Carnitine Derivatives in Hereditary Cardiomyopathic Animals

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SUMMARY

BIO 14.6 Syrian hamsters and diabetic KK mice have been reported to develop hereditary cardiomyopathy spontaneously. In order to investigate the pathophysiological role of carnitine metabolism in hereditary cardiomyopathy, tissue levels of carnitine derivatives and the histology of the heart, liver and skeletal muscles from BIO 14.6 hamsters and KK mice were studied.

Free carnitine levels in the heart of the BIO 14.6 hamsters (287.0 ± 27.0 n mole/g wet tissue) were significantly lower than in the control group (348.8 ± 83.8, $p<0.05$). Short chain acylcarnitine (197.0 ± 56.0 n mole/g wet tissue) and total carnitine (667.6 ± 136.4 n mole/g wet tissue) in the hearts of the BIO 14.6 hamsters were significantly lower than in the control group (short: 425.2 ± 54.8, total: 1023.6 ± 81.4, $p<0.001$). There was no significant difference in the levels of various carnitine derivatives of the liver and skeletal muscles from the BIO 14.6 hamsters and control hamsters. On the other hand, carnitine derivatives in KK mice did not change significantly compared with those in the heart, liver and skeletal muscles of the control mice.

Histological findings showed that heart muscle degeneration and necrosis were found in both cardiomyopathic animals. Coagulative necrosis was found in both animals, whereas myocytolytic necrosis was found only in the BIO 14.6 hamsters. In KK mice, the right ventricle, especially tissue under the epicardium, was severely affected compared with the left ventricle. In the BIO 14.6 hamsters, however, lesions were scattered over both ventricles

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with a predilection for the left ventricle.

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LONG chain fatty acids are unable to penetrate the inner mitochondrial membrane. Carnitine is essential if fatty acyl derivatives are to penetrate the inner mitochondrial membrane and be transported to the oxidation sites in the mitochondria. As free fatty acids are the main source of energy in the normal myocardium, normal cardiac metabolism depends upon carnitine.[1] Systemic carnitine deficiencies, which take the form of cardiomyopathy and other lesions, have been reported in humans.[2)-6)] While the reason for carnitine deficiency in this disease is poorly understood, a defect in renal and probably in gastrointestinal transport of carnitine is considered a likely cause.[3),6)]

It has been also reported that oral carnitine therapy improved cardiomyopathy and muscle strength.[2),3),5),6)]

The BIO 14.6 hamsters provide a good model for the experimental study of cardiomyopathy because they develop cardiomyopathy and muscular dystrophy in a reproducible and predictable fashion.[7)-11)] It has been reported that the myocardium of the BIO 14.6 hamsters showed depressed fatty acid oxidation[12),13)] and decreased carnitine concentration[14),15)] compared with that of normal hamsters.

Recently, it has also been reported that KK mice, which develop overt diabetes, have spontaneous cardiomyopathy.[16)-20)]

In order to investigate the pathophysiological role of carnitine metabolism in cardiomyopathy, free carnitine, short chain acylcarnitine and long chain acylcarnitine levels in the myocardium, skeletal muscle and liver of the BIO 14.6 hamsters and KK mice were measured.

Materials and Methods

Cardiomyopathic animals used in the present study are BIO 14.6 Syrian hamsters (Mesocricatus auratus) and KK mice (Mus musculus). The BIO 14.6 hamsters were obtained from the Central Institute for Experimental Animals, Kawasaki, Japan. The normal Syrian golden hamsters used as controls were purchased from Shizuoka Laboratory Animal Center, Hamamatsu, Japan. The KK mice were originally maintained and C57BL/6J mice were used as normal controls. All animals were bred by full-sib mating

in our Institute for Experimental Animals. The animals were housed in polycarbonate cages with plane-chip bedding and given commercial rodent food and tap water *ad libitum*. Only male hamsters of the BIO 14.6 and control strains were studied at between 80 and 100 days of age, as were only male mice of the KK and C57BL/6J strains at between 140 and 175 days of age, as all BIO 14.6 hamsters and KK mice have been reported to develop cardiomyopathy during this period.[11),16),19),20)] The animals were killed by cervical dislocation in light ether anesthesia. The heart, liver and gastrocnemius muscles were excised and frozen immediately in liquid nitrogen and then stored at −80°C. Some of these tissues were preserved for histological examination.

Free carnitine was determined enzymatically using carnitine acetyltransferase by a modification of the method of Marquis et al.[21)] Short chain acylcarnitine and long chain acylcarnitine were assayed as free carnitine using alkaline hydrolysis by the method of Pearson et al.[22)] Values were expressed as the concentration for wet tissue weight and shown as mean±SD. Statistical analysis was performed by non-paired Student's t-test and probabilities of <0.05 or less were taken as the level of significance.

Hearts were sliced transversely at the midportion of the ventricle. Slices 5 mm thick were immediately fixed in 10% buffered formalin for 24–48 hours and embedded in paraffin. Sections 3 μm thick were stained with hematoxylin-eosin, Mallory-Azan and von Kossa's method for calcium salts. Slices of the liver, lungs and gastrocnemius muscles were treated similarly.

Results

Myocardial tissue levels of carnitine derivatives:

BIO 14.6 Syrian hamsters

Fig. 1 shows changes in the myocardial tissue levels of various carnitine metabolites in the BIO 14.6 and normal hamsters.

Free carnitine in the BIO 14.6 hamsters (287.0±26.0 n mole/g wet tissue) was significantly lower than in the control hamsters (384.8±83.8, $p<0.05$). Short chain acylcarnitine (197.0±50.6) and total carnitine (667.6 ±136.4) in the BIO 14.6 hamsters was also lower than in the control group (425.0±54.8 and 1023.6±81.4, respectively, $p<0.001$). There was no significant difference in the levels of long chain acylcarnitine between the BIO 14.6 and control hamsters.

KK mice

The data summarized in Fig. 2 indicate the changes in the myocardial tissue levels of various carnitine metabolites in the KK mice. The tissue

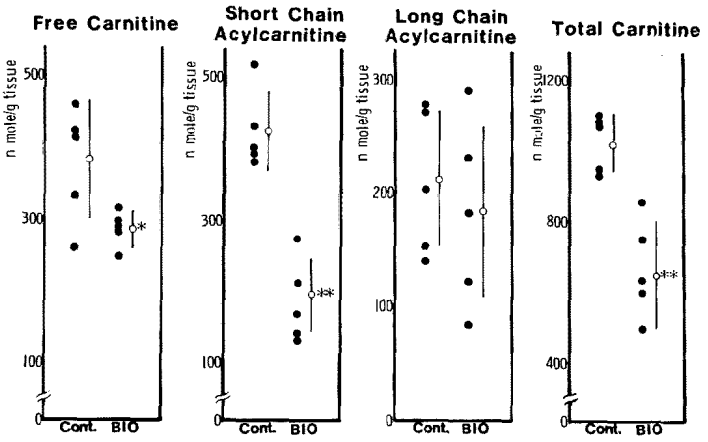

Fig. 1. Carnitine derivatives in the hearts of the BIO 14.6 and control hamsters. Values are expressed as mean±SD per gram wet tissue weight. P values represent the difference between the BIO 14.6 and the control as assessed by non-paired Student's t-test. * $p<0.05$, ** $p<0.001$.

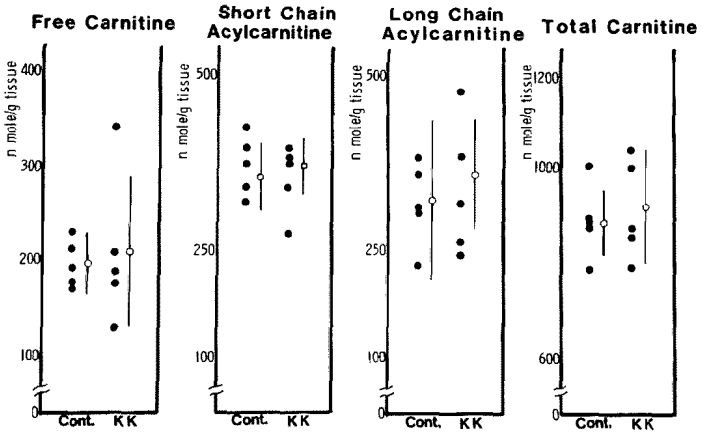

Fig. 2. Carnitine derivatives in the hearts of KK and control mice. Values are expressed as mean±SD per gram wet tissue weight. There is no significant difference between the KK and control mice.

levels of various carnitine derivatives in the KK mice (free carnitine: 210.6 ±80.5 n mole/g wet tissue, short chain acylcarnitine: 354.4±48.4 n mole/g wet tissue, long chain acylcarnitine: 357.2±81.2 n mole/g wet tissue, total carnitine: 923.2±127.8) were not significantly different from those in the control mice (free carnitine: 199.2±25.1, short chain acylcarnitine: 371.2± 41.8, long chain acylcarnitine: 322.6±65.0, total carnitine: 893.0±64.0).

Carnitine derivatives in the liver and skeletal muscles:

BIO 14.6 Syrian hamsters

There were no differences in the levels of free carnitine or short chain

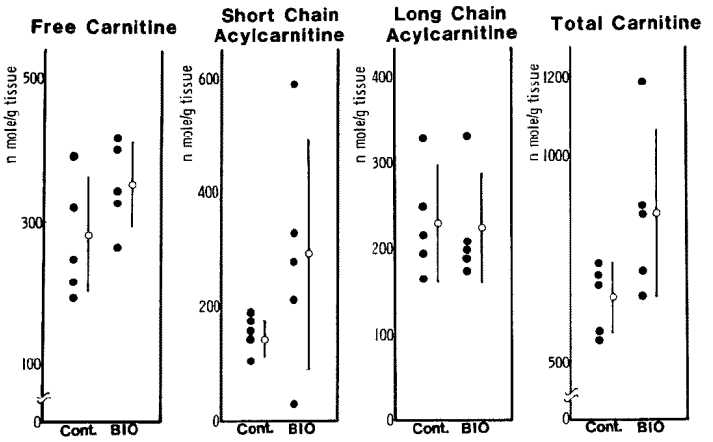

Fig. 3. Carnitine derivatives in the liver of BIO 14.6 and control hamsters. Values are expressed as mean±SD per gram wet tissue weight. There is no significant difference between the BIO 14.6 and control hamsters.

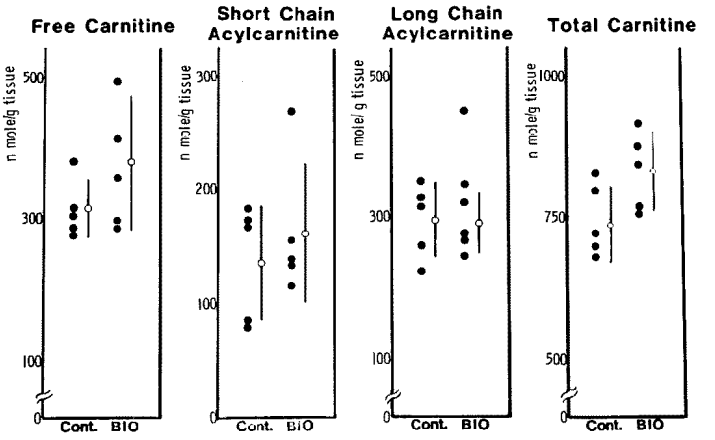

Fig. 4. Carnitine derivatives in the skeletal muscle of the BIO 14.6 and control hamsters. Values are expressed as mean±SD per gram wet tissue weight. There is no significant difference between the BIO 14.6 and control hamsters.

and long chain acylcarnitine in the livers of the BIO 14.6 (free carnitine: 351.4±61.1, short chain acylcarnitine: 291.0±201.6, long chain acylcarnitine: 222.8±63.4) and control hamsters (free carnitine: 282.0±78.6, short chain acylcarnitine: 144.6±30.4, long chain acylcarnitine: 230.8±68.6). However, total carnitine in the livers of the BIO 14.6 hamsters (864.8±204.2) was higher than that in the control hamsters (657.0±82.9, $p<0.1$) (Fig. 3).

No significant differences in the carnitine concentration of the skeletal muscle were observed between the BIO 14.6 (free carnitine: 377.6±95.6,

short chain acylcarnitine: 162.2±61.7, long chain acylcarnitine: 292.0±41.2, total carnitine: 831.4±70.4) and control hamsters (free carnitine:

Table I. Changes in Tissue Levels of Carnitine Derivatives of the Liver and the Skeletal Muscle in KK Mice

		Free carnitine (n mole/g)	Short chain acylcarnitine (n mole/g)	Long chain acylcarnitine (n mole/g)	Total carnitine (n mole/g)
Control (C57BL/6J)	Liver	318.2±186.9	133.6±137.7	247.7±65.2	699.2±119.2
	S. K.	142.4±64.1	129.2±82.0	394.0±63.0	665.6±117.9
KK mice	Liver	377.6±70.9	86.8±170.6	357.2±81.2	923.2±127.8
	S. K.	161.8±41.0	123.4±170.4	559.3±192.4	845.2±235.3

S. K.=skeletal muscle.
Values are expressed per gram wet tissue weight and represented as mean±SD.
Statistical analyses are performed using non-paired t-test.
There is no significant difference between the control and the KK mice.

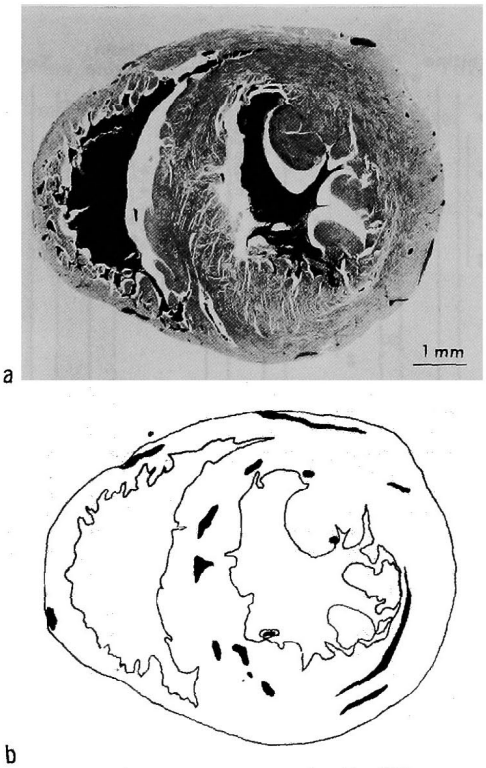

Fig. 5. a: The heart of a BIO 14.6 Syrian hamster cut transversely at the midportion of the ventricle. Hematoxylin and eosin stain; scale 1 mm. b: Schema showing the distribution of fibrosis and calcification in Fig. 5a. The lesions of fibrosis with or without calcification are scattered over both ventricles with a predilection for the left. The dark area shows the fibrotic and calcified lesions in the myocardium.

314.4±40.8, short chain acylcarnitine: 137.8±51.8, long chain acylcarnitine: 295.8±52.2, total carnitine: 735.6±64.7) (Fig. 4).

KK mice

Carnitine derivatives in the liver and skeletal muscles showed no significant changes between the KK and control mice (Table I).

Histological findings:

BIO 14.6 Syrian hamsters

Scattered myocardial lesions were found in both ventricles, but there appeared to be a predilection for the left ventricle compared with the right ventricle (Fig. 5). Discrete foci of fibrosis and calcification were found in the myocardium. Muscle fibers undergoing myocytolytic necrosis were scattered over the ventricle (Fig. 7) and were sometimes accompanied by focal infiltration of macrophages. The liver and lungs showed moderate congestion.

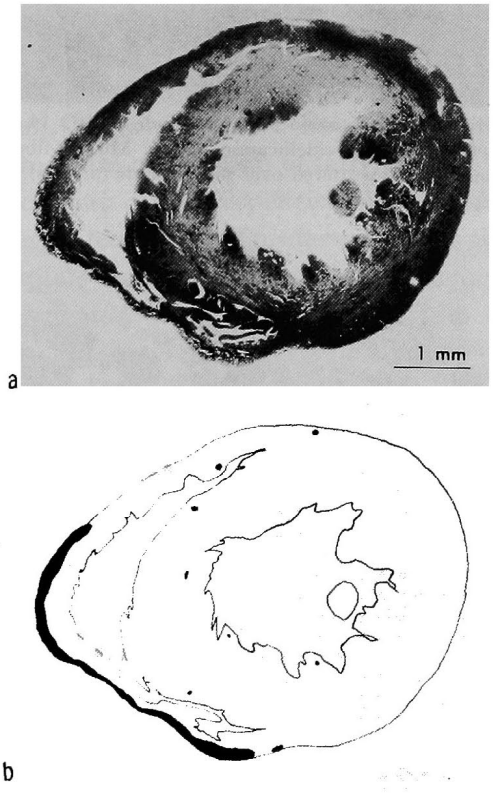

Fig. 6. a: The heart of a KK mouse cut transversely at the midportion of the ventricle. Hematoxylin and eosin stain; scale 1 mm. b: Schema showing the distribution of fibrosis and calcification in Fig. 6a. Zonal fibrosis with severe calcification is found under the epicardium of the antero-lateral right ventricle. The dark area shows the fibrotic and calcified lesions in the myocardium.

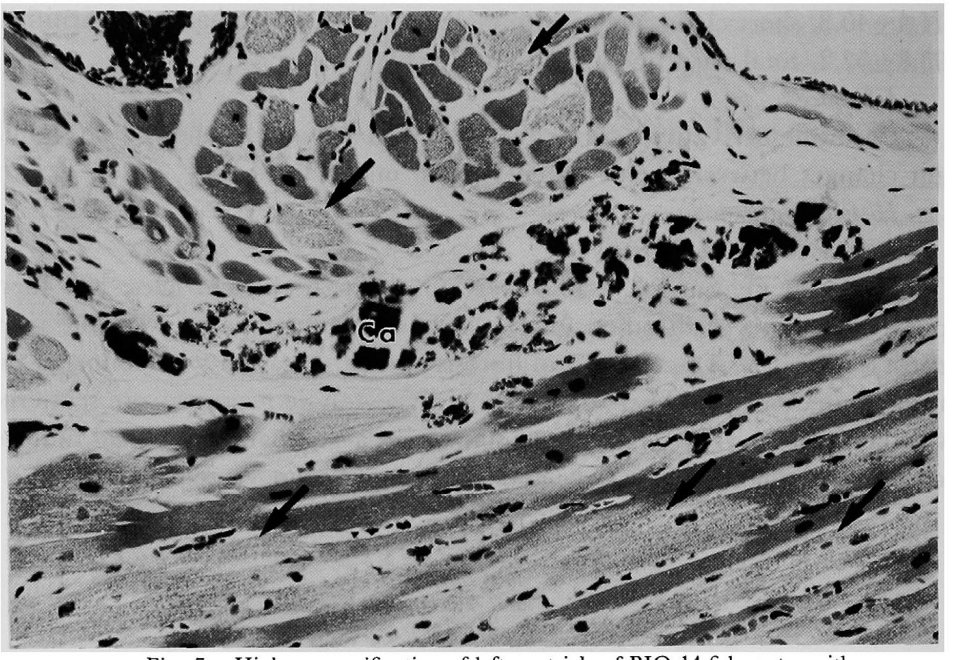

Fig. 7. Higher magnification of left ventricle of BIO 14.6 hamster with a small zone of fibrosis and calcification (Ca). Muscle fibers undergoing myocytolytic necrosis are scattered over the ventricle (arrows). Hematoxylin and eosin stain; scale 40 μm.

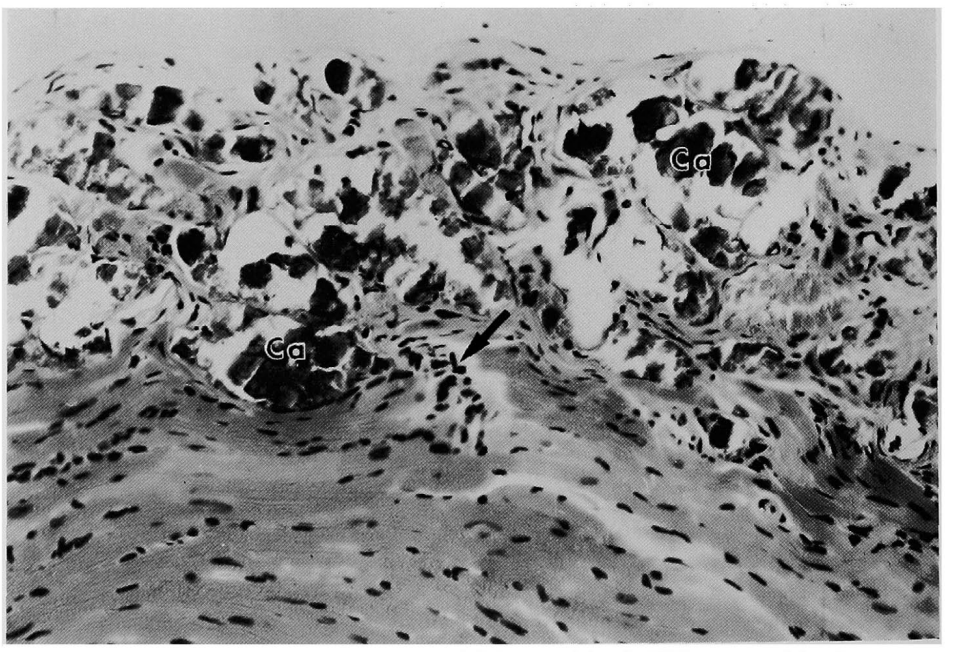

Fig. 8. Higher magnification of right ventricle of a KK mouse. Muscle fibers under the epicardium show marked degeneration and necrosis with extensive fibrosis and calcification (Ca). Focal infiltration with macrophages is observed (arrow). Hematoxylin and eosin stain; scale 40 μm.

KK mice

The hearts of the KK mice showed myocardial degeneration and necrosis. Scattered myocardial lesions were found in both ventricles and the interventricular septum and papillary muscles, especially in the right ventricle under the epicardium (Fig. 6). Muscle fibers under the epicardium of the right ventricle showed marked degeneration and necrosis with extensive fibrosis and calcification (Fig. 8). Degenerated muscle cells showed increased eosinophilia or vascular degeneration. Focal fibrosis with or without calcification was found in the left ventricle. Macrophage infiltration was found focally around the degenerated muscle fibers. The liver and lungs showed slight congestion.

Discussion

There have been many reports of biochemical investigations in the BIO 14.6 and other cardiomyopathic lines of Syrian golden hamsters that have shown alterations in several functions such as oxidative phosphorylation and lipid metabolism. However, there have been few reports describing changes in the intermediate metabolism of fatty acids.[1),12),13),23)] It has also been reported that cardiac carnitine deficiency is found throughout the life span of cardiomyopathic hamsters. This fact indicates that cardiac carnitine deficiency is not simply secondary to advanced cardiomyopathy.[15)]

This study investigated the concentrations of the carnitine derivatives and the histological changes in the hearts of BIO 14.6 hamsters and KK mice suffering from hereditary cardiomyopathy.

As shown in Fig. 1, in the BIO 14.6 cardiomyopathic hamsters, free carnitine, short chain acylcarnitine and total carnitine were significantly lower than in the control group as reported by Hoppel et al.[14)] However, long chain acylcarnitine did not change significantly compared with that in the control hamsters in contrast to the reported results from Hoppel et al.[14)] There were no significant differences in the livers and skeletal muscles of the BIO 14.6 and control hamsters.

Carnitine synthesized in the liver is released into plasma for transfer to peripheral tissues, where it is taken up against a concentration gradient by means of a transport system which is not completely understood. York and Carol have reported that cardiac carnitine-binding protein prepared from the hearts of the cardiomyopathic hamsters was characterized by both a reduced maximal binding of carnitine and an increased dissociation constant compared with the cardiac carnitine-binding protein prepared from the hearts of normal hamsters.[15),24)] They thought that in the cardiomyo-

pathic hamsters the transport of carnitine into the cardiac muscle is altered and that this might be one of the causes of spontaneous hereditary cardiomyopathy which develops in the BIO 14.6 Syrian hamsters.

In this study, levels of carnitine derivatives in the heart decreased, but those in the liver and skeletal muscles did not change significantly. These data support their hypothesis.

On the other hand, it has been reported that diabetic KK mice have various myocardial lesions and show a remarkable increase in calcium and a decrease in magnesium content in the myocardium.[16),19),20)] Fogel and Neely have reported that the tissue levels of carnitine were decreased in hearts from diabetic animals.[25)]

Concentrations of carnitine derivatives in the hearts of KK mice have not been reported. In this investigation none of the tissues examined showed any significant difference in levels of the metabolites of various carnitine derivatives between KK mice and controls, as shown in Fig. 2 and Table I.

There were two histological differences between the BIO 14.6 hamsters and the KK mice. One is a degenerative change in the cardiac muscle. Briefly, the enlargement of sarcoplasma with the disappearance of myofibrils which was found only in BIO 14.6 hamsters, was not noted in the KK mice.

The other difference is in the distribution of the myofibrils with severe degenerative changes. In the KK mice, severe zonal lesions were located under the right ventricle with widespread fibrosis with calcification, and spotty fibrosis was found in the left ventricle. In BIO 14.6 hamsters, however, the right ventricle was not as severely affected. The lesions with fibrosis were scattered throughout both ventricles. These histological differences might indicate that the pathogenesis of cardiomyopathy might vary from one species to the other. Ruber et al have reported that the pathologic feature of diabetic cardiomyopathy is microangiopathy, i.e., intimal and subendothelial thickening of arteriolar walls by deposition of periodic acid-Sciff (PAS) positive staining mucopolysaccharide material.[26)] Though hyperglycemia is observed in KK mice, no PAS positive staining material is detected around the blood vessels of myocardium.[20)] We do not think that the histological changes in the myocardia of KK mice are secondary to diabetis mellitus.

These results suggest that lowered levels of carnitine derivatives in the myocardium may play an important pathophysiologic role in the development of cardiomyopathy in BIO 14.6 hamsters, but not in KK mice.

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