The benefit of medium-chain triglyceride therapy on the cardiac function of SHRs is associated with a reversal of metabolic and signaling alterations

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1Department of Physical Education, International Pacific University, Okayama, Okayama; 2Center for Tsukuba Advanced Research Alliance, 3Institute of Clinical Medicine, and 4Institute of Health and Sport Sciences, University of Tsukuba, Tsukuba, Ibaraki; and 5Tsukuba Research Laboratory, NOF Corporation, Tsukuba, Ibaraki, Japan

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Iemitsu M, Shimojo N, Maeda S, Irukayama-Tomobe Y, Sakai S, Ohkubo T, Tanaka Y, Miyauchi T. The benefit of medium-chain triglyceride therapy on the cardiac function of SHRs is associated with a reversal of metabolic and signaling alterations. Am J Physiol Heart Circ Physiol 295: H136–H144, 2008. First published May 2, 2008; doi:10.1152/ajpheart.01417.2006.—The spontaneously hypertensive rat (SHR) is a model of cardiomyopathy that displays a genetic defect in cardiac fatty acid (FA) translocase/CD36, a plasma membrane long-chain FA transporter. Therapy with medium-chain FAs, which do not require CD36-facilitated transport, has been shown to improve cardiac function and hypertrophy in SHRs despite persistent hypertension. However, little is known about the underlying molecular mechanisms. The aim of this study was to document the impact of medium-chain triglyceride (MCT) therapy in SHRs on the expression level and activity of metabolic enzymes and signaling pathways. Four-week-old male SHRs were administered MCT (SHR-MCT) or long-chain triglyceride (SHR-LCT) for 16 wk. We used Wistar-Kyoto (WKY) rats as controls (WKY-MCT and WKY-LCT). The SHR-MCT group displayed improved cardiac dysfunction [as assessed by left ventricular (LV) end-diastolic pressure and activity levels of mRNA, protein, and activity levels in key enzymes and transporters. Additionally, SHR-MCT group showed decreased expression and transcriptional regulation of myocardial peroxisome proliferator-activated receptor-α, which regulates the transcription of LCHAD and MCAD genes, corresponding to the changes seen in those enzymatic activities. Furthermore, SHR intake caused inhibition of JNK activation in SHR hearts. Collectively, the observed changes in the myocardial activity of metabolic enzymes and signaling pathways may contribute to the improved cardiac dysfunction and hypertrophy in SHR-MCT groups.

In this study, we documented metabolic and signaling alterations in SHR hearts. We tested the hypothesis that the benefits of long-term administration of medium-chain triglycerides (MCT) to SHRs may be associated with a reversal of these alterations. SHRs and their controls, Wistar-Kyoto (WKY) rats, were administered either MCT or long-chain triglycerides (LCT) for 16 wk beginning at 4 wk of age. In addition to tracking blood pressure and cardiac function, we evaluated 1) the myocardial levels of mRNA, protein, and activity levels of selected metabolic enzymes relevant to carbohydrate and FA oxidation and 2) the activity of signaling pathways relevant to development of LVH.

METHODS

Animals and protocol. Experimental protocols were approved by the Committee on Animal Research at the University of Tsukuba. Male 4-wk-old WKY rats and SHRs were obtained from Charles River Laboratories, Inc. (Wilmington, MA). Rats were housed in a 12-h light-dark cycle at constant temperature (21°C) with free access to standard laboratory chow and tap water. All efforts were made to minimize the number of animals used and their suffering. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Hypertensive rats (SHRs) fed a LCT diet (SHR-LCT group) were homogenized in Isogen (50 mg tissue/1 ml Isogen) using a Polytron tissue homogenizer (model PT10SK/35, Kinematica, Lucerne, Switzerland). Total tissue RNA was isolated with Isogen reagent (Nippon Gene, Toyama, Japan) as previously described (31). Single-stranded cDNA was synthesized from prepared RNA (2 μg) using omniscript reverse transcriptase (QIAGEN) with an oligo(dT) primer at 37°C for 15 s.

dDNA was diluted in a 1:10 ratio, and 1 μl was used for PCR. Each PCR mixture contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 200 μM of each dNTP, 0.5 μM of each gene-specific primer, and 0.025 U/μl Taq polymerase (Takara, Shiga, Japan). The following gene-specific primers for CD36 (1), MADC (23), LCHAD (24), and β-actin (25) were synthesized according to published cDNA sequences: CD36, sense 5'-GTGCAAAGAGGAAAGAC3' and antisense 5'-CATCCTCATCTCAACCC-3'; MCAD, sense 5'-GATTGCGCATCTAACCAG-3' and antisense 5'-TAGAGGCAAAGTACGGTTCGG-3'; LCHAD, sense 5'-CCCTCCAGATGGCCCTCCTG-3' and antisense 5'-GGTGGCCAGATGGCCAGACG-3'; and β-actin, sense 5'-GAAGATCTCTGAGCCGCTG-3' and antisense 5'-CGTACTCCCTGTTGTAGTCC-3'.

PCR was performed using a PCR thermal cycler (model TP-3000, Takara). The cycle profile included denaturation for 15 s at 94°C followed by annealing and extension. The annealing was performed for 15 s at 55°C for CD36, 15 s at 60°C for MCAD and LCHAD, and 15 s at 72°C for β-actin. The extension time at 72°C was 45 s for CD36, MCAD, and LCHAD and 60 s for β-actin.

Amplified PCR products were electrophoresed on 1.2% agarose gels, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. Photographs of gels were scanned using a CanoScan 600 scanner (Canon, Tokyo, Japan); band intensities were quantified using MacBAS software (Fuji Film, Tokyo, Japan) as previously described (10–12).

Electrophoretic separation analysis for measurements of MHC isoforms. Electrophoretic separation analysis on an SDS-polyacrylamide gel was performed as previously described (11). Stacking and separating gels contained 4% and 8% N,N'-methylene-bis-acrylamide, respectively, at a ratio of 50:1. After gels had been stained with Coomassie brilliant blue, we scanned them to evaluate the quantities of α-MHC and β-MHC isoforms.

Muscle oxidative enzymatic activity. Heart tissues (50 mg) were homogenized in 20 volumes of 175 mM KCl, 2 mM EDTA, and 10 mM glutathione on ice using a Teflon homogenizer. Homogenates were centrifuged at 1,000 × g for 10 min at 4°C, and the supernatant was diluted in 200 volumes of 10 mM Tris-HCl (pH 7.0). To determine

![Graph showing time course of changes in arterial systolic blood pressure using tail-cuff sphygmonanometer in Wistar-Kyoto (WKY) rats](http://ajpheart.physiology.org/)}
LCHAD enzymatic activity, 50 μl of each sample were incubated for 5 min at 30°C in a 925-μl incubation mixture containing 125 μM Tris•HCl (pH 7.0), 167 mM triethanolamine, 25 mM EDTA, and 4.5 mM NADH. Reactions were initiated by the addition of 25 μl of 1 mM acetoacetyl-CoA. Enzymatic activity was determined spectrophotometrically at 340 nm for 10 min (12). To determine PFK-1 enzymatic activity, 50 μl of each sample were combined with 1.5 μl of 1 M potassium fluoride and incubated for 5 min at 30°C in a 950-μl incubation mixture containing 25 mM HEPES (pH 7.6), 0.1 mM ATP, and 0.1 mM NADH. These reactions were assessed spectrophotometrically at 340 nm for 15 min (21).

Heart tissues (50 mg) were homogenized in 20 volumes of 200 mM HEPES (pH 7.6) with 100 mM EDTA on ice using a Teflon homogenizer. Homogenates were centrifuged at 7,000 rpm for 10 min at 4°C. MCAD enzymatic activity in the supernatant was determined by an incubation of 10 μl of each sample for 5 min at 37°C in a 440-μl incubation mixture containing 100 mM HEPES (pH 7.6), 0.1 mM EDTA, 200 μM ferrocenium hexafluorophosphate, and 0.5 mM sodium tetrathionate. Reactions were initiated by the addition of 50 μl of 0.5 mM octanoyl-CoA. Samples were assessed spectrophotometrically at 300 nm for 5 min (20).

Electrophoresis and immunoblot analysis. Western blot analysis of PPAR-α (12), JNK (13), and ERK (13) proteins was performed as previously described. Briefly, samples were separated on SDS-polyacrylamide gels (10%) and transferred to polyvinylidene difluoride membranes (Millipore, Tokyo, Japan) at 3 mA/cm² for 60 min. Membranes were treated with blocking buffer [PBS containing 0.1% Tween 20 (PBS-T) and 5% skim milk] in the presence of polyclonal anti-PPAR-α antibody (1:500 dilution in blocking buffer, Santa Cruz Biotechnology, Santa Cruz, CA), G9 monoclonal anti-phospho-JNK (Thr183/Tyr185) antibody (1:1,000 dilution in blocking buffer, Cell Biotechnology, Santa Cruz, CA), and monoclonal anti-phospho-ERK (Thr202/Tyr204) antibody (1:1000 dilution in blocking buffer, Santa Cruz Biotechnology, Santa Cruz, CA). Blots were incubated with these antibodies followed by secondary antibodies and incubated with Envision detection reagents (Amersham-Pharmacia Biotech). Bands were scanned using a Gel Assistance scanner (Bio-Rad, Hercules, CA) and quantitated using the ImageJ software (Wayne Rasband, NIH).

Table 1. Selected characteristics of the WKY-LCT, WKY-MCT, SHR-LCT, and SHR-MCT groups

<table>
<thead>
<tr>
<th></th>
<th>WKY-LCT</th>
<th>WKY-MCT</th>
<th>SHR-LCT</th>
<th>SHR-MCT</th>
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<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>7</td>
<td>7</td>
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<tr>
<td>BW, g</td>
<td>429.0±9.5</td>
<td>379.2±4.0*</td>
<td>430.2±6.8</td>
<td>419.3±6.9</td>
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<td>LVW, g</td>
<td>0.95±0.02</td>
<td>0.92±0.05</td>
<td>1.28±0.03†</td>
<td>1.16±0.02‡</td>
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<td>LVW/BW,g</td>
<td>2.22±0.02</td>
<td>2.43±0.11</td>
<td>3.00±0.04†</td>
<td>2.78±0.07†</td>
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<td>Myocyte area, μm²</td>
<td>543.3±5.9</td>
<td>538.3±5.4</td>
<td>603.2±9.6†</td>
<td>578.5±4.3‡</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>253.2±12.2</td>
<td>236.1±18.5</td>
<td>410.9±12.7†</td>
<td>378.9±14.6‡</td>
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<tr>
<td>Blood pressure, mmHg</td>
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<td></td>
<td></td>
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<tr>
<td>Systolic</td>
<td>105.8±2.0</td>
<td>107.5±2.1</td>
<td>199.6±6.6†</td>
<td>206.2±10.6†</td>
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<tr>
<td>Diastolic</td>
<td>70.1±2.7</td>
<td>69.5±3.1</td>
<td>149.6±5.1†</td>
<td>152.3±3.7†</td>
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<tr>
<td>Mean</td>
<td>82.0±2.4</td>
<td>82.1±2.8</td>
<td>166.2±5.5†</td>
<td>170.2±8.3‡</td>
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Values are means ± SE; n is the number of rats per group. WKY-LCT and WKY-MCT, Wistar-Kyoto rats fed a long-chain triglyceride (LCT) or medium-chain triglyceride (MCT) diet, respectively; SHR-LCT and SHR-MCT, spontaneously hypertensive rats fed a LCT or MCT diet, respectively; BW, body weight; LVW, left ventricular weight. *P < 0.05 vs. WKY-LCT, SHR-LCT, and SHR-MCT groups; †P < 0.05 vs. WKY-LCT and WKY-MCT groups; ‡P < 0.05 vs. WKY-MCT and SHR-MCT groups; †P < 0.05 vs. the SHR-LCT group.

Fig. 2. Left ventricular (LV) systolic pressure (A), LV diastolic pressure (B), positive derivative of LV pressure/P value (+dP/dt; C), and negative first derivative of LV pressure/P value (−dP/dt; D) in WKY-LCT (n = 6), WKY-MCT (n = 6), SHR-LCT (n = 7), and SHR-MCT (n = 7) groups. Values are expressed as means ± SE. *P < 0.05 vs. WKY-LCT and WKY-MCT groups; #P < 0.05 vs. the SHR-LCT group.
Signaling, Beverly, MA), polyclonal anti-JNK antibody (1:1,000 dilution in blocking buffer, Cell Signaling), polyclonal anti-phospho-ERK (Thr\textsuperscript{202}/Tyr\textsuperscript{204}) antibody (1:1,000 dilution in blocking buffer, Cell Signaling), or polyclonal anti-ERK antibody (1:1,000 dilution in blocking buffer, Cell Signaling) for 12 h at 4°C. Membranes had been washed six times in PBS-T, PPAR-\gamma/H\textsubscript{9251} buffer, Cell Signaling), for 1 h at room temperature. After membranes had been washed six times in PBS-T, PPAR-\alpha, phospho-JNK, JNK, phospho-ERK, and ERK were detected using the ECL plus system technology, anti-rabbit antibody (1:2,000 dilution in blocking buffer, Santa Cruz Bio-technology), anti-mouse antibody (1:1,000 dilution in blocking buffer, Santa Cruz Biotechnology), or polyclonal anti-ERK antibody (1:1,000 dilution in blocking buffer, Cell Signaling), or anti-mouse IgG (1:2,000 dilution in blocking buffer, Cell Signaling), for 1 h at room temperature. After membranes had been washed six times in PBS-T, PPAR-\alpha, phospho-JNK, JNK, phospho-ERK, and ERK were detected using the ECL plus system (Amersham Biosciences, Piscataway, NJ) by exposure to Hyper film (Amersham Biosciences).

Gel mobility shift assays. Heart tissues were homogenized with 10 volumes of 10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1.5 mM MgCl\textsubscript{2}, 10 mM NaF, 1 mM Na\textsubscript{3}VO\textsubscript{4}, 1 mM DTT, 20 mM \beta-glycerophosphate, 0.5 mM PMSF, 60 \mu g/ml aprotinin, and 2 \mu g/ml leupeptin on ice using a Teflon homogenizer. After the addition of Nonidet P-40 (to 0.6%), the homogenate was rotated for 30 min at 4°C and centrifuged at 3,000 \times g for 10 min at 4°C. The precipitated nuclear fraction was resuspended in 20 mM HEPES (pH 7.9), 0.4 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1.5 mM MgCl\textsubscript{2}, 10 mM NaF, 1 mM Na\textsubscript{3}VO\textsubscript{4}, 0.2 mM DTT, 20 mM \beta-glycerophosphate, 0.5 mM PMSF, 60 \mu g/ml aprotinin, 2 \mu g/ml leupeptin, and 20% glycerol and centrifuged at 18,000 \times g for 10 min at 4°C. The protein concentration was determined in the supernatant. Gel mobility shift assays using the myocardial nuclear extracts were performed as previously described (12, 15). Briefly, the samples of LV nuclear extracts (15 \mu g protein) were incubated with 50,000 counts/min of a \textsuperscript{32}P-labeled double-stranded oligonucleotide probe containing the consensus PPAR-\alpha binding sequence (5'-TGACCTTTGACCTAGTTTGTG-3') of the promoter regions of LCHAD and MCAD at room temperature for 20 min in 10 \mu l binding buffer consisting of 10 mM Tris\textcdot HCl (pH 7.5), 50 mM NaCl, 0.5 mM EDTA, 1 mM MgCl\textsubscript{2}, 0.5 mM DTT, 4% glycerol, and 0.05 mg/ml poly(dl-Idc). DNA-protein complexes were electrophoresed on a 4% nondenaturing polyacrylamide gel, and the gel was then dried, subjected to autoradiography, and analyzed with a bioimaging analyzer (BAS-5000, Fuji Film, Tokyo, Japan).

Statistical analysis. Values are expressed as means \pm SE. Statistical analysis used ANOVA followed by Scheffe’s F-test for multiple comparisons. P values of <0.05 were accepted as significant.

RESULTS

Arterial blood pressure. Arterial systolic blood pressure measured using a tail-cuff sphygmonanometer increased significantly in the SHR-LCT and SHR-MCT groups after 2 wk of LCT or MCT diets compared with the WKY-LCT and WKY-MCT groups, but there were no significant differences between the SHR-LCT and SHR-MCT groups (Fig. 1).

Cardiac hypertrophy and function. Whereas body weights were significantly lower in the WKY-MCT group than in the WKY-LCT group, there were no significant differences between the SHR-MCT and SHR-LCT groups (Table 1). LV weight was significantly higher in the SHR-LCT group than in either the WKY-LCT or WKY-MCT groups. Whereas LV weight in the SHR-MCT group was reduced compared with the SHR-LCT group, the LV mass index for body weight did not differ between the SHR-LCT and SHR-MCT groups (P = 0.065). The myocyte cross-sectional area of the SHR-LCT group was significantly higher than either the WKY-LCT or WKY-MCT groups, whereas that in the SHR-MCT group was reduced compared with the SHR-LCT group. Whereas arterial systolic, diastolic, and mean blood pressures and heart rate measured using a catheter were significantly greater in the SHR-LCT group than in the WKY-LCT group, there were no significant differences between the SHR-MCT and SHR-LCT groups (Fig. 1).

MHC isoforms

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Fig. 3. Expression of CD36 mRNA in hearts (LVs) from the WKY-LCT (n = 6), WKY-MCT (n = 6), SHR-LCT (n = 7), and SHR-MCT (n = 7) groups. Top: representative RT-PCR analysis of CD36 mRNA. Expression of \beta-actin mRNA served as an internal control. Bottom: results of densitometric analysis of CD36 mRNA expression levels. Images of PCR product gel electrophoresis were examined by densitometry. CD36 mRNA levels were normalized to those of \beta-actin mRNA. Values are expressed as means \pm SE. *P < 0.05 vs. WKY-LCT and WKY-MCT groups.

Fig. 4. Expression of \alpha-myosin heavy chain (MHC) and \beta-MHC isoforms in hearts (LVs) from the WKY-LCT (n = 6), WKY-MCT (n = 6), SHR-LCT (n = 7), and SHR-MCT (n = 7) groups. Top: representative images of the electrophoretic separation of \alpha-MHC and \beta-MHC isoforms. Bottom: results of densitometric analysis of \beta-MHC/\alpha-MHC isoform expression levels. Values are expressed as means \pm SE. *P < 0.05 vs. WKY-LCT and WKY-MCT groups; #P < 0.05 vs. the SHR-LCT group.
significantly lower in the SHR-MCT group than in the SHR-LCT group (Fig. 2). LVSP, however, did not differ between animals of the same genetic background regardless of diet. The \( \frac{dP}{dt}/P \) value, which corrects the observed changes in pressure by the LVSP, was significantly lower in the SHR-LCT group than in the WKY-LCT group from either WKY-LCT or WKY-MCT groups. In the SHR-MCT group, these enzymatic activities were significantly higher than those observed in the SHR-LCT group (Fig. 5, A and B). The activity of the glycolytic enzyme PFK-1 was significantly higher in the SHR-LCT group than in WKY-LCT, WKY-MCT, or SHR-MCT groups (Fig. 5, C).

Myocardial energy metabolic enzyme activity. Activity of FA \( \beta \)-oxidation enzymes LCHAD and MCAD were significantly lower in hearts from the SHR-LCT group than those from either WKY-LCT or WKY-MCT groups. In the SHR-MCT group, these enzymatic activities were significantly higher than those observed in the SHR-LCT group (Fig. 5, A and B). The activity of the glycolytic enzyme PFK-1 was significantly higher in the SHR-LCT group than in WKY-LCT, WKY-MCT, or SHR-MCT groups (Fig. 5, C).

Cardiac PPAR-\( \alpha \) protein expression and transcriptional regulation of PPAR-\( \alpha \) target genes. As revealed by immunoblot analysis of PPAR-\( \alpha \) in WKY-LCT, WKY-MCT, SHR-LCT, and SHR-MCT groups, cardiac expression levels of PPAR-\( \alpha \) in the SHR-LCT group were significantly lower than in both the WKY-LCT and WKY-MCT groups. Cardiac levels could be observed between SHR-LCT and SHR-MCT groups. CD36 mRNA expression was readily identifiable in the hearts from the WKY-LCT and WKY-MCT groups, with no significant differences attributable to diet. These results agree with the report of Aitman et al. (1) identifying mutant transcripts in SHR heart tissue.

Myocardial MHC isoforms. We analyzed the ratio of \( \beta \)-MHC to \( \alpha \)-MHC expression in the heart by electrophoretic separation of MHC isoforms following isolation from WKY-LCT, WKY-MCT, SHR-LCT, and SHR-MCT groups (Fig. 4). This ratio was significantly higher in the SHR-LCT group than in the SHR-MCT group. SHRs given MCT exhibited a lower ratio than those given LCT, indicating a shift from \( \alpha \)-MHC to \( \beta \)-MHC isoforms in the heart.

Myocardial CD36 mRNA expression. Cardiac mRNA expression of CD36, which is involved in the uptake of LCFA, was barely detected in both the SHR-LCT and SHR-MCT groups (Fig. 3). No significant differences in CD36 mRNA levels could be observed between SHR-LCT and SHR-MCT groups.
of PPAR-α in the SHR-MCT group were significantly higher than those in the SHR-LCT group (Fig. 6). Moreover, mRNA expression levels of MCAD and LCHAD, which are target genes of transcriptional regulation through PPAR-α, were significantly lower in the SHR-LCT group than in either the WKY-LCT or WKY-MCT groups (Fig. 7, A and B). However, there were no significant differences in MCAD and LCHAD mRNA levels between WKY-LCT or WKY-MCT and SHR-MCT groups. Additionally, we performed gel mobility shift assays for myocardial PPAR-α DNA binding to examine whether the change in myocardial PPAR-α expression affected PPAR-α binding to the PPRE, which is the PPAR-α binding domain of FA metabolic enzyme genes such as LCHAD, MCAD, etc. The activity of myocardial PPAR-α DNA binding using PPRE oligonucleotide in the SHR-LCT group was significantly lower than those in either WKY-LCT or WKY-MCT groups (Fig. 7; C); however, it was significantly higher in the SHR-MCT group than in the SHR-LCT group.

**Phosphorylation of cardiac JNK.** The PPAR-α agonist fenofibrate downregulates the biological activity of JNK, a member of the MAPK family that functions in cardiac hypertrophy-induced signaling to inhibit the progression of pathological cardiac hypertrophy (15). We examined JNK phosphorylation in hearts from the WKY-LCT, WKY-MCT, SHR-LCT, and SHR-MCT groups by immunoblot analysis. Phosphorylation of cardiac JNK was significantly higher in the SHR-LCT group than in either WKY-LCT or WKY-MCT groups, whereas levels in the SHR-MCT group were significantly lower than those in the SHR-LCT group (Fig. 8). The phosphorylation of cardiac ERK, another member of the MAPK family, was also significantly increased in the SHR-LCT group than in either WKY-LCT or WKY-MCT groups but did not differ between SHR-LCT and SHR-MCT groups (Fig. 8).

**DISCUSSION**

We demonstrated that long-term administration of MCT beginning in the early stages of hypertension improves cardiac diastolic and contractile function in SHRs with concomitant prevention of the associated increases in the cardiac β-MHC-MHC-.
to-α-MHC isoform ratio. In addition, the compensatory cardiac hypertrophy seen in SHRs was mildly reduced following MCT ingestion without abrogation of arterial blood pressure and heart rate elevations. These results are similar to those of previous studies (30, 33). In this study, MCT administration increased the activities of LCHAD and MCAD, important FA metabolic enzymes, and inhibited increases in the activity of PFK-1, a critical glycolytic enzyme, in the hearts of SHRs. These findings suggest that MCT administration to SHRs prevents the shift in energy supply from FA metabolism to glycolysis, which is a compensatory adaptation to pathological cardiac hypertrophy. A MCT-enriched diet fed to SHRs also enhanced myocardial PPAR-α protein expression and transcriptional upregulation of LCHAD and MCAD enzymes. These findings suggest that long-term MCT administration, when initiated in the early stages of hypertension in SHRs, improves cardiac function by increasing the effective energy supply from FA metabolism to glycolysis, which is a compensatory adaptation to pathological cardiac hypertrophy. A MCT-enriched diet fed to SHRs also enhanced myocardial PPAR-α protein expression and transcriptional upregulation of LCHAD and MCAD enzymes. These findings suggest that long-term MCT administration, when initiated in the early stages of hypertension in SHRs, improves cardiac function by increasing the effective energy supply from FA metabolism to glycolysis, which is a compensatory adaptation to pathological cardiac hypertrophy. A MCT-enriched diet fed to SHRs also enhanced myocardial PPAR-α protein expression and transcriptional upregulation of LCHAD and MCAD enzymes. These findings suggest that long-term MCT administration, when initiated in the early stages of hypertension in SHRs, improves cardiac function by increasing the effective energy supply from FA metabolism to glycolysis, which is a compensatory adaptation to pathological cardiac hypertrophy.
FA metabolic homeostasis (4, 16). Mice lacking PPAR-α (PPAR-α−/− mice) exhibit decreased enzymatic activity and protein and mRNA expression of FA metabolic enzymes in cardiac tissue (3, 35). Therefore, PPAR-α has a critical role in the regulation of target genes encoding FA metabolic (β-oxidation) enzymes, such as LCHAD, MCAD, and CPT-I (3, 4, 35). In this study, PPAR-α protein expression in the heart was reversed by MCT administration to SHRs. Additionally, the MCT-enriched diet improved the decreases in activity of myocardial PPAR-α DNA binding to the PPRE and that tended to upregulate MCAD and LCHAD gene expression in the hearts of SHRs. Thus, activation of PPAR-α signaling in the heart by MCT treatment should upregulate myocardial metabolic capacity. Our MCT mixture included MCFAs, which are composed of 70% caprylic acid and 30% capric acid. Capric acid is not a good activator of PPAR-α compared with LCFAs (7); it is unclear if caprylic acid is a PPAR-α ligand or activator. Future studies will be necessary to clarify this issue and examine the effect of caprylic acid on PPAR-α activity.

In this study, long-term MCT administration to SHRs did not alter the typical increases in arterial blood pressure and heart rate seen in these animals but did mildly inhibit the compensatory cardiac hypertrophy. Whereas MCT inhibited the activation of JNK, ERK activation was unaffected. An activator of PPAR-α, fenofibrate, also prevented the compensatory cardiac hypertrophy without any observable reductions in arterial blood pressure after abdominal aortic banding (15). Thus, MCT (as does fenofibrate) may influence both JNK and tachycardia, even in the absence of hypertension, may induce sufficient mechanical stress to the heart to increase enzymatic activity.

A recently report by Labarthe et al. (19) demonstrated an increase in the contribution of exogenous FAs to energy production following MCT (octanoate = caprylic acid) supplementation when perfused SHR hearts were exposed to adrenergic stress. Although long-term MCT administration to SHRs increased the activity and expression levels of enzymes in a metabolic pathway, this change may not necessarily indicate alterations in substrate flux through the pathway. Future studies should attempt to examine substrate utilization directly in the hearts of MCT-fed SHRs. Whereas this study revealed the effect of MCT supplementation on SHR hearts, there may also be systemic effects that will be important to identify. It may also be informative to examine the effect of MCT on cardiac myocytes in culture. Wu et al. (36) reported that basal protein expression levels of PPAR-α in the heart did not differ between SHRs and WKY rats, whereas this study identified significant decreases in PPAR-α protein levels in hearts from the SHR-LCT group. The previous report showed decreased body weights in SHRs compared with WKY rats. On the other hand, we did not observe any such difference between the groups. However, the reason for this difference is unclear. Further studies should clarify this issue and examine the effect of body weight on basal PPAR-α protein levels in SHR and WKY hearts. In the hypertrophied heart, administration of the PPAR-α agonist WY-14,643 inhibited skeletal α-actin gene expression and prevented the switching of substrate utilization from FAs to glucose in the heart, resulting in the depression of compensatory adaptations, which is essential for the maintenance of cardiac function (37). This study examined MCT administration to SHRs in the early stages of hypertension, prior to the induction of compensatory cardiac adaptations. Therefore, MCT-induced increases in PPAR-α expression may improve cardiac function by upregulating FA metabolic gene expression, which would mitigate the hypertensive-induced compensatory cardiac hypertrophy with JNK activation. It will be necessary to examine this issue in greater detail at different stages throughout the process of compensatory adaptations in SHRs.

In conclusion, the benefit of MCT therapy in SHRs on cardiac function, which was observed despite the absence of any effects on blood pressure, is associated with the following changes: 1) increased activity and mRNA levels of selected enzymes involved in FA utilization, including PPAR-α-regulated metabolic genes, and 2) decreased activity of hypertrophic JNK signaling.

REFERENCES

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