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Attenuation of Cardiac Hypertrophy in Carnitine-Deficient Juvenile Visceral Steatosis (JVS) Mice Achieved by Lowering Dietary Lipid

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We examined the development of cardiac hypertrophy in juvenile visceral steatosis (JVS) mice, a model of systemic carnitine deficiency, by varying the amount of lipid in the diet. Cardiac hypertrophy was markedly attenuated by decreasing soy bean oil (SBO) from 5% (w/w) to 1%. Triglyceride contents of the ventricles of JVS mice fed 1% SBO were significantly lower than in JVS mice fed 5% SBO. The addition of medium-chain triglycerides metabolically utilized by JVS mice did not affect the development of cardiac hypertrophy. On the other hand, the mRNA levels of atrial natriuretic peptide and skeletal α-actin, which are related to cardiac hypertrophy, were also attenuated by decreasing lipid in the diet. Adenylate energy charge and creatine phosphate in the heart of JVS mice at the early stage of hypertrophy were not significantly different from control mice given the same laboratory chow (4.6% of lipid). Although urinary prostaglandin $F_{2\alpha}$ levels were found to be increased in JVS mice at 15 days of age when they developed cardiac hypertrophy, administration of aspirin was not efficacious. We, therefore, propose that the proportion of lipid in the diet is important in the development of cardiac hypertrophy in carnitine-deficient JVS mice, and that this is not related to prostaglandin formation.

Key words: atrial natriuretic peptide, carnitine deficiency, carnitine deficiency–associated gene expressed in ventricle-1, prostaglandin $F_{2\alpha}$.

Abbreviations: ANP, atrial natriuretic peptide; BW, body weight; CDV-1, carnitine deficiency–associated gene expressed in ventricle-1; EC, energy charge; ELISA, enzyme-linked immunosorbent assay; FFA, free fatty acid; HW, heart weight; JVS, juvenile visceral steatosis; MCT, medium-chain triglyceride; $PGF_{2\alpha}$; prostaglandin $F_{2\alpha}$; SBO, soy bean oil; Sk-Act, skeletal α-actin.

Juvenile visceral steatosis (JVS) mice, which suffer from fatty liver, growth retardation, hypoglycemia and hyperammonemia (*1*), are systemically deficient in carnitine (*2*), but all the symptoms are relieved by carnitine treatment (*3*). Carnitine deficiency is caused by a defect in the plasma membrane transport of carnitine (*4*, *5*) executed by Octn2, which is encoded by Slc22a5 (*6*, *7*). Carnitine-deficient JVS mice suffer from cardiac hypertrophy, which is associated with increases in cardiac protein, DNA and cardiac cell volume (*8*). The gene expression pattern of the hypertrophied ventricles of JVS mice differs from that in pressure-loaded models: there is no increase in the ratio of β-myosin heavy chain mRNA to α-myosin heavy chain mRNA in JVS mice, in contrast to the ratio in hypertrophy caused by aortic coarctation in rats and mice (*9–11*). Yoshida *et al.* (*12*) showed that the level of cardiotrophin-1 mRNA is lower in the ventricles of JVS mice. These results show the uniqueness of the cardiac hypertrophy caused by carnitine deficiency as compared with hypertrophy produced by pressure overload. To define the characteristic pathogenesis and pathophysiology of the hypertrophy in carnitine-deficient JVS mice, we applied differential display analysis and found three novel carnitine deficiency–associated genes expressed in ventricles (CDV-1, -2, and -3) (*13–15*) that were expressed differently in the hypertrophied ventricles of JVS mice. CDV-1, which is specifically expressed in the heart and specifically suppressed in the hypertrophied ventricles of JVS mice, encodes a protein with an α-helical coiled-coil structure (*13*, *14*).

Theoretically, it is possible to speculate that cardiac hypertrophy caused by carnitine deficiency is related to the accumulation of long-chain fatty acids and/or a reduction in ATP production since carnitine is an indispensable factor in the entry of long-chain fatty acids into the mitochondria (*16–18*). However, the precise mechanism of the hypertrophy still remains to be elucidated. From our previous reports, during the suckling period and during starvation when lipid metabolism is accelerated, JVS mice show severe fatty liver and altered gene expression of the urea cycle enzyme gene (*19–21*). The heart is one of the most active organs in fatty acid metabolism, even in the fed state (*22*, *23*). In view of this, we decided to evaluate the toxicity of dietary fats involved in the development of cardiac hypertrophy.

Recently, both prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and F_2-isoprostanes, which are produced from arachidonic acid,

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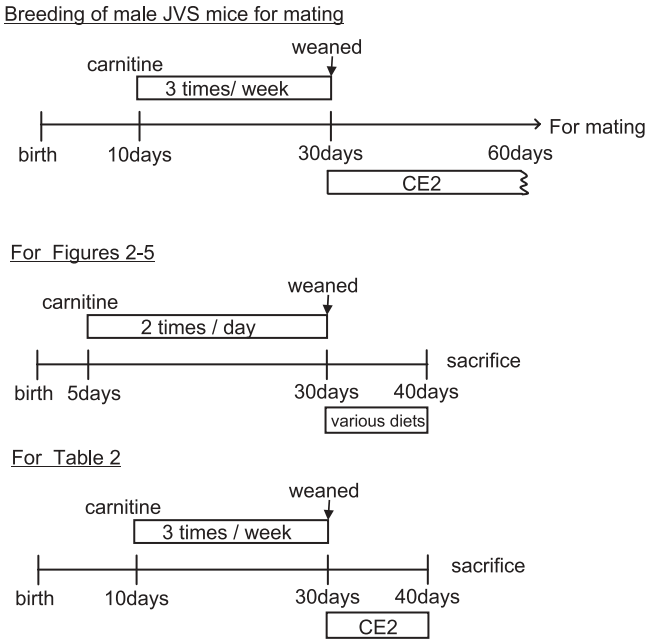

Fig. 1. **Schedule of carnitine treatment and diet for JVS mice.** Male JVS mice after 2 months of age were used for mating. Laboratory chow (CE2 from CLEA Japan) was provided in the cage in which the mother lived with her pups before weaning at 30 days.

have been found to induce cardiac hypertrophy (*24–26*). We also examined the possible involvement of these prostaglandins in the development of cardiac hypertrophy.

MATERIALS AND METHODS

Animals and Dietary Conditions—Homozygous mutant JVS (jvs/jvs) and heterozygous (+/jvs) mice were produced by mating a heterozygous female and a homozygous male injected intraperitoneally with carnitine (5 μmol/animal) three times a week (Fig. 1 and Ref. *3*). Heterozygous mice that showed no symptoms of cardiac hypertrophy within three months of age were used as controls (*8*).

All control and JVS mice used for the experiments shown in Figs. 2–5 were treated intraperitoneally with 5 μmol/animal carnitine twice a day from 5 days till 30 days (Fig. 1), and were supplied with laboratory chow (protein 25.2% and fat 4.6%; CE2 from CLEA Japan) before weaning. The mice were weaned at 30 days, and various diets (Table 1; Nosan Co., Japan) were given as indicated in Fig. 1. The study was approved by the Animal Experimentation Committee, Kagoshima University, and was carried out according to guidance from the committee.

Biochemical Analysis—The mice were sacrificed at 30 or 40 days under pentobarbital anesthesia (100 mg/kg of body weight, BW). Heparinized blood was taken for analysis. Plasma free fatty acids (FFA) were assayed with a Determiner-NEFA (Kyowa Medix Co., Ltd, Japan). For the determination of adenine nucleotide and creatine phosphate in the heart (Table 2), mice were fed a CE2 diet and treated with carnitine (5 μmol/animal) three times a week, until 30 days (Fig. 1). At 40 days, the mice were anesthetized with pentobarbital (100 mg/kg of BW). Immediately

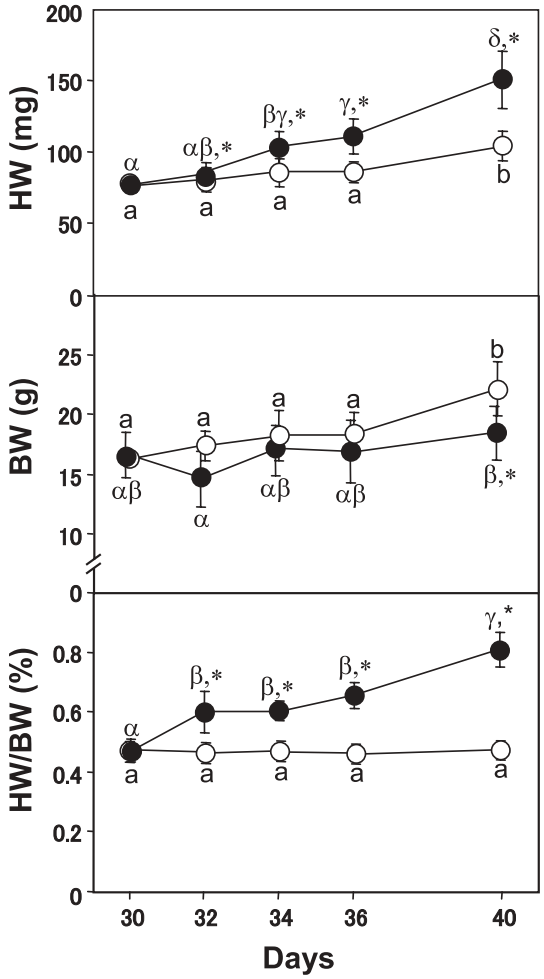

Fig. 2. **Time dependent changes in heart weight (HW), body weight (BW) and HW/BW ratio after cessation of carnitine administration.** Mice were fed a 5% SBO diet; experimental procedures were performed as described in "MATERIALS AND METHODS." Open and closed circles show HW, BW and HW/BW in control and JVS mice, respectively. Numbers of mice were more than 5 at each age. Results are shown as mean ± SD; values sharing identical superscripts (alphabetical and Greek letters) are not significantly different, $P < 0.05$. *$P < 0.05$ *versus* control at the same age by un-paired Student's *t* test analysis.

after opening the chest, a part of the heart was removed and freeze-clamped, pulverized under liquid nitrogen, and homogenized with 6% (w/v) perchloric acid. The perchloric acid extract was neutralized with 1 N KOH, and creatine phosphate and adenine nucleotides in the neutralized sample were quantified by enzymatic analysis (*27*) and by HPLC analysis (*28*), respectively. The HPLC analysis was done essentially according to the method described by Sellevold *et al.* (*28*) with a Wakosil 5C18-200 column (4.6 mm × 250 mm; Wako Pure Chemical Industries, Ltd., Japan) eluted with 0.1 M KH_2PO_4 adjusted with 1 N KOH to pH 6.0 at a flow rate of 0.5 ml/min. Protein content was determined by the Lowry method (*29*) after dissolving the perchloric acid precipitate with 1 N NaOH. Adenylate energy charge (EC) was calculated as the ratio of (ATP + ADP/2)/(ATP + ADP + AMP). Carnitine content in the diets (CE2 and SBO) was measured by a photometric

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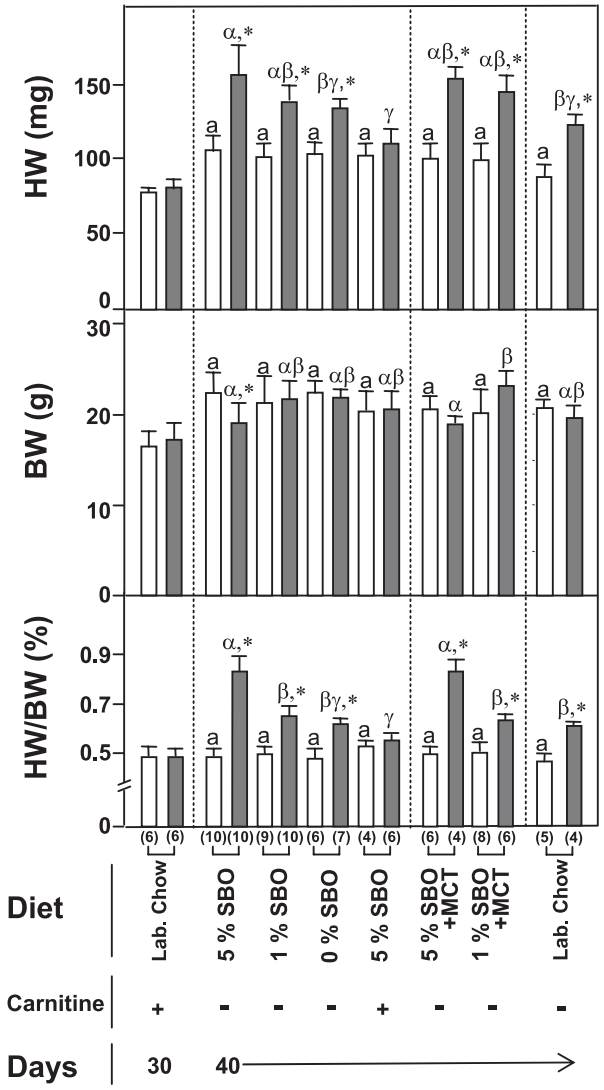

Fig. 3. **Effects of carnitine, amount of dietary lipid and MCT on cardiac hypertrophy in JVS mice.** Open and closed columns show HW, BW and HW/BW in control and JVS mice, respectively, at 30 and 40 days of age. Mice were fed each diet for 10 days as described in "MATERIALS AND METHODS." (+) and (–) denote with and without carnitine treatment. The diets listed in Table 1 are shown in the figure. The numbers of mice are shown in parentheses. Results are shown as mean ± SD; values sharing identical superscripts (alphabetical and Greek letters) are not significantly different, $P < 0.05$. *$P < 0.05$ *versus* control with the same treatment by un-paired Student's *t* test analysis.

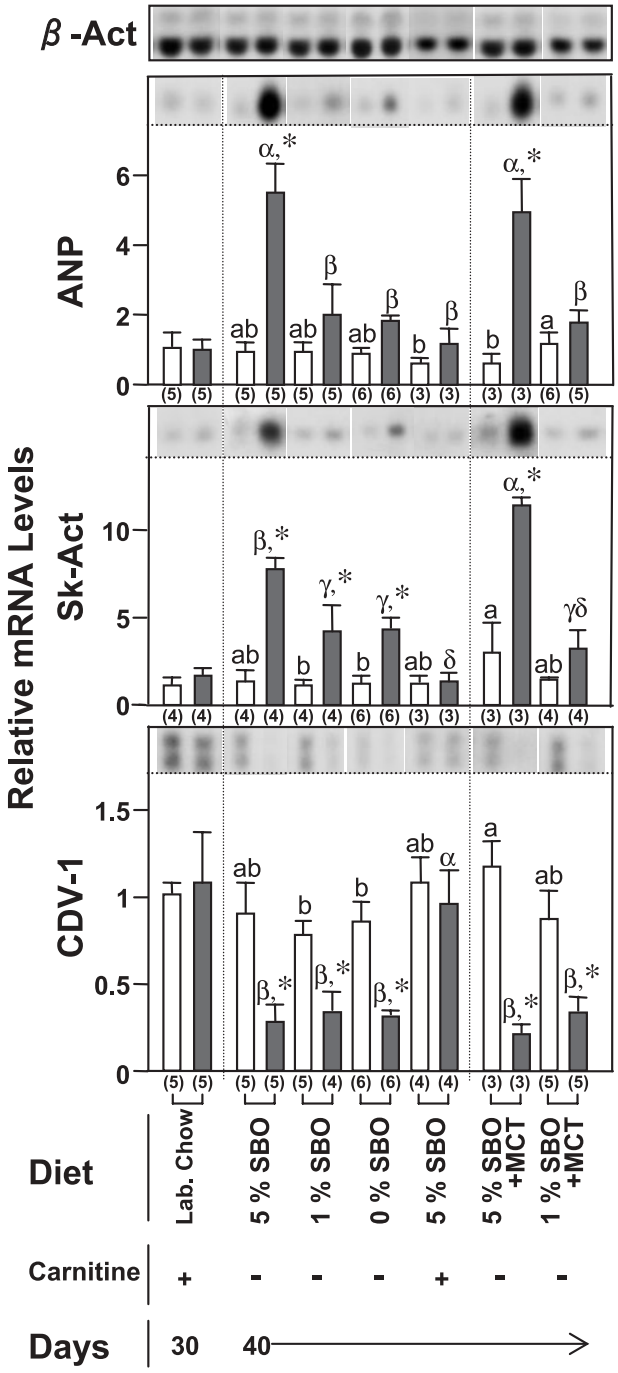

Fig. 4. **Effects of carnitine, amount of dietary lipid and MCT on gene expression.** Open and closed columns show the mRNA levels of ANP, Sk-Act and CDV-1 in control and JVS mice, respectively, at 30 or 40 days of age. In the upper part of each figure, representative data from Northern blot analysis are shown. The amount of mRNA detected by Northern blot analysis was quantified densitometrically. β-Actin was used as an internal standard (Northern data are shown at the top) and the mRNA levels relative to β-actin were calculated, with the value of control mice at 30 days set at 1.0. The numbers of mice are shown in parentheses. (+) and (–) denote with and without carnitine treatment. The diets listed in Table 1 are shown in the figure. Results are shown as mean ± SD; values sharing identical superscripts (alphabetical and Greek letters) are not significantly different, $P < 0.05$. *$P < 0.05$ *versus* control with the same treatment by un-paired Student's *t* test analysis.

assay kit (*30*) after treatment in accordance with the published procedure (*31*).

For lipid analysis, the ventricles were removed from anesthetized mice as described above, treated by freeze-drying, and the lipids were extracted with chloroform/methanol solution according to the method of Bligh and Dyer (*32*). Triglycerides and total cholesterol in the extracted lipids were measured using commercial kits (Wako, Osaka, Japan).

In the aspirin experiments (Table 3), 1 mg aspirin dissolved in H_2O and neutralized with 10 N NaOH was administered twice per day intraperitoneally to mice

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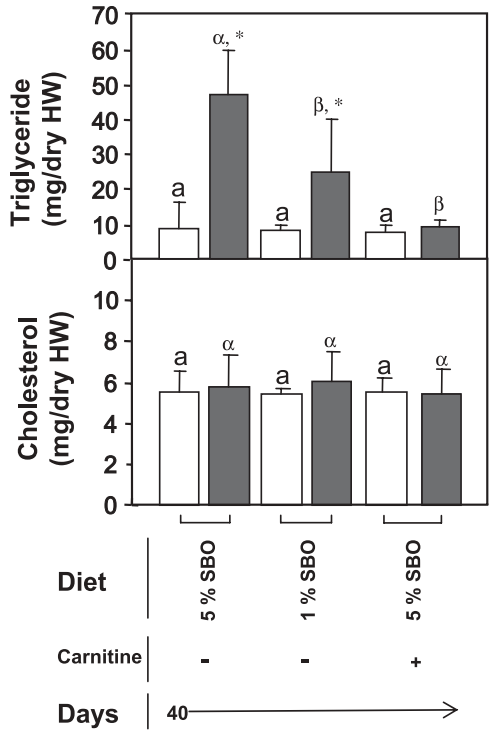

Fig. 5. **Effects of carnitine and amount of dietary lipid on the triglyceride and cholesterol contents of ventricles.** Open and closed columns show triglycerides and cholesterol in control and JVS mice, respectively ($N = 5$). (+) and (–) denote with and without carnitine treatment. The diets listed in Table 1 are shown in the figure. Results are shown as mean ± SD; values sharing identical superscripts (alphabetical and Greek letters) are not significantly different, $P < 0.05$. *$P < 0.05$ *versus* control at the same days of age by un-paired Student's t test analysis.

Table 1. **Composition of diets [% (w/w)].**

	5% SBO	1% SBO	0% SBO	5% SBO +MCT	1% SBO +MCT
Casein	20	20	20	20	20
α-Corn starch	55.5	59.5	60.5	51.5	55.5
Sucrose	10	10	10	10	10
Cellulose	5	5	5	5	5
SBO	5	1	0	5	1
MCT	0	0	0	4	4
Mineral mixture	3.5	3.5	3.5	3.5	3.5
Vitamin mixture	1	1	1	1	1
Total	100	100	100	100	100

SBO denotes soy bean oil. MCT (medium-chain triglyceride) consists of C_8:C_{10} (75:25).

from 5 days to 15 days of age. Urine samples were collected at 15 days, and heart and body weights were measured. The dose of aspirin (1 mg/animal) was chosen according to the results reported for mice and neonatal rats (*33*, *34*). Urinary $PGF_{2\alpha}$ and 15-isoprostane F_{2t} (8-iso-$PGF_{2\alpha}$) were assayed with competitive ELISA systems provided by Cayman Chemical (Ann Arbor, USA) and Oxford Biochemical Research (Oxford, USA), respectively.

RNA Analysis—Hearts were taken from mice under pentobarbital anesthesia (100 mg/kg of BW) and dissected into two parts transversely at the bottom of the auricles.

Table 2. **Content of adenine nucleotide, adenylate energy charge and creatine phosphate in the hearts of control and JVS mice.**

	Control mice (8)	JVS mice (6)
Adenine nucleotide (nmol/mg protein)		
ATP	23.2 ± 6.0	20.7 ± 3.8
ADP	7.5 ± 2.1	8.1 ± 1.5
AMP	2.0 ± 0.7	2.4 ± 0.8
Total	32.7 ± 8.2	31.2 ± 5.5
Energy charge	0.824 ± 0.024	0.795 ± 0.029
CP (nmol/mg protein)	27.2 ± 12.1	30.3 ± 14.7
Body weight (g)	17.9 ± 1.0	17.9 ± 2.0
Heart weight (mg)	82 ± 7	168 ± 18*
HW/BW (%)	0.48 ± 0.01	0.95 ± 0.11*

All mice (numbers are shown) were fed CE2 and sacrificed at 40 days of age. Energy charge was calculated as the ratio of (ATP + ADP/2)/(ATP + ADP + AMP). CP denotes creatine phosphate. HW/BW denotes ratio of heart weight to body weight. Values are presented as mean ± SD. *Denotes $P < 0.05$ versus control.

Table 3. **Urinary $PGF_{2\alpha}$ and 15-isoprostane F_{2t} of control and JVS mice at 15 days of age with and without aspirin treatment.**

	Control mice	JVS mice
Heart weight (mg)		
without aspirin	47 ± 3 (9)	61 ± 1 (3)*
with aspirin	47 ± 3 (6)	57 ± 8 (7)*
Body weight (g)		
without aspirin	8.5 ± 0.3 (9)	7.5 ± 1.1 (3)*
with aspirin	7.4 ± 0.6 (6)†	6.2 ± 0.9 (7)*
Urinary $PGF_{2\alpha}$ (ng/mg Cr)		
without aspirin	8.7 ± 0.9 (4)	18.1 ± 2.6 (5)*
with aspirin	1.8 ± 0.5 (5)†	2.3 ± 1.1 (5)†
Urinary 15-isoprostane F_{2t} (ng/mg Cr)		
without aspirin	16.7 ± 5.2 (5)	23.0 ± 12.8 (5)

Numbers of animals are given in parentheses. Cr denotes creatinine. Values are presented as mean ± SD. *Denotes $P < 0.05$ *versus* control mice. †Denotes $P < 0.05$ *versus* mice without aspirin.

The lower part was used as the ventricle (*9*). The ventricles were weighed and frozen at –80°C for further mRNA analysis. Total RNA was extracted from frozen ventricles by the method of Chomczynski and Sacchi (*35*). For the detection of atrial natriuretic peptide (ANP), skeletal α-actin (Sk-Act), CDV-1 and β-actin mRNAs, Northern blot analysis was performed using 20 μg of total RNA with each specific probe as described previously (*9*, *13*, *36*). The amount of mRNA detected by Northern blot analysis was quantified densitometrically with a Molecular Image Analyzer (Bio-Rad, USA). β-Actin mRNA was used as an internal standard.

Statistical Analysis—Values are shown as mean ± standard deviation (SD). Data (Figs. 2–5) were evaluated by unpaired Student's t test or one-way analysis of variance (ANOVA) followed by the Tukey-Kramer method (*37*) for multiple comparison. A value of $P < 0.05$ was considered statistically significant. JMP software (SAS Institute, Japan) was used for the statistical analysis. In the figures, values not sharing identical superscripts are significantly different, $P < 0.05$. The remaining data (Tables 2 and 3) were analyzed statistically by the

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Student's *t* test (StatView version 5.0, SAS Institute, USA) with the level of significance set at $P < 0.05$.

RESULTS

Cardiac Hypertrophy after Cessation of Carnitine Treatment in JVS Mice—We treated control and JVS mice with carnitine (5 μmol/animal twice a day) until 30 days of age when the mice were weaned (Fig. 1). At 30 days, there was no significant difference in heart weight (HW), body weight (BW) or HW/BW between the carnitine treated JVS and control mice (Fig. 2). After cessation of carnitine and weaning from the mothers, 5% soy bean oil (SBO) diet was supplied to the mice. The CE2 diet (laboratory chaw) contains carnitine (137 ± 15 nmol/g, $n = 5$), but the SBO (5%) diet does not (not detectable: $n = 5$). To observe the effect of dietary fat, we used the SBO diet, which does not contain carnitine, and whose composition is well defined (Table 1). We first estimated how many days the diet would need to be given to the JVS mice to allow an accurate evaluation of the effect of dietary fat content on the development of cardiac hypertrophy. Figure 2 shows that the HW of JVS mice increased at a faster rate than controls. The HW/BW of JVS mice also increased to higher levels than in controls. From these results, we concluded that it was suitable to feed mice for 10 days to evaluate the effects of the diets.

Effect of Carnitine and Dietary Lipid on Cardiac Hypertrophy and Gene Expression in JVS Mice—To test the effect of carnitine and the amount of dietary lipid, mice were fed synthetic diets containing 5% (w/w), 1% or 0% SBO diet without carnitine injection, or a 5% SBO diet with carnitine injection (twice a day). Continuous carnitine treatment caused no difference in HW, BW, HW/BW and mRNA levels of ANP, Sk-Act and CDV-1 between control and JVS mice at 30 or 40 days (Figs. 3 and 4). There was almost no variation in HW, BW, or HW/BW among control mice fed the diets described above. On the other hand, JVS mice fed a 5% SBO diet for 10 days without carnitine treatment showed lower BW and developed marked cardiac hypertrophy as judged from HW and HW/BW. The HW and HW/BW of JVS mice fed the 5% SBO diet were higher than those of JVS mice fed CE2 (4.6% lipid) as shown in Fig. 3. This is probably due to the difference between the carnitine contents of the diets. Since a mouse eats 3–4 g of food per day, CE2 supplies about 0.5 μmol of carnitine, which is equivalent to 1/10th of the carnitine used for treatment. The HW/BW of JVS mice fed a diet containing 1% SBO was significantly lower than those of JVS mice fed the 5% SBO diet. The JVS mice on the 0% SBO diet showed slightly, but not significantly, lower HW and HW/BW than JVS mice fed the 1% SBO diet. JVS mice fed the 0% SBO diet showed significantly lower HW and HW/BW than JVS mice fed the 5% SBO diet. The HW and HW/BW of JVS mice fed the 0% SBO diet were still significantly higher than control mice fed the same diet.

As shown in Fig. 4, the level of ANP mRNA in JVS mice fed the 5% SBO diet without carnitine injection increased to about 5 to 6 times the control level, and the Sk-Act mRNA level was about 7 times the control level. The amount of CDV-1 mRNA decreased to about one-third. The ANP and Sk-Act mRNA levels of JVS mice fed the 1% SBO or 0% SBO diets were significantly lower than in those fed the 5% SBO diet. The Sk-Act mRNA levels of JVS mice fed the 1% SBO or 0% SBO diets were higher than those of control mice fed the same diets. In the case of CDV-1 mRNA, the levels in JVS mice fed the 5%, 1% and 0% SBO diets were significantly lower than in controls fed the same diets. The mRNA levels in JVS mice fed the above diets were statistically the same, indicating that the down-regulation of CDV-1 in JVS mouse heart does not occur in proportion to dietary fat. These results suggest that CDV-1 mRNA is a sensitive marker for hypertrophy, although further studies are required to determine whether it is applicable to hypertrophied hearts in other animal models.

We examined whether mice show alterations in fatty acid metabolism when fed these various dietary lipid amounts. Plasma FFA levels in JVS mice fed laboratory chow and treated with carnitine at 30 days were not significantly different from those of control mice (0.96 ± 0.53 *versus* 1.07 ± 0.38 mEq/liter, number of sacrificed mice, 5). Ten days after changing the diet from laboratory chow to a synthetic diet without carnitine treatment, the FFA levels of JVS mice fed the 5% SBO diet (1.55 ± 0.56 mEq/liter, $N = 5$) were significantly higher than those of JVS mice fed the 1% SBO diet (0.88 ± 0.20 mEq/liter, $N = 5$), as well as those of control mice fed the 5% and 1% SBO diets (0.76 ± 0.25 and 0.88 ± 0.22 mEq/liter, $N = 5$). The 0% SBO diet did not further decrease the FFA of JVS mice (0.80 ± 0.27 mEq/liter, $N = 5$) or controls (1.24 ± 0.40 mEq/liter, $N = 5$).

The triglyceride contents of the ventricles of JVS mice fed the 5% SBO diet were significantly higher than those of JVS mice fed the 1% SBO diet or carnitine-treated JVS mice fed the 5% SBO diet (Fig. 5). The triglyceride contents of JVS mice fed the 5% or 1% SBO diets were significantly higher than those of control mice fed the same diets. On the other hand, the cholesterol levels showed no statistical differences among control and JVS mice under these conditions.

Effect of a diet containing medium-chain triglycerides (MCT) on cardiac hypertrophy and gene expression in JVS mice—We evaluated the effect of MCT, which has been used for the treatment of systemic carnitine deficiency (*16*, *17*), on hypertrophy in JVS mice. The addition of 4% MCT to the 5% SBO diet neither suppressed nor enhanced the cardiac hypertrophy of JVS mice (Fig. 3). Again, when 4% MCT was added to the 1% SBO diet, HW, BW and HW/BW did not differ from those of mice fed the 1% SBO diet. The level of Sk-Act mRNA in JVS mice was significantly increased by the addition of MCT to the 5% SBO diet (Fig. 4). Apart from this, the addition of MCT to the 5% or 1% SBO diets did not change the mRNA levels of ANP, Sk-Act or CDV-1 from the basal levels in JVS mice fed the 5% or 1% SBO diets.

Content of Adenine Nucleotide, Adenylate Energy Charge and Creatine Phosphate in the Hearts of Control and JVS Mice—To evaluate whether energy deficit is involved in the development of cardiac hypertrophy, we measured the content of adenine nucleotides and creatine phosphate in the hearts of control and JVS mice. As we wanted to know the possible involvement of energy deficit at the early stage of the cardiac hypertrophy, we measured these substances 10 days (40 days of age) after the cessation of carnitine treatment. JVS mice treated with

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carnitine (5 μmol/animal, three times a week) until 30 days, and thereafter fed laboratory chow showed cardiac hypertrophy at 40 days. The content of total adenine nucleotide, the energy charge and creatine phosphate in the hearts of JVS mice did not differ from those of control mice at 40 days of age. The energy charge was comparable to that reported for perfused rat working heart (0.89 ± 0.01) (*28*), suggesting that there is no remarkable energy deficit in JVS mouse heart under these conditions.

Effect of Aspirin on the Urinary Concentrations of $PGF_{2\alpha}$ and 15-Isoprostane F_{2t}—To elucidate the mechanism of hypertrophy attenuation by decreased dietary lipid, we tested whether any lipid metabolites, such as prostaglandins, are involved in the development of hypertrophy. In this experiment, after the administration of aspirin or saline to mice for 10 days from 5 days of age, urinary levels of $PGF_{2\alpha}$ and 15-isoprostane F_{2t}, an F_2-isoprostane, were measured at 15 days. As shown in Table 3, in control experiments without aspirin, the $PGF_{2\alpha}$ level was much higher in the urine of JVS mice that showed marked cardiac hypertrophy than in control mice. On the other hand, there was no significant difference in the 15-isoprostane F_{2t} level in urine between JVS and control mice. As shown in Table 3, aspirin treatment markedly decreased the urinary $PGF_{2\alpha}$ of both control and JVS mice, and the BWs of JVS and control mice were lowered by this treatment. The HWs of JVS mice, however, continued to be higher than those of control mice with aspirin, and were unaffected by aspirin. These results suggest that prostaglandin formation is not related to hypertrophy in JVS mice.

DISCUSSION

Carnitine is an essential cofactor in the oxidation of long-chain fatty acids (*16–18*). Theoretically, cardiac hypertrophy caused by carnitine deficiency may be related to an accumulation of long-chain fatty acids and/or a decrease in ATP production. However, the precise mechanism of the hypertrophy remains to be elucidated.

In the present study, we examined the effect of dietary lipid on the development of cardiac hypertrophy. When the amount of lipid was decreased from 5% to 1%, the cardiac hypertrophy of JVS mice was greatly attenuated, suggesting that lipid is the causative factor for cardiac hypertrophy under carnitine deficiency. However, cardiac hypertrophy was not completely normalized by the elimination of lipid in the no-fat (0% SBO) diet. This may be because the elimination of lipid from the diet does not reduce the FFA levels in the plasma to levels lower than those of JVS mice fed the 1% SBO diet.

We also tested whether the energy deficit in the heart of carnitine-deficient JVS mice is related to cardiac hypertrophy. First, we measured the adenine nucleotide content and calculated the energy charge in the heart of control and JVS mice to evaluate the energy state. In addition, creatine phosphate was measured because heart tissue contains high creatine kinase activity to maintain ATP levels. As shown in Table 2, we could not find any decrease in total adenine nucleotide and creatine phosphate contents, or in the energy charge of the hearts of JVS mice. Kuwajima *et al.* (*38*) reported a lower adenine nucleotide content in JVS mouse heart without any treatment at 4 or 8 weeks of age. This discrepancy may be due to the different durations of the carnitine-deficient state. We kept mice without carnitine treatment for only 10 days after day 30, compared with 4 or 8 weeks in the experiments of Kuwajima, et al. Second, we used MCT, which is composed of medium-chain fatty acids, because JVS mice metabolize MCT in the carnitine-deficient state (*16–18*), and mammalian hearts can use MCT effectively as an energy source (*39*). There were no significant differences in the functional parameters of hearts perfused with octanoate and palmitate (*39*). As shown in Figs. 3 and 4, when MCT (4%) was administered with the 5% SBO diet, the HW, HW/BW, ANP and the Sk-Act mRNA levels retained the high values of JVS mice. The addition of 4% MCT to the 1% SBO diet also caused no changes. The present results, together with the considerations stated above, suggest that the cardiac hypertrophy of JVS mice is not caused by an energy deficit, but by long-chain fatty acids derived from dietary lipids.

Some possible mechanisms may be considered in which cardiac hypertrophy is caused by dietary fatty acids that are not degraded by mitochondrial β-oxidation. The dietary fatty acids may (i) be metabolized to some kind of effector, such as prostaglandin, thus inducing cardiac hypertrophy (*24–26*); (ii) activate the PKC and/or PPAR signaling pathways (*40*, *41*); or (iii) activate the Ca channel located on the plasma membrane of cardiac cells (*42*). In view of these possible mechanisms, we examined whether prostaglandin(s) are involved in the cardiac hypertrophy caused by carnitine deficiency. Urinary $PGF_{2\alpha}$ levels were markedly higher in JVS mice than in controls. However, the administration of aspirin, which inhibits the synthesis of prostaglandin, did not affect the cardiac hypertrophy of JVS mice at all, although the $PGF_{2\alpha}$ levels decreased sharply. These results suggest that prostaglandin formation is not related to hypertrophy in JVS mice. As to the second possibility, Saburi et al. (*43*) recently reported that JVS mice showed a higher concentration of 1,2-diacylglycerol, an endogenous activator of PKC, in the heart. The same group more recently reported the beneficial effects of fish oil on hypertrophy, and concluded that this effect involves PKC proteins (*44*). This appears to support our results indicating that the hypertrophy is caused by dietary lipids and/or metabolites. During the suckling period and during starvation when lipid metabolism is accelerated, JVS mice show severe fatty liver and high plasma FFA levels (*19*, *20*). The accumulation of fatty acids has been shown to play a role in the suppression of urea cycle enzyme gene expression (*19–21*). As shown in Fig. 5, the triglyceride contents of the ventricle are co-related to cardiac hypertrophy in JVS mice. Dietary fatty acids and/or their metabolites may be involved in cardiac hypertrophy as well as in urea cycle enzyme gene suppression. Concerning this view, Chiu et al have reported that an accumulation of cardiotoxic lipid species leads to cardiac hypertrophy and the induction of programmed cell death (*45*). Further research is needed to elucidate the signaling cascade downstream of fatty acids and the metabolites relevant to cardiac hypertrophy. As to the third possibility, serum FFA level in JVS mice fed the 5% SBO diet were significantly higher than in JVS mice fed the 1% SBO diet (see "RESULTS"). It is interesting that extracellular lipids modulate the function of membrane proteins in the cardiac cells of JVS mice. For example,

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the activation of Ca channels by various fatty acids should be examined in JVS mice.

At present, it is not clear what kinds of fatty acids or metabolites are the most relevant to hypertrophy. However, SBO contains a high proportion of linoleic acid (about 54% of total fatty acids), and the CE2 diet we used is also high in linoleic acid at 49%. We tried adding 4% linoleate (C18:2), stearate (C18:0), oleate (C18:1), or linolenate (C18:3) to the 1% SBO diet for JVS and control mice. In the case of linoleate (C18:2), the control and JVS mice avoided the diet. Therefore, we could not evaluate the effect of this fatty acid on hypertrophy. Non-esterified linoleic acid has been reported to decrease food intake, probably because it is less palatable and has a greater satiation effect (*46*). In the cases of the other fatty acids, the JVS mice fed C18:0, C18:1 or C18:3 showed no significant difference in HW/BW as compared to JVS mice fed the 1% SBO diet (data not shown). There may be a problem in the method of supplement with fatty acids. Due to their different palatabilities and metabolisms, fatty acids may have to be supplied as triglycerides. Further searches for causative fatty acid(s) are needed.

Finally, although further studies are needed to elucidate the mechanism of hypertrophy in JVS mice, we have shown that restricting dietary lipids, especially long chain-fatty acids, is beneficial for the attenuation of cardiac hypertrophy in carnitine deficiency.

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