Differential effects of high-fat diet on myocardial lipid metabolism in failing and nonfailing hearts with angiotensin II-mediated cardiac remodeling in mice

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Pellieux C, Montessuit C, Papageorgiou I, Pedrazzini T, Lerch R.
Differential effects of high-fat diet on myocardial lipid metabolism in failing and nonfailing hearts with angiotensin II-mediated cardiac remodeling in mice. Am J Physiol Heart Circ Physiol 302: H1795–H1805, 2012. First published March 9, 2012; doi:10.1152/ajpheart.01023.2011.—Normal myocardium adapts to increase of nutritional fatty acid supply by upregulation of regulatory proteins of the fatty acid oxidation pathway. Because advanced heart failure is associated with reduction of regulatory proteins of fatty acid oxidation, we hypothesized that failing myocardium may not be able to adapt to increased fatty acid intake and therefore undergo lipid accumulation, potentially aggravating myocardial dysfunction. We determined the effect of high-fat diet in transgenic mice with overexpression of angiotensinogen in the myocardium (TG1306/R1). TG1306/R1 mice develop ANG II-mediated left ventricular hypertrophy, and at one year of age approximately half of the mice present heart failure associated with reduced expression of regulatory proteins of fatty acid oxidation and reduced palmitate oxidation during ex vivo working heart perfusion. Hypertrophied hearts from TG1306/R1 mice without heart failure adapted to high-fat feeding, similarly to hearts from wild-type mice, with upregulation of regulatory proteins of fatty acid oxidation and enhancement of palmitate oxidation. There was no myocardial lipid accumulation or contractile dysfunction. In contrast, hearts from TG1306/R1 mice presenting heart failure were unable to respond to high-fat feeding by upregulation of fatty acid oxidation proteins and enhancement of palmitate oxidation. This resulted in accumulation of triglycerides and ceramide in the myocardium, and aggravation of contractile dysfunction. In conclusion, hearts with ANG II-induced contractile failure have lost the ability to enhance fatty acid oxidation in response to increased fatty acid supply. The ensuing accumulation of lipid compounds may play a role in the observed aggravation of contractile dysfunction.

fatty acid metabolism; lipotoxicity

PHENOTYPIC MODIFICATION of cardiac myocytes in chronic heart failure involves changes in myocardial substrate use. In animal models of heart failure, regulatory proteins of the fatty acid oxidation pathway are reduced in the myocardium, associated with a shift from fatty acid to glucose oxidation (11, 18, 21, 31, 33, 40–41). Reduced expression of fatty acid oxidation enzymes has also been observed in failing human hearts (37, 41). However, the role of reduction of fatty acid oxidation in the progression of left ventricular (LV) remodeling and heart failure remains controversial (43). Some authors consider reduction of fatty acid oxidation an adaptive response, which may protect the myocardium by potentially favorable effects of stimulation of glucose oxidation in stressed cardiac myocytes (45). On the other hand, reduction of fatty acid oxidation may contribute to progression of contractile dysfunction by accumulation of toxic lipid intermediates in the cytoplasm of cardiac myocytes (5, 42). If this putative mechanism applies to failing hearts, enhancement of dietary fatty acid intake may further compromise myocardial contractile function.

Normal myocardium adapts to prolonged exposure to high circulating levels of fatty acids by upregulating regulatory proteins of the fatty acid oxidation pathway, largely through fatty acid-induced activation of the nuclear transcription factors peroxisome proliferator-activated receptors (PPAR) α and β/δ (2). Transgenic mice with impaired ability to increase fatty acid oxidation capacity by deletion of the PPARα gene respond to fasting-induced elevation of circulating fatty acid by accumulation of lipids in the myocardium (19). Compatible with a role of lipid accumulation in contractile dysfunction, PPARα-deficient mice exhibit reduced contractile reserve (16, 24). Similarly, deletion of the PPARβ/δ gene results in lipotoxic cardiomyopathy (5). These observations in mice with transgenic reduction of the fatty acid oxidation pathway support the hypothesis that persistent limitation of fatty acid oxidation may contribute to cardiac dysfunction by lipid accumulation. However, it is presently not known whether and to which extent the myocardium of failing hearts with acquired downregulation of the fatty acid oxidation pathway is still able to adapt to increased fatty acid supply by enhancement of fatty acid oxidation.

Therefore, we determined in the present study the effect of high-fat diet on myocardial contractile function and substrate metabolism in mice with angiotensin II (ANG II)-induced hypertrophic ventricular remodeling and heart failure. For this purpose, we have used transgenic mice carrying multiple copies of the rat angiotensinogen gene under control of the cardiac α-myosin heavy chain promoter, which results in elevated myocardial angiotensinogen and ANG II levels without hypertension (TG1307/R1 mice) (26). The model has been extensively characterized (13, 25–26, 36) and was shown to reproducibly generate ventricular hypertrophy evolving into heart failure associated with downregulation of the fatty acid oxidation pathway (33).

METHODS

Animal model. The study was conducted in male transgenic mice with targeted overexpression of angiotensinogen in the heart (TG1306/R1) (25) and their wild-type (WT) littermates. TG1306/R1 mice are a one-rein gene strain with a C57BL/6 background harboring a transgene composed of the cardiac-specific α-myosin heavy chain promoter fused to a rat angiotensigen cDNA (25). During the first year of life, all mice were fed ad libitum standard diet chow. After 1 yr, both WT and TG1306/R1 mice were randomly assigned to either standard diet or high-fat diet. After 8 wk on the assigned diet ad libitum, mice were
anesthetized with 10 mg sodium thiopental intraperitoneally, and hearts were excised. Cardiac ventricles were separated from atria, and ventricles were frozen in liquid nitrogen for tissue analysis. Heart weight, determined before freezing, refers to ventricular weight without atria and was normalized to tibia length and body weight. Experimental procedures in animals were approved by the Ethical Committee for Animal Experimentation of the University of Geneva and the local animal protection authorities. The investigation conforms with the American Physiological Society (APS) Guiding Principles for the Care and Use of Vertebrate Animals in Research and Training (revision approved by the APS Council on July 16, 2010).

Diet. Standard diet (purified diet no. 2125; Provimi Kliba Research) derived 10.1% of metabolizable energy from fat, 65.6% from carbohydrates, and 20.4% from proteins. The source of lipids of the standard diet was a mixture of lard (4.0% of metabolizable energy) and soybean oil (5.4% of metabolizable energy) providing 1.7% of metabolizable energy as palmitate, 3.0% as oleate, and 3.6% as linoleate (only fatty acids with contribution ≥1% mentioned). High-fat diet (purified diet no. 2127; Provimi Kliba Research diets) derived 60.0% of metabolizable energy from fat, 17.1% from carbohydrates, and 19.3% from proteins. The lipid sources were lard (53.7% of metabolizable energy) and soybean oil (5.6% of metabolizable energy) providing 14.2% of metabolizable energy as palmitate, 7.8% as stearate, 1.7% as palmitoleate, 24.4% as oleate, 8.4% as linoleate, and 1.0% as linolenate. For both diets, the source of protein was casein, and the sources of carbohydrate were sucrose, maltodextrin, and cornstarch. The diets had low sodium content (<0.3% by weight) and were closely matched for content of mineral elements, trace elements, and vitamins.

Blood analysis. Blood samples were collected in sodium thiosulfate-anticoagulated mice after 4 h of food deprivation. After centrifugation of blood samples (10 min at 600 g; 4°C), free fatty acid concentration was measured using a diagnostic kit based on enzymatic reaction (Abcam). Enzyme-linked immunosorbent assays were used for measurement of serum concentrations of insulin and leptin (Miltiopore). Serum concentration of glucose was measured enzymatically using a kit from Cayman Chemicals.

RT-PCR. RNA extraction and RT-PCR were performed as in previous studies (33–34). For relative quantitation, the number of transcripts of the target gene per transcript of cyclophilin was determined. 18S rRNA was used as an alternative housekeeping gene for normalization with identical results. Primers and probes used for RT-PCR analysis of cyclophilin, 18S, tumor necrosis factor-α (TNF-α), atrial natriuretic factor, brain natriuretic peptide (BNP), PPARα, PPARβ/δ, PPARγ, fatty acid translocase (FAT/CD36), medium-chain acyl-CoA dehydrogenase (MCAD), and muscle-type carnitine palmitoyltransferase-I (mCPT-I) have been published previously (34). For additional genes, the following forward primers, reverse primers, and probes were used: GLUT1 (forward: 5′-GGT GTG CAG CAC CCT GTG TA-3′; reverse: 5′-GAC GAA CAG CGA CAC CAC AGT-3′; probe: 5′-FAM-CCA TCG CTT CGG GTA TCA ACA C- TAMARA-3′), GLUT4 (forward: 5′-CCC CCG ATA CCT CAT CAT C-3′; reverse: 5′-GCA TCA GAC ACA TCA GCC CAG-3′; probe: 5′-FAM-CTG CCC GAA AGA GTC GTA AAC GCC T- TAMARA-3′), and pyruvate dehydrogenase kinase 4 (PDFK4; forward: 5′-TTC ACA CCT TCA CCA GTG GC-3′; reverse: 5′-AAC GGG CGG TTT TCT TGA TGA TG-3′; probe: 5′-FAM-CTT GCC GCT CAT GGC ATT CTT G-TAMARA-3′). mRNA expression is given as the percentage of the mean value measured in WT mice receiving standard diet.

Western blot analysis. Preparation of protein extracts of ventricular myocardium and Western blot analysis were performed using standard protocols (33–34). Western blot analysis was performed using polyclonal antibodies against PPARα, PPARβ/δ, PPARγ, FAT/CD36, and MCAD from Cayman Chemical, TNF-α from Brunschwig Chemicals, and mCPT-I, a gift from Dr. Gebre Woldegiorgis. Specific signals were detected by chemiluminescence (Amersham). Results are expressed as the percentage of average signal intensity measured for samples from standard diet-fed WT mice that were loaded on the same gel.

Myocardial triglyceride and ceramide measurement. For lipid extraction using a modified Bligh and Dyer method (20), ventricular samples (90 mg of tissue) were homogenized in 1.9 ml of chloroform-methanol-1 mol/NaCl [1:2:0.4 (vol/vol/vol)], and the suspension was vortexed vigorously. After the subsequent addition of 500 µl chloroform and 500 µl of 1 mol/l NaCl, phases were separated by centrifugation at 3,000 rpm (2,000 g) for 5 min. The organic phase containing cardiac lipids was dissolved in methanol (final dilution 1.9 vol/vol).

Myocardial ceramides were measured by enzyme-linked immunosorbent assay (ELISA). For this purpose, microtiter plates (P96 MicroWell Nunc) were washed in methanol and then air-dried before coating. Microplate wells were coated with cardiac lipid extracts at 4°C overnight. Plates were then dried at 65°C to allow methanol to evaporate. Remaining binding sites on the wells were blocked with 3% BSA in PBS at pH 7.2 for 2 h at room temperature and washed three times, at room temperature, in PBS containing 0.05% Tween 20. Mouse anti-ceramide IgM (1:10; Sigma) was added to wells and allowed to react for 1 h. Excess primary antibody was removed by washing as described above, and the plate was subsequently incubated for 1 h with goat anti-mouse IgG peroxidase (1:1,000; Cell Signaling Technology) diluted in PBS. After washing, Trimethoxybenzic acid substrate solution was added. The reaction was stopped after 10 min with 4 N H2SO4. Results were read at 450 nm by spectrophotometer. Ceramide concentration was determined using the relationship obtained from linear regression of standard curves. Triglyceride content was measured in lipid extracts using an enzymatic assay kit (Cayman).

Histological analysis. Myocardial lipid deposition was assessed by oil red O staining of ventricular sections. Ventricles were fixed in 4% formal, embedded in optimal cutting-temperature compound, frozen on dry ice, and then stored at −80°C until sectioning. Serial 15-µm-thick sections were obtained using a cryostat (HMS25 Cryostat microscope; Microm, Waldorf, Germany). Ventricular sections were stained with oil red O and counterstained with hematoxylin. Images of heart sections were obtained by a Carl Zeiss Axioskop microscope equipped with an Axioscime color charge-coupled device camera (Carl Zeiss) and acquired with the AxioVision software (Carl Zeiss). Oil red O-stained droplets were quantified using MetaMorph 7 analysis software from Molecular Devices.

Isolated working mouse heart perfusion. Cardiac function and metabolism were measured in isolated working mouse hearts as described previously (33). Mice were heparinized (100 units ip) and anesthetized with 10 mg sodium thiopental intraperitoneally. The heart was subsequently excised and placed in ice-cold Krebs-Henseleit bicarbonate (KHB) buffer (pH 7.4) consisting of (in mmol/l): 118 NaCl, 25 NaHCO3, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 2.5 CaCl2, 0.5 EDTA, and 5 glucose. The aorta was cannulated with an 18-gauge plastic cannula, and retrograde perfusion at 37°C was initiated in the Langendorff mode with KHB buffer at a perfusion pressure of 60 mmHg. All perfusates were continuously gassed with 95% O2-5% CO2. After cannulation of the left atrium with a 16-gauge steel cannula, hearts were perfused in the working mode at a preload pressure of 7.5 mmHg and an afterload pressure of 55 mmHg. The total volume of the recirculating system was 40 ml. Lactate accumulation during the 40-min perfusion period remained negligibly low (0.5 ± 0.07 mmol/l; n = 6). The buffer used for the working heart perfusion was KHB buffer supplemented with 50 µU/ml insulin (Novo Nordisk) and containing the following metabolic substrates: 0.5 mmol/l [9,10-3H]palmitate (Perkin Elmer) bound to 5% BSA and 5 mmol/l [1-U14C]glucose (Perkin Elmer).

A 2-Fr micromanometer-tipped catheter (Millar Instruments, Houston, TX) was inserted in the left ventricle through the apex. Cardiac output was obtained from the sum of aortic and coronary flows. Cardiac power was calculated as the product of LV developed pressure and cardiac output.
Myocardial oxygen consumption (MV\(\dot{O}_2\)) was measured using a fiber-optic oxygen probe (FOXY-AL300; Ocean Optics, Duiven, Germany) placed in the pulmonary trunk as described previously (17). Cardiac efficiency was calculated as the ratio of cardiac power to MV\(\dot{O}_2\). Glucose oxidation was determined by measuring \(^{14}\)CO\(_2\) (17). Cardiac efficiency was calculated as the ratio of cardiac power to MV\(\dot{O}_2\). Glucose oxidation was determined by measuring \(^{14}\)CO\(_2\) (17). 

Cardiac hypertrophy and markers of heart failure in 14-month-old transgenic TG1306/R1 mice. To assess the influence of heart failure on metabolic adaptation, we assessed, as previously (33), TG1306/R1 mice on the basis of the lung weight (LW) to groups without (TG) or with (TG-PC) heart failure, whereby LW exceeding the mean value measured in WT mice by more than two SDS was considered to indicate pulmonary congestion (PC). Correlation of LW with cardiac power measured during ex vivo working heart perfusion (Fig. 1A) and with myocardial BNP mRNA expression (Fig. 1B) confirmed clustering of TG1306/R1 mice in two nonoverlapping groups. In one group, comprising all TG mice without heart failure, cardiac power, BNP mRNA expression, and LW were similar to those measured in hearts from WT mice. In the second group, comprising only TG-PC mice, cardiac power was reduced, and both BNP mRNA expression and LW were increased.

Among transgenic mice fed standard diet, 19 out of 37 TG1306/R1 mice (51%) presented increased LW (Table 1). Indexes of contractile function, including LV developed pressure, LV dp/dt\(\text{max}\), aortic flow, cardiac output, and cardiac power, did not differ between hearts isolated from standard diet-fed WT mice and TG mice without heart failure (Fig. 2 and Table 2). In contrast, all parameters of contractile function were significantly reduced in hearts isolated from TG-PC mice (Fig. 2 and Table 2). Myocardial BNP mRNA was increased sixfold in the TG-PC group compared with WT mice (Fig. 3A), but not in TG mice with normal LW. Similarly, TNF-\(\alpha\) mRNA and protein were increased in TG-PC mice, but not in TG mice without signs heart failure (Fig. 3, B and C).

Ventricular hypertrophy was present in TG1306/R1 mice, both without (TG group) and with (TG-PC group) heart failure, with an increase of heart weight normalized to tibia length by 22 and 55%, respectively (Table 1). Effect of high-fat diet on body mass, serum levels, heart weight, and function in WT and TG1306/R1 mice with and without heart failure. High-fat diet increased body weight in all mice, but without a difference between WT, TG, and TG-PC.

### Table 1. Body weight, heart weight and lung weight

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<th>Standard Diet</th>
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<th>High-Fat Diet</th>
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<tr>
<td></td>
<td>WT</td>
<td>TG-PC</td>
<td>WT</td>
<td>TG-PC</td>
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<tr>
<td>n</td>
<td>31</td>
<td>18</td>
<td>19</td>
<td>28</td>
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<tr>
<td>Body wt, g</td>
<td>36.0 ± 0.9</td>
<td>37.7 ± 0.8</td>
<td>39.3 ± 2.3</td>
<td>43.6 ± 1.7†</td>
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<tr>
<td>Heart wt, mg</td>
<td>161.1 ± 4.7</td>
<td>201.0 ± 8.0*</td>
<td>247.0 ± 23.8*</td>
<td>161.1 ± 5.9</td>
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<td>Heart wt/tibia length, mm/mm</td>
<td>9.5 ± 0.25</td>
<td>11.6 ± 0.5*</td>
<td>14.7 ± 1.4*</td>
<td>9.3 ± 0.3</td>
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<tr>
<td>Heart wt/body wt, mg/g</td>
<td>4.5 ± 0.1</td>
<td>5.3 ± 0.2*</td>
<td>6.3 ± 0.2‡</td>
<td>3.7 ± 0.1†</td>
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<tr>
<td>Lung wt, mg</td>
<td>202.4 ± 8.7</td>
<td>212.5 ± 5.1</td>
<td>264.3 ± 8.7‡</td>
<td>210.4 ± 3.2</td>
</tr>
<tr>
<td>Lung wt/tibia length, mm/mm</td>
<td>12.0 ± 0.3</td>
<td>12.2 ± 0.2</td>
<td>15.7 ± 0.4‡</td>
<td>12.1 ± 0.2</td>
</tr>
<tr>
<td>Lung wt/body wt, mg/g</td>
<td>5.6 ± 0.1</td>
<td>5.7 ± 0.1</td>
<td>6.7 ± 0.11‡</td>
<td>4.8 ± 0.1†</td>
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Values are means ± SE in wild-type mice (WT) or transgenic mice without (TG) and with (TG-PC) increased lung weight; \(n\), no. of mice. *\(P < 0.05\) vs. WT mice on the same diet. †\(P < 0.05\) vs. corresponding group on standard diet. ‡\(P < 0.05\) vs. TG mice without heart failure on the same diet.
mice (Table 1). Serum concentrations of fatty acid, glucose, insulin, and leptin did not differ among the groups fed standard diet (Table 3). Fasting serum concentrations of free fatty acid and leptin were significantly higher after high-fat feeding in WT, TG, and TG-PC mice compared with the corresponding group fed standard chow. Furthermore, glucose and insulin concentrations were increased after high-fat feeding, suggesting development of insulin resistance.

None of the mice died during the high-fat-feeding period. Eight weeks of high-fat diet did not alter the proportion of TG1306/R1 mice presenting increased LW (18 out of 36 mice; 50%) compared with TG1306/R1 mice fed standard diet (Table 1). This suggests that high-fat diet did not trigger the onset of heart failure. However, BNP mRNA was 11 times higher in TG mice presenting increased LW (18 out of 36 mice; 50%) compared with TG1306/R1 mice fed standard diet (Table 2). Consistent with this interpretation, high-fat feeding (Fig. 3) increased myocardial protein levels of FAT/CD36, mCPT-I, and MCAD. As observed previously (33), in mice fed standard diet, myocardial protein levels of FAT/CD36, mCPT-I, and MCAD did not differ from WT mice in TG mice without heart failure, achieving comparable levels among groups (Fig. 5, A and C). mRNA expression was concomitantly increased after high-fat feeding (Table 2).

Enhancement of fatty acid oxidation by high-fat diet is abolished in failing hearts but not in hearts with compensated hypertrophy. To determine whether metabolic adaptation to high-fat diet is preserved during ANG II–induced cardiac remodeling, we measured myocardial expression and indexes of contractile function in hearts from WT mice and hearts from TG mice without heart failure (Fig. 4, A and B). However, palmitate oxidation was markedly reduced in TG-PC hearts, by 38%, compared with the mean value measured in WT mice, associated with an increase of glucose oxidation by 21%. High-fat diet markedly increased palmitate oxidation in hearts from WT mice and nonfailing hearts from TG mice, by 244 and 239%, respectively, compared with corresponding groups of hearts from mice fed standard chow (Fig. 4A). Glucose oxidation was concomitantly reduced by 25 and 23%, respectively (Fig. 4B).

In contrast to WT and nonfailing TG hearts, palmitate oxidation was not stimulated by high-fat diet in failing TG-PC hearts and remained unaltered low (Fig. 4A). Correspondingly, glucose oxidation remained increased, without change (Fig. 4B). As reported previously (33), MV˙O2 was slightly higher in TG-PC hearts, but high-fat diet did not modify myocardial MV˙O2 in any of the three groups (Fig. 4C). Cardiac efficiency was reduced in TG-PC hearts from mice fed standard diet and was further decreased by high-fat feeding (Table 2).

High-fat diet–induced enhancement of regulatory proteins of fatty acid metabolism is lost in failing hearts with ANG II–mediated hypertrophy. To determine whether the blunted activation of palmitate oxidation by high-fat feeding in failing hearts is related to an altered response of expression of regulatory proteins of fatty acid metabolism, we measured myocardial mRNA and protein expression of FAT/CD36, mCPT-I, and MCAD. As observed previously (33), in mice fed standard diet, myocardial protein levels of FAT/CD36, mCPT-I, and MCAD did not differ from WT mice in nonfailing hearts from TG mice, by 244 and 239%, respectively, compared with corresponding groups of hearts. mRNA expression was concomitantly increased two- to fivefold, suggesting enhanced transcription during high-fat feeding (Fig. 5, D, E, and F). In contrast, high-fat diet...
did not modify mRNA or protein expression of regulatory proteins of fatty acid metabolism in failing TG-PC hearts.

Fatty acid-induced activation of regulatory genes of fatty acid metabolism involves activation of PPARs. We therefore measured myocardial expression of the three PPAR isoforms, PPARα, PPARβ/δ, and PPARγ (Fig. 6). In mice fed standard diet, both mRNA and protein expression of all three PPAR isoforms were reduced by ~50% in the TG group, and by >70% in the TG-PC group, as reported previously (33). High-fat diet increased mRNA and protein expression of all three isoforms in WT and TG mice without heart failure (Fig. 6). Because each PPAR isoform transactivates its own expression (3), enhancement of PPAR mRNA expression in the WT and TG group indirectly suggests activation of transcriptional activity of PPARs by high-fat feeding. However, neither mRNA nor protein expression of any of the three PPAR isoforms was induced by high-fat diet in failing TG-PC hearts (Fig. 6). Taken together, the results suggest that the transcriptional activation of regulatory proteins of fatty acid metabolism in response to high-fat diet is compromised in TG-PC mice with heart failure.

mRNA expression of selected regulatory proteins of glucose metabolism are shown in Fig. 7. As observed previously in 1-yr-old TG1306/R1 mice (33), there occurred a shift in glucose transporter expression with a decrease of insulin-regulated GLUT4 and an increase of GLUT1. The changes were more pronounced in failing TG-PC hearts. mRNA expression of PDK4, a PPARα-regulated enzyme that mediates inactivation of pyruvate dehydrogenase, was unchanged in myocardial expression of the three PPAR isoforms, which was significantly more pronounced in hearts from high-fat-fed mice (Fig. 8A). Myocardium of hearts from TG mice without heart failure, fed either standard or high-fat diet, exhibited cardiac myocyte hypertrophy without detectable accumulation of neutral lipids in the cytoplasm. In contrast, hearts from TG-PC mice, there was accumulation of neutral lipids, which was significantly more pronounced in hearts from high-fat-fed mice (Fig. 8B and C). Consistent with histological data, myocardial triglyceride levels, measured in lipid extracts, were not altered by high-fat feeding in WT and TG hearts without heart failure (Fig. 8C). However, myocardial triglyceride content was more than doubled after high-fat feeding in failing TG-PC hearts.

Because ceramide is considered to mediate cytotoxic effects of neutral lipid accumulation (32), we measured ceramide content in myocardial lipid extracts (Fig. 8D). Myocardial ceramide levels were comparable in WT and nonfailing TG hearts and were not modified by the diet regimen. Similarly, myocardium of TG-PC mice fed standard diet exhibited no increase in ceramide levels. However, the amounts of myocardial ceramide were significantly higher (increased by 51%) in TG-PC mice under high-fat diet compared with the TG-PC group fed standard chow. The observations suggest that down-regulation of the myocardial fatty acid oxidation pathway in ANG II-induced heart failure is associated with accumulation of toxic lipid intermediates during high-fat feeding.

**Experiments in adult rat cardiac myocytes: Effect of TNF-α on fatty acid-mediated upregulation of regulatory proteins of fatty acid oxidation.** We have previously observed in adult rat cardiac myocytes that TNF-α reduces the expression of regul-

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**Table 3. Serum substrate and hormone concentrations**

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<th>Standard Diet</th>
<th>High-Fat Diet</th>
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<tr>
<td></td>
<td>WT</td>
<td>TG</td>
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<tr>
<td>Free fatty acids, mM</td>
<td>0.13 ± 0.01</td>
<td>0.13 ± 0.02</td>
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<tr>
<td>Glucose, mM/l</td>
<td>12.5 ± 0.7</td>
<td>12.7 ± 0.8</td>
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<tr>
<td>Insulin, ng/ml</td>
<td>0.64 ± 0.17</td>
<td>0.70 ± 0.18</td>
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<tr>
<td>Leptin, pg/ml</td>
<td>4.3 ± 2.4</td>
<td>4.8 ± 1.2</td>
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<td></td>
<td></td>
<td>0.22 ± 0.01†</td>
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<td>15.5 ± 0.3†</td>
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<td>1.79 ± 0.08†</td>
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<td>34.4 ± 2.6†</td>
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Values are means ± SE in WT, TG, and TG-PC mice; n = 6 mice for each value. *P < 0.05 vs. WT mice on the same diet. †P < 0.05 vs. corresponding group on standard diet. ‡P < 0.05 vs. TG mice without heart failure on the same diet.
latory genes involved in fatty acid oxidation (34). In view of this observation and given the increase of TNF-α in TG-PC hearts in the present study, we used adult rat cardiac myocytes to determine whether TNF-α alters the transcriptional response of genes encoding regulatory proteins of fatty acid oxidation to enhancement of fatty acid availability. In control cardiac myocytes, supplementation of the medium with 0.5 mmol/l fatty acid (0.25 mmol/l palmitate plus 0.25 mmol/l oleate) increased mRNA expression of PPARα and PPARβ/δ and of the target genes mCPT-I and MCAD two- to threefold (Fig. 9A). In cardiac myocytes cultured without fatty acid supplementation, addition of TNF-α (10 μg/l) resulted in marked reduction of mRNA expression of all investigated genes. In the presence of TNF-α, supplementation of the medium with 0.5 mmol/l fatty acid still increased mRNA expression, but values remained markedly lower compared with control cardiac myocytes cultured at the same fatty acid concentration without TNF-α.

To determine whether TNF-α alters DNA binding of PPARs, we measured in nuclear extracts from adult rat cardiac myocytes binding of PPARα and PPARβ/δ to peroxisome proliferator-responsive element (PPRE)-containing oligonucleotides by an ELISA. TNF-α markedly reduced DNA binding of PPARα and PPARβ/δ after 3 days of culture (Fig. 9B). The results obtained in adult rat cardiac myocytes are consistent with the hypothesis that TNF-α may be involved in the impairment of the transcriptional response to fatty acid stimulation of regulatory genes of fatty acid oxidation.

![Fig. 4](image_url) Effects of high-fat diet on cardiac energy metabolism in WT mice and TG1306/R1 mice with ANG II-induced ventricular hypertrophy. Palmitate oxidation (A), glucose oxidation (B), and myocardial oxygen consumption (MVO₂) (C), were measured during ex vivo perfusion of hearts in the working mode as described in METHODS. Open bars, values measured in hearts isolated from standard diet-fed WT, TG, and TG-PC mice; filled bars, corresponding values obtained in hearts from mice fed high-fat diet for 8 wk; n ≥ 7 hearts for each value. Values are means ± SE. *P < 0.05 vs. WT mice on the same diet. †P < 0.05 vs. corresponding group on standard diet. ‡P < 0.05 vs. TG mice without heart failure on the same diet.

![Fig. 5](image_url) High-fat diet increases protein and mRNA expression of regulatory proteins of fatty acid metabolism in nonfailing hearts, but not in failing hearts from TG1306/R1 mice. Bars represent protein expression of fatty acid translocase (FAT/CD36, A), muscle-type carnitine palmitoyltransferase-I (mCPT-I, B), and medium-chain acyl-CoA dehydrogenase (MCAD, C) measured by Western blot in ventricles from WT, TG, and TG-PC mice after feeding standard diet (open bars) or high-fat diet (filled bars) for 8 wk. Values are expressed as a percentage of values measured in samples from standard diet-fed WT mice. Corresponding mRNA values measured by RT-PCR with cyclophilin as the housekeeping gene are also displayed for FAT/CD36 (D), mCPT-I (E), and MCAD (F). mRNA values are expressed as a percentage of the mean value measured in hearts from WT mice fed standard diet; n ≥ 5 hearts for each value. Values are means ± SE. *P < 0.05 vs. WT mice on the same diet. †P < 0.05 vs. corresponding group on standard diet. ‡P < 0.05 vs. TG mice without heart failure on the same diet.
DISCUSSION

Normal myocardium exhibits pronounced metabolic flexibility and adjusts to prolonged elevation of fatty acid supply by increased myocardial expression of genes encoding regulatory proteins of fatty acid oxidation (4, 46). In the present study, we demonstrate that, contrary to normal hearts, failing hearts from TG1306/R1 mice have, in addition to reduced baseline levels, entirely lost the ability to increase expression of regulatory proteins of fatty acid metabolism and consequently to adapt to high dietary fatty acid supply. High-fat feeding elicited in failing TG1306/R1 hearts myocardial accumulation of lipid compounds and further deterioration of LV contractile function.

Metabolic adaptation of TG1306/R1 mice to high-fat diet. Although many studies have demonstrated reduction of fatty acid oxidation and of regulatory proteins of the fatty acid oxidation pathway in myocardium of failing hearts (11, 18, 21, 31, 33, 40–41), little is known whether the myocardium still is able to adapt to increased nutritional fatty acid supply. Therefore, we have in this study challenged with high-fat diet 1-yr-old mice presenting targeted overexpression of angiotensinogen in the myocardium (TG1306/R1 mice). TG1306/R1 mice develop LV hypertrophy in response to chronic elevation of ANG II in the myocardium (26). After the first year of life, approximately half of TG1306/R1 mice present signs of heart failure. We have previously observed (33) that, in 1-yr-old TG1306/R1 mice without signs of heart failure, myocardial protein expression of regulatory proteins of fatty acid metabolism did not differ from WT mice, resulting in unaltered fatty acid and glucose oxidation during ex vivo perfusion. In contrast, in failing hearts from TG1306/R1 mice, regulatory proteins of fatty acid metabolism were severely reduced, associated with marked reduction of fatty acid oxidation and enhancement of glucose oxidation (33). The present study
extends these observations and demonstrates a profound difference between failing and nonfailing hearts from TG1306/R1 mice in the metabolic adaptation to high-fat feeding. The metabolic response of hypertrophied hearts from TG1306/R1 mice without signs of heart failure did not differ from that of hearts from WT mice. Both WT and nonfailing TG1306/R1 hearts displayed after high-fat feeding an identical increase of protein expression of investigated regulatory proteins of fatty acid metabolism (FAT/CD36, mCPT-I, AND MCAD) and of palmitate oxidation. In contrast, high-fat feeding restored neither expression of regulatory proteins of fatty acid metabolism nor palmitate oxidation in hearts from TG1306/R1 mice presenting heart failure.

The increase of protein expression of regulatory proteins of fatty acid metabolism in nonfailing hypertrophied hearts from TG1306/R1 mice was associated with an increase of mRNA expression, indicating preserved capability to respond to high-fat diet by enhancement of transcription. This is consistent with previous reports demonstrating upregulation of mRNA expression of regulatory proteins of fatty acid metabolism by high-fat diet.

**Fig. 8.** High-fat diet enhances lipid accumulation in the myocardium of TG1306/R1 mice presenting heart failure, but not during compensated hypertrophy. A: representative histological sections showing accumulation of oil red O-stained lipid droplets in failing hearts of WT1306/R1 mice (TG-PC), which increases after high-fat feeding. Sections of nonfailing hearts (TG) from TG1306/R1 mice exhibit myocyte hypertrophy compared with hearts from WT mice, but no accumulation of lipid droplets. B: bars showing morphometric analysis of oil red O-stained sections. Myocardial content of triglycerides (C) and ceramide (D) was determined in lipid extracts. Open bars, values measured in hearts of mice fed standard diet; filled bars, values measured in mice fed high-fat diet. Values are means ± SE of at least 6 hearts. *P < 0.05 vs. WT mice on the same diet. †P < 0.05 vs. corresponding group on standard diet. ‡P < 0.05 vs. TG mice without heart failure on the same diet.

**Fig. 9.** TNF-α reduces expression of regulatory proteins of fatty acid oxidation (A) and DNA-binding activity of PPARα and PPARβ/δ (B) in isolated adult rat cardiac myocytes. mRNA expression of PPARα, PPARβ/δ, CPT-I, and MCAD (A) is increased 2- to 3-fold in control adult rat cardiac myocytes (open bars) cultured for 7 days in medium containing 0.5 mmol/l fatty acid (0.25 mmol/l palmitate plus 0.25 mmol/l oleate) compared with cardiac myocytes cultured with medium without fatty acid supplementation. The addition of TNF-α (filled bars) markedly reduced expression of regulatory proteins at either fatty acid concentration. mRNA expression is displayed as percent of values measured in cardiac myocytes cultured without TNF-α. *P < 0.05 vs. same conditions without TNF-α. †P < 0.05 vs. corresponding value without fatty acid supplementation. DNA binding of PPARα and PPARβ/δ (B) was impaired by TNF-α. DNA binding was measured in nuclear extracts of adult rat cardiac myocytes immediately after isolation and after 3, 7, and 10 days of culture by an enzyme-linked immunosorbent assay. The graph displays the time course of reduction of DNA binding expressed as a percentage of values measured in cardiac myocytes cultured without TNF-α. Values are means ± SE of at least 3 determinations for each data point.
diet in normal myocardium (28) and in rodent models of ventricular hypertrophy (1, 30). The most likely mechanism of high-fat diet-induced stimulation of transcription of regulatory proteins of fatty acid metabolism in nonfailing hearts is activation of PPARα and PPARβ/δ both of which are implicated in transcription of FAT/CD36, mCPT-I, and MCAD. Consistent with this interpretation, mRNA and protein expression of PPARα and PPARβ/δ, but also of PPARy, were significantly increased by high-fat feeding in both WT and TG1306/R1 mice without heart failure. In contrast, both mRNA and protein expression of all three PPAR isoforms were severely reduced in hearts from TG1306/R1 mice with heart failure, without a change during high-fat diet. The mechanism underlying the apparent refractoriness of PPAR-mediated upregulation of genes of fatty acid metabolism in failing hearts of TG1306/R1 mice is presently not known. One possibility is that TNF-α, which was increased in the myocardium of failing hearts independently of the dietary regimen, contributed to persistent reduction of PPAR isoforms and regulatory proteins of fatty acid metabolism. We have previously reported that, in adult rat cardiac myocytes in long-term culture, ANG II-induced reduction of protein levels of all PPAR isoforms and of regulatory proteins of fatty acid metabolism in nonfailing hearts is activated by 10.220.33.3 on October 15, 2017 http://ajpheart.physiology.org/ Downloaded from may cause myocardial lipid accumulation (22). The results of our study indicate that, in this model, obesity alone is not sufficient to cause significant myocardial lipid accumulation, but lipid accumulation is induced by the combined effects of high-fat feeding and ANG II-mediated reduction of fatty acid oxidation in the myocardium.

Effect of high-fat feeding on contractile function. In this study, high-fat feeding resulted in lower LV contractile function of hearts isolated from TG1306/R1 mice presenting heart failure. Myocardial expression of BNP was also further increased, which is consistent with the interpretation that high-fat feeding aggravated heart failure.

The effect of high-fat diet on contractile function has recently been investigated in a number of rodent models of chronic cardiac overload, which have provided inconsistent results. Indexes of contractile function were found unchanged (6–7, 27, 29), improved (8, 30, 38–39), or deteriorated (1) after high-fat feeding. In the present study in mice with ANG II-induced remodeling, the effect of high-fat diet on contractile function measured during ex vivo perfusion differed between mice without and with heart failure.

Our observations in TG1306/R1 mice are compatible with the hypothesis that impairment of fatty acid oxidation may contribute to contractile dysfunction in failing hearts by favoring accumulation of lipid intermediates in the cytoplasm. However, our results do not allow identification of a potential link between lipid accumulation and contractile dysfunction. Nevertheless, in our study, accumulation of lipid droplets and triglycerides in failing TG1306/R1 hearts was associated with increased myocardial content of ceramide, which is elevated in models of cardiac lipotoxicity (9–10, 14), and has documented lipotoxic effects (32).

Study limitations. There are a number of limitations of this study that need to be addressed. First, loss of metabolic flexibility in failing hearts of TG1306/R1 mice may be model-specific and not apply to other etiologies of heart failure. The model has been selected because chronic myocardial ANG II stimulation seems to play a central role in a number of clinically relevant conditions leading to heart failure (44). Second, mice were not examined at 12 mo for determination of metabolic and functional status before the dietary intervention and for validation of homogenous assignment to both diet groups. We had previously characterized myocardial metabolism and function in 1-yr-old TG1306/R1 mice by identical methods and observed that close to half of mice exhibited heart failure. Although skewed assignment to diet groups cannot be excluded, the number of 1-yr-old TG1306/R1 mice included for randomization was selected rather high (73 TG1306/R1 mice) to keep this risk low. However, the cross-sectional design of the present study does not provide information on the time course of observed modifications. Third, contractile function was not monitored in vivo. Although indexes of contractile function obtained during ex vivo perfusion offer the advantage to reflect alteration of myocardial function without interference of differing loading conditions, the relevance of observed diet-induced differences for in vivo conditions is not known. Nevertheless, deterioration of ex vivo contractile indexes in failing TG1306/R1 hearts after high-fat diet was paralleled by a further increased myocardial BNP expression indirectly, suggesting further impairment of contractile function in vivo. Fourth, palmitate oxidation was measured during working heart perfusion.

Lipid accumulation in failing hearts. The histological findings indicate that heart failure in TG1306/R1 mice is associated with accumulation of lipids in the cardiac myocytes. Our data are compatible with the interpretation that reduction of myocardial fatty acid oxidation is causally involved in this phenomenon because increased fatty acid supply by high-fat feeding substantially enhanced lipid accumulation in failing hearts, but not in nonfailing TG1306 hearts, which were able to adapt to increased fatty acid supply by transcriptional expansion of fatty acid oxidation capacity.

After 8 wk of high-fat feeding, both WT and TG1306/R1 mice exhibited features of diet-induced obesity, including increased body weight (by ~25%), elevation of serum concentration of fatty acids and leptin, as well as increased serum concentration of glucose and insulin, suggesting insulin resistance. It is currently debated whether feeding-induced obesity...
at a perfusate palmitate concentration of 0.5 mmol/l. Stimulation of palmitate oxidation in failing TG1306/R1 hearts at higher perfusate palmitate concentration cannot be excluded. However, the selected palmitate concentration is comparatively high considering serum concentrations measured in vivo during high-fat diet in the present study and in reported studies (1, 6, 38). Finally, the results of the present study do not provide information on the effect of high-fat diet on global mitochondrial function. Fatty acid and glucose oxidation are an integral part of energy metabolism in mitochondria, which undergo a number of functional and structural alterations during the development of heart failure (15).

In conclusion, we provide evidence that, in heart failure induced by prolonged exposure to ANG II, the myocardium has lost the ability to adapt to high-fat diet by increasing expression of regulatory proteins of fatty acid oxidation and stimulation of fatty acid oxidation. This may contribute to aggravation of lipid accumulation and contractile dysfunction seen during increased nutritional fat supply in these hearts. The observation may have implications for progression of heart failure under conditions of high circulating levels of fatty acid, including diabetes.

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DISCLOSURES

There are no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS


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