Diabetic State-Induced Modification of Ca, Mg, Fe and Zn Content of Skeletal, Cardiac and Smooth Muscles

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Abstract

The metal content of diaphragm, gastrocnemius, ventricle, and bladder muscles in genetically obese diabetic KK-CAy and alloxan-diabetic ddY mice was compared with that in prediabetic KK-CAy and normal ddY mice, because the muscles of the diabetic KK-CAy mice had morphological abnormalities, such as atrophy, disappearance of Z-band, disturbed myofibrils and swollen sarcoplasmic reticulum. The amounts of calcium (Ca) in gastrocnemius, ventricle and bladder muscles from the prediabetic KK-CAy mice were significantly 7.7, 98.3, and 36.9% greater, respectively, than those in normal ddY mice. In contrast, the magnesium (Mg) content of the diaphragm, gastrocnemius, and ventricle in the prediabetic mice was 8.6, 7.4, and 4.3% lower, respectively, than in the ddY mice. The iron (Fe) content of the diaphragm, gastrocnemius, and ventricle muscles in the prediabetic mice was 29.2, 43.6, and 44.6% greater, respectively, than in the ddY mice. The Ca content in the gastrocnemius muscles of the diabetic KK-CAy mice and the alloxan-diabetic mice was 19.8 and 11.7% higher, respectively, than in the prediabetic and normal mice. The Ca content of the ventricle muscle was increased only in the alloxan-mice. The gastrocnemius Mg was also 9.0 and 5.5% greater in the KK-CAy and the alloxan-mice. The Fe content of the diaphragm and the gastrocnemius muscles from the KK-CAy mice was 27.3 and 23.2% greater, respectively, than in the prediabetic mice. The zinc (Zn) content of the gastrocnemius and the bladder was 16.4 and 18.0% higher, but the ventricle Zn was 13.4% lower, respectively, than in the prediabetic control. The changes in metal content induced by the diabetic state may be related to the morphological abnormalities.

The male KK-CAy mouse has a genetically predisposed syndrome similar to human type 2 diabetes mellitus (NIDDM) and includes hyperglycemia, hyperinsulinenia, obesity, and insulin resistance (Kimura et al., 1979). Our previous studies demonstrated that the diabetic state enhances the sensitivity to succinylcholine, a depolarizing neuromuscular blocker, of acetylcholine receptors (AChR) at the neuromuscular synapse of the gastrocnemius

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Muscle (Kimura et al., 1986b; 1988). The hypersensitivity of the diabetic muscles may be caused partly by changes in the muscle membranes surrounding AChR and is thought to depend on a deficiency of insulin action, not on an increased level of blood glucose. We have also reported that intracellularly released calcium ions (Ca^{2+}) from diaphragm plasma membranes are enhanced in alloxan-diabetic mice (Kimura et al., 1986a). In addition, the increase in the activity of the muscle Ca^{2+}-activated proteinase in alloxan-injected rats is consistent with the proposed role of protease in initiating metabolic turnover of myofibrillar proteins (Brooks et al., 1983).

Magnesium (Mg) acts essentially as a cofactor in several enzyme systems. Ca often reverses these effects of Mg. The concentration of Mg is related to carbohydrate metabolism in several ways. For instance, insulin increases the uptake of Mg in muscles (Aikawa, 1960; Mellerup, 1974). Some patients with IDDM are reported to have a lower Mg content in striated muscles and plasma (Sjögren et al., 1986). Plasma Mg levels in diabetes are closely dependent on the concentration of blood glucose (Mather et al., 1982).

Zinc (Zn) has a strong affinity for insulin, and is associated with insulin in the islets of Langerhans (Engelbart and Kief, 1970). Insulin secretagogues stimulate the release of Zn from the islets (Formby et al., 1984). Zn is also capable of modulating insulin action. Some investigators have reported that hepatic binding of insulin was enhanced by Zn (Arquilla et al., 1978) and that lipogenesis in the adipocytes is stimulated by synergism between insulin and Zn (Coulston and Dandona, 1980). Iron (Fe) is the most abundant of the trace elements in animals. Hemoglobin contains two-thirds of the total amount of Fe. Skeletal muscles also contain Fe in ferritin, hemosiderin and myoglobin. The Fe level in skeletal muscles from streptozotocin-diabetic rats has been reported to be greater than that in the control tissues (Failla and Kiser, 1981; Johnson and Evans, 1984). However, information concerning the influence of diabetes mellitus on the metabolism of these trace metals is conflicting. Different amounts of these metals have been reported in diabetic mice (Levine et al., 1983; Failla and Kiser, 1981; Johnson and Evans, 1984).

In the present study, our aim was to determine morphological changes in the diaphragm of the diabetic KK-CA^y mouse and compare them with Ca, Mg, Fe, and Zn levels in skeletal, cardiac, and smooth muscles of the diabetic KK-CA^y, and the alloxan-diabetic mice.

Materials and Methods

**Animals**

Male genetically obese diabetic KK-CA^y (28-48 wks old, with blood glucose levels of 381±14 mg/dl) and prediabetic KK-CA^y mice (8-12 wks old, with blood glucose levels of 134±4 mg/dl) were bred in our laboratory. ddY strain male mice (7-10 wks old, with blood glucose levels of 121±2 mg/dl) (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were used. Alloxan-induced diabetic ddY mice were used 1, 2, and 4 wks after an injection of 85 mg of alloxan (Nakarai Chemicals Co., Kyoto, Japan) per kg in 0.9% saline into the tail veins of ddY strain male mice. Levels of blood glucose in these alloxan-mice were 430±18, 455±10, and 434±22 mg/dl, respectively. The alloxan-diabetic and normal control ddY mice were sacrificed when they were 8 wks old. Blood glucose levels were measured by the glucose oxidase method with a glucose analyzer (type 2, Beckman Instruments INC, Fullerton, CA).

**Preparation of sections of diaphragms for light microscopy**

Diaphragm muscles of the diabetic KK-CA^y, the prediabetic KK-CA^y, and the ddY mice were fixed with Bouin’s solution (saturated picric acid solution [15 vol.], 36% formaldehyde solution [5 vol.], and glacial acetic acid [1 vol.]), embedded in paraffin, and sectioned. Sections
were stained with hematoxylin-eosin, and observed under a light microscope.

**Preparation of sections of diaphragms for electron microscopy**

Diaphragm muscles of the diabetic KK-CA\(^{v}\) and ddY mice were extended in saline, and fixed in 1\% paraformaldehyde, 3\% glutaraldehyde and 100 mM sodium cacodylate buffer (Nakarai) (pH 7.4) (Karnovsky’s fixative) for 1 h at 4°C. The muscles were washed for 2 h at 4°C with 200 mM cacodylate buffer (pH 7.4) containing 5\% sucrose (CBS). The muscles were post-fixed for 1 h at 4°C with 1\% osmium tetroxide (Bio. Rad, Richmond, CA) in 100 mM cacodylate buffer, washed with CBS, dehydrated with ethanol and propylene oxide (Nakarai), and embedded in epoxy resin (Oken Shoji Co., Tokyo, Japan). The muscles were cut into 60 to 100 nm thick sections with an Ultramicrotome (Reichert, Vienna, Austria) and collected on 150 mesh copper grids (Nissin EM Co., Tokyo). The muscles were then stained with saturated uranyl acetate solution and 0.4\% citric lead solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and examined with a 200 CX electron microscope (JEOL, Tokyo) at 80 KV.

**Determination of calcium, magnesium, iron, and zinc content in muscles**

Isolated diaphragm, gastrocnemius, ventricle, and bladder muscles were washed with saline, weighed and dissolved in 60\% nitric acid (Nakarai) overnight at room temperature. The amounts of Ca, Mg, Fe, and Zn were determined by the procedure of Parker (1963) with some modifications as follows: Samples dissolved in 60\% nitric acid were diluted with 30.4\% strontium chloride solution (Nakarai) to a final concentration of 1N nitric acid and 10,000 p.p.m. strontium for determination of Ca, Fe, and Zn. The sample for measuring Mg content was diluted ten fold. The metal content was determined with an AA-640-13 atomic absorption spectrophotometer (Shimadzu Co., Kyoto) which was equipped with a double beam background corrector and an air acetylene flame for atomization.

**Expression of data and statistical significance**

The data represent the mean values±SE of the metal content in 100 g wet weight muscles measured in 5–35 experiments. The statistical significance of difference in values for the diabetic KK-CA\(^{v}\) mice from values in the prediabetic KK-CA\(^{v}\) mice or of of difference in values for the prediabetic KK-CA\(^{v}\) mice from the normal ddY mice was determined by Student’s unpaired t-test. The significance of difference in values for the alloxan ddY mice from the normal ddY mice was determined by Dunnett’s multiple comparison test.

**Results**

**Morphological observation of diaphragm of diabetic KK-CA\(^{v}\) mice**

The diaphragm muscles from diabetic KK-CA\(^{v}\) mice were compared morphologically with those from prediabetic KK-CA\(^{v}\) and ddY mice. Light micrographs showed atrophy and reduction in size of muscle fibers in the diaphragms of the diabetic KK-CA\(^{v}\) mice (Fig. 1C) in comparison with those of the prediabetic KK-CA\(^{v}\) and the ddY mice (Fig. 1B and A). Ultrastructures of the diaphragm muscles were further examined with an electron microscopy. Electron micrographs at different magnifications showed the disappearance of Z-bands, markedly disturbed arrangement of myofibrils, and swollen sarcoplasmic reticula in the diaphragm muscles of the diabetic mice (Fig. 2B and D), as compared with those of the prediabetic KK-CA\(^{v}\) and the ddY mice (Fig. 2A and C). These observations indicate that the diaphragm muscles of KK-CA\(^{v}\) mice were morphologically changed by the diabetic state.

**Wet weight of various kinds of muscle in the alloxan-diabetic ddY mice**

The wet weight of diaphragm, gastrocnemius, ventricle and bladder muscles of alloxan-diabetic mice was compared with those of age-matched normal ddY mice (Table 1). The wet weight of the diaphragm, gastrocnemius and ventricle muscles
Fig. 1. Light micrographs of diaphragms of normal (A), prediabetic (B), and diabetic (C) mice. The bar represents 10 μm. Note that the diaphragm of the diabetic KK-CAY mouse shows atrophy of the muscle fibers.
Fig. 2. Electron micrographs of diaphragms of normal ddY (A, C) and diabetic K.K.-CA' (B, D) mice. The bars represent 1 μm. Note that the diaphragm of the diabetic K.K.-CA' mouse shows the disappearance of the Z-bands, disarrangement of myofibrils and a swollen sarcoplasmic reticulum.
Table 1. Wet weight of various kinds of muscle in alloxan-diabetic and normal ddY mice.

<table>
<thead>
<tr>
<th>Mice</th>
<th>Diaphragm (mg)</th>
<th>Gastrocnemius (mg)</th>
<th>Ventricle (mg)</th>
<th>Bladder (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>55.2±0.7</td>
<td>159.9±4.9</td>
<td>124.3±3.3</td>
<td>24.7±1.0</td>
</tr>
<tr>
<td>Alloxan-Diabetic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 week elapsed</td>
<td>46.4±1.0**</td>
<td>122.2±2.9**</td>
<td>104.1±3.7**</td>
<td>28.3±2.2</td>
</tr>
<tr>
<td>2 weeks elapsed</td>
<td>45.0±1.2**</td>
<td>113.2±5.5**</td>
<td>101.0±3.5**</td>
<td>37.6±2.5*</td>
</tr>
<tr>
<td>4 weeks elapsed</td>
<td>43.6±2.3**</td>
<td>110.4±6.2**</td>
<td>101.4±5.5**</td>
<td>40.6±3.6**</td>
</tr>
</tbody>
</table>

Values represent the means±SE of 9–13 experiments. *P<0.05, **P<0.01: Significantly different from those in the normal ddY mice.

was decreased in a time-dependent fashion after alloxan injection. In contrast, the weight of the bladder muscle was increased. Four wks after the alloxan injection, the weights of the diaphragm, gastrocnemius, ventricle, and bladder muscles were 79.0, 69.0, 81.6, and 164.2% of the corresponding muscle from the normal mice. The ratio of wet weight of bladder muscle to dry weight was significantly increased by the alloxan injection from 3.99±0.05 to 4.64±0.14. But these ratios in other muscles were not influenced by alloxan.

**Calcium and magnesium content of muscles from the diabetic KK-CAy and the alloxan-diabetic mice**

The amounts of Ca and Mg in the diaphragm, gastrocnemius, ventricle, and bladder muscles of the diabetic KK-CAy and the alloxan mice were compared with those in the prediabetic KK-CAy and the normal ddY mice. The Ca content of the gastrocnemius, ventricle and bladder muscles in the prediabetic KK-CAy mice (8–12 wks old) was significantly higher than in the ddY mice (7–10 wks old) (Fig. 3A). The effect of a strain difference on the Ca content was clearly observed in the ventricle > the bladder > the gastrocnemius muscles in that order. The Ca content of these muscles in age-matched prediabetic KK-CAy and normal ddY mice was compared. The amounts of Ca in the diaphragm, gastrocnemius, ventricle, and bladder muscles in the prediabetic mice (8 wks old) were 6.8±0.4, 8.9±0.1, 6.6±1.6, and 9.3±0.5 mg/100g wet tissues, respectively. In comparison with the 5.1±0.1, 7.6±0.2, 3.9±0.1, 5.0±0.2mg/100g in the corresponding muscle of the age-matched normal ddY mice, those of the diaphragm, the gastrocnemius, and the bladder muscles were also significantly high (P<0.05). The Ca content of the gastrocnemius muscles from the diabetic KK-CAy mice was significantly higher by 19.8% than that from the prediabetic mice (Fig. 3A). The diaphragm Ca tended to be increased, but the difference was not significant. The Ca content of the gastrocnemius muscles from the alloxan mice was also significantly increased in a time-dependent fashion by 2 wks after the alloxan injection, compared with that from the age-matched normal mice (Fig. 3B). These results indicate that the gastrocnemius muscle Ca is clearly influenced by the diabetic state in both the KK-CAy and the alloxan mice. Similar results were also obtained from data expressed as the Ca content in dried muscles (data not shown). The ventricle Ca content in the alloxan mice was significantly higher, by 10.6%, than that of the control muscles, but it was not changed in the diabetic KK-CAy mice.

The Mg content in the diaphragm, the gastrocnemius, and the ventricle muscles...
Ca, Mg, Fe AND Zn IN DIABETIC MUSCLES

from the prediabetic mice (8–12 wks old) was significantly lower than in the normal ddY mice (7–10 wks old) (Fig. 4A). To investigate whether these results were due to aging and/or a strain difference, the Mg content of the muscles was compared in age-matched prediabetic KK-CA\(^2\) and ddY mice. The Mg content in the diaphragm, gastrocnemius, ventricle and bladder muscles in the prediabetic mice (8 wks old) was 23.1 ± 0.7, 27.4 ± 0.5, 20.9 ± 0.3, and 13.3 ± 0.4 mg/100 g wet tissues, respectively. The Mg content in the corresponding muscle in the ddY mice was 24.1 ± 0.3, 27.8 ± 0.4,

![Fig. 3. (A) Ca content of diaphragm, gastrocnemius, ventricle, and bladder muscles of normal ddY (open columns), prediabetic KK-CA\(^2\) (sparsely dotted columns) and genetically obese diabetic KK-CA\(^2\) (densely dotted columns) mice. (B) Ca content of various muscles in alloxan-diabetic mice 1 to 4 wks after alloxan injection (dotted columns) and in normal ddY mice (open columns). The values represent means ± SE (n=6–35). *P <0.05, **P <0.01; Significant difference in values for the diabetic mice from the corresponding value for the prediabetic KK-CA\(^2\) mice, difference in values for the prediabetic KK-CA\(^2\) mice from the ddY mice, and difference in values for the alloxan-diabetic mice from the control ddY mice.](image-url)
22.2 ± 0.3, and 14.9 ± 0.3 mg/100 g wet tissues. As compared with those data, only the Mg content in the bladder of the prediabetic mice was significantly lower (p < 0.05). These results indicate that the decrease in Mg in the diaphragm, the gastrocnemius, and the ventricle of the prediabetic mice is induced by the age factor more than a strain difference. In contrast, the Mg content was significantly increased, by 9.0%, in the gastrocnemius from the diabetic KK-CAY mice compared with the prediabetic control, but it was not changed in the diaphragm muscle (Fig. 4A). The Mg content in the gastrocnemius from age-matched alloxan mice was also significantly

Fig. 4. (A) Mg content of various muscles in normal ddY (open columns), prediabetic KK-CAY (sparsely dotted columns), and genetically diabetic KK-CAY (densely dotted columns) mice. (B) Mg content of various muscles in alloxan-diabetic mice 1 to 4 wks after alloxan injection (dotted columns) and in normal ddY mice (open columns). The values represent the means ± SE (n = 5-34). * P < 0.05, ** P < 0.01: Significant difference in values for the diabetic KK-CAY mice from the corresponding value in the prediabetic KK-CAY mice, difference in values for the prediabetic KK-CAY mice from the ddY mice, and difference in values for the alloxan-diabetic mice from the control ddY mice.
increased, by 4.5–5.5%, 1–2 wks after the injection of alloxan (Fig. 4B).

The ratios of Ca content to Mg content were compared in the four kinds of muscle of the diabetic, prediabetic KK-CA\(^{\gamma}\) and the normal ddY mice (Table 2). The Ca/Mg ratios of the four kinds of muscle in the prediabetic KK-CA\(^{\gamma}\) mice were significantly higher than those of the normal ddY mice. In addition, the Ca/Mg ratio of the diabetic gastrocnemius muscle was significantly higher than that of the prediabetic control.

**Iron and Zinc content of muscles of the diabetic KK-CA\(^{\gamma}\) mice**

The amounts of trace metals in the four kinds of muscle of the KK-CA\(^{\gamma}\) mouse in the normal state and the diabetic state were compared. The Fe content of the diaphragm, the gastrocnemius, and the ventricle muscles from the prediabetic KK-CA\(^{\gamma}\) mice was significantly increased, as compared with the corresponding muscles in the normal ddY mice (Fig. 5). The Fe content was also significantly increased, by 27.3 and 23.2% in the diaphragm and the

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**Table 2. The Ca/Mg ratio in various kinds of muscle of normal ddY, prediabetic and diabetic KK-CA\(^{\gamma}\) mice.**

<table>
<thead>
<tr>
<th>Mice</th>
<th>Diaphragm</th>
<th>Gastrocnemius</th>
<th>Ventricle</th>
<th>Bladder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal ddY</td>
<td>0.24±0.01</td>
<td>0.29±0.01</td>
<td>0.18±0.01</td>
<td>0.39±0.01</td>
</tr>
<tr>
<td>Prediabetic</td>
<td>0.29±0.01*</td>
<td>0.34±0.01**</td>
<td>0.38±0.06**</td>
<td>0.55±0.05*</td>
</tr>
<tr>
<td>KK-CA(^{\gamma})</td>
<td>0.29±0.02</td>
<td>0.37±0.01§§</td>
<td>0.38±0.04</td>
<td>0.49±0.04</td>
</tr>
</tbody>
</table>

Values represent the means±SE (N=5–34). * P<0.05, ** P<0.01: Significantly different from values in normal ddY mice. §§ P<0.01: Significantly different from values in the prediabetic KK-CA\(^{\gamma}\) mice.

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**Fig. 5.** Fe content of muscles in normal ddY (open columns), prediabetic KK-CA\(^{\gamma}\) (sparsely dotted columns) and genetically diabetic KK-CA\(^{\gamma}\) (densely dotted columns) mice. The values represent the means±SE (n=6–14). ** P<0.01: Significant difference in values for the diabetic KK-CA\(^{\gamma}\) mice from the corresponding value for the prediabetic mouse or the difference in values for the prediabetic KK-CA\(^{\gamma}\) mice from the ddY mice.
gastrocnemius muscles, respectively, from the diabetic mice, as compared with that of the prediabetic mice (Fig. 5). These results indicate that the Fe content of both skeletal muscles in the KK-CAβ mice was increased by the diabetic state.

The Zn content of the ventricle muscle of the prediabetic mouse was found to be significantly higher, and the bladder Zn to be lower than the corresponding muscle in the ddY mouse (Fig. 6). However, the Zn content was significantly decreased, by 13.4%, in the ventricle muscle, and increased, by 16.4 and 18.0%, in the gastrocnemius and the bladder muscles, respectively, from the diabetic KK-CAβ mice in comparison with that of the corresponding muscles from the prediabetic mice.

Discussion

The present study demonstrates that ultrastructures of the diaphragm of the KK-CAβ mouse are changed by the diabetic state, as indicated atrophy, disappearance of Z-bands, disarrangement of myofibrils, and a swollen sarcoplasmic reticulum. This observation is supported by some reports that patients with diabetes mellitus develop diabetic amyotrophy characterized by a pattern of muscle fiber atrophy, and an increase in the thickness of the basement membranes of the muscle fibers (Bloodworth and Epstein, 1967; Shafiq et al., 1968).

Since the morphological changes in dystrophic muscles are thought to be caused by the increased Ca²⁺ content (Maunder-Sewry et al., 1980; Bertorini et al., 1982), we investigated the Ca, Mg, and trace metal content of the diaphragm, gastrocnemius, ventricle and bladder muscles in diabetic mice. By comparing the Ca content of the diabetic muscle with that of the normal control muscle, the influence of the diabetic state in the KK-CAβ mice and the alloxan-mice was observed. Both diabetic states increase the Ca content of the gastrocnemius muscle (Fig. 3A, B). The Ca content of the diaphragm from the KK-CAβ tended to be increased, although it

Fig. 6. Zn content of muscles in normal ddY (open columns), prediabetic KK-CAβ (sparsely dotted columns), and genetically diabetic KK-CAβ (densely dotted columns) mice. The values represent mean±SE (n=6–14). * P<0.05, ** P<0.01: Significant difference in values for the diabetic KK-CAβ mice from the corresponding value for the prediabetic mice or difference in values for the prediabetic mice from the ddY mice.
did not differ significantly from that of the prediabetic mice. The ventricle Ca is only increased in the alloxan-mice. The higher Ca content in the diabetic muscle may probably be due to increasing intracellular Ca pools. This possibility is also indicated by some studies showing that intracellular Ca binding protein is increased in the muscle of diabetic mice (Morley et al., 1982), and that calcium-activated neutral protease is activated in the diabetic mouse skeletal muscle (Brooks et al., 1983; Kobayashi et al., 1989).

The Mg content in these muscles is also influenced by the diabetic state. The Mg content in the gastrocnemius muscle was increased by the diabetic state in both the KK-CA	extsuperscript{v} and the alloxan-mice (Fig. 4A, B). This increased Mg content is positively correlated with the Ca content in the diabetic muscle. Since Mg is mostly intracellular, increased Mg in the diabetic muscles could be due to changing intracellular pools. These data lend no support to the assumption that intracellular Mg deficiency is also promoted by the diabetic state (Wallach and Verch, 1987).

The strain difference between the prediabetic KK-CA	extsuperscript{v} and the normal ddY mice also affected the amounts of Ca and Mg. The amount of Ca in the gastrocnemius, the ventricle and the bladder muscles of the prediabetic KK-CA	extsuperscript{v} mice was significantly higher than that in the normal ddY mice. When compared with age-matched ddY mice, the Ca content in the diaphragm, the gastrocnemius, and the bladder muscles in the prediabetic mice (8 wks) was also significantly higher. These results demonstrate that the increased amount of Ca in these muscles is due mainly to a strain difference but not the age factor. These results are supported by the report that the muscle Ca content does not change with age in normal subjects (Bertorini et al., 1982). In contrast, we observed greater reciprocal decrease in Mg in these muscles except the bladder from the prediabetic mice than in those from the normal ddY mice. However, Mg in these muscles except the bladder in the prediabetic mice (8 wks) does not significantly differ from that in age-matched normal ddY mice. These results mean that the amount of Mg is influenced by factors of age in addition to a strain difference.

The abnormality of Ca and Mg content in the diabetic muscles became more evident when the results were expressed as the ratio of the Ca to Mg (Ca/Mg) (Table 2). The Ca/Mg ratio for the gastrocnemius muscle from the diabetic mouse was significantly higher than that of the prediabetic mouse. These results suggest that the increase in the Ca in the gastrocnemius muscles, especially in Ca/Mg ratio, plays a role in the pathogenesis of diabetes mellitus. The Ca/Mg ratio for the four kinds of muscle from the prediabetic KK-CA	extsuperscript{v} mouse was significantly higher than that for the corresponding normal muscle. The effect of a strain difference on the Ca/Mg ratio is clearly observed in the ventricle > the bladder > the diaphragm > the gastrocnemius muscles in this order.

The Ca and Mg content was compared with values reported previously to justify our experimental procedure. The Ca content of heart muscle was 4.0 or 5.0 mg/100 g wet tissues in ICR mouse (Hamuro et al., 1970), and 3.0 or 3.2 mg/100 g wet tissues in rat (Wallach and Verch, 1987). Our finding in the ventricle in the normal ddY mouse was 3.9 or 4.1 mg Ca/100 g, and is almost similar to these published values. Our Mg reading in the ddY mice was 23.9 mg/100 g wet diaphragm, 27.6 mg/100 g wet gastrocnemius, and 22.2 mg/100 g wet ventricle. These values are also similar to values already reported in rat muscles (Abraham et al., 1981), rabbit muscles (Aikawa, 1960), rat heart (Wallach and Verch, 1987; Abraham et al., 1981), and rabbit heart muscle (Aikawa, 1960). These Ob-
servations indicate that our experimental procedure for the measurement of cellular metal ions is justified. Our results also reveal marked changes in the amounts of the trace metals Fe and Zn in these muscles of the diabetic KK-CA\textsuperscript{v} mice. The Fe is contained in ferritin, hemosiderin and myoglobin in the skeletal muscle cells. The Fe content of the diaphragm and the gastrocnemius muscles of the diabetic KK-CA\textsuperscript{v} mouse was significantly increased by the diabetic state (Fig. 5). These results are supported by studies in streptozotocin (STZ)-diabetic rats (Failla and Kiser, 1981; Johnson and Evans, 1984). The Fe content of these muscles except the bladder in prediabetic KK-CA\textsuperscript{v} mice was also higher than that of normal ddY mice. The mechanism of Fe metabolism in the diabetic state is not yet clear.

The Zn content of the gastrocnemius muscle in the KK-CA\textsuperscript{v} mouse was significantly increased by the diabetic state, but the ventricle Zn content was significantly decreased (Fig. 6). This result in the gastrocnemius muscles is supported by studies in STZ-diabetic rats (Failla and Kiser, 1981). Failla and Kiser reported that tissue Zn content is correlated with cytosol Zn content (1981). The bladder Zn in the KK-CA\textsuperscript{v} mice was also increased by the diabetic state. These results may be related to some reports that hyperzincuria is observed in various forms of diabetes mellitus (Kinlaw et al., 1983; Levine et al., 1983; Lau and Failla, 1984) and resulted from glucose-mediated processes (Lau and Failla, 1984). In addition, the Zn content in the ventricle is increased, and the bladder Zn is decreased by strain and/or age.

In conclusion, the amounts of Ca and Mg in the gastrocnemius muscles were increased by the diabetic state of the KK-CA\textsuperscript{v} mice, although these abnormal metabolic processes in the diabetic mice were also induced by inherent properties. In addition, the diabetic state increased the Fe content of the diaphragm and gastrocnemius muscles.

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