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Am J Physiol Heart Circ Physiol 295:H136-H144, 2008. First published 2 May 2008;
doi: 10.1152/ajpheart.01417.2006

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

Motoyuki Iemitsu,^{1,2} Nobutake Shimojo,³ Seiji Maeda,^{2,4} Yoko Irukayama-Tomobe,⁴ Satoshi Sakai,³ Takeshi Ohkubo,⁵ Yukihisa Tanaka,⁵ and Takashi Miyauchi^{2,3}

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

Submitted 27 December 2006; accepted in final form 25 April 2008

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 SHR 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 SHR 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 SHR on the expression level and activity of metabolic enzymes and signaling pathways. Four-week-old male SHR 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 the positive and negative first derivatives of LV pressure/*P* value], a shift in the β -myosin heavy chain (MHC)-to- α -MHC ratio, and cardiac hypertrophy compared with the SHR-LCT group without an effect on blood pressure. Administration of MCT of SHR reversed the LCT-induced reduction in the cardiac FA metabolic enzymatic activities of long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) and medium-chain acyl-CoA dehydrogenase (MCAD). In the SHR-MCT group, the protein expression and transcriptional regulation of myocardial peroxisome proliferator-activated receptor- α , which regulates the transcription of LCHAD and MCAD genes, corresponded to the changes seen in those enzymatic activities. Furthermore, MCT intake caused an 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 following MCT therapy.

peroxisome proliferator-activated receptor- α ; CD36; c-Jun NH₂-terminal kinase; myosin heavy chain; spontaneously hypertensive rats

UNDER PHYSIOLOGICAL CONDITIONS, fatty acids (FAs) account for 60–70% of the oxygen consumption used for energy production in the heart (8, 28). In FA metabolism, long-chain FAs (LCFAs) are the most important lipid substrates for energy production in the heart (34). Spontaneously hypertensive rats (SHRs), which develop hypertension from as early as 4 wk of age, gradually acquire hypertensive cardiac hypertrophy leading to deteriorations in cardiac function (18, 29, 38). SHRs

exhibit a shift in myocardial energy production from the use of FAs toward the use of glycolysis (5). Pathological cardiac hypertrophy in SHRs also results in an abnormal regulation of protein and mRNA levels in key enzymes and transporters involved in myocardial energy metabolism (5, 10).

CD36 plays an important role as a LCFA-binding/transport protein that shuttles FAs from the extracellular compartment into the cytoplasm (1, 22). CD36 knockout mice demonstrate attenuated LCFA uptake and utilization in the heart (6). The SHR is a model of cardiomyopathy that develops hypertension, left ventricular (LV) hypertrophy (LVH), and cardiac dysfunction between 8 and 12 wk of age (18, 38). These rats are characterized by a restricted utilization of exogenous LCFAs for energy production, which has been attributed to a genetic defect in CD36. Numerous studies have demonstrated that the administration of medium-chain FAs (MCFAs), which do not require CD36-facilitated transport, increases exogenous FA oxidation and reverses cardiac dysfunction and hypertrophy development (9, 14, 19, 30, 33). While several studies have documented the benefits of MCFAs treatment in SHRs, little is known about the underlying molecular mechanisms. In contrast to LCFAs, MCFAs, which have chain lengths of 8–14 carbons, are rapidly taken up by cells. They are then activated directly in mitochondria prior to β -oxidation through a pathway that is not subject to regulation by carnitine palmitoyl transferase (CPT)-I (26, 27). There is little if any incorporation of MCFAs into triglycerides.

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

Address for reprint requests and other correspondence: T. Miyauchi, Cardiovascular Div., Institute of Clinical Medicine, Univ. of Tsukuba, Tsukuba, Ibaraki 305-8575, Japan (e-mail: t-miyauc@md.tsukuba.ac.jp).

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River Japan (Yokohama, Japan). Animals were cared for according to the Guiding Principles for the Care and Use of Animals based on the Helsinki Declaration of 1964. The basal diet was standard chow CLEA rodent (CE-7, CLEA Japan, Tokyo, Japan). LCT or MCT dietary supplements were prepared by adding 5% oil by mass. The LCT diet supplements contained 100% LCT, 11% stearic (C_{18:0}), 51% linoleic (C_{18:2}), and 38% other (e.g., C_{16:0}, C_{16:1}, and C_{18:1}) oils (Soybean shirashimne oil, Nisshin Oil, Tokyo, Japan), whereas the MCT diet supplements used a mixture of 81.5% MCT and 18.5% LCT (Panasate 810, NOF, Tsukuba, Japan). Oils at a final concentration of 5% were mixed with 95% basal diet using a mechanical food blender. Panasate 810 was composed of 70% caprylic (C₈) and 30% capric (C₁₀) oils. SHR and WKY rats were started on the LCT or MCT diets 4 wk after being weaned. SHR and WKY rats were fed either the MCT diet (SHR-MCT and WKY-MCT) or the LCT diet (SHR-LCT and WKY-LCT) for 16 wk. Rats were maintained on a 12:12-h light-dark cycle and received food (MCT or LCT diets) and water ad libitum. We measured the resting systolic arterial pressure of animals every 2 wk with a tail-cuff sphygmomanometer (model MK-1030, Muromachi Kikai, Tokyo, Japan). After the body weight and hemodynamic parameters of the animals had been measured, the heart was removed and rinsed in ice-cold saline to remove contaminating blood. The LV was then separated from the right ventricle and atria, weighed, and frozen in liquid nitrogen. LV samples were stored at -80°C until the determination of expression levels of CD36, long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD), and medium-chain acyl-CoA dehydrogenase (MCAD) mRNA by RT-PCR analysis; analysis of the enzymatic activities of LCHAD, MCAD, and phosphofructokinase-1 (PFK-1); determination of the proportions of α -myosin heavy chain (MHC) and β -MHC isoforms; analysis of the activity of peroxisome proliferator-activated receptor (PPAR)- α DNA binding to the peroxisome proliferator response elements (PPRE) by gel mobility shift assay; and determination of protein levels of PPAR- α , JNK, and ERK by Western blot analysis.

Hemodynamic measurements. Hemodynamic parameters in anesthetized rats were measured as previously described (31) with minor modifications. Rats were anesthetized with an intraperitoneal injection of sodium thiobarbital (50 mg/kg body wt). A microtip pressure transducer catheter (model SPC-320, Millar Instruments, Houston, TX) was inserted into the left carotid artery. Arterial blood pressure and heart rate were monitored as the catheter was advanced into the LV; LV systolic pressure (LVSP) and LV end-diastolic pressure (LVEDP) were determined as previously described (31). These hemodynamic parameters were recorded through the use of a polygraph system. The positive and negative first derivatives of LV pressure ($\pm dP/dt_{max}$) were derived from active analog differentiation using a pressure signal differentiation amplifier. The body temperature of the rat was maintained at 37°C using a small animal warmer.

Myocyte cross-sectional area. For the determination of myocyte cross-sectional area, frozen heart tissues were cut into 8- μ m-thick sections, and hematoxylin and eosin-stained slides were then prepared using standard methods (32). At least 16 sections were taken from each sample and at least 64 microscopic fields were examined at $\times 400$ magnification. The myocyte scan area was calculated using ImageJ 1.33 software (National Institutes of Health) as previously described. Only those myocytes in which the nucleus was centrally located within the cell were digitized and analyzed to ensure uniformity for the measurement of cross-sectional area.

RT-PCR. Total tissue RNA was isolated with Isogen reagent (Nippon Gene, Toyama, Japan) as previously described (10–13). Briefly, tissues were homogenized in Isogen (50 mg tissue/1 ml Isogen) using a Polytron tissue homogenizer (model PT10SK/35, Kinematica, Lucerne, Switzerland). Total RNA was extracted in chloroform, precipitated with isopropanol, and washed in 75% (vol/vol) ethanol. Total RNA was treated with RNase-free DNase (QIAGEN, Tokyo, Japan) and then further purified using an RNeasy mini kit (QIAGEN). Single-stranded cDNA was

synthesized from prepared RNA (2 μ g) using omniscrypt reverse transcriptase (QIAGEN) with an oligo(dT) primer at 37°C for 60 min.

cDNA was diluted in a 1:10 ratio, and 1 μ l was used for PCR. Each PCR mixture contained 10 mM Tris·HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 μ M of each dNTP, 0.5 μ M of each gene-specific primer, and 0.025 U/ μ l *Taq* polymerase (Takara, Shiga, Japan). The following gene-specific primers for CD36 (1), MCAD (23), LCHAD (24), and β -actin (25) were synthesized according to published cDNA sequences: CD36, sense 5'-GTGCAAAGAAGGAAAGCC-3' and antisense 5'-CATCACTACTCCAACACC-3'; MCAD, sense 5'-GTATTGACGCGATCTAACCCAG-3' and antisense 5'-TAGAGGCAAAGTACGTGTTCCG-3'; LCHAD, sense 5'-CCTTCCAGATGGCCTTCCTG-3' and antisense 5'-GTTGCCAGATAGCGCAGAGC-3'; and β -actin, sense 5'-GAAGATCCTGACCGAGCGTG-3' and antisense 5'-CGTACTCCTGCTTGCTGATCC-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

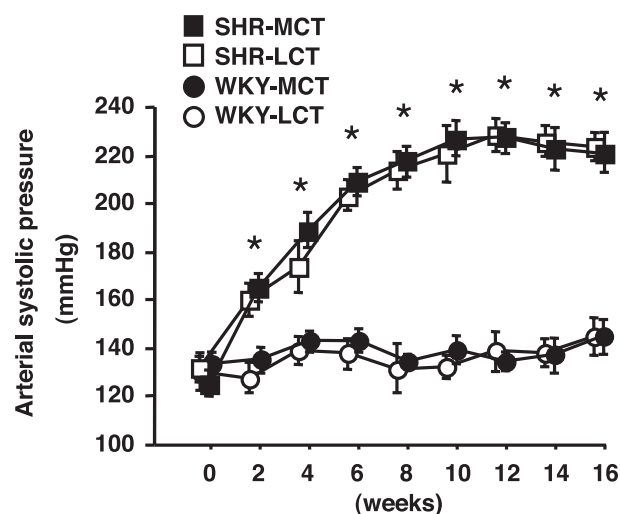


Fig. 1. Time course of the changes in arterial systolic blood pressure using tail-cuff sphygmomanometry in Wistar-Kyoto (WKY) rats fed a long-chain triglyceride (LCT) diet (WKY-LCT group; $n = 6$), WKY rats fed a medium-chain triglyceride (MCT) diet (WKY-MCT group; $n = 6$), spontaneously hypertensive rats (SHRs) fed a LCT diet (SHR-LCT group; $n = 7$), and SHRs fed a MCT diet (SHR-MCT group; $n = 7$) from 4 to 20 wk old. Values are expressed as means \pm SE. * $P < 0.05$ vs. WKY-LCT and WKY-MCT groups.

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

	WKY-LCT	WKY-MCT	SHR-LCT	SHR-MCT
<i>n</i>	6	6	7	7
BW, g	429.0±9.5	379.2±4.0*	430.2±6.8	419.3±6.9
LVW, g	0.95±0.02	0.92±0.05	1.28±0.03†	1.16±0.02†‡
LVW/BW, mg/g	2.22±0.02	2.43±0.11	3.00±0.04†	2.78±0.07†
Myocyte cross-sectional area, μm ²	543.3±5.9	538.3±5.4	603.2±9.6†	578.5±4.3†‡
Heart rate, beats/min	253.2±12.2	236.1±18.5	410.9±12.7†	378.9±14.6†
Blood pressure, mmHg				
Systolic	105.8±2.0	107.5±2.1	199.6±6.6†	206.2±10.6†
Diastolic	70.1±2.7	69.5±3.1	149.6±5.1†	152.3±7.3†
Mean	82.0±2.4	82.1±2.8	166.2±5.5†	170.2±8.3†

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. the SHR-LCT group.

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 β-glycerophosphate, 100 μM DTT, 12 mM glycylglycine, 0.2 mM fructose-6-phosphate, 0.5 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 *g* 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

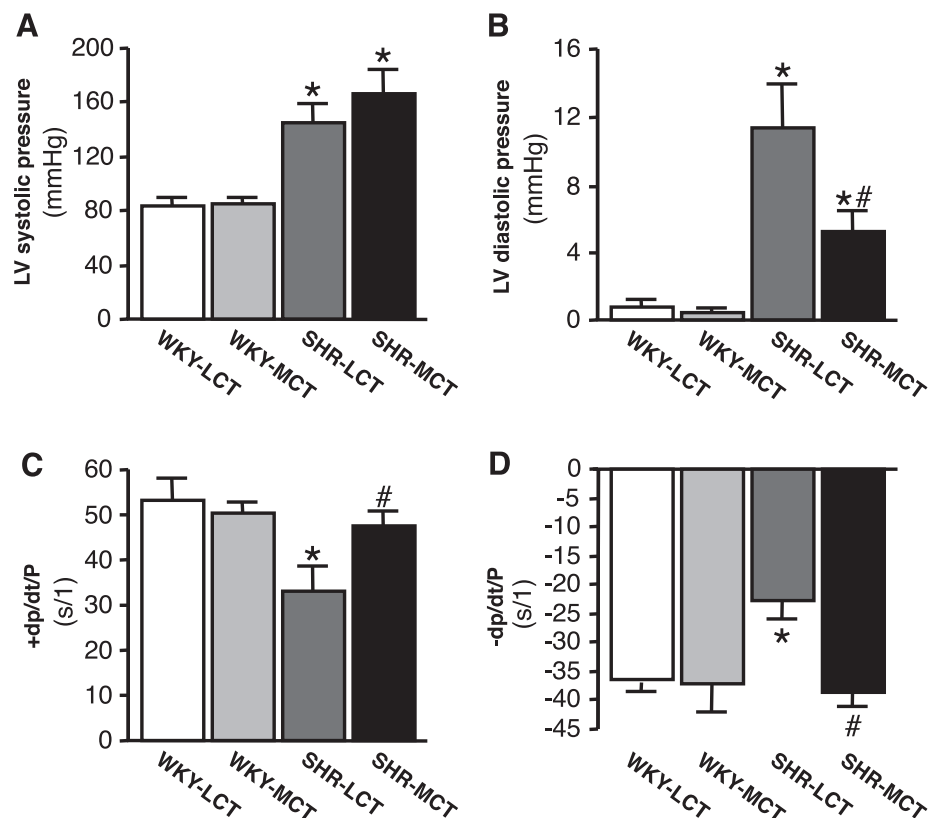


Fig. 2. Left ventricular (LV) systolic pressure (A), LV diastolic pressure (B), positive derivative of LV pressure/*P* value (+*dp/dtP*; C), and negative first derivative of LV pressure/*P* value (−*dp/dtP*; D) in WKY-LCT (*n* = 6), WKY-MCT (*n* = 6), SHR-LCT (*n* = 7), and SHR-MCT (*n* = 7) groups. Values are expressed as means ± SE. **P* < 0.05 vs. WKY-LCT and WKY-MCT groups; #*P* < 0.05 vs. the SHR-LCT group.

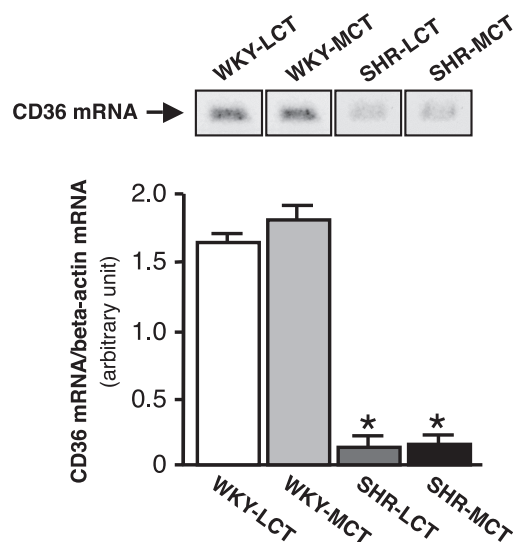


Fig. 3. Expression of CD36 mRNA in hearts (LVs) from the WKY-LCT ($n = 6$), WKY-MCT ($n = 6$), SHR-LCT ($n = 7$), and SHR-MCT ($n = 7$) groups. *Top*: representative RT-PCR analysis of CD36 mRNA. Expression of β -actin mRNA served as an internal control. *Bottom*: results of densitometric analysis of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

Signaling, Beverly, MA), polyclonal anti-JNK antibody (1:1,000 dilution in blocking buffer, Cell Signaling), polyclonal anti-phospho-ERK (Thr²⁰²/Tyr²⁰⁴) 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 were washed three times in PBS-T and then incubated with the appropriate horseradish peroxidase-conjugated secondary antibody, donkey anti-goat antibody (1:1,000 dilution in blocking buffer, Santa Cruz Biotechnology), anti-rabbit antibody (1:2,000 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- α , 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 MgCl₂, 10 mM NaF, 1 mM Na₃VO₄, 1 mM DTT, 20 mM β -glycerophosphate, 0.5 mM PMSF, 60 μ g/ml aprotinin, and 2 μ 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 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 MgCl₂, 10 mM NaF, 1 mM Na₃VO₄, 0.2 mM DTT, 20 mM β -glycerophosphate, 0.5 mM PMSF, 60 μ g/ml aprotinin, 2 μ g/ml leupeptin, and 20% glycerol and centrifuged at 18,000 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 μ g protein) were incubated with 50,000 counts/min of a ³²P-labeled double-stranded oligonucleotide probe containing the consensus PPAR- α binding sequence (5'-TGACCTTTGACCTA-GTTTTG-3') of the promoter regions of LCHAD and MCAD at room temperature for 20 min in 10 μ l binding buffer consisting of 10 mM Tris·HCl (pH 7.5), 50 mM NaCl, 0.5 mM EDTA, 1 mM MgCl₂, 0.5 mM DTT, 4% glycerol, and 0.05 mg/ml poly(di-dC). 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 Scheffé'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 sphygmomanometer 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 were 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-LCT and SHR-MCT groups. LVSP and LVEDP were significantly higher in the SHR-LCT group than in the WKY-LCT group. LVEDP was

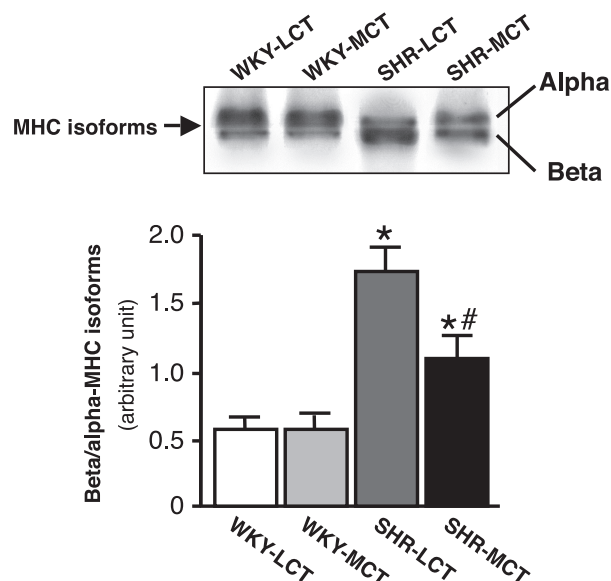


Fig. 4. Expression of α -myosin heavy chain (MHC) and β -MHC isoforms in hearts (LVs) from the WKY-LCT ($n = 6$), WKY-MCT ($n = 6$), SHR-LCT ($n = 7$), and SHR-MCT ($n = 7$) groups. *Top*: representative images of the electrophoretic separation of α -MHC and β -MHC isoforms. *Bottom*: results of densitometric analysis of β -MHC/ α -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.

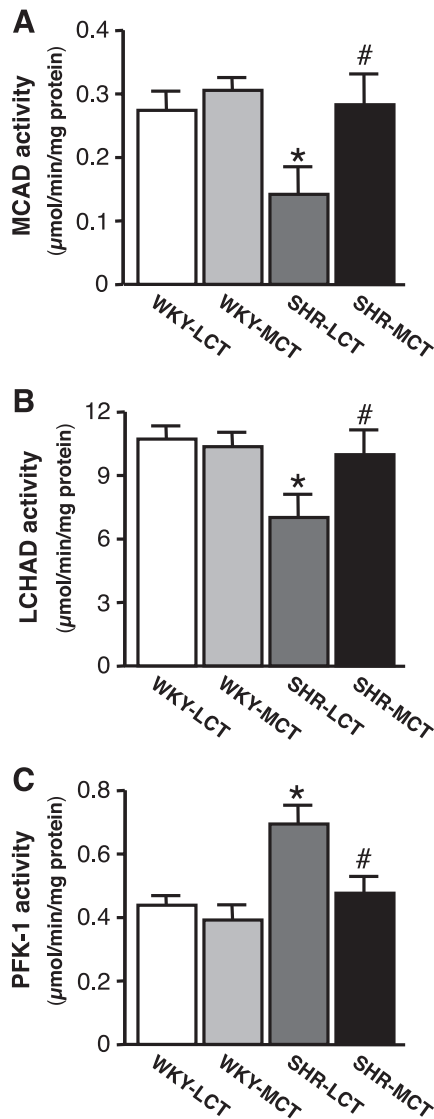


Fig. 5. Enzymatic activities of medium-chain acyl-CoA dehydrogenase (MCAD; *A*), long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD; *B*), and phosphofructokinase-1 (PFK-1; *C*) in hearts from the WKY-LCT ($n = 6$), WKY-MCT ($n = 6$), SHR-LCT ($n = 7$), and SHR-MCT ($n = 7$) groups. Values are expressed 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 $+dP/dt/P$ 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, whereas that of the SHR-MCT group was significantly higher than that in the SHR-LCT group. In contrast, $-dP/dt/P$ value, which was also corrected by the LVSP, was significantly higher in the SHR-LCT group than in the WKY-LCT group, whereas that in the SHR-MCT group was significantly lower than that in the SHR-LCT group.

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. 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 β -MHC to α -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 WKY-LCT group. SHRs given MCT exhibited a lower ratio than those given LCT, indicating a shift from α -MHC to β -MHC isoforms in the heart.

Myocardial energy metabolic enzyme activity. Activity of FA β -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- α protein expression and transcriptional regulation of PPAR- α target genes. As revealed by immunoblot analysis of PPAR- α in WKY-LCT, WKY-MCT, SHR-LCT, and SHR-MCT groups, cardiac expression levels of PPAR- α in the SHR-LCT group were significantly lower than in both the WKY-LCT and WKY-MCT groups. Cardiac levels

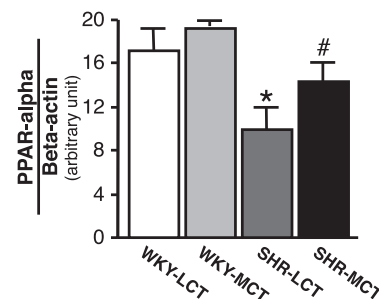
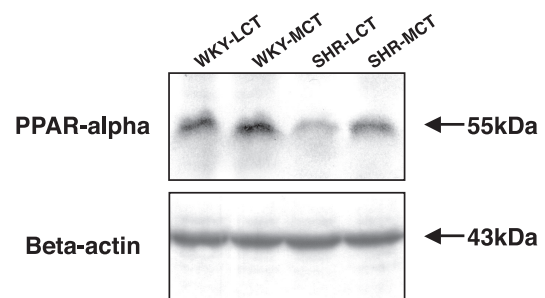


Fig. 6. Peroxisome proliferator-activated receptor (PPAR)- α protein expression in hearts (LVs) from the WKY-LCT ($n = 6$), WKY-MCT ($n = 6$), SHR-LCT ($n = 7$), and SHR-MCT ($n = 7$) groups. *Top* and *middle*: representative immunoblots of PPAR- α and β -actin proteins. Arrows indicate the specific bands corresponding to PPAR- α protein (55 kDa) and β -actin protein (43 kDa). *Bottom*: results of densitometric analysis of PPAR- α protein expression levels. PPAR- α protein levels were normalized to those of β -actin protein 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.

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 DNA 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. 7C); 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 mem-

ber 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-

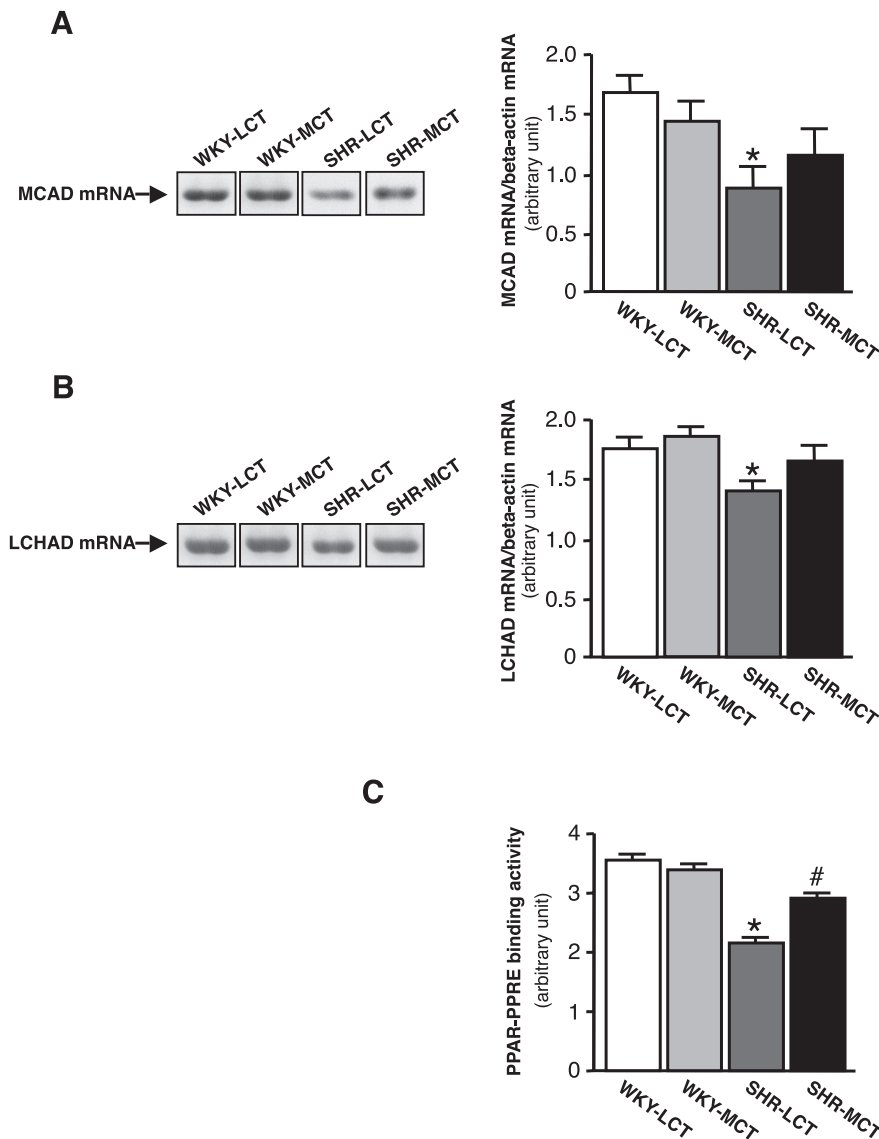


Fig. 7. A and B: mRNA expression levels of MCAD (A) and LCHAD (B) in hearts (LVs) from the WKY-LCT ($n = 6$), WKY-MCT ($n = 6$), SHR-LCT ($n = 7$), and SHR-MCT ($n = 7$) groups. *Left*, representative RT-PCR analysis of MCAD and LCHAD mRNA. Expression of β -actin mRNA served as an internal control. *Right*, results of densitometric analysis of MCAD and LCHAD mRNA expression levels. Images of PCR product gel electrophoresis were examined by densitometry. MCAD and LCHAD mRNA levels were normalized to that of β -actin mRNA. C: activity of PPAR- α DNA binding to the peroxisome proliferator response element (PPRE) in hearts (LV nuclear extracts) from the WKY-LCT ($n = 6$), WKY-MCT ($n = 6$), SHR-LCT ($n = 7$), and SHR-MCT ($n = 7$) groups. Results of the statistical analysis of the level of PPAR- α DNA binding activity in the heart were measured by a densitometer. 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.

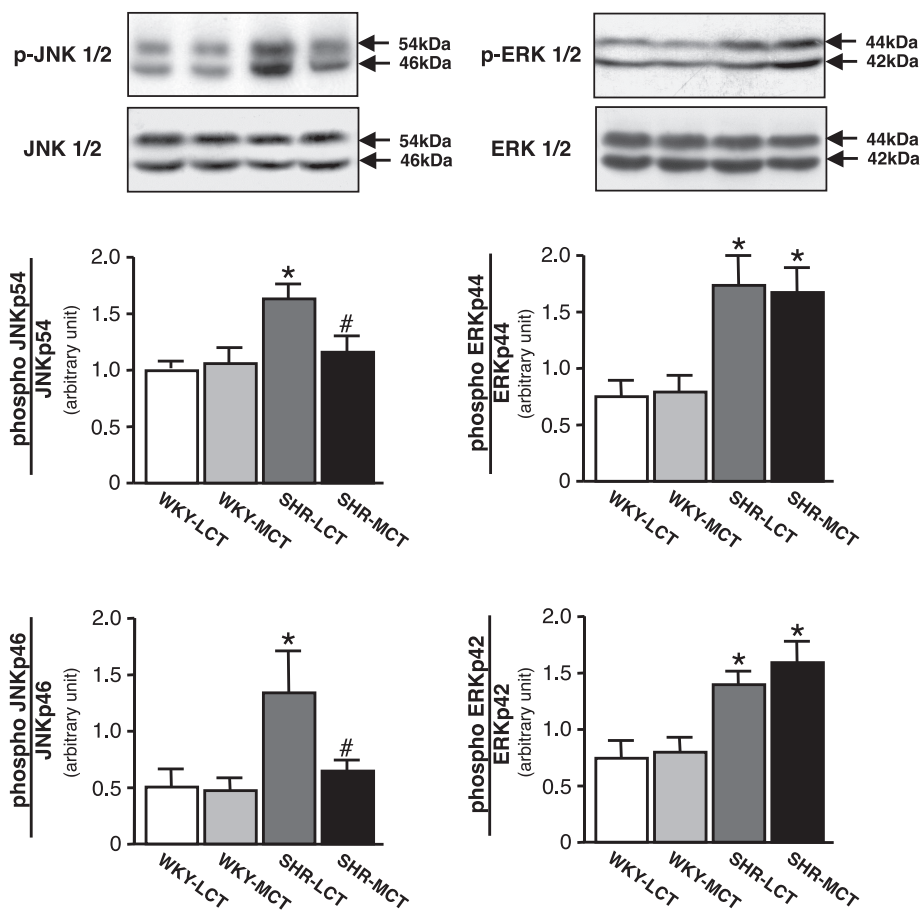


Fig. 8. Changes in the activity of JNK1/2 (p54 and p46) and ERK1/2 (p44 and p42) in hearts (LVs) from the WKY-LCT ($n = 6$), WKY-MCT ($n = 6$), SHR-LCT ($n = 7$), and SHR-MCT ($n = 7$) groups. *Top*: representative immunoblots of phosphorylated (p-)JNK1/2 and JNK1/2 (*left*) and p-ERK1/2 and ERK1/2 (*right*) proteins. Arrows indicate the specific bands corresponding to JNK1 and JNK2 (p54 and p46) proteins (54 and 46 kDa) and ERK1 and ERK2 (p44 and p42) proteins, respectively. *Middle and bottom*: results of densitometric analysis of JNK1/2 [p54 (*middle left*) and p46 (*bottom left*)] and ERK1/2 [p44 (*middle right*) and p42 (*bottom right*)] activations. 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.

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 via the upregulation of myocardial PPAR- α -mediated transcription of target genes, such as FA metabolic enzymes. Interestingly, MCT administration inhibited the hypertrophy-induced activation of cardiac JNK signaling in SHRs. Thus, MCT-induced increases in cardiac metabolic enzyme activity, improvements in cardiac function, and hypertrophic JNK signaling may result in the mitigation of the compensatory cardiac hypertrophy typically seen in SHRs.

In this study, long-term MCT administration to SHRs up-regulated the enzymatic activity of LCHAD and MCAD and prevented the increases in glycolytic enzymatic activity seen in

the hearts of SHRs. The decrease in FA oxidation is accompanied by an increase in glycolysis and accelerated activity of glycolytic enzymes involved in anaerobic metabolism (2, 17, 19). Several studies have suggested that alterations in myocardial energy metabolism contribute to the depression of cardiac contractile function seen in this animal model (9, 19, 33). Our results suggest that the upregulation of myocardial FA metabolism induced by MCT administration may help to prevent the progression of cardiac dysfunction typically seen in SHRs. In our previous report (10) using similarly aged SHRs, mRNA expression of several FA metabolizing enzymes, such as CPT-I, CPT-II, and acyl-CoA synthase, increased in cardiac tissue from SHRs given LCT. In addition, the levels of myocardial LCHAD activity in SHRs with LCT have been previously reported to be similar to those seen in WKY rats fed the same diet (33). These reports suggest that in SHRs, cardiac tissue may develop compensatory adaptations to provide supplemental energy; the breakdown of these compensatory adaptations may have been faster in this study than that in previous reports. Therefore, our results would be affected by the different time course of cardiac hypertrophy progression in SHRs. Further studies will be required to clarify this issue and examine the effect of different cardiac conditions on MCT treatment efficacy in SHRs.

The molecular mechanisms underlying the MCT-induced enhancement of cardiac LCHAD and MCAD enzymatic activities remain unclear. PPAR- α complexes with retinoid X receptor bind to the PPRE upstream of several genes involved in

FA metabolic homeostasis (4, 16). Mice lacking PPAR- α (PPAR- $\alpha^{-/-}$ 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 PPARE 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 PPAR- α signaling in the heart independently. Additionally, the MCT-enriched diet did not impact the mRNA expression by SHRs of cardiac markers of hypertrophy, including endothelin-1, angiotensin-converting enzyme, brain natriuretic peptide, and skeletal α -actin (33). ERK, which is activated by mechanical stress in SHRs, may remain unaffected by the administration of a MCT-enriched diet, because hypertension and tachycardia, even in the absence of hypertrophy, 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.

GRANTS

This work was supported by Ministry of Education, Culture, Sports, Science and Technology of Japan Grants-In-Aid for Scientific Research 18300215, 18650186, and 20700563 and a grant from Tsukuba Advanced Research Alliance Project of the University of Tsukuba.

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