Recruitment of Compensatory Pathways to Sustain Oxidative Flux With Reduced Carnitine Palmitoyltransferase I Activity Characterizes Inefficiency in Energy Metabolism in Hypertrophied Hearts

Natalia Sorokina, PhD; J. Michael O’Donnell, PhD; Ronald D. McKinney, BS; Kayla M. Pound, BS; Gebre Woldegiosgiris, PhD; Kathryn F. LaNoue, PhD; Kalpana Ballal, PhD; Heinrich Taegtmeyer, MD, DPhil; Peter M. Buttrick, MD; E. Douglas Lewandowski, PhD

Background—Transport rates of long-chain free fatty acids into mitochondria via carnitine palmitoyltransferase I relative to overall oxidative rates in hypertrophied hearts remain poorly understood. Furthermore, the extent of glucose oxidation, despite increased glycolysis in hypertrophy, remains controversial. The present study explores potential compensatory mechanisms to sustain tricarboxylic acid cycle flux that resolve the apparent discrepancy of reduced fatty acid oxidation without increased glucose oxidation through pyruvate dehydrogenase complex in the energy-poor, hypertrophied heart.

Methods and Results—We studied flux through the oxidative metabolism of intact adult rat hearts subjected to 10 weeks of pressure overload (hypertrophied; n=9) or sham operation (sham; n=8) using dynamic $^{13}$C–nuclear magnetic resonance. Isolated hearts were perfused with [2,4,6,8,10,12,14,16-$^{13}$C$_8$] palmitate (0.4 mmol/L) plus glucose (5 mmol/L) in a 14.1-T nuclear magnetic resonance magnet. At similar tricarboxylic acid cycle rates, flux through carnitine palmitoyltransferase I was 23% lower in hypertrophied ($P<0.04$) compared with sham hearts and corresponded to a shift toward increased expression of the L–carnitine palmitoyltransferase I isoform. Glucose oxidation via pyruvate dehydrogenase complex did not compensate for reduced palmitate oxidation rates. However, hypertrophied rats displayed an 83% increase in anaplerotic flux into the tricarboxylic acid cycle ($P<0.03$) that was supported by glycolytic pyruvate, coincident with increased mRNA transcript levels for malic enzyme.

Conclusions—in cardiac hypertrophy, fatty acid oxidation rates are reduced, whereas compensatory increases in anaplerosis maintain tricarboxylic acid cycle flux and account for a greater portion of glucose oxidation than previously recognized. The shift away from acetyl coenzyme A production toward carbon influx via anaplerosis bypasses energy, yielding reactions contributing to a less energy-efficient heart. (Circulation. 2007;115:2033-2041.)

Key Words: fatty acids ■ glucose ■ hypertrophy ■ isotopes ■ metabolism

Hypertrophied myocardium demonstrates reductions in both bioenergetic potential1–4 and fatty acid oxidation.5–8 Recent studies report increased glycolysis in hypertrophied hearts.5–12 However, glucose oxidation via pyruvate dehydrogenase complex (PDC) does not keep pace, suggesting an apparent uncoupling between glycolysis and glucose oxidation.10,11 The findings of reduced fatty acid oxidation without matched increases in glucose oxidation to support energy flux raise the question of how tricarboxylic acid (TCA) cycle flux can be maintained under these conditions. The present study addresses this question, providing new evidence for compensatory recruitment of alternative intermediary pathways to maintain flux through the energy-providing pathways in hypertrophic myocardium.

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Despite the reductions in free fatty acid oxidation, transport of long-chain free fatty acids into mitochondria by carnitine palmitoyltransferase I (CPT1) versus overall TCA cycle flux rates has not been well characterized in the intact hypertrophied heart. Furthermore, altered expression of the CPT1 M (muscle) and L (liver) isoforms has been suggested in chronically stimulated cell cultures, but the functional impact of potential shifts in M- and L-CPT1 expression on long-
chain fatty acid oxidation in intact, hypertrophied hearts has not been examined.\textsuperscript{13} Reductions in lipid entry into the mitochondria have been noted and would alter energy transfer mechanisms, suggesting a need for compensatory changes in the mode of energy provision such as increased fueling of oxidative metabolism from glucose.

Therefore, we hypothesized that in the event of altered long-chain fatty acid oxidation in hypertrophied myocardium and in the absence of increased glucose oxidation through PDC to accommodate enhanced glycolysis, compensatory pathways must sustain carbon flux through the TCA cycle for energy synthesis. Such adjustments in reducing equivalent use within the mitochondria must involve intermediate exchange between the mitochondria and cytosol and recruitment of compensatory intermediary pathways that sustain TCA cycle flux. Furthermore, the potential for alternative routes of glucose oxidation such as carboxylation of pyruvate for “anaplerotic” flux of carbon into the second span, or 4-carbon span, of the cycle must also be considered.

The results of the present study offer surprising new information on the recruitment of compensatory intermediary pathways that, although less efficient in energy synthesis, maintain TCA cycle activity in the hypertrophied heart and allow potential rerouting of glucose oxidation into the TCA cycle.

\section*{Methods}

\subsection*{Pressure-Overload Hypertrophy}

Pressure-overload cardiac hypertrophy (HYP) was produced in male Sprague-Dawley rats (Harlan, Indianapolis, Ind) by constriction of the transverse aorta.\textsuperscript{14} In sham surgical rats (sham), the aorta was isolated but not constricted. Hypertrophic state was confirmed via assay for atrial natriuretic factor mRNA.\textsuperscript{15} At 10 weeks after banding, atrial natriuretic factor and ratio of heart weight to body weight were elevated above those of sham by 77% and 63%, respectively. The protocols are approved by the Animal Care Policies and Procedures Committee at University of Illinois at Chicago (Institutional Animal Care and Use Committee accredited), and animals were maintained in accordance with the \textit{Guide for the Care and Use of Laboratory Animals} (National Research Council, revised 1996).

\subsection*{Isolated Perfused Rat Heart}

Isolated hearts were perfused in retrograde fashion as previously described with modified Krebs-Henseleit buffer (116 mmol/L NaCl, 4 mmol/L KCl, 1.5 mmol/L CaCl\textsubscript{2}, 1.2 mmol/L MgSO\textsubscript{4}, and 1.2 mmol/L NaH\textsubscript{2}PO\textsubscript{4} equilibrated with 95% O\textsubscript{2}/5% CO\textsubscript{2} with 0.4 mmol/L unlabeled palmitate/albumin complex [3:1 molar ratio] and 5 mmol/L glucose).\textsuperscript{16,17} A water-filled latex balloon in the left ventricle was set to a diastolic pressure of 5 mm Hg provided hemodynamic recordings (Powerlab, AD Instruments, Colorado Springs, Colo). Left ventricular developed pressure and heart rate were recorded continuously. Hearts were maintained at 37°C. Pulmonary artery effluent was collected for measurement of oxygen consumption.

\subsection*{Experimental Protocols}

Hearts were initially supplied buffer containing unlabeled palmitate/albumin and glucose for 10 minutes to ensure metabolic equilibrium and to allow collection of \textsuperscript{13}C–magnetic resonance (NMR) background signals of naturally abundant \textsuperscript{13}C (1.1%). At this time, energetic state was monitored by \textsuperscript{31}P-NMR (phosphocreatine and ATP content). At the start of each enrichment protocol, the perfusate was switched to buffer (1 L) containing [2,4,6,8,10,12,14,16-\textsuperscript{13}C\textsubscript{1}] palmitate (0.4 mmol/L) (Isotec, Inc, Miamisburg, Ohio) plus unlabeled glucose (5 mmol/L) (HYP, n = 9; sham, n = 8). Perfusion with \textsuperscript{13}C-enriched media continued for 40 minutes with collection of sequential \textsuperscript{13}C-NMR spectra (every 2 minutes).\textsuperscript{16–18} Two additional groups of isolated hearts were perfused with unlabeled palmitate (0.4 mmol/L) plus [1,6,\textsuperscript{13}C\textsubscript{1}] glucose (5 mmol/L) (HYP, n = 7; sham, n = 5) to evaluate glucose metabolism from tissue extracts. After each protocol, hearts were freeze-clamped for biochemical analysis.

\subsection*{NMR Spectroscopy}

Perfused hearts were situated in a 20-mm NMR probe within a vertical 9-mm-bore, 9.4-T magnet. NMR data from isolated, beating hearts were obtained with a Bruker 400 AVANCE NMR spectrometer (Bruker Daltonics, Billerica, Mass). \textsuperscript{31}P- and \textsuperscript{13}C-NMR measurements were acquired by methods described elsewhere.\textsuperscript{16–22} \textsuperscript{13}C-NMR spectra were collected in 2-minute time blocks over the 40-minute protocol, and \textsuperscript{31}P-NMR spectra were each collected over 2-minute intervals.

In vitro \textsuperscript{1}H- and \textsuperscript{13}C-NMR analysis was performed on spectra from heart tissue extracts at 14.1 T.\textsuperscript{16–22}

\subsection*{Kinetic Analysis of \textsuperscript{13}C-NMR Data}

Metabolic flux was determined during \textsuperscript{13}C palmitate oxidation in the intact, hypertrophied rat heart ex vivo using a previously described method for kinetic analysis of the progressive \textsuperscript{13}C enrichment of glutamate, as detected via NMR spectroscopy.\textsuperscript{16,18,23–24} \textsuperscript{13}C-labeled palmitate is transported across the mitochondrial membrane via CPT1 and oxidized through \beta oxidation, contributing to the formation of acetyl coenzyme A (CoA) enriched at the second carbon position. \textsuperscript{13}C–acetyl CoA enters the TCA cycle, subsequently enriching the 4-carbon position of \alpha-ketoglutarate (Figure 1). Transport and chemical exchange between \alpha-ketoglutarate and cytosolic glutamate result in labeling at the glutamate 4-carbon. As the carbon label recycles back into the first span of the TCA cycle, \textsuperscript{13}C incorporates at the 2- or 3-carbon of \alpha-ketoglutarate and ultimately glutamate.\textsuperscript{16,18,20,22} The rate of isotope exchange between these metabolic compartments has been reported.\textsuperscript{16,18,21–23}

A recently described model was applied for a simultaneous, 3-parameter, least-squares fit to the \textsuperscript{13}C-enrichment curves of the 3- and 4-carbon positions of glutamate (MATLAB, The MathWorks Inc, Natick, Mass).\textsuperscript{16} Nonlinear fitting of the model to \textsuperscript{13}C-NMR data provided (1) TCA cycle flux, (2) the interconversion rate between cytosolic glutamate and mitochondrial \alpha-ketoglutarate via the oxoglutarate-malate carrier (OMC), and (3) \textsuperscript{13}C-palmitate entry into the mitochondria as an index of CPT1 flux. Anaplerotic flux is the product of TCA cycle flux and the ratio of anaplerotic to citrate synthase determined at steady state.\textsuperscript{16,22}

\subsection*{Tissue Biochemistry}

Assays for glutamate, aspartate, citrate malate, \alpha-ketoglutarate, and lactate in perchloric acid extracts of frozen left ventricle were determined spectrophotometrically and fluorometrically.\textsuperscript{15,25} Alanine content was determined by in vitro \textsuperscript{1}H-NMR.\textsuperscript{26} The percent of labeled acetyl CoA entering the TCA cycle was determined from in vitro \textsuperscript{13}C-NMR spectra.\textsuperscript{26}

Atrial natriuretic factor expression was determined after total RNA isolation by single extraction with an acid guanidinium thiocyanate–phenol-chloroform mixture.\textsuperscript{15,27}

For Western blotting of CPT1 isoforms, hearts excised from both sham-operated (\(n = 5\)) and aortic-banded (\(n = 5\)) rats were perfused with ice-cold muscle-specific enolase media containing protease inhibitors. The left ventricle was minced and homogenized in 1 mL ice-cold muscle-specific enolase media and centrifuged at 4000 rpm for 15 minutes. Total homogenate protein concentration was determined from a standard curve (Bradford assay), and equivalent samples (80 to 100 \(\mu\)g protein) were dissolved in Laemmli buffer, loaded, and separated on a 7.5% Tris-HCl gel. Gels were transferred onto nitrocellulose, and Western blots were performed according to standard techniques using human heart M-CPT1 and rat liver L-CPT1 antibody.\textsuperscript{29,30} Semiquantitative densitometric analysis was
performed with the Bio-Rad Universal Hood (Bio-Rad, Hercules, Calif) and Quantity One software (Bio-Rad).

Assays for anaplerotic enzymes were performed on sham hearts (n/H11005 5) and HYP hearts (n/H11005 5) as previously described.31,32 Briefly, streptavidin blots for pyruvate carboxylase and propionyl-CoA carboxylase assays were prepared with streptavidin-horseradish peroxidase conjugate (1:1000; Biomedica, Foster City, Calif).31 Acetyl CoA carboxylase was probed with anti–acetyl CoA carboxylase antibody (1:2000; Cell Signaling Technology, Inc, Danvers, Mass).

For RNA extraction and real-time quantitative polymerase chain reaction of mRNA transcripts for pyruvate carboxylase and malic enzyme, total RNA was prepared with TRI reagent (Molecular Research Center, Inc, Cincinnati, Ohio) and tested for purity (UV absorption ratio, A260/A280 1.8). With previously published sequences for probes and primers, real-time quantitative polymerase chain reaction was performed and normalized by total RNA concentration.31,32

Statistical Analysis

Results are reported as mean±SE unless indicated otherwise. Comparison of mean values between sham and HYP groups was performed with Student t test. Functional measurements over time were compared through the use of repeated-measures ANOVA. Statistical significance was determined at values of P<5%.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Function and Bioenergetic State

Functional measurements (Figure 2) were similar between the HYP and sham hearts for the first 20 minutes of the isotope-enrichment phase, which dominates the flux measurements.18,22,23 HYP hearts displayed compromised energetics, as shown by a 30% lower ratio of phosphocreatine to ATP than in sham hearts (Table 1). Oxygen consumption was not significantly different between groups (HYP, 17±1 μmol·min⁻¹·g⁻¹ dry weight; sham, 21±2 μmol·min⁻¹·g⁻¹ dry weight).

Metabolic Flux Rates and Long-Chain Fatty Acid Oxidation

The resulting ¹³C-enrichment scheme is shown in Figure 1. Representative ¹³C-NMR spectra from a heart oxidizing labeled palmitate are displayed in Figure 3. Figure 4 illustrates mean isotope-enrichment curves for the relative carbon positions of glutamate in both experimental groups.

Despite similar TCA cycle flux rates (Table 1), the entry of palmitate into the oxidative pathways was significantly reduced in HYP hearts (Figure 5A). In contrast, in vitro analysis of the final, steady-state enrichment of acetyl CoA from ¹³C-enriched palmitate demonstrated no difference between groups. These data indicate that acetyl CoA formation was not supplemented by other substrates, despite reduced rates of palmitate oxidation. Figure 5B displays the percentage of labeled carbon entering the TCA cycle as acetyl-CoA derived from ¹³C palmitate versus unlabeled substrates. These enrichment data demonstrate that the contribution of unlabeled glucose to the formation of acetyl-CoA, via the action of PDC, was not different between sham and HYP hearts.
This finding was directly confirmed by experiments performed with 13C-enriched glucose as described below. Tissue metabolite content is presented in Table 2.

**CPT1 Isoform Expression**

Hypertrophied myocardium displayed a significant shift in the relative isoform distribution of CPT1 isoforms. L-CPT1 expression dramatically increased relative to M-CPT1 expression in the HYP rat heart (Figure 6).

**Compensatory Changes in Oxidative Intermediary Pathways: Activation of Anaplerotic Flux**

Actual anaplerotic flux was 2-fold higher in HYP hearts than in sham-operated hearts (Figure 7). In the absence of increased glucose oxidation through PDC and despite lowered palmitate oxidation rates in hypertrophied hearts, these data demonstrate the compensatory response for maintaining TCA cycle flux rates that are similar to those of the sham hearts. Increased influx of unlabeled carbon into the second span of the TCA cycle provided additional carbon to support flux within the TCA cycle of the hypertrophic hearts. Aspartate and malate were increased in hypertrophied myocardium, consistent with increased anaplerotic flux (Table 2).

Increased anaplerosis alone will not sustain TCA cycle flux,31,33 and the 2 spans of the cycle must be kept in equilibrium. In HYP hearts, this balance was evident by the increased exchange of α-ketoglutarate in and out of the first span: sham OMC flux, 1.6±0.3; HYP OMC flux, 2.5±0.2 (P<0.05).

**Metabolic Fate of Glucose in the Hypertrophied Myocardium: Decarboxylation Versus Carboxylation of Pyruvate**

Experiments with 13C-glucose and unlabeled palmitate showed no increased oxidation of glycolytic end products via PDC. The percentage of glucose, among all substrates, contributing to acetyl CoA production was similar in HYP and nonhypertrophied hearts (15±1% for HYP; 13±1% for sham). Nonetheless, the glycolytic end products lactate and alanine were increased with hypertrophy (Table 2). Taken

### Table 1. Physical and Metabolic Characteristics of Hypertrophied Hearts

<table>
<thead>
<tr>
<th>Group</th>
<th>Heart Weight, g</th>
<th>Body Weight, g</th>
<th>Ratio of Heart Weight to Body Weight, mg/g</th>
<th>PCR/ATP</th>
<th>Vtca, (\mu)mol · min (^{-1}) · g (^{-1}) · dw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>1.80±0.2</td>
<td>409±18</td>
<td>4.3±0.3</td>
<td>2.1±0.1</td>
<td>12±1</td>
</tr>
<tr>
<td>Banded</td>
<td>2.6±0.1*</td>
<td>366±12*</td>
<td>7.0±0.5*</td>
<td>1.5±0.1*</td>
<td>10±1</td>
</tr>
</tbody>
</table>

Values are mean±SE. PCR indicates phosphocreatine; Vtca, TCA cycle flux; and dw, dry weight. *P<0.05.

![Figure 3. Dynamic-mode 13C-NMR spectra of intact beating heart. Selected 13C spectra, each collected in 2 minutes (from bottom to top), from an isolated, hypertrophied rat heart oxidizing [2,4,6,8,10,12,14,16-13C8] palmitate in the presence of unlabeled glucose. Peak assignments are glutamate carbon-2 (GLU-C2), glutamate carbon-3 (GLU-C3), and glutamate carbon-4 (GLU-C4). TG indicates 13C-enriched triglycerides from storage of 13C-palmitate.](image)

![Figure 4. Isotopic enrichment curves of glutamate (Glu) in rat heart. Representative 13C-enrichment curves for glutamate, as detected by 13C-NMR, from grouped experiments. Solid line represents least-squares fitting of the kinetic analysis to the enrichment data (mean±SD). For actual data analysis, the fitting was performed on the curve from each individual heart. Top, Control hearts of age-matched, sham-operated rats. Bottom, Hypertrophied hearts. Note the relative differences between 13C enrichment at the 3-carbon position of glutamate vs that of the 4-carbon between sham-operated hearts (top) and hypertrophied hearts (bottom). ● Indicates 4-carbon of glutamate; ○, 3-carbon of glutamate.](image)
together, these findings provide evidence of increased glycolytic activity in the absence of increased in glucose oxidation via PDCA.9–11 Thus, despite the reduction in palmitate entry into mitochondrial oxidative pathways, the oxidation of glucose via PDC did not make up the difference to support the TCA cycle with carbon entry at acetyl CoA.

In contrast to the lack of compensatory increases in acetyl CoA production from 13C-enriched glucose, the extent of 13C enrichment in the total tissue glutamate pool was markedly increased in the HYP hearts (6.1±1.4% of total glutamate pool) versus shams (2.2±0.2; P<0.05), indicating increased carbon entry from glucose into the TCA cycle. Therefore, a surprising finding of the present study is that although glucose oxidation through PDC did not increase, another route for glucose entry into the TCA cycle, anaplerosis, provided a measure of compensatory flux necessary to maintain TCA cycle flux in the HYP rat heart.

**Anaplerotic Enzyme Expression**

As shown in Figure 8, mRNA transcript levels of malic enzyme were >2-fold greater in HYP hearts than sham hearts, but the mRNA transcript levels for pyruvate carboxylase, propionyl-CoA carboxylase, and acetyl CoA carboxylase (data not shown).

**Discussion**

In this study of hypertrophied rat hearts, the rate of palmitate entry into oxidative metabolism was reduced 23% compared with normal hearts despite similar TCA cycle flux rates. The key finding is that this reduced rate of palmitate oxidation was balanced by a compensatory increase in anaplerotic flux. The data further suggest that the increased anaplerosis in hypertrophic hearts is fueled in part by increased carboxylation of the glycolytic pyruvate. This latter finding is supported by data showing increased 13C enrichment of glutamate from oxidation of 13C-enriched glucose despite no change in the 13C enrichment of acetyl CoA produced by the activity of PDC on pyruvate. Such increased entry of pyruvate into the TCA cycle via anaplerosis accounts for some of the mismatch observed in the known increase in glycolytic activity within the hypertrophic myocardium that occurs in the absence of any coupled increase in oxidation of glycolytic end products via the action of PDC.10,11

Notably, the contribution of carbohydrate oxidation in the hypertrophic hearts, via the formation of acetyl CoA from glycolytic end products through PDC, did not increase to compensate for the drop in the rate of palmitate oxidation. Indeed, the relative contributions of palmitate and glucose to acetyl-CoA formation were not different between hypertrophic hearts and controls. Therefore, with a reduction in the actual rate of palmitate oxidation in the hypertrophic myocardium, glucose oxidation via PDC did not increase to support the rate of oxidative energy synthesis. This finding is consistent with previous studies showing no elevation in glucose oxidation through PDC despite increased glycolysis in the hypertrophied heart.10,11 We report evidence of increased glycolysis in hypertrophic rat hearts, but we also report a 2.8-fold increase in the amount of 13C-enriched glucose entering the oxidative metabolism of the hypertrophied heart without an increase through PDC.

The findings indicate that rather than an increase in pyruvate oxidation through PDC, pyruvate oxidation increased via carboxylation and anaplerosis in the hypertrophied hearts. Although not all of the carbon flux through anaplerosis is supplied by pyruvate, the contribution from 13C-labeled pyruvate is likely to occur via increases in either the malic enzyme or pyruvate carboxylase.34 This is the first study to demonstrate an increased mRNA level in the hypertrophied myocardium of malic enzyme, which catalyzes the carboxylation of pyruvate to form malate. Consistent with

**TABLE 2. Steady-State Metabolite Content in Intact Hearts**

<table>
<thead>
<tr>
<th>Group</th>
<th>Glutamate</th>
<th>α-Ketoglutarate</th>
<th>Aspartate</th>
<th>Citrate</th>
<th>Malate</th>
<th>Lactate</th>
<th>Alanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>14±2</td>
<td>0.26±0.05</td>
<td>2.9±0.3</td>
<td>1.02±0.30</td>
<td>0.12±0.02</td>
<td>2.4±0.7</td>
<td>1.6±0.4</td>
</tr>
<tr>
<td>Hypertrophy</td>
<td>17±1</td>
<td>0.14±0.04</td>
<td>4.7±0.4*</td>
<td>1.04±0.31</td>
<td>0.23±0.03*</td>
<td>6.0±0.9*</td>
<td>3.5±0.6*</td>
</tr>
</tbody>
</table>

Values are given as µmol/g dry weight. Values are mean±SE.
*P<0.05.
this mechanism is the elevated malate content of hypertrophied hearts. Thus, anaplerotic activity is increased in the hypertrophic myocardium, and increased expression of malic enzyme may provide an alternative route for the entry of glycolytic end products, in part compensating for the loss of carbon entry into the TCA cycle that results from reduced rates of long-chain fatty acid oxidation.

Interestingly, the drop in the rate of palmitate oxidation coincided with increased expression of L-CPT1. This increased L-CPT1 is consistent with reversion to fetal isoform distribution, which in neonatal heart coincides with low fatty oxidation. At first, the isoform shift may appear counterintuitive because the L-CPT1 isoform is less responsive to inhibition by malonyl-CoA. However, we did not note a particular drop in M-CPT1 expression in hypertrophic versus normal hearts (Figure 6), so this elevation of L-CPT1 may represent yet another of many compensatory changes to counter the general reduction in the rate of palmitate entry into the oxidative metabolism. Findings by Doh et al demonstrate that long-chain fatty acid oxidation rates in tissues containing predominantly M-CPT1 were directly proportional to protein content but not similarly proportional to the CPT1 activity in tissues containing L-CPT1. Thus, increased expression of L-CPT1 in the hypertrophic rat hearts may not be expected to correlate with the reduced flux through CPT1. The elevation of the L-CPT1 in hypertrophied myocardium may be a compensatory response to the reduction of palmitate oxidation and the more general downregulation of gene expression for the long-chain fatty acid oxidation enzymes.

The 2 spans of the TCA cycle must maintain the carbon mass balance under equilibrium conditions for net forward flux. Thus, carbon influx into the second span of the TCA cycle via anaplerosis must be balanced by a comparable exchange within the first span. This exchange occurs via α-ketoglutarate influx/efflux from the mitochondria through the bidirectional OMC. The elevation in both the mean rates of α-ketoglutarate exchange and anaplerotic flux in hypertrophic hearts amounted to 0.9 and 1.0 μmol · min⁻¹ · g⁻¹ dry weight, respectively, over that of the sham hearts. Therefore, the increase in anaplerotic flux into the second span of the TCA cycle was matched, within error, by increased α-ketoglutarate exchange from the first span of the cycle, thus maintaining equilibrium within the TCA cycle.

However, these adjustments to oxidative energy synthesis are far from optimal and consistent with the energy-poor condition of the hypertrophied myocardium. Reduced entry of carbon from fatty acids into the TCA cycle at the citrate

Figure 6. CPT1 isoform expression. Western blots for M-CPT1 isoform (top left) and L-CPT1 isoform (bottom left). Right, Normalized results. Note the significant increase in L-isoform expression in HYP hearts vs sham. Gray bar denotes sham hearts; striped bar, HYP hearts. *P<0.05.
synthase reaction limits the supply of key 2-carbon chain moieties to sustain net forward cycle flux, which can become limited and lead to energetic failure. Increased entry of carbon moieties at either side of the TCA cycle without an overall increase in cycle flux creates the potential for an altered distribution of metabolic intermediates in the mitochondria (Table 2). This redistribution of carbon mass within the TCA cycle and other metabolites of cycle intermediates may limit substrate availability at the energy-yielding, redox-sensitive dehydrogenase reactions of the TCA cycle because cycle intermediates are rerouted to exchange with metabolite pools outside of the cycle. A specific example of such inefficiency in the hypertrophic heart is provided by the increased exchange of \( ^13C \)-ketoglutarate across the mitochondrial membrane via OMC, which competes for this substrate with the \( ^13C \)-ketoglutarate dehydrogenase that would otherwise oxidize \( ^13C \)-ketoglutarate and produce the NADH necessary for fueling oxidative phosphorylation. Another example of inefficiency in the intermediary metabolism of the hypertrophied myocardium is the limited ability of the hypertrophied heart to accommodate increased production of glycolytic end products by oxidation through PDC, another source of NADH. Therefore, the adjustments observed in the intermediary metabolism of the hypertrophic heart are key changes that may compensate for a lack of normal activity through CPT1 and PDC but are ultimately inefficient in supporting the energy-yielding reactions of the TCA cycle.

**Study Limitations**

The present study, like many others, is subject to the inherent limitations of the isolated, perfused heart in studying a diseased state. The pressure-overloaded, hypertrophied hearts in this study displayed contractile function similar to that of sham hearts during the measurements of metabolic flux with isotope enrichment but did not sustain this same level of contractile function throughout the entire protocol. Although in vivo hearts do not display a short-term functional decline, one advantage of the many published ex vivo experiments is that the data reflect inherent, adaptive changes in the myocardium in the absence of the pressure overload.

No direct measure of glycolytic flux was intended for analysis of a potential mismatch between glycolytic rate and pyruvate oxidation. Nevertheless, with normal physiological palmitate concentration (0.4 mmol/L), we report an increase in anaplerosis of 0.9 mmol·L\(^{-1}\)·min\(^{-1}\)·g\(^{-1}\) dry weight, which converts to 0.45 µmol·min\(^{-1}\)·g\(^{-1}\) glucose. This rate represents a significant fraction of the increase in unoxidized glucose from glycolysis. It is tempting to speculate that strategies to activate PDC and to counter increased anaplerosis may prove therapeutic.

**Conclusions**

Cardiac hypertrophy produced by pressure overload in this rat model resulted in significant changes in intermediary metab-
olism. The reduced contractile function, impaired bioenergetic potential, and reduced palmitate oxidation are all consistent with previous reports.¹⁻⁸ Having confirmed the comparability of this hypertrophic heart model with others, the present study is the first to establish a shift in CPT1 isoform expression toward an increase in L-CPT1 in hypertrophic myocardium. Furthermore, although the study supports previous reports of increased glycolysis in the absence of a commensurate increase in glucose oxidation via PDC,¹⁰⁻¹² we provide the first evidence of increases in both anaplerotic flux and increased oxidation of glycolytic end products through anaplerosis in hypertrophied hearts. The recruitment of anaplerosis and increased mRNA levels for malic enzyme can be considered adaptive responses of the hypertrophied cardiomyocyte to maintain relatively normal TCA cycle flux rates under the limiting conditions of reduced fatty acid oxidation and limited oxidation of glucose through PDC. However, these shifts in intermediary metabolism indicate a less efficient mode of carbon use for fueling energy synthesis in the myocardium. Indeed, the metabolic characteristics of the hypertrophic heart are deviations from the normal, efficient mechanisms used to maintain oxidative ATP synthesis and contractile function and thereby may be potential factors in the transition from a compensated adaptation to overt heart failure.

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Disclosures

None.

References

CLINICAL PERSPECTIVE

Adjusting the balance of fuel use for energy synthesis in the heart between fat and carbohydrate has gained momentum as a potential therapeutic strategy in strengthening the failing heart. Shifting away from fat oxidation to that of glucose has theoretical benefits that range from influencing the intracellular environment for pH and redox state to optimizing the relative costs of oxygen consumption for “burning” these fuels. In this report, we describe additional and potential maladaptive changes in the fat/carbohydrate fuel balance during the onset of pressure overload–induced cardiac hypertrophy as a result of altered expression of metabolic enzymes. A shift toward reduced rates of long-chain fatty acid oxidation by the hypertrophied heart with increased reliance on glucose is a well-known but poorly understood characteristic of the hypertrophied heart. The present study demonstrates that in the face of reduced rates of fatty acid oxidation, the pressure-overloaded, hypertrophied heart recruits a compensatory metabolic pathway, referred to as anaplerosis (or “filling up” of the tricarboxylic acid cycle), thereby providing an alternative route for glucose oxidation. However, increased oxidation of carbohydrates via anaplerosis is an inherently inefficient process for energy synthesis compared with the normal route of glucose oxidation via pyruvate dehydrogenase complex. Thus, the potential salutary impact of therapeutic protocols for cardiomyopathy that augment glucose oxidation through pyruvate dehydrogenase complex may derive not only from a shift away from fatty acid oxidation but also from diverting glucose away from the less efficient oxidation via anaplerosis toward the more normal route of pyruvate dehydrogenase complex.
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