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Inability of selenium to affect creatine metabolism in rat muscle*

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1. Two groups of adult rats were placed in a metal-free environment and pair-fed with selenium-supplemented and Se-deficient diets.

2. After 5 months the animals were killed and skeletal muscle concentrations of creatine, creatine phosphate, ATP, protein and Se were determined.

3. Se deficiency was indicated by the low Se content of the skeletal muscle from the deficient animals, but no changes were found in the amounts of the other components.

4. These results suggest that Se may not be involved in creatine metabolism and that Se deficiency may not be concerned independently in the development of nutritional dystrophy, where changes are found in the levels of protein, creatine, creatine phosphate and ATP.

Free creatine taken up from the bloodstream by skeletal muscle is partially converted to creatine phosphate. Energy stored in this compound is the direct source of ATP for muscle when energy is needed during exercise. Fitch & Rahmanian (1969) have reported that the creatine content of skeletal muscle is lowered in hereditary muscular dystrophy in mice, even though the entry of creatine into dystrophic muscle exceeds that of normal tissue. Vignos & Warner (1963) found that creatine phosphate and ATP levels are low in progressive muscular dystrophy in humans. These findings suggest that creatine is not retained by dystrophic muscle, possibly the result of a defect in creatine metabolism. Schwarz & Foltz (1957) have shown that selenium is an essential trace element in mammals. Muth, Oldfield, Shubert & Remmert (1959) found that this element had a curative effect on white muscle disease in lambs and calves, and that the creatinuria which accompanied this myopathy disappeared following recovery. These findings indicate that lesions in creatine metabolism may be involved in the aetiology of myopathy and that Se may be involved in creatine metabolism.

A preliminary report from this laboratory (Barak, Beckenhauer & Blotcky, 1972) showed that the total creatine content of skeletal muscle did not change in rats fed on a Se-deficient diet for 3 months. As total creatine is composed of both free creatine and creatine phosphate it is possible that the concentrations of these substances could change with respect to each other and not be reflected in total creatine values. The present study was done to determine whether deficiency of Se in the

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Table 1. *Composition (g/kg) of Se-deficient diet* fed to rats*

Torula yeast	300
Sucrose	590
Lard, tocopherol-free	50
Salt mixture (Hubbell, Mendel & Wakeman, 1937)	50
Vitamin mixture†	10

* Burk, Whitney, Frank & Pearson (1968); purchased from Teklad Inc., Mamouth, Illinois, USA.
† Supplying (mg/kg diet): cyanocobalamin 0·1, biotin 1, calcium pantothenate 2, menaphthone 1, riboflavin 2·5, thiamin HCl 0·4, retinol 14, ergocalciferol 8, D-α-tocopheryl acetate 200.

rat affected the individual concentrations of creatine metabolites for the muscle, and also that of ATP, an important product of creatine metabolism in this tissue. Although Se deficiency was obtained in the original study (Barak *et al.* 1972) in the present study the deficiency was extended to test the effect of a longer period of Se-deficiency on creatine metabolism in muscle.

EXPERIMENTAL

Twelve adult male rats of the Sprague–Dawley strain (SASCO Animal Breeders, Omaha, Nebraska, USA) were used in this study. These rats were separated into two groups of six each and were pair-fed. The experimental rats were given the Se-deficient (Torula yeast) diet of Burk, Whitney, Frank & Pearson (1968), the composition of which is shown in Table 1, and which was purchased from Teklad Inc., Mamouth, Illinois, USA. The control rats received the same diet to which was added 0·4 mg Se as sodium selenite/kg diet. The rats received these diets for a period of 5 months during which they were housed in a metal-free environment. The tocopherol content of the diet was sufficient to prevent muscular dystrophy in both groups during this period. All animals were given *ad lib.* demineralized water from glass drinking containers.

The rats were anaesthetized with diethyl ether and the gastrocnemius muscle was removed and freeze-dried at −70°. The muscle was then dissected, weighed rapidly using a torsion balance, and placed in a stainless-steel percussion mortar, previously cooled to −70°, and powdered. Perchloric acid (0·6 M) was added (3·2 ml/g tissue powder) and this mixture was frozen at −70°, powdered, transferred to a 5 ml homogenizing flask and warmed to 2° using an ice bath. The mixture, in an ice bath, was homogenized at medium speed for 30 s using a Model 45 homogenizer (The VirTis Co., Gardiner, New York, USA). The homogenate was centrifuged at 8000 ***g*** for 10 min at 0°, and the ATP and creatine phosphate contents of the supernatant fraction were estimated. The amounts of both components were estimated enzymically using a single incubation procedure which was a combination of those described by Lamprecht & Trautschold (1965) and Lamprecht & Stein (1965). It was also convenient to estimate the glucose-6-phosphate (G-6-P) content using the same procedure. The reaction sequence was followed spectrophotometrically by

Table 2. *Concentrations of creatine compounds (μmol/g protein) for gastrocnemius muscle from adult male rats pair-fed with selenium-deficient and Se-supplemented (0·4 mg/kg diet) diets*

(Mean values with their standard errors for six rats/group)

Diet*	Free creatine	Creatine phosphate	Total creatine
Se-supplemented (control)	1280 ± 89	146 ± 10	1426 ± 92
Se-deficient	1467 ± 74	171 ± 7	1630 ± 64

* For details, see Table 1.

monitoring the change in extinction at 340 nm associated with the reduction of NADP. The sequence began with the addition of glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49) and the G-6-P content was estimated from the amount of NADPH produced. Glucose was then added and a new 'zero level' was established for the extinction at 340 nm. Hexokinase (*EC* 2.7.1.1) was added and the ATP content was estimated from the amount of NADPH produced with hexokinase. ADP was then added to the incubation mixture, another new 'zero level' was established and creatine kinase (*EC* 2.7.3.2) was added to convert creatine phosphate to creatine, and ADP to ATP. The creatine phosphate content was determined from NADPH production at this stage of the reaction sequence. In this sequence of reactions, 1 μmol G-6-P, ATP or creatine phosphate produced 1 μmol NADPH.

The gastrocnemius muscle from the other hind leg of the rat was dissected and used for the analysis of total creatine, Se and protein. Total creatine content was estimated using the method of Ennor & Rosenberg (1952) and the amount of free creatine was calculated by subtracting the value obtained for creatine phosphate. Se content was determined by the neutron-activation method of Blotcky, Arsenault & Rack (1973). Protein content was determined using the method of Lowry, Rosebrough, Farr & Randall (1951). The concentrations of each component were expressed on a protein basis (μmol/g muscle protein). The statistical method used for analysis of the results was Student's *t* test.

RESULTS

The mean initial body-weight for the rats was 200 g. At the end of the experiment the mean body-weight for the control rats was 453 (SD 21)g and the corresponding value for the Se-deficient rats was 417 (SD 19)g; the difference was not significant. Table 2 shows the concentrations of free creatine and creatine phosphate for skeletal muscle from rats fed on Se-deficient and Se-supplemented diets. The results suggested that the inclusion of 0·4 mg Se/kg diet had no effect on the concentrations of creatine metabolites for rat muscle.

The results in Table 3 indicated that dietary Se maintained a normal level of this element for muscle from Se-supplemented rats and that the Se-deficient diet produced a 7-fold decrease in Se concentration for this tissue. Although Se content

Table 3. *Concentrations of protein, selenium, ATP and glucose-6-phosphate (G-6-P) for gastrocnemius muscle from adult male rats pair-fed with Se-deficient and Se-supplemented (0·4 mg/kg diet) diets*

(Mean values with their standard errors for six rats/group)

Diet†	Protein (mg/g muscle)	Se (μg/g protein)	ATP (μmol/g protein)	G-6-P (μmol/g protein)
Se-supplemented (control)	143 ± 9·0	1·45 ± 0·15	43·7 ± 2·3	8·0 ± 1·2
Se-deficient	132 ± 9·5	0·21 ± 0·16*	49·8 ± 4·2	8·9 ± 1·0

* Significantly lower than the control value: $P < 0·001$.
† For details, see Table 1.

was lowered, the concentrations of protein, G-6-P and ATP, a product of creatine metabolism in muscle, were unchanged.

DISCUSSION

It has been generally accepted that vitamin E and Se are intimately related in intermediary metabolism in muscle, although their function in the maintenance of the integrity of muscle is not understood. Although the diet used in this study was Se-deficient, it contained adequate amounts of α-tocopheryl acetate (200 mg/kg), and after 5 months on the diet the animals did not develop any symptoms of nutritional dystrophy. After this period of Se deficiency, the changes found in the amounts of muscle constituents were not those reported for various dystrophies. The reported changes in protein content for the skeletal muscle from mice with hereditary dystrophy (Weinstock, Epstein & Milhorat, 1958; Fitch & Rahmanian, 1969) and creatine levels for muscle from the same animals (Fitch & Rahmanian, 1969) were not found in this study. Also the changes in muscle creatine phosphate and ATP contents found by Vignos & Warner (1963) during progressive muscular dystrophy in humans and by Barak, Allison & Sorrell (1974) in hereditary muscular dystrophy in mice were not found in the Se-deficiency of this study. These results suggest that Se may not be linked to creatine metabolism in rats and also that Se is less likely to have an independent function in the aetiology of myopathy in animals. The process by which Se relieves nutritional myopathies in domestic animals remains unclear.

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