w/v) in 8% phosphoric acid (w/v) for 10 s, dried with a hair dryer, and baked for 2 min at 200 °C. For quantification, the TLC plates were scanned at 300 × 300 dots/inch with a HP flatbed 4cT scanner (Hevellert Packard, Copenhagen, Denmark). The digitized images were analyzed with Multi-analyst software (Bio-Rad). All of the samples were analyzed twice on different TLC plates. A linear regression analysis of the TLC-based cardiac triglyceride measurements versus enzymatic cardiac triglyceride measurements yielded an r² = 0.91 (n = 80).

**RNA Purification**—Frozen heart and liver biopsies (40–50 mg) were homogenized with a Polytron PT1200CL (Buch & Holm, Herlev, Denmark) in TRIzol reagent (Invitrogen). Total RNA was extracted according to the manual by the manufacturer and suspended in RNase-free H2O. The RNA concentration was calculated from the absorbance at 260 nm (A260) (18). The RNA integrity was assured always by electrophoresis on a 1% agarose gel.

**Synthesis and Amplification of cDNA**—First strand cDNA was synthesized from 1 µl of total RNA with Moloney murine leukemia virus reverse transcriptase (40 units, Roche A/S, Avedore, Denmark) and random hexamer primers in 10-µl reactions. The primers for amplification of mouse apoB, MTP, glyceraldehyde-3-phosphate dehydrogenase, and β-actin are described elsewhere (18). The primers for brain natriuretic peptide (BNP) (5′-CTGAAAGTCTGGTCGCCAGAT-3′ and 5′-GTTCTTTTGGGTTGGCCTTTGG-3′) and human apoB (5′-GGGACTGTCGATCGAGGGATC-3′ and 5′-AGTGCTTTCGCTCCTTCCCAT-3′) were obtained from Sigma-Geneoys (Pampisford, United Kingdom). The human apoB transcript could not be amplified from wild type mouse liver cDNA, and the mouse apoB transcript could not be amplified from human HepG2 cell cDNA. The specificity of each PCR was further confirmed by DNA sequencing of upper and lower strands of PCR transcripts (18). We never observed any amplification of genomic DNA from the cDNA preparations.

**mRNA Quantification**—Quantitative real-time PCR analysis of mRNA expression was done with a LightCycler and the DyNAmeter SYBR Green kit (Roche A/S). The PCR reactions (20 µl) contained 2 µl of SYBR Green I mixture, 2–3 µM MgCl2, 10 pmol of each primer, cDNA synthesized from 20 ng of total RNA, and PCR grade H2O. For each mRNA transcript in each tissue biopsy, the time point of the log-linear increase in amplified DNA during the PCR was determined with the fit-point option of the LightCycler software. The relationship between that time point and the relative concentration of an mRNA transcript was determined by analyzing in each run the dilution series of cDNA from wild type mouse livers or HepG2 cells (cDNA synthesized from 100, 20, 2, and 0.2 ng of total RNA). All differences among groups were analyzed with Student’s t test.

**RESULTS**

**Effects of STZ on Blood Glucose and Plasma Lipids**—Injection of the pancreatic β-cell toxin STZ conferred a pronounced and sustained increase in blood glucose levels and a corresponding decrease in plasma insulin levels in male mice (Table I). However, in female mice, the diabetic response after STZ treatment was marginal (Table I). The blood glucose concent-
FIG. 1. mRNA levels of MTP, mouse apoB, and human apoB in heart and liver of male mice. A, heart (left panel) and liver (right panel) MTP mRNA in wild type and apoB transgenic mice. Closed bars represent STZ-treated mice. Open bars represent vehicle-treated control mice. B, heart (left panel) and liver (right panel) mouse apoB mRNA in wild type and apoB transgenic mice. Closed bars represent STZ-treated mice. Open bars represent vehicle-treated control mice. C, heart (left panel) and liver (right panel) human apoB mRNA in apoB transgenic mice. Closed bars represent STZ-treated mice. Open bars represent vehicle-treated control mice. The mRNA levels were quantified by real-time PCR and normalized by the glyceraldehyde-3-phosphate dehydrogenase mRNA level in each sample. Similar results were obtained without the normalization (data not shown). The values for MTP and mouse apoB mRNA are expressed as a fraction of the expression in livers pooled from three 5–7-week-old C57Bl6 mice. The values for human apoB are expressed as a fraction of expression in cultured HepG2 cells. The p values for two-group comparisons indicated by brackets are shown in the figure. Values are the mean ± S.E. (n = 10 in each group). ApoBTg, apoB transgenic.
Heart ApoB Expression and Cardiac Function

Cardiac lipids were measured by densitometry after separation by TLC. Hepatic lipids and free glycerol were measured with enzymatic tests.

**FIG. 2.** Cardiac triglyceride and free glycerol content of male mice. A, heart triglycerides in wild type and apoB transgenic mice. Closed bars represent STZ-treated mice. Open bars represent vehicle-treated control mice. B, heart glycerol in wild type and apoB transgenic mice. Closed bars represent STZ-treated mice. Open bars represent vehicle-treated control mice. The triglyceride and glycerol contents were determined with an enzymatic method. The p values for two-group comparisons indicated by brackets are shown in the figure. Values are the mean ± S.E. (n = 10 in each group except in the STZ-treated apoB transgenic group where n = 9) (one sample was excluded due to a technical error). ApoB
tg, apoB transgenic.

**TABLE II**

Heart and liver lipids in male mice

Cardiac lipids were measured by densitometry after separation by TLC. Hepatic lipids and free glycerol were measured with enzymatic tests. Values are the mean ± S.E. ww, wet weight.

|                  | Wild type  | Control  | ApoB transgenic  | Control  |
|------------------|------------|----------|------------------|----------|
| **Heart**        |            |          |                  |          |
| Triglycerides    | 5.1 ± 0.8  | 3.5 ± 0.3| 3.7 ± 0.4        | 3.9 ± 0.4|
| Free cholesterol | 2.7 ± 0.2  | 2.7 ± 0.2| 2.7 ± 0.2        | 2.7 ± 0.1|
| Sphingomyelin    | 1.2 ± 0.1  | 1.1 ± 0.1| 1.2 ± 0.1        | 1.1 ± 0.1|
| Phosphatidylcholine | 16 ± 2   | 17 ± 2   | 16 ± 1           | 17 ± 1   |
| Phosphatidylserine | 1.0 ± 0.1 | 1.1 ± 0.1| 1.1 ± 0.1        | 1.0 ± 0.04|
| Phosphatidylinositol | 1.1 ± 0.1 | 1.1 ± 0.1| 1.1 ± 0.1        | 1.1 ± 0.1|
| Phosphatidylethanolamine | 13 ± 1 | 14 ± 1 | 13 ± 1 | 14 ± 1 |
| Cardiolipin      | 4.5 ± 0.3  | 4.8 ± 0.2| 4.5 ± 0.2        | 4.7 ± 0.2|
| **Liver**        |            |          |                  |          |
| Triglycerides    | 11.0 ± 0.8 | 9.8 ± 0.7| 10.3 ± 1.1       | 14.4 ± 1.2|
| Total cholesterol| 11.2 ± 1.2 | 13.2 ± 1.1| 13.0 ± 0.6       | 14.7 ± 0.9|
| Free glycerol    | 2.0 ± 0.1  | 1.4 ± 0.1| 4.6 ± 0.4        | 7.2 ± 0.7|

*p = 0.07 compared with control mice of the same genotype.

*p < 0.05 compared with control mice of the same genotype.

*p < 0.001 compared with control mice of the same genotype.
To assess whether the overexpression of the apoB transgene in the heart affected cardiac stores of other lipid classes than triglycerides, we measured a series of cardiac lipids in addition to triglycerides (Table II). There was no effect of STZ treatment or apoB overexpression on cardiac contents of cholesterol or phospholipids in male mice (Table II) or in female mice (data not shown). The TLC analysis confirmed the normalization of cardiac triglycerides in the STZ-treated apoB transgenic male mice (Table II).

Liver triglyceride and total cholesterol stores were not affected by STZ treatment in wild type male mice. The hepatic triglyceride content was lower in STZ-treated apoB transgenic male mice compared with control apoB transgenic male mice (Table II). A reduction of hepatic triglyceride stores in STZ-treated diabetic mice has been described previously (19).

**Effects of Diabetes and ApoB Overexpression on Cardiac Function—**Echocardiography and Doppler flow analyses showed that STZ-induced diabetes affects cardiac function of wild type male mice (Fig. 3, A–C, and Table III). In accordance with previous findings in rats (11, 15), the heart rate was slightly decreased in STZ-treated mice, although this effect was not statistically significant. The indexes of systolic function (e.g. circumferential shortening (Fig. 3B) and diameter of the left ventricle at the end of the systole (Table III)) and diastolic function (E-wave deceleration time (Fig. 3C)) were affected negatively by STZ at the base-line recordings. For each of these parameters, the effect of STZ treatment was less pronounced and/or absent in apoB transgenic mice. In apoB transgenic male mice, the heart rate tended to be increased, whereas circumferential shortening, diameter of the left ventricle at the end of the systole, and E-wave deceleration time at base line were not significantly affected after STZ treatment (Fig. 3, B and C).

Increased BNP expression is a sensitive indicator of cardiac dysfunction (20). STZ-treated diabetic rats have increased BNP gene expression in the heart (21). In this study, STZ-treated wild type male mice had increased cardiac BNP mRNA levels compared with vehicle-treated wild type mice (Fig. 3D). In contrast, BNP gene expression was not affected by STZ treatment in the apoB transgenic male mice (Fig. 3D). Diabetes has also been associated with apoptosis of cardiac myocytes (22). Terminal deoxynucleotidyltransferase dUTP nick-end (TUNEL) staining of histological sections from the apical section of each mouse heart in this study revealed very few apoptotic cells with similar occurrence in STZ-treated and vehicle-treated mice (data not shown), and STZ treatment did not affect heart weight. **Fig. 3.** Interfaces of cardiac function and heart BNP mRNA expression in male mice. Cardiac function was evaluated by echocardiography and Doppler flow analysis by an experienced echocardiography analyst (E. Bollano) without knowledge of mouse genotype or previous treatment. A, representative two dimensionally M-mode-guided echocardiograms of the left ventricle from an STZ-treated and a control wild type male mouse. The dotted lines indicate end-diastolic diameter, the solid lines indicate end-systolic diameter. Note the increased end-systolic diameter in the STZ-treated mouse. B, circumferential shortening of the left ventricle at base-line in wild type and apoB transgenic mice. Closed bars represent STZ-treated mice. Open bars represent vehicle-treated control mice. Circumferential shortening was determined as a fractional shortening of the left ventricle (i.e. left ventricular end-diastolic minus left ventricular end-systolic diameter divided by left ventricular end-diastolic diameter) divided by the emptying time of the left ventricle. C, E-wave deceleration time in wild type and apoB transgenic mice. Closed bars represent STZ-treated mice. Open bars represent vehicle-treated control mice. E-wave deceleration time was measured by Doppler flow analysis as the time from the maximal E-wave velocity (i.e. early filling of the left ventricle) to zero velocity. A decrease in E-wave deceleration time might reflect increased stiffness of the ventricular wall. D, heart BNP mRNA levels in wild type and apoB transgenic mice. Closed bars represent STZ-treated mice. Open bars represent vehicle-treated control mice. The BNP mRNA level was normalized by the glyceraldehyde-3-phosphate dehydrogenase mRNA level in each sample. Similar results were obtained without the normalization (data not shown). The BNP mRNA values are expressed as a fraction of the expression in hearts pooled from chow-fed C57Bl6 mice. The p values for two-group comparisons indicated by brackets are shown in the figure. Values are the mean ± S.E. ApoBTg, apoB transgenic.
Heart ApoB Expression and Cardiac Function

Biomechanical cardiac characteristics of male mice

Cardiac performance was examined 12 weeks after STZ treatment with echocardiography and Doppler flow analysis. Blood pressure was measured with a tail cuff. Cardiac stress was induced with dobutamine after the baseline recordings had been made. Fractional shortening was determined as the difference between left ventricular end-diastolic and left ventricular end-systolic diameters divided by left ventricular end-diastolic diameter. Circumferential shortening was determined as fractional shortening divided by the emptying time of the left ventricle. Left ventricular outflow velocity and E/A were determined by Doppler flow analysis. E indicates early diastolic filling wave. A, indicates late diastolic filling wave. Values are mean ± S.E.

|                          | Wild type |                   | ApoB transgenic |                   |
|--------------------------|-----------|-------------------|-----------------|-------------------|
|                          | STZ       | Control           | STZ             | Control           |
| Heart weight (mg/g body weight) | 4.3 ± 0.1 | 4.5 ± 0.1         | 4.4 ± 0.1       | 4.2 ± 0.1         |
| Left ventricular diameter in diastole (mm) | 3.9 ± 0.1 | 3.8 ± 0.1         | 4.0 ± 0.1       | 4.0 ± 0.1         |
| **Base line**             |           |                   |                 |                   |
| Heart rate (min⁻¹)        | 341 ± 15.2| 385 ± 22.2        | 369 ± 17.5      | 339 ± 13.3        |
| Blood pressure (mm Hg)    | 134 ± 6   | 135 ± 6           | 132 ± 4         | 136 ± 4           |
| Left ventricular diameter in systole (mm) | 2.3 ± 0.1  | 1.9 ± 0.1         | 2.3 ± 0.1       | 2.1 ± 0.1         |
| Fractional shortening (%) | 41 ± 2ґ   | 51 ± 1            | 43 ± 2ґ         | 49 ± 1            |
| Left ventricular outflow velocity (m/sec) | 0.52 ± 0.03 | 0.55 ± 0.03 | 0.50 ± 0.02 | 0.50 ± 0.03 |
| E/A                      | 2.3 ± 0.27| 1.9 ± 0.11        | 1.9 ± 0.05      | 2.3 ± 0.19        |
| **Stress**                |           |                   |                 |                   |
| Heart rate (min⁻¹)        | 645 ± 32  | 644 ± 24          | 663 ± 21        | 649 ± 26          |
| Blood pressure (mm Hg)    | 151 ± 4   | 160 ± 4           | 153 ± 4         | 153 ± 4           |
| Fractional shortening (%) | 58 ± 2ґ   | 66 ± 1            | 57 ± 2ґ         | 67 ± 1            |
| Circumferential shortening (circ/sec) | 8.8 ± 0.3ґ | 10.4 ± 0.6 | 9.2 ± 0.5ґ | 11.1 ± 0.7 |
| Left ventricular outflow velocity (m/sec) | 0.49 ± 0.03ґ | 0.68 ± 0.04 | 0.52 ± 0.03 | 0.57 ± 0.03 |

* p < 0.005.
* * p < 0.0005 compared with control mice of the same genotype.
* * * p = 0.05.

**DISCUSSION**

The metabolism of fat is essential for the function of the heart. The present results suggest that accelerated lipoprotein formation by cardiac myocytes can remove excess triglycerides from the diabetic heart. This idea is in accordance with a recent seminal study (23), which demonstrated that hearts from apoB transgenic mice secrete more triglycerides in apoB-containing lipoproteins than control mice. Also, the overexpression of apoB in the heart decreased triglyceride stores in mice with a genetic defect in the metabolism of long chain fatty acids that results in cardiac triglyceride accumulation during prolonged fasting (23).

MTP is rate-limiting for the production and secretion of apoB-containing lipoproteins from the liver (24–26). Previous studies (26–29) have shown a tight correlation among MTP mRNA, protein, and activity levels in vitro and in vivo. Because the MTP mRNA expression levels were increased in the heart of diabetic mice, this study is compatible with the idea that lipoprotein secretion from the heart might be regulated. This notion has also gained support from studies of heart biopsies from patients undergoing cardiac surgery. Those studies suggest that the MTP mRNA expression is increased in hypoxic human left ventricle compared with normoxic human left ventricle, and that the MTP mRNA level is negatively associated with cardiac triglyceride storage in patients with ischaemic heart disease. An increase of lipoprotein secretion rates from cardiac myocytes during increased fat load (such as in diabetes with increased flux of free fatty acids into the heart and cardiac ischemia with decreased β-oxidation of fatty acids) could contribute to maintain a constant (low) level of triglycerides in cardiac myocytes. Interestingly, in this study, we only observed increased MTP gene expression in the heart and not in the livers of STZ-treated diabetic mice. The latter observation is in accordance with a more elaborate study of MTP in diabetic mouse livers (18). Further studies are needed to determine whether it might be possible to increase lipoprotein secretion from the heart without increasing the secretion from the liver.

We were able to test the functional consequences of cardiac triglyceride accumulation in the diabetic mouse heart, because cardiac triglyceride stores in diabetic mice were increased in wild type mice but were normal in apoB transgenic mice. Importantly, the blood levels of major cardiac fuel substrates (i.e., glucose, free fatty acids, and triglycerides) were similarly affected by STZ treatment in wild type and in apoB transgenic mice. Moreover, in the heart, the effect of apoB overexpression on cardiac lipids was confined to triglycerides, whereas there was no effect of apoB overexpression on cardiac cholesterol or phospholipid content. The assessments of cardiac BNP gene expression as well as biomechanical cardiac function suggested that a diabetes-specific affection of the heart in STZ-treated male mice is attenuated by a normalization of cardiac triglyceride stores. In apoB transgenic mice, several echocardiographic measures reflecting both systolic and diastolic function were either unaffected by STZ-induced diabetes or less affected than in wild type mice. This finding implies that lipoprotein secretion from the heart plays an integrated physiological role in cardiac function. Still, some parameters of systolic function during dobutamine stress were decreased by STZ treatment in both wild type and apoB transgenic mice. This indicates that the decline in systolic function of STZ-treated mice was not entirely related to cardiac triglyceride accumulation.

A causal link of cardiac triglyceride accumulation and cardiac dysfunction has been proposed recently (8). The normalization of cardiac triglycerides in genetically obese fa/fa rats with a peroxisome proliferator-activated receptor γ agonist attenuates the development of cardiac dysfunction (30). Cardiac overexpression of peroxisome proliferator-activated receptor α (31) or long chain acyl-CoA synthetase (32) in mouse hearts both confer an increase in triglyceride stores and a concomitant decrease of cardiac function. This study supports the idea that a normalization of cardiac triglyceride stores improves cardiac function in diabetic mice. This conclusion may add evidence to an emerging concept (8, 32, 33) that the accumulation of neutral lipids in cardiac myocytes causes “lipotoxic” heart disease. It is also conceivable that the accelerated removal of triglycerides by increased lipoprotein secretion affects intracellular fluxes of free fatty acids or perhaps cardiac fuel substrate utilization. An effect of apoB overexpression on intracellular intermediate metabolites in cardiomyocytes is compatible with the finding that cardiac free glycerol levels were increased in

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control apoB transgenic mice versus control wild type.

In conclusion, this study supports the idea that lipoprotein formation by the heart plays an integrated role in cardiac lipid homeostasis and affects cardiac physiology in diabetic mice.

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