Muscular Metabolism in Aged Rats Under Exhaustive Exercise: Effect of a Modified Alkaline Supplementation

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ABSTRACT

A modified alkalizing supplementation (MAS) was tested on skeletal muscle metabolism in aged rats undergoing exhaustive exercise. Aged Wistar rats were allocated into two groups: saline (A) and saline added with 16 mg of MAS (B) before treadmill exercise. Blood and gastrocnemius and soleus muscle were analyzed after exercise for succinate dehydrogenase (SDH), acetyl carnitine (ALCAR), and glycogen. Lactic acid (LA), creatin-phosphokinase (CPK), and gas analysis were tested in the blood. Exercise caused a significant increase of LA and CPK and muscle glycogen fall. Arterial desaturation at exhaustion was prevented in the B group (p < 0.05). Exercise-induced increase of SDH and ALCAR was further enhanced in B rats (p < 0.05). This study suggests that MAS can improve fast and endurance muscle metabolism in aged rats by increasing cellular acetyl group availability and tricarboxylic acid turnover.

INTRODUCTION

Increasing blood bicarbonate concentration has long been cited as a potential ergogenic mechanism improving contractile function¹ although there are scanty reports in aged animals. Despite the fact that skeletal muscle is relatively impermeable to bicarbonate ions, the increased extracellular base concentration resulting from bicarbonate administration is believed to buffer hydrogen ions released by muscle during contraction, thus beneficially modifying the pH gradient between muscle and surrounding extracellular fluid. Another mechanism by which bicarbonate may improve contractile function is by increasing the availability of acetyl-CoA towards the tricarboxylic acid cycle.² The aim of this study was to test whether a modified alkalizing supplementation could improve muscular metabolism and adaptation to exhaustive exercise in aged rats by examining either the gastrocnemius (fast muscle related to instantaneous force) and the soleus (slow muscle related to endurance).

MATERIALS AND METHODS

Animals and treatment

Male Wistar rats (14 months old) were individually housed in a temperature-controlled room, under a 12 h light/dark cycle for 1 week before use in the experiment. All animals were familiarized with a motor-driven treadmill for 3 days, 5 min/day, on a
5% grade. A total of 20 rats were randomly divided into two groups (10/group): the control and alkaline-supplemented groups. Rats in both the control group and the alkaline-supplemented one were subjected to treadmill exercise. Five unexercised rats represented the internal resting control. The exercise-loaded rats were made to run on a mechanical treadmill apparatus (Seibukusho Biolectric, Tokyo, Japan), consisting of a wide, endless belt riding on metal rollers. An acrylic box, partitioned into ten individual compartments, was suspended to cover the belt, providing a limited running area for each rat. Motivation was provided by an electric shock needle plate at the rear of each compartment. The belt was set at an angle of 10 degrees, running at a constant speed of 20 m/min. Alkaline supplementation was administered orally through a naso-gastric tube at a dose of 16 mg (calcium bicarbonate, sodium bicarbonate, magnesium and potassium carbonate, and bisodium phosphate; Basenpulver, Named, Italy) 30 min before the exercise while the control group was administrated distilled water orally. All rats were killed by cervical dislocation either at rest (control) or at the point of exhaustion, scheduled at the same time of the day to eliminate diurnal effects and 4 h after their last meal (fed state). The intensity of the acute bout of exhaustive exercise (25 m/min and 10% grade) was selected to represent a relative workload of 70–75% of their maximal oxygen uptake. Exhaustion was defined as the inability of the rats to run on the treadmill despite mild electric shocks and to upright themselves when placed on their back. Blood and gastrocnemius and soleus samples were collected immediately after the exercise loading. Blood was taken from the abdominal aorta using a heparinized syringe with the rat anesthetized.

**Blood biochemistry**

A 50 μL aliquot of the pre- and post-exercise blood samples was immediately mixed with 200 μL of ice-cold 7% perchloric acid and centrifuged at 1500 g for 10 min at 4°C. The supernatant was analyzed enzymatically in duplicate for lactate content. The serum CK activity was determined by a colorimetric assay, at 340 nm, and was expressed in U/L, where 1 U resulted in the phosphorylation of 1 mmol of creatine per min at 25°C.

**Tissue preparation and assay of ALCAR and glycogen**

After cervical dislocation, hind limb muscles were rapidly removed. Portions of red and white gastrocnemius and soleus were immediately frozen (within 2 s) in liquid N2, dissected free of nonmuscular components, connective tissue, and blood, and stored until used for biochemical analysis. An aliquot of freeze-dried muscle powder was then extracted in 0.5 M perchloric acid containing 1 mM EDTA; the supernatant was neutralized with 2.2 M KHCO3 and was then used for the spectrophotometric determination of acetyl-carnitine and muscle glycogen.3 Muscles were boiled in 30% KOH saturated with Na2SO4 until homogenization (usually 30 min). Homogenates were kept on ice, and glycogen was precipitated by addition of a 1.2 volume of 95% ethanol. Samples were centrifuged for 30 min at 840 g, and pellets were resuspended in H2O. Assays were conducted on aliquots against appropriate blanks at 490 nm. Results were determined from a standard curve generated at the same time and are expressed in milligrams of glycogen per gram of tissue. Separate gastrocnemius and soleus samples were removed from the hind limb and frozen immediately in liquid nitrogen for measurement of succinate dehydrogenase (SDH) activity.

**Determination of SDH activity**

Frozen gastrocnemius and soleus samples were homogenized for 2 min with 5 volumes of 0.3 M phosphate buffer (pH 7.4). Sodium succinate was added to yield a final concentration of 17 mM and mixed for 2 min. Then, sodium cyanide, cytochrome c, aluminum chloride, and calcium chloride were added to final concentrations of 1, 17, 0.4, and 0.4 mM, respectively. This mixture was measured within a few minutes at 550 nm. SDH activity was calculated from the ferric-cytochrome c concentration and protein content.
Protein assay

Protein contents were determined by the method of Lowry et al.,4 with bovine serum albumin as a standard.

Statistical evaluation

Statistical analysis was performed using Student's *t*-test and one way analysis of variance (one way-ANOVA). The accepted level of significance was preset as *p* < 0.05. Data are represented as means ± SEM.

RESULTS

Body weight changes were not influenced by either exercise or Basenpulver treatment (data not shown) and food consumption was similar between the groups of rats.

Blood lactate and CPK

Compared to resting animals, exercise rats showed a significant increase of LA and CPK in both plasma and muscle (*p* < 0.001), and there was no difference between groups at the muscular level. This phenomenon was partly mitigated in alkaline supplement-fed rats (*p* < 0.05).

Muscle glycogen and ALCAR concentration

A major decrease in glycogen concentration occurred, with around 56% reductions in the control-exercise group compared with the resting control group (*p* < 0.01), with no difference among the two groups (data not shown). As shown in Figure 1, ALCAR concentration in the muscle samples was not affected by exercise. However, alkalizing supplementation brought a significant increase in both muscle samples (*p* < 0.05).

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**FIG. 1.** Effect of alkaline supplementation on SDH, CPK, ALCAR, and lactate. BP, Basenpulver. *p* < 0.01, **p* < 0.05.
**SDH activity in skeletal muscle**

SDH activity in skeletal muscle was measured as an index of tricarboxylic acid cycle turnover. As shown in Figure 1, SDH activity in the gastrocnemius and soleus muscle of the exercise group was found to be significantly higher in comparison with baseline \((p < 0.01)\). Alkaline supplementation significantly upregulated this increase compared with the control group, and such effect was more evident in the gastrocnemius muscle \((p < 0.01)\).

**Blood gas analysis**

Arterial desaturation and pH at exhaustion was significantly lower compared to resting rats \((pO_2, 87.6 \text{ vs. } 98.7; \text{pH } 7.32 \pm 0.02; \text{bicarbonate, } 7.51 \pm 0.01; p < 0.01)\) but this phenomenon was prevented in the B group \((p < 0.05)\) (data not shown). There was no effect of alkalinizing supplementation on pCO\(_2\) value.

**DISCUSSION**

It is known that muscle can produce 30–32 molecules of ATP *via* the tricarboxylic acid cycle in the presence of an adequate oxygen supply and only two through anaerobic glucose metabolism when the supply of oxygen is insufficient. Moreover, it has been reported that endurance training increases the activities of enzymes involved in both aerobic glucose metabolism and the tricarboxylic acid cycle.\(^5,6\) This study suggests that a specifically balanced alkaline supplementation is able to beneficially influence muscle metabolism by increasing cellular acetyl group availability and tricarboxylic acid turnover also in aged rats during exhaustive exercise. This is in agreement with an *in vitro* study proposing that any strategy that increases the mitochondrial availability of acetyl groups and concomitantly increases blood bicarbonate content may improve the maintenance of contractile function.\(^2\) This may also explain the observed increase of acetyl carnitine,\(^5\) which is known to fluctuate during contraction according to acetyl-CoA availability. In agreement, Hollidge-Horvat et al.\(^6\) have reported a trend increase of acetyl-carnitine content in human skeletal muscle following sodium bicarbonate ingestion. Interestingly, it has been shown that the cellular carnitine pool has to be near maximally acetylated (~80%) to raise mitochondrial acetyl group availability to the extent whereby it can match the demands of the tricarboxylic acid cycle and overcome the acetyl group deficit at the onset of contraction.\(^2\) Taken together, these preliminary data suggest that a balanced alkaline supplementation proves to be beneficial in fast and endurance muscle metabolism in aged rats with a likely ergogenic effect.

**REFERENCES**


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