Cardiomegaly in the juvenile visceral steatosis (JVS) mouse is reduced with acute elevation of heart short-chain acyl-carnitine level after L-carnitine injection

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Abstract The long-term administration of L-carnitine was very effective in preventing cardiomegaly in juvenile visceral steatosis (JVS) mice, which was confirmed by heart weight as well as the lipid contents in heart tissue. After i.p. injection of L-carnitine, the concentration of free carnitine in heart remained constant, although serum free carnitine level increased up to 80-fold. On the other hand, a significant increase in short-chain acyl-carnitine level in heart was observed. These results suggest that increased levels of short-chain acyl-carnitine, not free carnitine, might be a key compound in the protective effect of L-carnitine administration in JVS mice.

Key words: Cardiomegaly; Carnitine acetyltransferase; Carnitine transporter; Juvenile visceral steatosis mouse

1. Introduction

In primary carnitine deficiency, cardiomegaly is a common symptom. Heart carnitine levels in untreated patients are lower than those in normals and the oral administration of L-carnitine reduces cardiomegaly. During such a treatment, it would be expected that the carnitine level in heart would be corrected when the serum carnitine concentration is restored to normal [1–7]. However, there are, in fact, no data to support this conclusion.

Juvenile visceral steatosis (JVS) mice, discovered in 1988, are currently thought to serve as a valuable animal model for primary carnitine deficiency [8–11]. Heart carnitine levels are low in JVS mice and they show cardiomegaly which responds to L-carnitine treatment [12–17]. These findings for JVS mice are identical to the reported features of primary carnitine deficiency in humans.

The present study was undertaken to examine the issue of whether or not heart carnitine levels are altered as a result of L-carnitine treatment.

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Abbreviations: ANOVA, analysis of variance; JVS, juvenile visceral steatosis; CAT, carnitine acetyltransferase

2. Materials and methods

2.1. Animals

The JVS mice used were B6/JVS mice [18,19], which were obtained by back-crossing the autosomal recessive mutant gene from C3H/JVS mice [9]. C57BL/6J mice served as controls. All animals were maintained under specific pathogen-free conditions.

JVS and control mice were studied at 2, 4 and 8 weeks of age for long-term experiments. Most JVS mice died within 5 weeks after birth unless they had received an intraperitoneal injection of L-carnitine. Thus, the mice were killed at 4 weeks after birth (JVS/N at 4 weeks group). Since only a small number of animals survived to 8 weeks if they did not receive treatment, L-carnitine was injected intraperitoneally at a dose of 1 μmol/g body weight every morning from 10 to 20–25 days after birth. The animals were killed at 8 weeks without subsequent injection (JVS/N at 8 weeks group). For the animals in the treatment group, intraperitoneal injection of L-carnitine which began from day 10 after birth was continued until they were killed at 4 or 8 weeks (JVS/T at 4 weeks and JVS/T at 8 weeks, respectively). For the short-term experiments after intraperitoneal injections of L-carnitine, mice aged 8–9 weeks were used. The heart was removed after an injection of sodium pentobarbital (50 μg/g i.p.), immediately frozen in liquid nitrogen and stored until use.

The heart weight and the body weight were determined and the heart weight relative to body weight was calculated.

2.2. Carnitine levels in heart during L-carnitine treatment

The hearts of the JVS mice were removed at ages 2, 4 or 8 weeks 24 h after an intraperitoneal injection of L-carnitine. The heart carnitine levels were determined in order to analyze the long-term effect of L-carnitine treatment.

2.3. Acute change of carnitine levels in serum and heart after an intraperitoneal injection of L-carnitine

After an intraperitoneal injection of L-carnitine at a dose of 1 μmol/g body weight, blood was collected from cut tails. The time points were 3, 5, 7, 9, 11, 13, 15, 22.5, 30 and 45 min, 1, 2 and 4 h (all times accurate to ±30 s). Serum was separated and its free carnitine levels were determined. The time course of free carnitine levels in blood was analyzed by a one-compartment model with first order absorption [20]. To analyze the change of carnitine concentration in heart after L-carnitine injection, the hearts were removed at 15 and 30 min by the following method.

After an L-carnitine injection, at 8 min, sodium pentobarbital (50 μg/g) was injected intraperitoneally. At 11 min, heparin (30 unit/30 μl) was injected into the femoral vein to prevent blood coagulation. At 14 min, cold saline (4°C) was infused from the left ventricle to wash the heart in situ for 1 min [21], and the heart was immediately removed and placed in liquid nitrogen.

After an L-carnitine injection, pentobarbital was injected at 23 min, heparin was injected at 26 min, cold saline was infused at 29 min for 1 min and the heart was treated as described above.

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2.4. Biochemical analysis

Free carnitine levels in serum were measured by radioisotope assay and photometric assay using a carnitine assay kit [22,23]. Heart was extracted with 6% perchloric acid [17]. The acid-soluble fraction was used for the determination of free carnitine and short-chain acyl-carnitine. The acid-insoluble fraction was used for the determination of long-chain acyl-carnitine [22,24]. Then both these acyl-carnitine fractions were treated with alkaline, and the free carnitine was assayed spectrophotometrically. Folch's method was used to extract lipids from the heart [25]. Total lipid, triacylglycerol, total cholesterol and total phospholipid were quantified using Inui's method [26]. Phospholipid fractions were quantified using two-dimensional thin-layer chromatography [27].

2.5. Reagents

L-Carnitine (β-hydroxy-trimethylaminobutyric acid) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of reagent grade and obtained from Sigma or Wako (Osaka, Japan). The carnitine assay kit was purchased from Kainos (Japan).

2.6. Statistics

Results are expressed as mean ± S.D. Differences between the two groups were tested by the unpaired *t*-test, and differences among the three groups were tested by one-way ANOVA (analysis of variance) followed by post hoc Fisher's PLSD. Replicated measurements were analyzed by repeated ANOVA. A value of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Effects of L-carnitine treatment on heart weight, body weight and the heart weight/body weight ratio

The heart weights at ages 2 and 8 weeks were significantly greater in untreated JVS mice than in control mice (Table 1). Cardiomegaly was less marked in treated JVS mice than in untreated JVS mice after 8 weeks of treatment.

The heart weight at age 4 weeks was lower in the untreated JVS mouse group than in the control group and the treated JVS mouse group. Treatment of JVS mice with L-carnitine resulted in a fall in heart weight to a level which was still significantly different from that of the control group at 8 weeks.

The body weights of the untreated JVS mouse group at ages 4 and 8 weeks were significantly lower than those of the control group. Treatment of JVS mice with L-carnitine resulted in a significant increase in body weight in comparison with untreated JVS mice. The heart weight/body weight ratio, which is an indicator of cardiomegaly, at ages 2, 4 and 8 weeks was significantly higher in the untreated JVS mouse group than in the control group. This ratio decreased after 4 and 8 weeks of treatment with L-carnitine.

3.2. Effects on heart lipid levels

The amounts of total lipid, triacylglycerol, total phospholipid, phosphatidylinositol, phosphatidylethanolamine and cardiolipin contained in the heart of the 8-week-old JVS mouse group were significantly greater than those in the control heart (Table 2). Treatment of JVS mice with L-carnitine resulted in a decrease in these amounts which were not significantly different from those of the control group.

3.3. Carnitine levels in heart during L-carnitine treatment

Heart free carnitine, short-chain acyl-carnitine and long-chain acyl-carnitine levels in untreated JVS mice were significantly lower than in the control mice (Fig. 1). Treatment of JVS mice with L-carnitine did not result in a significant rise in these parameter.

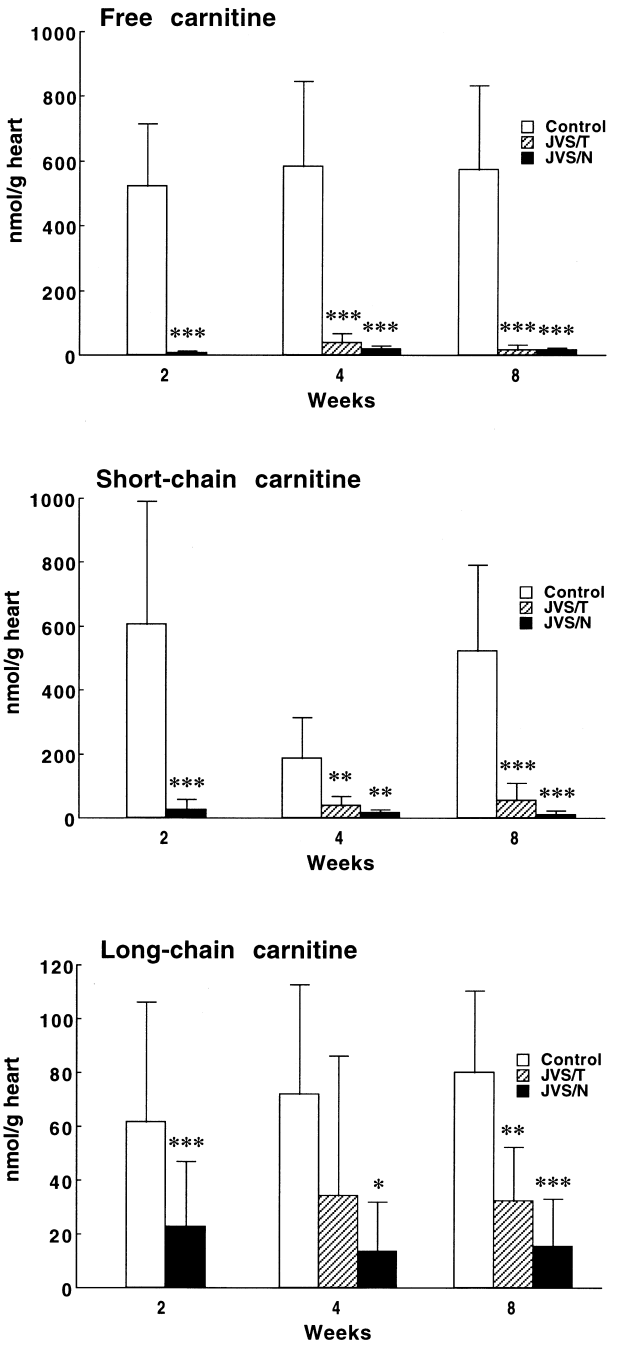

Fig. 1. Myocardial carnitine contents in control, treated JVS and non-treated JVS mice at 2, 4 and 8 weeks of age. JVS mice were treated with L-carnitine from day 10 after birth until 4 weeks or 8 weeks of age, respectively. Concerning the data at 4 weeks of age, JVS mice received no treatment, whereas JVS/N mice at 8 weeks of age were treated with L-carnitine from day 10 after birth until day 21–25. Results are means ± S.D. of 4–19 independent measurement. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared with control.

3.4. Time course of blood free carnitine levels after an intraperitoneal injection of L-carnitine

The time course of free carnitine levels after an intraperitoneal injection of L-carnitine was simulated in control (Fig. 2A)

Table 1
Heart weight, body weight and heart weight/body weight ratio for JVS and control mice at 2, 4 and 8 weeks of age

	(A) Control	(B) JVS/T	(C) JVS/N	*P*		
				A vs. B	A vs. C	B vs. C
Heart weight (g)						
2 weeks	0.05 ± 0.01 (11)	–	0.09 ± 0.01 (4)	–	< 0.01	–
4 weeks	0.09 ± 0.02 (11)	0.11 ± 0.02 (7)	0.07 ± 0.04 (12)	n.s.	< 0.05	< 0.01
8 weeks	0.12 ± 0.03 (9)	0.16 ± 0.02 (9)	0.23 ± 0.01 (7)	< 0.01	< 0.001	< 0.001
Body weight (g)						
2 weeks	7.31 ± 1.21 (11)	–	6.84 ± 0.66 (4)	–	n.s.	–
4 weeks	16.04 ± 3.49 (11)	16.14 ± 3.22 (7)	5.00 ± 1.63 (12)	n.s.	< 0.001	< 0.001
8 weeks	23.39 ± 4.44 (9)	22.29 ± 3.61 (9)	18.45 ± 1.76 (7)	n.s.	< 0.05	< 0.05
Heart weight/body weight $\times 10^{-3}$						
2 weeks	6.9 ± 1.1 (11)	–	12.0 ± 1.4 (5)	–	< 0.001	–
4 weeks	5.7 ± 0.6 (11)	7.1 ± 1.1 (7)	13.1 ± 2.1 (12)	n.s.	< 0.001	< 0.001
8 weeks	5.1 ± 0.6 (9)	7.3 ± 0.8 (9)	12.5 ± 0.9 (7)	< 0.001	< 0.001	< 0.001

JVS/T: JVS mice were treated with L-carnitine from day 10 after birth until 4 weeks or 8 weeks of age. JVS/N: concerning the data at 4 weeks of age, JVS mice received no treatment, whereas JVS/N at 8 weeks of age were treated with L-carnitine from day 10 after birth until days 21–25.
Data are means ± S.D.
The number of animals used is shown in parentheses.

and JVS mice (Fig. 2B). Before injection, free carnitine levels in plasma were 46.63 nmol/ml in control mice and 19.74 nmol/ml in JVS mice. The peak concentration was 1548 nmol/ml at 11 min in control mice and 1570 nmol/ml at 11 min in JVS mice. The half-time for the disappearance was 19 min in control mice and 22 min in JVS mice.

3.5. Short-term changes in heart carnitine levels after an intraperitoneal injection of L-carnitine

Free carnitine, short-chain acyl-carnitine and long-chain acyl-carnitine levels in hearts were significantly lower in the JVS mouse group, compared to the control group, when tested using repeated ANOVA ($P < 0.001$) (Fig. 3). In the case of the control group, the free carnitine levels tended to decrease after the injection. The decrease was statistically significant at 30 min after the injection. The long-chain acyl-carnitine level increased with time after the injection, and the increase was statistically significant at 15 and 30 min after the injection. The total carnitine level remained unchanged.

Among JVS mice, the free carnitine level and the long-chain acyl-carnitine level remained unchanged after the injection. The short-chain acyl-carnitine level and the total carnitine level were significantly higher at 15 min after the injection, compared to their pre-injection levels.

4. Discussion

In an earlier study, we reported that the C3H/JVS mouse develops cardiomegaly, as characterized by hypertrophy of both ventricles and the septum [17]. In addition, it has previously been reported that administration of L-carnitine to this JVS mouse resulted in reduction of cardiomegaly [16].

In the present study, we found the B6/JVS mouse also develops a similar cardiomegaly and also responds to carnitine therapy. In short, this study clearly demonstrates that a mouse with a different background, if only the mutant gene is defective, is capable of exhibiting cardiomegaly and has the potential to respond to therapeutic intervention.

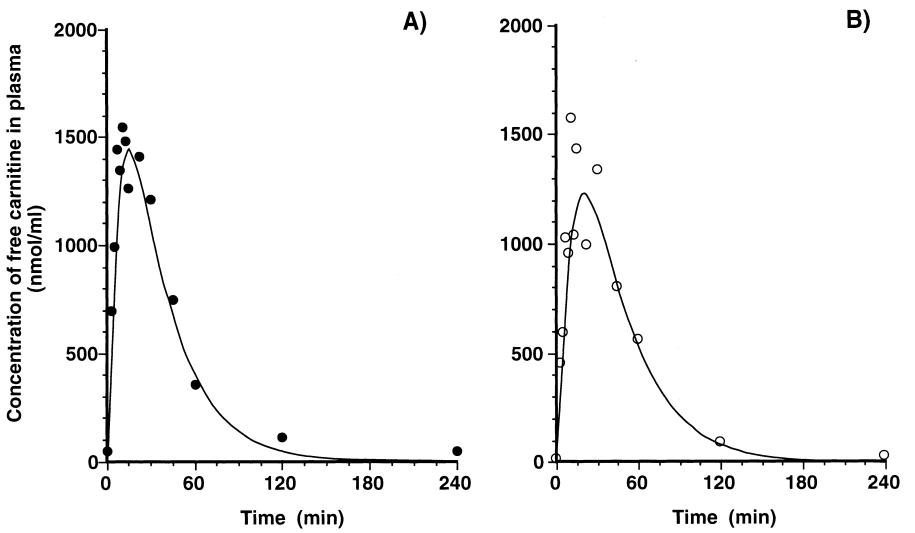

Fig. 2. Time courses of free carnitine levels in plasma after an intraperitoneal injection of L-carnitine. L-Carnitine was administered to control (A) or JVS mice (B) at a dose of 1 μmol/g body weight. Mean values from 3–10 samples are plotted. Solid curves are simulated by a one-compartment model with first order absorption [20].

Table 2
Profile of lipids in heart tissue of control and JVS mice at 8 weeks of age

	(A) Control	(B) JVS/T	(C) JVS/N	*P*		
				A vs. B	A vs. C	B vs. C
Total lipid	26.98 ± 7.26 (6)	40.12 ± 10.18 (6)	57.96 ± 19.39 (6)	n.s.	< 0.01	< 0.05
Triacylglycerol	5.24 ± 3.63 (7)	8.18 ± 4.77 (9)	23.39 ± 20.44 (9)	n.s.	< 0.05	< 0.05
Cholesterol	4.13 ± 0.80 (10)	3.65 ± 0.58 (8)	4.03 ± 0.33 (8)	n.s.	n.s.	n.s.
Total phospholipid	16.65 ± 6.68 (11)	20.78 ± 6.18 (10)	29.35 ± 9.06 (10)	n.s.	< 0.001	< 0.05
Phosphatidylinositol	0.62 ± 0.14 (9)	0.88 ± 0.33 (6)	1.18 ± 0.45 (6)	n.s.	< 0.01	n.s.
Phosphatidylserine	0.68 ± 0.33 (9)	0.77 ± 0.46 (6)	0.69 ± 0.47 (6)	n.s.	n.s.	n.s.
Phosphatidylcholine	8.36 ± 2.45 (9)	8.86 ± 3.09 (6)	11.97 ± 3.67 (6)	n.s.	n.s.	n.s.
Phosphatidylethanolamine	5.78 ± 1.92 (9)	7.62 ± 3.34 (6)	12.73 ± 4.39 (6)	n.s.	< 0.001	< 0.05
Cardiolipin	2.81 ± 0.93 (9)	3.33 ± 1.15 (6)	6.23 ± 2.42 (6)	n.s.	< 0.01	< 0.05
Sphingomyelin	0.67 ± 0.17 (9)	0.40 ± 0.28 (6)	0.50 ± 0.14 (6)	n.s.	n.s.	n.s.

Units: μg/mg weight. Data are means ± S.D.
JVS/T: JVS mice were treated with L-carnitine from day 10 after birth until 8 weeks of age. JVS/N: JVS mice were treated with L-carnitine from day 10 after birth until days 21–25, after which no treatment was received.
The number of animals used is shown in parentheses.

Since free carnitine is a substrate for carnitine acetyltransferase (CAT) and carnitine palmitoyltransferase, a decrease in the free carnitine level within the myocardium would be predicted to result in a disturbance in intracellular lipid metabolism [28–31]. We therefore analyzed the total lipid content and its composition. The results showed that the JVS mouse, compared to the control, had higher levels of total lipid, triacylglycerol, total phospholipid, phosphatidylinositol, phosphatidylethanolamine and cardiolipin. The levels of all of these tended to decrease to normal control levels as the result of administration of L-carnitine.

Since the administration of L-carnitine was found to have such an obvious preventive effect, we then examined whether any increase in the level of carnitine within the heart existed. Initially, untreated JVS mice, compared to the control, were found to have significantly lower contents of free carnitine,

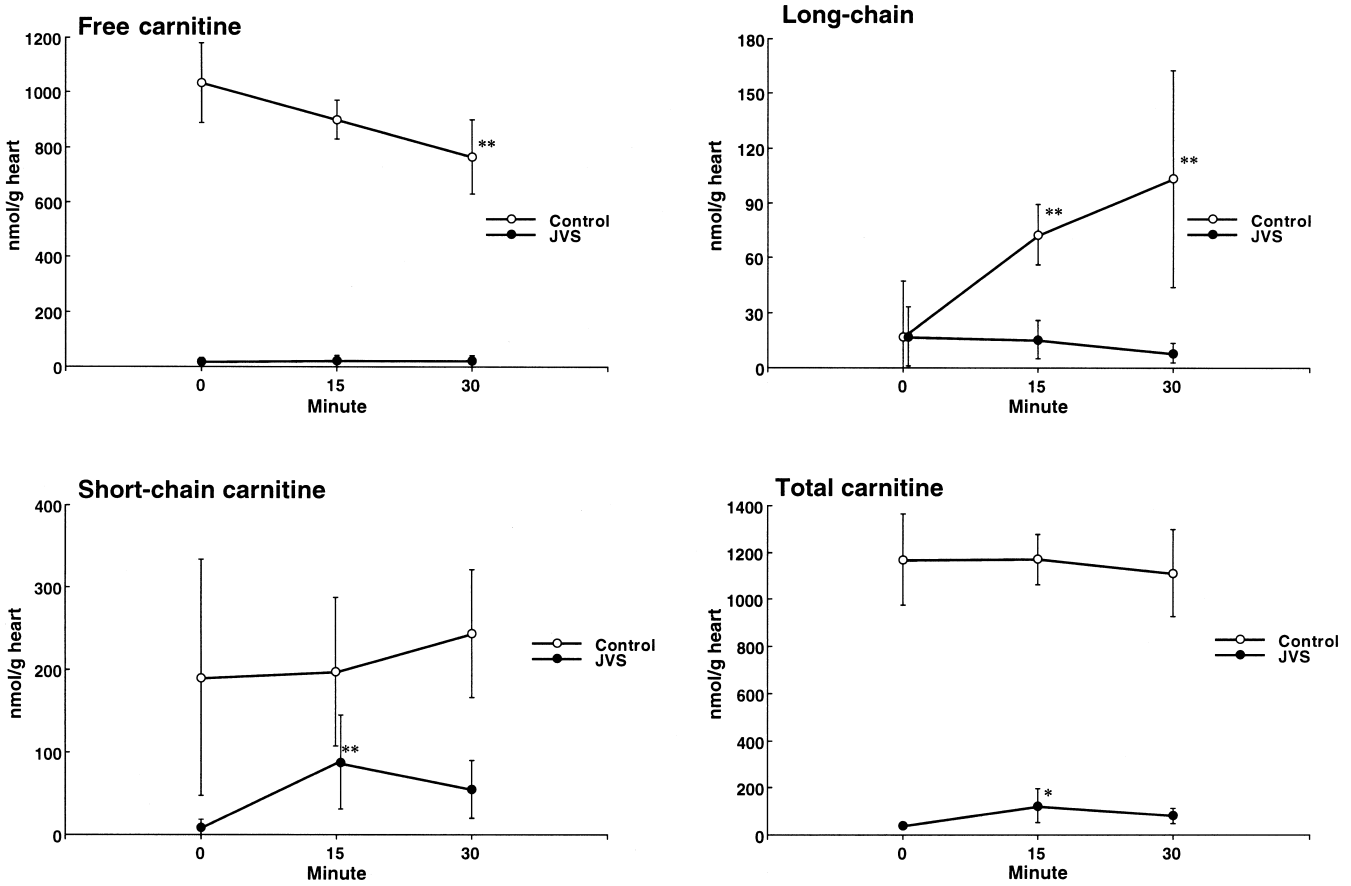

Fig. 3. Time courses of heart carnitine levels after an intraperitoneal injection of L-carnitine. Carnitine contents in control mice and JVS mice are plotted at 0 min, 15 min and 30 min after an intraperitoneal injection of L-carnitine at a dose of 1 μmol/g body weight. Results are means ± S.D. of 4–10 independent measurements. *$P < 0.05$; **$P < 0.01$ compared with control.

short-chain acyl-carnitine, and long-chain acyl-carnitine at weeks 2, 4 and 8. This result was the same as observed in the C3H/JVS mouse [10].

The heart carnitine content of the JVS mouse during the treatment tended to be higher at 24 h after the final administration, compared to the untreated JVS mouse, but this increase was not statistically significant. To examine the acute effect of L-carnitine, the free carnitine levels in heart was measured at 15 and 30 min after the injection of L-carnitine, when the free carnitine level in the blood was still maintained at a higher level (Fig. 2). As shown in Fig. 3, the level of free carnitine in heart was not altered significantly before and after administration. It is possible to assume that the free carnitine level in the heart is increased when the administration of L-carnitine prevents cardiomegaly in a JVS mouse. However, contrary to this prediction, as shown in Fig. 3, the increase of free carnitine level in the heart was negligible even in the case of animals which showed a high level of free carnitine after injection. These results question the direct role of free carnitine in its cardioprotective effect in JVS mice.

In contrast, the short-chain acyl-carnitine level was increased as shown in Fig. 3. This increase was not predicted. Short-chain acyl-carnitine is a general name for acyl-carnitine which contain an acyl group of less than 10 carbons [24]. Although we did not analyze the composition of short-chain acyl-carnitine in the present study, short-chain acyl-carnitine eventually serves as a good substrate for the TCA cycle, thereby contributing to the amelioration of the disorders in heart energy metabolism. For example, acetyl-carnitine and propionyl-carnitine enter the mitochondria via transport through the mitochondrial membrane, these compounds are finally converted to citrate and succinate, respectively [32]. In particular, it has been well demonstrated, in numerous experimental models of heart failure resulting from coronary artery ligation [33], pressure overload [34] and volume overload [35], that the administration of L-propionyl-carnitine improves cardiac function. L-Acetyl-carnitine can also improve the recovery of cardiac output in the isolated perfused rat heart [36]. Thus, an intraperitoneal injection of L-carnitine is expected to result in a beneficial effect. With daily injection of L-carnitine as were used in the treated group, this effect is repeated and might lead to the prevention of cardiomegaly. CAT, the biosynthetic enzyme for short-chain acyl-carnitine, is abundant in heart [32,37]. Therefore, the JVS mouse heart offers an environment in which short-chain acyl-carnitine level can be increased. However, additional studies will be required in order to elucidate the precise mechanism for the increase in short-chain acyl-carnitine levels.

In summary, the administration of L-carnitine to the JVS mouse, an animal model of primary carnitine deficiency, resulted in an increase in the short-chain acyl-carnitine levels within the heart. This compound appears to be a key compound which might explain the simultaneous manifestation of therapeutic effects.

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