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Effects of 6-days nitrate supplementation on $[Ca^{2+}]$ and CRT in skeletal muscle of exhausted rats

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Objective There are many active ingredients in sports nutrition, and nitrate is gradually being valued by sports nutrition and product developers. Supplementation of nitrate is a practical method to increase circulating plasma nitrite, thereby increasing NO bioavailability. However, the existing research has rarely reported the dose effect between nitrate supplementation and changes in exercise efficiency and capacity. The mechanism of action of nitrate is not fully understood yet. The aim of this study was to analyze the effects of different doses of nitrate on the exercise capacity of rats, as well as the detection of $[Ca^{2+}]$ and calreticulin (CRT) expression in the gastrocnemius, soleus and extensor digitorum longus, trying to figure out the effects of different doses of nitrate supplementation on calcium homeostasis in different types of muscle fibers.

Methods 40 SD rats (8-week-old) weighing 270-290 grams were randomly divided into control group (C group, 8), exercise control group (EC group, 8), exercise with low-dose supplementation group (ELN group, 8), exercise with medium-dose supplementation group (EMN group, 8) and exercise with high-dose supplementation group (EHN group, 8). Sodium nitrate was used as exogenous nitrate for oral gavage. The intragastric concentration was 0.3 mmol/day/kg body weight in the ELN group, 0.7 mmol/day/kg body weight in the EMN group, and 1.0 mmol/day/kg body weight in the EHN group. The others were orally administered with normal saline. All exercise groups (EC, ELN, EMN and EHN) underwent 3 days adaptive low-intensity treadmill training with slope 0°, speed 16 meter/min, and the time is 5 min, 10 min and 15 min incrementally. 24 hours after the end of the last gavage, a one-time exhaustion treadmill experiment was started. The running platform slope was -16°, the speed was 16 meter/min. Exhaustive experiment participants were not aware of the grouping of rats. Immediately after the end of exhaustive exercise, the rats were weighed and anesthetized with sodium pentobarbital solution. Blood is collected for testing $[NO_2^-]$. The gastrocnemius, soleus and extensor digitorum longus were collected for testing NOS activity, tissue $[Ca^{2+}]$ and CRT expression.

Results (1) Compared with the EC group, the exhaustion time of the other exercise groups was prolonged. The exhaustion time of the EMN group was very significantly prolonged from that of the EC group ($P < 0.01$). The exhaustion time of the EMN group was significantly prolonged from that of the EC group ($P < 0.05$). At the same time, the difference between the EMN group and the ELN group was statistically significant ($P < 0.05$). (2) Serum $[NO_2^-]$ in the supplemented nitrate groups (ELN, EMN and EHN) was higher than that in the EC group, and the difference was statistically significant ($P < 0.01$). The serum $[NO_2^-]$ in different groups (ELN, EMN and EHN) raised with the increase of nitrate concentration, and the difference between each adjacent concentration group was statistically significant ($P < 0.05$). (3) In the gastrocnemius, soleus and extensor digitorum longus respectively, there were no significant differences in TNOS, iNOS and cNOS activities between the groups ($P > 0.05$). (4) In the gastrocnemius, compared with the EC group, the $[Ca^{2+}]$ ($P < 0.05$) and CRT expression ($P < 0.05$) in the EMN group were significantly decreased. There was no difference between the ELN vs EC group ($P > 0.05$) and the EHN vs EC group ($P > 0.05$). (5) In the soleus, the $[Ca^{2+}]$ ($P < 0.05$) and CRT expression ($P < 0.05$) in the EMN group were significantly decreased. There was no difference between the ELN vs EC group ($P > 0.05$) and the EHN vs EC group ($P > 0.05$). (6) In the extensor digitorum longus, compared with the EC group, the $[Ca^{2+}]$ ($P < 0.05$) and CRT expression ($P < 0.05$) in

the EHN group were significantly decreased. There was no difference between ELN and EC group ($P>0.05$). The expression of CRT in EMN group was significantly decreased ($P<0.05$), but there is no difference of $[Ca^{2+}]$ between EMN and EC group ($P<0.05$).

Conclusions (1) 6-day sodium nitrate supplementation is a reliable method to increase serum $[NO_2^-]$ concentration. (2) 6-day sodium nitrate supplementation can prolong the duration of one-time exhaustive exercise in rats, and the dose of 0.7mmol/kg/d is the best. (3) Sodium nitrate supplementation can affect the $[Ca^{2+}]$ and the expression of CRT in skeletal muscle after one-time exhaustive exercise. Different concentrations of sodium nitrate have different effects on different types of muscle fibers.