Increased Glucose Uptake and Oxidation in Mouse Hearts Prevent High Fatty Acid Oxidation but Cause Cardiac Dysfunction in Diet-Induced Obesity

Jie Yan, MD, PhD; Martin E. Young, DPhil; Lei Cui, MD; Gary D. Lopaschuk, PhD; Ronglih Liao, PhD; Rong Tian, MD, PhD

Background—Shift of myocardial substrate preference has been observed in many chronic diseases such as diabetes and heart failure. This study was undertaken to elucidate the mechanisms underlying the chronic substrate switch in adult hearts and to determine the functional consequences of the switch.

Methods and Results—Transgenic mice with cardiac-specific overexpression of the insulin-independent glucose transporter GLUT1 (TG) were used to increase intracellular glucose in cardiac myocytes. A high-fat diet was used to increase the fatty acid supply to the heart. High-fat diet induced a 40% increase in fatty acid oxidation in wild-type hearts, whereas glucose oxidation was decreased to 30% of the control. In contrast, glucose oxidation was >2-fold higher in TG hearts, and the high-fat diet failed to upregulate fatty acid oxidation in these hearts. Glucose induced changes in the expression of multiple metabolic genes, including peroxisome proliferator-activated receptor-α (decreased by 51%), 3-oxoacid CoA transferase (decreased by 67%), and acetyl-CoA carboxylase (increased by 4-fold), resulting in a remodeling of the metabolic network to favor a shift of substrate preference toward glucose. Although TG mice on a normal diet maintained normal cardiac energetics and function, the inability to upregulate myocardial fatty acid oxidation in TG mice fed a high-fat diet resulted in increased oxidative stress in the heart, activation of p38 mitogen-activated protein kinase, and contractile dysfunction.

Conclusions—We have demonstrated that chronic increases in myocardial glucose uptake and oxidation reduce the metabolic flexibility and render the heart susceptible to contractile dysfunction. (Circulation. 2009;119:2818-2828.)

Key Words: cardiomyopathy ■ contractility ■ fatty acids ■ glucose ■ metabolism

The heart requires constant and substantial energy supply for continuous pumping and thus has developed an elaborate metabolic network for using all carbon substrates, including carbohydrates, fatty acids, ketones, and amino acids. During development, the substrate preference of the heart switches from primarily carbohydrate (fetal and neonatal stage) to predominantly fatty acids (adult). Although the adult heart uses fatty acids for >50% of its energy supply, the cardiac metabolic machinery is highly flexible, allowing rapid switch of substrate use in response to a variety of stresses such as exercise, fasting, and ischemia. Chronic shift of myocardial substrate preference also has been noted in many diseases such as diabetes mellitus and heart failure. However, the underlying mechanisms and the functional consequence of the shift are poorly understood.

Clinical Perspective on p 2828

We have previously shown that the adult mouse heart can adapt to sustained high intracellular glucose by switching to a fetal-like metabolic pattern for life with no adverse functional consequence. Here, we demonstrate that chronic increases in intracellular glucose altered expressions and activities of key regulatory proteins in fatty acid and ketone metabolism pathways. Such a remodeling allows a long-term shift of substrate preference toward glucose while maintaining cardiac energetic and function. However, in our mouse model of complete adaptation to a high-intracellular-glucose milieu, the heart fails to upregulate fatty acid oxidation during diet-induced obesity and suffers from increased oxidative stress and contractile dysfunction. Thus, the prevention of the
high fatty acid oxidation during high-fat (HF) diet–induced obesity predisposes the heart to functional impairment.

Methods

Animal Models

Transgenic mice overexpressing the insulin-independent glucose transporter GLUT1 in the heart (TG) were generated on FVB background as previously described.6 TG mice and their wild-type (WT) littermates (16 weeks old) were randomly assigned to an HF diet (45% energy from fat) or a nutrient-matched low-fat (LF) diet (12% energy from fat, both from TestDiet, Richmond, Ind) for 20 weeks. Mice were housed in a climate-controlled environment with a 12-hour light/dark cycle and free access to food and water. Animal experimental protocols were approved by the Harvard Medical Area Standing Committee on Animals. After 20 weeks of feeding, blood samples were drawn from mice for determination of glucose (One-Touch Glucose Monitor, Lifescan Inc, Milpitas, Calif), free fatty acids (Wako Chemicals, Richmond, Va), and insulin (Crystal Chemical Inc, Wakefield, Mass) levels using commercially available assay kits.

Isolated Perfused Heart Experiments and Nuclear Magnetic Resonance Spectroscopy

Mice were heparinized (100 U IP) and anesthetized by sodium pentobarbital (150 mg/kg IP). The heart was excised and perfused at a constant pressure of 80 mm Hg at 37°C as previously described.7 The perfusate contained the following (in mmol/L): NaCl 118, NaHCO3 25, KCl 5.3, CaCl2 2, MgSO4 1.2, EDTA 0.5, glucose 5.5, mixed long-chain fatty acids 0.4 (bound to 1% albumin), DL-β-hydroxybutyrate 0.38, lactate 1.0, and insulin 50 μU/mL, equilibrated with 95% O2 and 5% CO2 (pH 7.4). Hearts were paced at 7 Hz throughout the protocol. Isovolumic contractile function was estimated by the product of left ventricular (LV) developed pressure and heart rate (rate-pressure product [RPP]). Myocardial oxygen consumption (MVO2) was measured by determining the arteriovenous differences in O2 saturation as previously described.8

After a 30-minute equilibration period, hearts were maintained at baseline workload or challenged with high workload by increasing the CaCl2 concentration from 2 to 4 mmol/L in the perfusate for 30 minutes. Dynamic changes in cardiac high-energy phosphate content and intracellular pH (pHi) were monitored by 31P nuclear magnetic resonance (NMR) spectroscopy simultaneously with a continuous mass). Data from each heart were normalized by background fluorescence intensity to calculate the DCFDA intensity.

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction

Total RNA extraction and quantitative real-time polymerase chain reaction of heart samples were performed as previously described.10 Primer and probe sequences for these assays are presented in Table I of the online-only Data Supplement. Standard RNA was made for all assays by the T7 polymerase method (Ambion, Austin, Tex) with total RNA isolated from mouse hearts. Additional details are provided in the online-only Data Supplement.

Biochemical Assays

Myocardial lipids were extracted from unperfused hearts by chloroform/methanol, and triglyceride content was assayed with a Sigma (St Louis, Mo) kit (TR0100) as described previously.11 Detection and quantification of myocardial malonyl-CoA level were performed by extracting CoA esters from powdered tissue into 6% perchloric acid and measuring with a modified high-performance liquid chromatography as described.12 Cardiac glycogen content was determined by an alkaline extraction procedure to separate glycogen and exogenous glucose in the tissue. Glucose released from glycogen was measured with a glucose assay kit (GAHK-20, Sigma).

Immunoblotting

Western blotting was performed with cardiac tissue lysates. Total protein extracts (40 μg) were resolved by SDS-PAGE and transferred to polyvinylidene fluoride membranes. Overnight incubation (4°C) was performed with the primary antibodies. Further details are provided in the online-only Data Supplement.

Transthoracic Echocardiography

Mice were anesthetized with an Acuson Sequoia C-256 echocardiograph machine (Siemens, Malvern, Pa) and a 15-MHz probe as described previously.7

Measurements of Cell Shortening and Intracellular Ca2+ Transients

Freshly isolated cardiac myocytes were placed in a chamber mounted on the stage of a Nikon TS 100 microscope and superfused with Tyrode buffer containing 1.8 mmol/L CaCl2, pH 7.4, at 37°C. Myocytes were field stimulated at 5 Hz, and cell shortening and relengthening were assessed with a video edge detector and specialized data acquisition software (SoftEdge Acquisition System and IonWizard, IonOptix, Milton, Mass). For simultaneous measurement of intracellular Ca2+ transient, myocytes were preloaded with 1 μmol/L fura-2/AM (Molecular Probes, Carlsbad, Calif) for 15 minutes, and fluorescence intensity was recorded with a dual-excitation fluorescence photomultiplier system (IonOptix) as previously described.13

Analysis of Tissue Reactive Oxygen Species Level

Tissue levels of lipid peroxides in heart homogenates were assessed by measuring the chromophore formed by the reaction of N-methylnicotinamide and methanesulfonic acid as described.14 To prevent sample oxidation during the assay, 5 mmol/L butylated hydroxytoluene was added before the homogenization. For histological assessments of tissue reactive oxygen species level, cryosections of the left ventricle were incubated for 1 hour at 37°C with 5-(6)-chloromethyl-2,7′-dichlorodihydrofluorescein diacetate (DCFDA; 4 μmol/L, Molecular Probes, Carlsbad, Calif) for 15 minutes, and fluorescence intensity was measured with a dual-excitation fluorescence photomultiplier system (IonOptix) as previously described.15

Statistical Analysis

All data are presented as mean±SEM. ANOVA with the Bonferroni post hoc test was performed for multiple-group comparisons, and an unpaired t test was used for 2-group comparisons. Analyses were performed with GraphPad Prism 4.0 (GraphPad Software, San Diego, Calif), and a value of P<0.05 was considered significant.

Results

Animal Models

We used TG mice as a model of increased intracellular glucose in cardiac myocytes. An HF diet was used to increase the fatty acid supply to the heart. Compared with normal-diet groups, both TG mice and their WT littermates fed an HF for 20 weeks exhibited an ≈50% increase in body weight (P<0.05 for both; Figure 1A). There was no difference in heart weight normalized to body weight or to tibia length among all groups (Table II in the online-only Data Supplement). Serum glucose and insulin levels also were significantly elevated but to the same extent in HFWT and HFTG mice (Figure 1B and 1C). These results

Downloaded from http://circ.ahajournals.org/ by guest on May 1, 2017
suggest that the WT and TG mice responded similarly to the HF diet, and both developed obesity, hyperglycemia, and insulin resistance.

Enhanced Myocardial Glucose Use in TG Mice Is Resistant to Modulation by HF Diet

To delineate the effects of increased glucose and fatty acid availability on the metabolic fate of each substrate, we examined the relative contributions of 5 classes of carbon substrates to the oxidative metabolism and MVO₂ in isolated perfused hearts from the 4 groups of mice using ¹³C NMR isotopomer analysis (Figure 2). Compared with WT, the contribution of glucose to oxidative metabolism in TG hearts was increased by 2-fold in mice fed a normal diet (P<0.05). The HF diet increased the contribution of fatty acids to oxidative metabolism by 40% in WT, whereas the contribution from glucose decreased to 30% of LFWT, suggesting an upregulation of myocardial fatty acid oxidation in mice with diet-induced obesity (P<0.05 versus LFWT for both). Interestingly, HF diet did not induce upregulation of myocardial fatty acid oxidation in TG mice, and the use of glucose remained high after 20 weeks of HF diet. Of note, none of the relative contributions from other substrates, ie, ketone, lactate, and endogenous substrates, in TG hearts was affected by HF diet regardless of diet (P<0.05 versus LFWT for both). Interestingly, HF diet did not induce upregulation of myocardial fatty acid oxidation in TG mice, and the use of glucose remained high after 20 weeks of HF diet. Of note, none of the relative contributions from other substrates, ie, ketone, lactate, and endogenous substrates, in TG hearts was affected by HF diet in isolated perfused hearts from the 4 groups of mice using ¹³C NMR isotopomer analysis (Figure 2).

When the hearts were challenged with high workload (cardiac performance increased by 60%), the relative oxidation of glucose increased and that of fatty acids decreased in WT hearts regardless of diet (Figure 2B), consistent with the previous studies showing that the relative contribution of carbon substrates to oxidative metabolism shifts to glucose under stress. In contrast, when TG hearts were challenged with high workload, there was no change in the substrate use profile compared with baseline in either diet group, suggesting a loss of metabolic flexibility. Because the ¹³C NMR isotopomer analysis provided relative oxidation of each substrate, we determined MVO₂ to assess total oxidative metabolism. MVO₂ was similar among the 4 groups at baseline and increased ~60% at high workload (Figure 2C and 2D). MVO₂ was slightly lower in HFTG at high workload, consistent with a lower contractile performance in the group (see below). MVO₂ normalized to workload was similar for all groups at each workload (Figure 2E and 2F).

Consistent with our previous observation, we found that myocardial glycogen content increased by 3-fold in TG hearts (Figure 2G). Although the HF diet increased glycogen content significantly (by 32%) in WT hearts, the glycogen content in TG hearts did not increase further with the HF diet. We also found a 2-fold increase in triglyceride content in TG hearts, which was in contrast to a modest increase (31%) caused by HF diet in WT hearts (Figure 2H). This was consistent with the observation of suppressed fatty acid contribution to oxidation in TG hearts. Collectively, the data showed that glucose use was reduced and fatty acid oxidation was increased in WT hearts by HF diet but not in TG hearts even though diet-induced obesity and insulin resistance developed in both groups.

Chronic Increases in Substrate Availability Remodeled the Metabolic Network at the Molecular Level

To determine the molecular mechanisms responsible for the changes of substrate preference caused by long-term alter-
ations of substrate availability to the heart, we measured the expression of a subset of genes known to play key roles in myocardial substrate use in the heart (Figure 3 and Figure I-II of the online-only Data Supplement). Peroxisome proliferator-activated receptor-α (PPARα), a key player in transcriptional control of cardiac fatty acid metabolism, was significantly downregulated in hearts with increased glucose or fatty acid availability (Figure 3A). The downregulation was more

Figure 2. Substrate use in the hearts of WT and TG mice fed an HF or LF diet. A and B, Relative contributions of each substrate to the oxidative metabolism in isolated mouse hearts perfused with 13C-labeled substrates during baseline (A) and high workload (B) (n=5 to 7 per group). C through F, Myocardial oxygen consumption and oxygen efficiency for contractile function during baseline (C and E) and high workload (D and F) (n=3 to 4 per group). Total glycogen (G) and triglyceride (H) content (n=5 to 7 per group) in the hearts of WT and TG mice fed a HF or LF diet for 20 weeks. Data are mean±SEM. *P<0.05 vs LFWT; #P<0.05 vs LFTG (see text for statistical significance for A and B).
pronounced in hearts with high glucose supply (TG) than in hearts with high fatty acid supply (HF diet). The expression of PPARα, another important regulator of fatty acid oxidation in cardiac tissue, decreased modestly only in the HFWT group (Figure IA of the online-only Data Supplement). The expression of retinoid X receptor–α (RXRα), the obligate partner of PPARs, was not different among the groups (Figure IB). Despite the marked decrease in PPARα expression, the expression of its target genes involved in fatty acid oxidation was mostly unsuppressed (online-only Data Supplement Figure IC through IH). However, one of the PPARα target genes, the fatty acid transporter CD36, was induced by HF diet in WT but not in TG hearts (Figure 3B).

We found significant changes in the expression of 3-oxoacid CoA transferase (SCOT) and acetyl-CoA carboxylase (ACC); neither of them was the PPARα target gene, but played important roles in ketone and fatty acid metabolism. SCOT expression was downregulated by 67% in TG hearts,
and the change was independent of the diet regimen (Figure 3C). SCOT catalyzes the rate-limiting step in the formation of acetyl-CoA from acetoacetate by transferring the CoA from succinyl-CoA and is therefore essential for ketone oxidation. The marked downregulation of SCOT predicts decreased ketone oxidation in TG hearts, which has been demonstrated by the 13C NMR study (Figure 2).

The expression of ACCα, mainly in the liver, and the expression of ACCβ, mainly in the heart, were both significantly induced in the LFTG and HFTG hearts (Figure 3D and 3E). The fact that ACC was not induced in HFWT and was not further induced by HF diet in TG hearts suggested that this change, similar to the downregulation of SCOT, was a glucose-dependent event. Consistent with the gene expression changes, Western blotting revealed significant increases in ACC protein in the hearts of TG mice on either diet regimen, and no change in ACC protein amount was found in the HFWT hearts (Figure 3F).

ACC catalyzes the synthesis of malonyl-CoA, a key metabolite in fatty acid metabolism that is degraded by malonyl-CoA decarboxylase, the expression of which was not altered in all groups (data not shown). Cardiac content of malonyl-CoA increased by 32% in the TG hearts (Figure 3G), suggesting that the total ACC activity was increased in these hearts. Similar to the pattern of ACC upregulation, cardiac malonyl-CoA content increased only in TG hearts and was not altered by HF diet in either the WT or TG group. Because malonyl CoA is a potent inhibitor of mCPT-1 in oxidative tissue, increased malonyl-CoA in TG hearts inhibits the rate-limiting step of fatty acids entering mitochondria, thus limiting the upregulation of fatty acid oxidation and increasing acyl-CoA content (Figure 4). In addition, malonyl CoA produced by ACCα in lipogenic tissue is an essential substrate for fatty acid synthesis, raising the possibility that the substantial upregulation of ACCα in TG hearts results in increased fatty acid synthesis and triglyceride accumulation, although fatty acid synthesis activity is considered very low in the heart.

These data collectively suggest that high intracellular glucose induced changes in the regulatory enzymes at the level of transcription in favor of glucose oxidation at the expense of fatty acids and ketone oxidation (Figure 4). Furthermore, the induction of ACC, together with downregulation of PPARα and consequently failure to upregulate CD36, prevented the upregulation of fatty acid oxidation despite diet-induced obesity.

**Impaired Contractile Function but Normal Energetics in TG Hearts During Diet-Induced Obesity**

To determine whether the altered substrate use profile had any functional significance, we assessed in vivo cardiac function by echocardiography in all mice at the end of 20 weeks of diet feeding (age of mice, 36 to 40 weeks). Compared with WT mice, LV fractional shortening was modestly but significantly decreased in TG mice fed an HF diet (57±2 versus 65±1; P<0.05). HFTG mice also showed modest increases in LV end-diastolic and end-systolic dimensions with no changes in wall thickness (Figure 5A through 5D). Thus, the inability to shift myocardial substrate use in TG mice fed an HF diet was associated with mild cardiac dysfunction in vivo.

We also assessed isovolumic contractile function of isolated perfused hearts from all groups to evaluate myocardial contractile function independently of neurohormonal regulation. At the baseline workload, all hearts were able to achieve and sustain an RPP of ≈50 000 mm Hg/min (Figure 6A). When subjected to high workload by doubling the Ca2+ concentration in the perfusion buffer, the RPP increased ≈60% in all groups. Importantly, all hearts except the HFTG group could sustain the high contractile performance. At the end of the high-workload period, the RPP of HFTG was decreased by 37% (Figure 6A), suggesting an impaired contractile reserve. This finding corroborated the in vivo function study and suggested that TG hearts, although protected from high fatty acid oxidation, were maladaptive to HF diet–induced obesity.

Because TG mice on the HF diet failed to increase myocardial fatty acid oxidation, we tested whether the inability to sustain high workload could be attributed to impaired myocardial energetic status resulting from inadequate ATP synthesis. Using 31P NMR spectroscopy, we measured dynamic changes in high-energy phosphate content in isolated perfused hearts at baseline and high workload. The concentrations of ATP and phosphocreatine, the energy reserve compound, and the intracellular pH were not significantly different in all groups during both baseline and high-workload challenge (Figure 6B through 6D), suggesting that myocardial energetic status was not impaired in these models. Thus, the contractile dysfunction associated with the failure to increase fatty acid oxidation in HFTG hearts was unlikely due to insufficient energy supply.

**Intracellular Calcium Homeostasis Is Maintained in Cardiac Myocytes of HFTG Mice**

Impaired calcium handling has been shown in diabetic cardiomyopathy.18 We thus determined whether abnormal
intracellular calcium handling contributes to the impaired contractile function in HFTG. We found a significant decrease in sarcoplasmic reticulum calcium ATPase (SERCA) protein in HFTG hearts (Figure 7A). Phospholamban, the inhibitory regulator of SERCA, was unchanged in total amount, but its phosphorylation was increased significantly (Figure 7B). Phosphorylation of phospholamban decreases the inhibition of SERCA, thus serving as a compensatory mechanism for maintaining the activity of SERCA protein. The sodium-calcium exchanger protein 1 was unchanged in all groups (Figure 7C). To directly assess the intracellular calcium cycling and its effect on cardiac myocyte contraction, we isolated cardiac myocytes from the 4 groups of hearts and measured intracellular calcium simultaneously with cell shortening (Figure 7D and 7E). The amplitude of cell shortening was decreased in myocytes isolated from HFTG hearts, but the cytosolic calcium level was unaltered at either the resting state or the peak of contraction. Thus, the myocyte study reproduced the contractile dysfunction observed both in isolated hearts and in vivo. Furthermore, it suggested that mechanisms independent of intracellular calcium handling were responsible for the impaired contraction of HFTG hearts.

Figure 5. Cardiac function assessed by transthoracic echocardiography. A, LV fractional shortening; B, LV end-systolic diameter; C, LV end-diastolic diameter; D, LV posterior wall thickness in WT and TG mice fed an HF or LF diet for 20 weeks. Data are mean±SEM (n=8 to 10 per group). *P<0.05 vs LFWT.

Figure 6. Contractile function and myocardial high-energy phosphate of isolated perfused mouse hearts measured by 31P-NMR spectroscopy. A, Cardiac contractile function estimated as RPP (n=6 to 12 per group). B through D, Myocardial concentrations of phosphocreatine (PCr), ATP, and intracellular pH (n=4 to 5 per group) in the hearts of WT and TG mice fed an HF or LF diet during baseline and high workload. Dotted vertical lines represent the switch from baseline to high workload. Data are mean±SEM. *P<0.05 vs LFWT.
Increased Oxidative Stress and Activation of p38 Mitogen-Activated Protein Kinase in HFTG Hearts

Previous studies have shown that activation of p38 mitogen-activated protein kinase (MAPK) exerts negative inotropic effects on cardiac myocytes independently of cytosolic calcium cycling.\(^{19-21}\) We examined the MAPK pathway and found increased phosphorylation of p38 MAPK in HFTG hearts (Figure 8A). The activation of p38 MAPK appeared to be unique because phosphorylations of other MAPKs such as extracellular signal-regulated kinase or Jun N-terminal kinase were increased only slightly and nondiscriminatory by the HF diet in WT and TG hearts. Using 2 independent methods to evaluate reactive oxygen species level in the heart, we found increased oxidative stress in the HFTG group. Cardiac malondialdehyde and 4-hydroxyalkenals levels, indicators of lipid peroxide content, were increased by >2.5-fold in the HFTG group (Figure 8B). Increased tissue reactive oxygen species load also was indicated by a higher intensity of DCFDA fluorescence in frozen tissue sections from HFTG hearts (Figure 8C). Concurrent increases in oxidative stress and p38 MAPK activation in HFTG suggest an important role of this pathway in the pathogenesis of contractile dysfunction during chronic metabolic derangement.

Discussion

Fatty acid oxidation supplies >50% of energy for a normal adult heart, whereas glucose and lactate are the main energy source for fetal hearts.\(^{1-3,5}\) Using genetic manipulations, we
have generated an adult mouse heart (TG) that uses carbohydrates (glucose and lactate) for 50% of its energy supply, whereas the use of fatty acids and ketone is suppressed, thus mimicking the fetal metabolic profile. The TG hearts have normal morphology and function, and the TG mice live a normal lifespan, suggesting a complete adaptation to the fetal-like cardiac metabolism. However, unlike the neonatal hearts that switch to fatty acid oxidation when circulating levels of fatty acid and oxygen supply increase after birth, glucose oxidation is sustained and high fatty acid oxidation is prevented in the adult TG hearts despite increased fatty acid availability during HF diet–induced obesity. We have identified a significant upregulation of ACC protein and activity in TG hearts that led to increased malonyl-CoA production, resulting in decreased fatty acid entry into mitochondria and increased lipid storage in the cytosol. Furthermore, the induction of ACC is glucose specific, is not affected by fatty acid availability, and thus represents a unique mechanism that cross-communicates glucose and fatty acid metabolism in the heart. A similar observation is made for SCOT, which accounts for inhibition of ketone oxidation in TG hearts.

The transcriptional program mediated by PPARα and PPARδ plays a central role in maintaining fatty acid oxidation in adult hearts. It also is responsible for the upregulation of fatty acid oxidation during increased fatty acid supply such as fasting or eating an HF diet.

Figure 8. p38 MAPK activation and increased reactive oxygen species production in the heart of TG mice fed an HF diet. A, Protein levels of MAPKs and their phosphorylation in the hearts of WT and TG mice fed an HF or LF diet for 20 weeks. Because p-P38 MAPK was nearly undetectable in the LF diet group, no quantitative comparisons among the groups were made. Lipid peroxide: malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) content (B) and frozen heart sections (C) stained with the green fluorescence dye DCFDA. Scale bar=20 μm. Data are expressed as mean±SEM from 4 to 5 hearts for each group. *P<0.05 vs LFWT.
ingly, sustained increases in the availability of either glucose or fatty acids result in a decrease in PPARα expression, although the expression of many PPARα target genes is not suppressed. The dissociation of PPARα expression and its activity in these models suggest that PPARα expression level alone may not be an appropriate marker of cardiac substrate metabolism in vivo. We speculate that increased fatty acid supply to the heart during an HF diet presents a higher amount of PPARα ligands that stimulate the transcriptional activity. It is possible that increased lipid storage in the TG hearts also stimulates PPARα activity. A similar observation has been made in hearts with suppressed fatty acid oxidation and increased lipid content. These observations indicate a very important role of feedback circuits in the network response to metabolic stress that would not have been predicted by the overexpression or deletion of PPARα. In addition, the substantial downregulation of PPARα expression (51% lower) in TG hearts may have played a key role in counteracting the stimulatory effects of fatty acids, eg, upregulation of the fatty acid transporter CD36, during the HF diet. We suggest that this mechanism adds to the ACC upregulation and renders the TG heart resistant to an HF diet–induced shift to fatty acid oxidation (Figure 4).

Despite the striking differences in substrate use pattern, cardiac energetics and function in WT mice subjected to an HF diet or in TG mice on regular diet are well sustained, supporting the notion that a normal heart is capable of using a wide range of energy substrates without compromising its function. These observations suggest that a simple shift of substrate preference toward carbohydrates or fatty acids is not sufficient to cause cardiac “glucotoxicity” or “lipotoxicity.” However, the adjustments of cardiac metabolic network to chronic changes in substrate availability deplete the system of its flexibility at the molecular level so that the reestablished homeostasis is vulnerable to further stresses. When the TG mice were subjected to an HF diet and developed obesity and insulin resistance, the absence of increased fatty acid oxidation was not protective but was associated with cardiac dysfunction. Thus, although high fatty acid oxidation in the heart has been associated with a number of pathological conditions, our findings suggest that maneuvers to sustain myocardial glucose oxidation and/or to prevent high fatty acid oxidation in obesity should be evaluated with great caution.

Altered substrate metabolism in the heart has been observed in multiple disease conditions, but its role in the pathogenesis of cardiac dysfunction has been elusive. It has been shown that a shift toward glucose preference is favorable for sustaining energy production under conditions of impaired ATP generation from fatty acid oxidation such as cardiac hypertrophy. However, we show here that a glucose-centered metabolic network in the adult heart increases the susceptibility to fatty acid overload despite a complete functional adaptation in the absence of additional metabolic perturbation. In diet-induced obesity, we find a higher level of reactive oxygen species in HFTG hearts when increased fatty acid availability failed to upregulate fatty acid oxidation. Activation of p38 MAPK, observed in HFTG hearts, is a known consequence of increased oxidative stress. Interestingly, chronic activation of p38 MAPK also has been shown to impair contractile function in the absence of abnormal calcium handling and is thus a likely mechanism for the cardiac dysfunction observed in HFTG.

The present study also has identified molecular responses in excitation-contraction coupling mechanisms during chronic changes of substrate availability. For example, increased oxidative stress in HFTG hearts is associated with a downregulation of SERCA protein at the posttranscriptional level. Interestingly, decreased SERCA protein is compensated for by inactivation of phospholamban in HFTG, yielding normal calcium transient. These mechanisms partially compensate for the impairment of cardiac function in chronic metabolic derangement. Indeed, the in vivo cardiac dysfunction observed in the present study is rather mild. Nevertheless, the study demonstrated that the underlying molecular remodeling in response to altered metabolism forms a basis for accelerated deterioration when other pathological conditions superimpose.

Acknowledgments

We would like to thank Dr Jun Yoshioka for his advice on the measurement of tissue reactive oxygen species, Dr Benbo Gao for his help with biochemical assays, and Drs Jianru Shi and Bo Wang for their help in the cardiac myocyte experiments.

Sources of Funding

This study is supported by National Institutes of Health fund R01 HL59246 (to Dr Tian). Dr Tian is an established investigator of the American Heart Association.

Disclosures

None.

References


12. Dyck JR, Hopkins TA, Bonnet S, Michelakis ED, Young ME, Watanabe M, Kawase Y, Jhiashe K, Lopaschuk GD. Absence of malonyl coenzyme...
A decarboxylase in mice increases cardiac glucose oxidation and protects the heart from ischemic injury. *Circulation*. 2006;114:1721–1728.


**CLINICAL PERSPECTIVE**

The heart requires constant and substantial energy supply for continuous pumping and thus has developed an elaborate metabolic network for using all carbon substrates, including carbohydrates, fatty acids, ketones, and amino acids. Chronic shift of myocardial substrate preference has been noted in many diseases such as diabetes and heart failure. However, the underlying mechanisms and the functional consequence of the shift are poorly understood. The present study demonstrates that chronic changes in substrate availability induce molecular remodeling of multiple metabolic pathways by altering expressions and activities of key regulatory proteins. The remodeling allows a long-term shift of substrate preference while maintaining cardiac energetic and function. However, the new homeostasis is established at the expense of network flexibility. In our study, the transgenic mouse model with enhanced glucose uptake, glycolysis, and glucose oxidation in the hearts shows complete adaptation to a high-intracellular-glucose milieu. However, failure to upregulate fatty acid oxidation in the transgenic hearts during diet-induced obesity results in increased oxidative stress and contractile dysfunction. Thus, this study cautions against the simple views that “high glucose oxidation is beneficial” and “high fatty acid oxidation is detrimental.” Instead, these observations suggest that inflexibility of the metabolic network in the heart caused by chronic metabolic diseases forms a basis for accelerated deterioration when other pathological conditions superimpose.
Increased Glucose Uptake and Oxidation in Mouse Hearts Prevent High Fatty Acid Oxidation but Cause Cardiac Dysfunction in Diet-Induced Obesity
Jie Yan, Martin E. Young, Lei Cui, Gary D. Lopaschuk, Ronglih Liao and Rong Tian

Circulation. 2009;119:2818-2828; originally published online May 18, 2009;
doi: 10.1161/CIRCULATIONAHA.108.832915

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/119/21/2818

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2009/05/18/CIRCULATIONAHA.108.832915.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/
Online Data Supplement

Supplemental Methods

RNA extraction and quantitative RT-PCR
Primer and probe sequences for these assays are presented in Data Supplement Table I. Standard RNA was made for all assays by the T7 polymerase method (Ambion, Austin, TX) with the use of total RNA isolated from mouse hearts. The correlation between the Ct (the number of PCR cycles required for the fluorescent signal to reach a detection threshold) and the amount of standard was linear over at least a five-log range of RNA for all assays. β-actin was used as control.

Immunoblotting
Western blotting was performed using cardiac tissue lysates. 40 µg of total protein extracts were resolved by SDS-PAGE and transferred to polyvinylidene fluoride membranes. Overnight incubation (4°C) were performed with the primary antibodies against acetyl-CoA carboxylase (ACC), phosphorylated ERK(p-ERK), phosphorylated JNK(p-JNK), phosphorylated p38 MAPK(p-p38 MAPK), ERK, JNK, p38 MAPK, β-Actin (Cell Signaling Technology), phosphorlamban (PLN, Upstate USA Inc), phosphorylated phospholamban(p-PLN, Upstate USA Inc), sodium-calcium exchanger protein 1 (NCX1, Abcam), and sarcoplasmic reticulum calcium ATPase (SERCA 2a, kindly provided by Federica del Monte, Harvard Medical School). Protein bands were visualized by chemiluminescent signal using ECL (Cell Signaling Technology) and quantified by densitometry using ImageQuant 5.0 (Molecular Dynamics, Sunnyvale, CA).
Supplemental Table I

Primer and probe sequences used in real time quantitative RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer/Probe</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Forward</td>
<td>5’-GGCCAGTGCTATGCTGAGAT-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-AGGGTGCAATGCTGCTCCA-3'</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>5’-FAM-CGTTTGAGCATAATGGGATGCAGCCAGATT-TAMRA-3'</td>
</tr>
<tr>
<td></td>
<td>Forward</td>
<td>5’-ACTTTTACCTACCCGCTGTG-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-CTGAGTGCCGGATATGGGC-3'</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>5’-FAM-TGCATCTTTTACCTTGAGGAGGCGCTAAG-TAMRA-3'</td>
</tr>
<tr>
<td></td>
<td>Forward</td>
<td>5’-ATGGGACATGATTATGGGCA-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-GATGGACCTCGAAATGTCAGA-3'</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>5’-FAM-AGATGCAGCCTCCTTTACACCTTTTGTAGGAGGGA-TAMRA-3'</td>
</tr>
<tr>
<td></td>
<td>Forward</td>
<td>5’-ATCAGTGATCCCGCCAAACT-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-GGGATGCGTGTAGGTGGAC-3'</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>5’-FAM-TCAAGCCGGGTGCATGGCACTGGG-TAMRA-3'</td>
</tr>
<tr>
<td></td>
<td>Forward</td>
<td>5’-GGGAGTAGAACGAAAGCGCAA-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-GCCAGACGATCCAGAGAT-3'</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>5’-FAM-CCATTAGTGAGAACAACCTGTCTTGTTAGGAGA-TAMRA-3'</td>
</tr>
<tr>
<td></td>
<td>Forward</td>
<td>5’-GGCATATGGGTTGACAGG-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-CCAAATACCTCTTTCTGAGTCA-3'</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>5’-FAM-AGGATCCGGCAAGAAATGTGTTGACCA-TAMRA-3'</td>
</tr>
<tr>
<td></td>
<td>Forward</td>
<td>5’-TGGGACACCATTTACCTCATAAA-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-GCCAGACATCCAAGACACCA-3'</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>5’-FAM-AGACTATACCCCCCTGTGTCCCTTGTAGGAA-TAMRA-3'</td>
</tr>
<tr>
<td></td>
<td>Forward</td>
<td>5’-TTCCACACCTCCACCATGC-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-AAAGGCGTTTCTGTTGAT-3'</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>5’-FAM-CGTTGGCCTCATGGCCATTCTTG-3'</td>
</tr>
<tr>
<td></td>
<td>Forward</td>
<td>5’-CAAGGCCCTCAGGTCACACT-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-TTGCAGTCCTCCGATCACCT-3'</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>5’-FAM-AGGCTGTAAAGGGCTTCTTTTCGGC-TAMRA-3'</td>
</tr>
<tr>
<td></td>
<td>Forward</td>
<td>5’-AGAGAGAAGTGCCATGGGT-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-CATCACAGCCCATCTGCAG-3'</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>5’-FAM-CTGGAGAGGTCTGCACAGGTCT-TAMRA-3'</td>
</tr>
<tr>
<td></td>
<td>Forward</td>
<td>5’-ACAGGACAGCGTCTCACAGT-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-TTGCAGTCCTCCGATCACCT-3'</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>5’-FAM-AGGCTGTAAAGGGCTTCTTTTCGGC-TAMRA-3'</td>
</tr>
<tr>
<td></td>
<td>Forward</td>
<td>5’-AACCGTAAAGGGCTTCTTTTCGGC-TAMRA-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-CCACGGTGATTTTCTGCGAC-3'</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>5’-FAM-SCATGGGGGGATGCTACACCTTTT-GAGTTCTGCACTTT-TAMRA-3'</td>
</tr>
<tr>
<td></td>
<td>Forward</td>
<td>5’-CCAACCAGAGGACAAAGATG-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-FAM-AGTTTGTCAGTAAACAGGTGAGACTCCAGCAA-TAMRA-3'</td>
</tr>
</tbody>
</table>
## Supplemental Table II

### General Characteristics of the Mice

<table>
<thead>
<tr>
<th></th>
<th>LFWT (n=7)</th>
<th>HFWT (n=9)</th>
<th>LFTG (n=9)</th>
<th>HFTG (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HW/BW, mg/g</td>
<td>4.07±0.15</td>
<td>3.85±0.26</td>
<td>4.29±0.15</td>
<td>3.96±0.08</td>
</tr>
<tr>
<td>HW/TL, mg/mm</td>
<td>7.53±0.54</td>
<td>7.95±0.26</td>
<td>8.67±0.60</td>
<td>8.73±0.45</td>
</tr>
<tr>
<td>Lung, wet/dry</td>
<td>4.04±0.12</td>
<td>4.07±0.08</td>
<td>4.11±0.07</td>
<td>4.13±0.07</td>
</tr>
<tr>
<td>Liver, wet/dry</td>
<td>3.00±0.04</td>
<td>2.92±0.07</td>
<td>2.91±0.03</td>
<td>2.92±0.04</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. HW/BW, heart weight normalized to body weight; HW/TL, heart weight normalized to tibia length.
Figure I. Expression of PPAR δ, RXRα and PPAR α target genes determined by real-time PCR. (A and B) Expression of PPAR δ and RXRα, (C-H) Expression of mCPT1, MCAD, LCAD, PDK4, UCP3 and Mte1 in the hearts of WT and TG mice fed a HF or LF diet for 20 weeks. Data are mean ± SEM (n=6 per group). *p < 0.05 vs LFWT.
Figure II

Figure II. mRNA expression levels of Glut1 and Glut4 in the hearts of WT and TG mice fed a HF or LF diet for 20 weeks. Data are mean ± SEM (n=3 per group). *p < 0.05 vs LFWT; #p < 0.05 vs LFTG.