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Cardiac hypertrophy in juvenile visceral steatosis (jvs) mice with systemic carnitine deficiency

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We have reported the clinical and biochemical findings in juvenile visceral steatosis (jvs) mice with systemic carnitine deficiency. This paper is the first report about cardiomyopathy in jvs mice. Adult jvs mice (at the age of 2–3 months) show cardiac hypertrophy which is caused by enlargement of the cardiac muscle cell associated with increases of non-collagen protein and DNA content. Carnitine administration (2 mg/head, twice a day, from 1 month of age) significantly suppresses the cardiac hypertrophy, showing that carnitine deficiency plays an important role in the development of the cardiac hypertrophy. The discovery of cardiac hypertrophy in carnitine-deficient jvs mice will lead to clarification of the pathophysiology of cardiomyopathy in systemic carnitine deficiency in human beings.

Cardiac hypertrophy; Systemic carnitine deficiency; Animal model; Juvenile visceral steatosis mice

1. INTRODUCTION

In 1988, Koizumi et al. reported a C3H-H-2° strain of mouse autosomal-recessively associated with microvesicular fatty infiltration of viscera [1]. The mice were later named juvenile visceral steatosis (jvs) mice [2]. Jvs mice have severe lipid accumulation in the liver, hypoglycemia, hyperammonemia and growth retardation. The lipid accumulation is found within 5 days of birth, but the hypoglycemia, hyperammonemia and growth retardation appear 3 weeks after birth [1,2]. Jvs mice are systemically deficient in carnitine [3]. Recently, we found that the primary defect of jvs mice is in the reabsorptional system for carnitine in the kidney (Horiuchi et al., manuscript in preparation).

Many cases of human systemic carnitine deficiency have been reported [4–7]. Recently, more than 20 cases of human systemic carnitine deficiency have been shown to have a defect of carnitine uptake into cultured fibroblasts and are suspected to have impaired renal conservation of carnitine. In these patients, the most common clinical manifestation is cardiomyopathy [5–7]. Unexpectedly, there have been only a few reports that give the detail informations about the cardiomyopathy. In clinical and pathological analysis, congestive heart failure, accumulations of triglycerides and increases of mitochondria have been only described [8,9].

We found that jvs mice suffer from cardiac hypertrophy associated with increases of cardiac proteins (cytosolic and mitochondrial proteins) and DNA content, but no recognizable triglyceride deposition. The suppression of the development of cardiac hypertrophy by carnitine administration shows the strong interrelationship between carnitine deficiency and cardiac hypertrophy in jvs mice. Therefore, we report here that jvs mice are a very valuable animal model for studying the pathophysiology in cardiomyopathy in systemic carnitine deficiency.

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Abbreviations: jvs, juvenile visceral steatosis; FBP, fructose 1,6-bisphosphate; GOT, glutamic oxaloacetic transaminase; SDH, succinate dehydrogenase; NCP, non-collagen protein.

2. MATERIALS AND METHODS

2.1. *Animals and carnitine administration*

Jvs mice were accidentally found in C3H-H-2° strain mice at Kanazawa University, Japan, in 1985 [1]. The unusually swollen whitish fatty liver is recognized through the abdominal wall 5 days after birth, at the latest, in homozygous mutants (jvs/jvs). Under our conditions for keeping jvs mice at Kagoshima University, about 50% of homozygous mutants die at 1 month of age, and almost all homozygous mutants die at 3–4 months of age without any treatment. We obtained three genetic types of the mice by the following procedures: homozygous mutants (jvs/jvs) and heterozygous mice (+/jvs) were produced by mating carnitine-treated homozygous mutant males with heterozygous females. Because heterozygous mice have no fatty liver, it is easy

to discriminate between homozygous mutants and heterozygous mice. Homozygous controls (+/+) were produced by mating homozygous controls with each other. Carnitine treatment was carried out to maintain surviving homozygous males for mating as reported previously [10]. In the present work, we used the surviving homozygous mutants without any treatment, except for those in Table III. The homozygous mutants and homozygous control mice used in Table III were injected with 5 μmol of L-carnitine from 11 days after birth till 20 days, because this treatment increases the survival rate. The carnitine-treated homozygous mutants in Table III were additionally injected with 10 μmol of L-carnitine twice a day from 30 days until 90 days after birth.

2.2. *Quantification of protein, DNA content and enzyme activities in heart*

The whole heart was homogenized with 9 vols. of 0.02 M $NaHCO_3$. The homogenate was treated with 3 vols. of 0.1 N NaOH to precipitate collagen and elastin according to the modified Lilienthal procedure [11]. The concentration of non-collagen protein in the supernatant extracted by alkali treatment was determined by Lowry's method [12]. The DNA concentration in the homogenates was assayed by using mithramycin intercalated with double strands of DNA [13]. Alternatively, DNA was extracted from the heart with phenol/chloroform after digestion with proteinase K [14], and the concentration of DNA was estimated by using an extinction coefficient (1 OD_{260nm} = 50 μg/ml of DNA). Aldolase activity for FBP as a substrate [15], GOT [16], and SDH [17] was assayed by the published procedures. We discriminatively assayed s-GOT and m-GOT activities by using an m-GOT assay kit (Eiken Kagaku Co., Tokyo). The homogenates were treated with anti-s-GOT antibody (swine) for 35 min at room temperature, and centrifuged at 3,000 rpm for 5 min. The GOT activity in the supernatant was determined as m-GOT activity. S-GOT was calculated as total GOT activity minus m-GOT activity. Serum carnitine was measured by using the isotope method [18].

2.3. *Statistics*

Data were analysed statistically by the unpaired *t*-test with the level of significance set at $P < 0.05$.

2.4. *Pathological examination*

Mice were sacrificed at 3 months of age. The fixation was carried out by 10% (v/v) formalin in 0.2 M sodium phosphate buffer (pH 7.4) for microscopic examination, and the specimens were stained with hematoxylin-eosin.

3. RESULTS

Table I shows that the wet weight of the adult heart and the ratio of heart weight to body weight in homozygous mutants (jvs/jvs) were about twice as high as those in heterozygous (+/jvs) and homozygous controls (+/+). There was no significant difference between homozygous controls and heterozygotes in heart weight and in the ratio of heart weight to body weight. The increase in heart weight was associated with increases in non-collagen protein content and DNA content per heart. The increase of total DNA content in jvs mice heart was reexamined by a determination of extracted DNA. Controls and homozygous mutants showed 78 ± 4 and 202 ± 58 μg/heart, respectively. There was no significant difference in the ratios of heart weight to body weight between male and female homozygous mutants (data not shown).

As shown in Fig. 1, microscopic examination revealed an increase in cardiac cell volume with deformation and enlargement of the nucleus and little vacuolation in the cytoplasms in homozygous mutants. Electronmicroscopic examination showed that there was no significant change in the size and number of lipid droplets, and that the number of mitochondria was larger in homozygous mutants than in controls (data not shown).

In homozygous mutants, non-collagen protein content was 2.5 times the control value (Table I). This increase was accompanied by increases not only in cytosolic enzymes, but also in mitochondrial enzymes as given in Table II. The increase of total enzyme activity per heart was about 2-fold in cytosolic enzymes (aldolase and s-GOT) and 3-fold in mitochondrial enzymes (m-GOT and SDH). These results indicate that homozygous mutants suffer from cardiac hypertrophy associated with increased cardiac protein content including cytosolic and mitochondrial proteins.

Since homozygous mutant mice were deficient in carnitine in the blood as shown in Table I, we assessed the effect of carnitine administration on cardiac hypertrophy in jvs mice (Table III). Mice were injected with L-carnitine (5 μmol once a day) from 11 days to 20 days after birth, and again injected (10 μmol twice a day) from 1 month to the time of sacrifice (3 months). There were no significant differences in heart weight to body

Table I

Heart weight, body weight, protein content, DNA content, and serum carnitine in control (+/+), heterozygotes (+/jvs), and homozygous mutants (jvs/jvs) at 2–3 months of age

	H.W. (g)	B.W. (g)	H.W./B.W. (%)	NCP/heart (mg)	DNA/heart (μg)	Serum carnitine (nmol/ml)
Control (+/+)	0.11 ± 0.02	21.6 ± 3.3	0.51 ± 0.03	10.7 ± 1.8	230 ± 70	45.7 ± 9.2
Heterozygotes (+/jvs)	0.10 ± 0.01	23.7 ± 4.7	0.43 ± 0.06	N.D.	N.D.	37.1 ± 5.9
Homozygous mutants (jvs/jvs)	0.23 ± 0.03**	20.8 ± 2.6	1.12 ± 0.12**	24.8 ± 3.7**	390 ± 100*	2.3 ± 0.8**

5 mice without carnitine treatment were used for each experiment from 70 days to 90 days after birth. Values are expressed as mean ± S.D. Statistically significant differences between homozygous mutants and controls: *$P < 0.05$; **$P < 0.01$. N.D., not determined. H.W., heart wet weight; B.W., body weight; NCP, non-collagen protein.

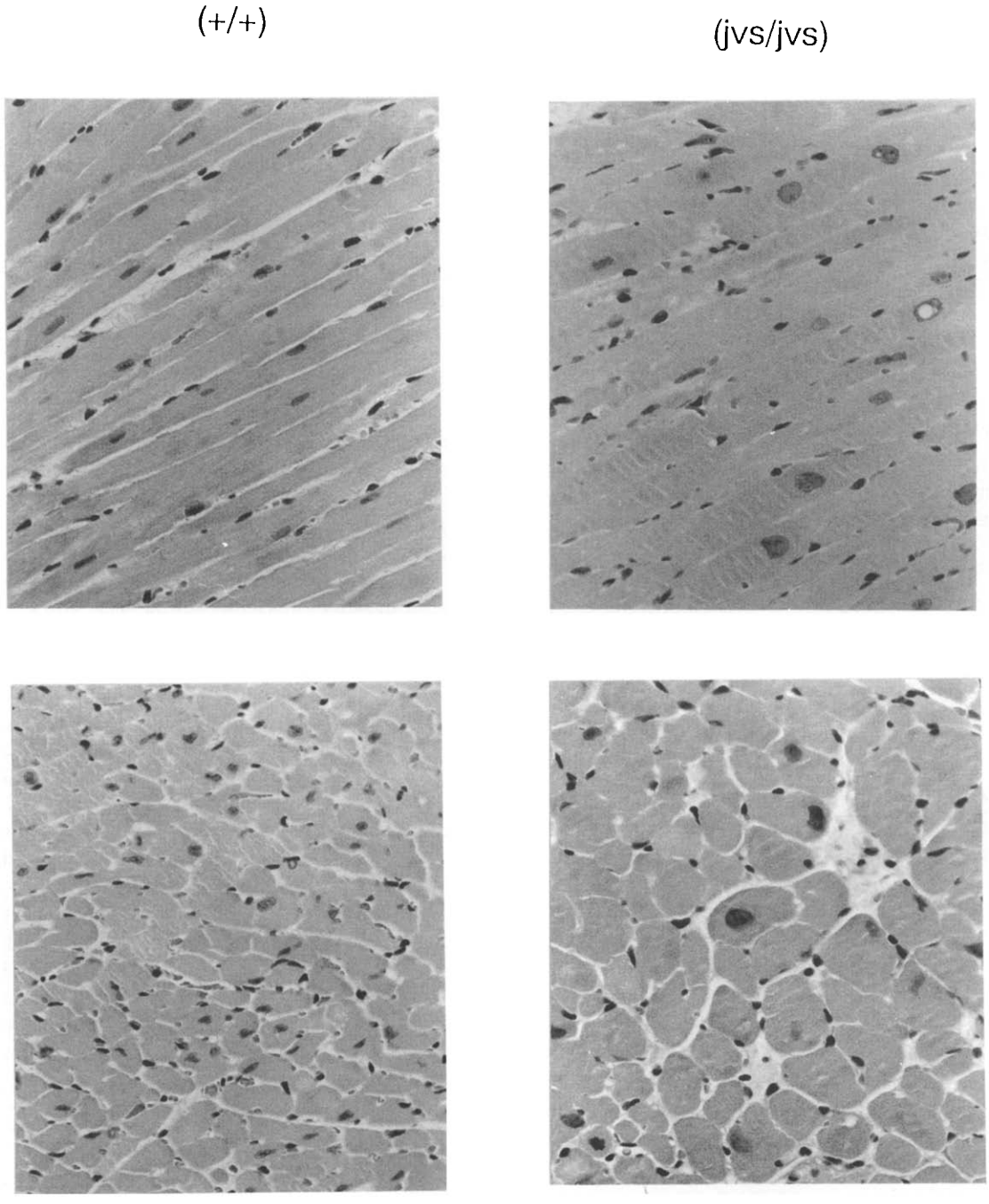

Fig. 1. Microscopic examination of hearts from control (+/+, left panels), and homozygous mutant (jvs/jvs, right panels) mice at 3 months of age. The upper part is longitudinal section and the lower part is cross section. Hematoxylin-eosin stain, × 300.

weight between homozygous mutants with and without short-term carnitine administration (from 11 days to 20 days after birth, Table I and Table III). On the other hand, long-term carnitine administered jvs mice (from 1 month to 3 months) showed significantly smaller heart weight and heart weight to body weight ratio than the homozygous mutants without additional long-term administration, and there was no significant difference in the ratios of heart weight to body weight between the long-term carnitine administered jvs mice and homozygous controls.

4. DISCUSSION

We found that jvs mice suffered from abdominal ascites at 3–4 months of age associated with congestive liver and enlargement of the heart. These conditions were diagnosed as heart failure. In order to understand

Table II

Cytosolic and mitochondrial enzyme activities in control (+/+) and homozygous mutant (jvs/jvs) mice at 2–3 months of age

	Cytosolic enzymes				Mitochondrial enzymes			
	Aldolase (FBP)		s-GOT		m-GOT		SDH	
	U/mg NCP	U/heart	U/mg NCP	U/heart	U/mg NCP	U/heart	U/mg NCP	U/heart
Control (+/+)	0.092 ± 0.027	0.93 ± 0.28	0.68 ± 0.05	7.0 ± 1.2	0.53 ± 0.03	5.5 ± 1.0	73 ± 16	0.75 ± 0.23
Homozygous mutants (jvs/jvs)	0.076 ± 0.022	1.91 ± 0.48**	0.63 ± 0.04	16.2 ± 3.3**	0.55 ± 0.07	14.1 ± 3.3**	94 ± 7*	2.4 ± 0.5**

4 mice were used for the experiments 70 days to 90 days after birth. Statistically significant differences between groups: $*P < 0.05$; $**P < 0.01$.

the pathophysiology of heart failure, we analysed the heart of jvs mice without abdominal ascites at 2–3 months of age, and found a remarkable increase in heart weight. This is probably caused by an increase in cardiac cell size associated with non-collagen protein content (Table I), without recognizable triglyceride deposition (Fig. 1). Although the DNA content was about twice that of homozygous controls, cardiac cell number did not seem to be increased in the pathological examination (Fig. 1). Therefore, we assume that the increase in DNA content is caused by stimulation of DNA replication without cell division, and that the increase in heart weight is mainly caused by cardiac cell hypertrophy associated with the increase of cytosolic and mitochondrial proteins (Table II).

As shown in Table III, this cardiac cell hypertrophy is prevented by long-term carnitine administration. Heterozygous mutants with sufficient carnitine concentration in the serum do not suffer from cardiac hypertrophy (Table I). Our previous report [10] and the present study show that carnitine deficiency plays an important role in the development of the pathological conditions of the liver and the heart in jvs mice. Carnitine deficiency causes an abnormality in organ-specific gene expression in the liver [19,20] and an abnormality in cell growth (hypertrophy) in the heart. How carnitine deficiency causes these various abnormalities in the heart and liver is a matter of great interest. Recently, Tomomura et al. [21] reported that the expression of the proto-oncogenes, c-jun and c-fos, is increased in the liver of homozygous mutants (jvs/jvs). The relationship between carnitine deficiency and the induction of the proto-oncogenes has not yet been elucidated. On the other hand, there are some reports about the induction of these proto-oncogenes in cardiac cells by factors which are related to cardiac hypertrophy, such as pressure overload [22], mechanical load [23], and β-adrenergic stimulation [24]. Therefore, in order to understand the pathophysiology of jvs mice, it is necessary to analyse the proto-oncogenes in the cardiac cells.

To our knowledge, 3 kinds of animal models for cardiac hypertrophy have been reported: spontaneously hypertensive rat (SHR) [25], syrian hamster with cardiomyopathy [26], and c-myc transgenic mice [27]. Although the mechanism causing cardiac hypertrophy in the former two animals is suspected to be in the abnormality of the cardiac cellular membrane [28,29], this is still not clear. The c-myc transgenic mice show cardiac hyperplasty, rather than cardiac hypertrophy. In any case, they are valuable animal models for cardiac abnormality in human beings. Our study revealed that jvs

Table III

Effect of carnitine administration on cardiac hypertrophy in jvs mice

Mouse	Carnitine treatment		B.W. (g)	H.W. (g)		H.W./B.W. (%)	
	11–20 days	30–90 days					
jvs/jvs (6)[a]	(+)[b]	(−)	25.0 ± 1.6	0.27 ± 0.05	**	1.07 ± 0.14	**
jvs/jvs (4)	(+)	(+)	25.0 ± 2.7	0.15 ± 0.03	*	0.59 ± 0.13	NS
+/+ (4)	(+)	(−)	23.5 ± 1.6	0.10 ± 0.01		0.45 ± 0.03	

[a] Numbers in parentheses indicate number of animals used for experiments.
[b] (+) and (−) indicate with and without carnitine administration, respectively.

Values are expressed as mean ± S.D. Statistically significant differences between groups: $*P < 0.05$; $**P < 0.01$. NS, not significant.

mice with systemic carnitine deficiency suffer from remarkable cardiac hypertrophy, and that the cardiac hypertrophy is strongly related to carnitine deficiency because it is treatable by carnitine administration (Table III). Therefore, jvs mice are a valuable animal model to clarify the mechanism of cardiac abnormality in systemic carnitine deficiency in humans.

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