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Effect of Hypophosphatemia on Muscle Metabolism after Exercise in Pigs

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Håglin, L. and B. Essén-Gustavsson: Effect of hypophosphatemia on muscle metabolism after exercise in pigs. Acta vet. scand. 1992, 33, 139-145. – Five Swedish Landrace pigs with a mean weight of 51±5 kg performed an exercise test on a treadmill at a speed of 1.8 m/s and a duration of 10 min. Hypophosphatemia was then induced in these pigs by addition of aluminium hydroxide (liquid antacid) to the normal feed. After 3 weeks, the exercise test was repeated when the mean weight of the pigs was 65±9 kg. Five other Swedish Landrace pigs with a mean weight of 72±4 kg performed a similar exercise test. Muscle biopsies from M. biceps and blood samples were taken from all pigs 3-5 days before and immediately after each exercise test. Hypophosphatemic pigs had significantly lower serum phosphate and higher aluminium levels than normophosphatemic pigs. In all pigs, glycogen content in muscle decreased significantly (-108 to -135 mmol/kg muscle) with exercise while no changes were seen in adenosine triphosphate, creatine phosphate or inorganic phosphate concentrations. In normophosphatemic pigs, glucose-6-phosphate and lactate concentrations increased significantly during exercise by 2-4 mmol/kg and 12.8-14.4 mmol/kg, respectively. However, in hypophosphatemic pigs, glucose-6-phosphate concentrations decreased significantly during exercise by 4.4 mmol/kg and lactate levels were unchanged. These results indicate that low serum inorganic phosphate levels influence muscle metabolism and glycolysis in connection with physical exercise.

adenosine triphosphate; creatine phosphate; glucose-6-phosphate; lactate; aluminium.

Introduction

Muscle weakness and myocardial dysfunctions in humans can be associated with hypophosphatemia induced by long-term treatment with antacids *(Boelens et al.* 1970, *Darsee & Nutter* 1978, *Lotz et al.* 1968). A reversible depression of myocardial performance and respiratory muscle weakness has also been observed in human patients who have low serum phosphate levels *(Davis et al.* 1988, *Gravelyn et al.* 1988, *O'Connor et al.* 1977).

From experimental animal studies it is known that hypophosphatemia can affect both cardiac and skeletal muscle function and metabolism. Diet induced phosphate depletion in dogs lowered stroke volume and peak blood flow *(Fuller et al.* 1978). With repletion of phosphorus the myocardial performance was improved *(Fuller et al.* 1978). Reversible changes in skeletal muscle composition and transmembrane electrical potential have been observed with moderate phosphorus depletion in dogs *(Fuller et al.* 1976). It has also been suggested that a subclinical myopathy in dogs may set the stage for rhabdomyolysis if acute, severe hypophosphatemia is superimposed *(Knochel et*

al. 1978). From a study on phosphate deficient mice it has been suggested that low levels of phosphate in serum and muscle give rise to a slower rate of ATP synthesis and delay recovery of skeletal muscle contractility *(Hettleman et al. 1983)*. Diet induced phosphate depletion in rats also showed that both serum phosphate and inorganic phosphate levels were low when an impaired energy metabolism was seen in myocardial and skeletal muscle *(Brautbar et al.* 1982, *Brautbar et al.* 1983a). However, ATP and creatine phosphate (CP) levels were unaltered in skeletal muscle but lowered in the myocardium. This difference was said to be attributed to the higher level of activity and need for continous energy production of heart as compared with skeletal muscle.

A high demand for energy production in skeletal muscles is required in connection with physical exercise. Is hypophosphatemia associated with an impaired availability of ATP in this situation? Hypophosphatemia can be induced in pigs with phosphate-binding antacids *(Håglin et al.* 1988). In this study both normo- and hypophosphatemic pigs exercised on a treadmill to determine if low phosphate levels influence skeletal muscle metabolism during physical exercise.

Materials and methods

Animals and experimental design

Swedish Landrace pigs kept in pens and fed the same diet were used in this study. Five pigs (group 1) were accustomed to a treadmill by walking on it for a few min on 5-6 separate days. These pigs then performed an exercise test on the treadmill at a speed of 1.8 m/sec for 10 min. The exercise test was repeated after 3 weeks when hypophosphatemia had been induced in these pigs by mixing phosphate binding antacid with their feed every day for 3 weeks. Aluminium hydroxide suspension (0.97 mmol $Al(OH)_3$ was given in a dose of 250 ml/kg feed which is equivalent to about 8-10 ml/kg body weight/day corresponding to 4.8-5.9 mg Al/kg body weight/day. After 3 weeks on this diet these pigs were hypophosphatemic (group 1-H). Five normophosphatemic pigs of similar weight (group 2) also performed an exercise test after becoming accustomed to the treadmill. Muscle biopsies from *M. biceps* and blood samples from the superior *vena cava* were taken on all pigs under anaesthesia with sodium pentobarbital 3-5 days before and as soon as possible (2-4 min) after each exercise test. The muscle biopsies were taken with a biopsy needle (diameter 5-6 mm). Blood samples were taken using aluminium-free syringes.

Muscle samples

The muscle biopsy samples were freeze-dried, dissected free of blood, fat and connective tissue and portions were weighed (1-5 mg). After boiling 1 portion of muscle tissue from each pig for 2 h in 1 ml of 1M hydrochloric acid glycogen was analyzed on the samples as glucose residues. Glucose was analyzed with a fluorimetric technique *(Lowry & Passonneau* 1973). Another portion of muscle tissue was homogenised in perchloric acid and neutralized with $KHCO_3$. After centrifugation inorganic phosphate, glucose-6-phosphate, adenosine triphosphate (ATP), creatinephosphate (CP) and lactate concentrations were determined on the supernatant with fluorimetric techniques according to *Lowry & Passonneau* (1973). The methodological error associated with these analyses was 5-7% expressed as the coefficient of variation for a single determination.

Blood samples

Whole blood was pipetted into perchloric

acid and lactate concentration was determined enzymatically (Boehringer Test combination No 124842). Serum phosphate was analyzed with a spectrophotometric method according to *Kallner (1975).* Aluminium was analyzed in serum by atomic absorption spectroscopy according to *Frech et al. (1982).* The methodological error for these analyses was <10% expressed as the coefficient of variation for a single determination.

Statistics

Conventional statistical methods were used to calculate means and standard errors (SE). Unpaired and paired Student's t-tests were used to evaluate differences between groups and between pre- and post- exercise samples. Statistical significance was declared at a probability of $p<0.05$.

Results

The mean body weight of the pigs in the normophosphatemic group 1 was 51±5 kg. These pigs became hypophosphatemic after 3 weeks of aluminium hydroxide treatment (group 1-H) and their mean body weight was significantly increased to 65±9 kg. The normophosphatemic pigs in group 2 had a mean weight of 72±4 kg.

Serum phosphate, blood lactate and serum aluminium levels before and after the exercise test are shown in Table 1. Aluminium hydroxide treatment of the pigs in group 1 during 3 weeks caused a significant reduction in serum phosphate concentration. Serum aluminium concentration was higher in hypophosphatemic than normophosphatemic pigs. Blood lactate levels were significantly increased in group 1 and group 1-H after the exercise tests (Table 1). Four of the 5 pigs had increased blood lactate levels in group 2 giving a significance level of $p<0.07$.

The concentrations of inorganic phosphate, glucose-6-phosphate, adenosine triphosphate, creatine phosphate, lactate and glycogen in skeletal muscle before and after the exercise tests are shown in Table 2.

Glycogen content had significantly decreased

Table 1. Serum phosphate, blood lactate and aluminium levels at rest and after treadmill exercise in normo- and hypophosphatemic pigs. Data are given as mean± SE.

Blood parameter	Time of blood sampling	Group 1 Normophosphatemic (n=5)	Group 1-H Hypophosphatemic (n=5)	Group 2 Normophosphatemic (n=5)
Serum phosphate (mmol/l)	Rest	3.1±0.1[b]	1.6±0.1	3.4±0.1[b]
	After exercise	3.3±0.1[b]	1.7±0.1	3.1±0.1[b]
Blood lactate (mmol/l)	Rest	2.7±0.1	2.6±0.3	4.4±0.7
	After exercise	6.6±0.8[a]	5.5±0.8[a]	7.4±1.2
Serum aluminium (µmol/l)	Rest	–	–	–
	After exercise	–	1.37±0.09	0.28±0.10

n = no. of animals.
a = significant difference as compared with resting value.
b = significant difference as compared with group 1-H.

Table 2. Muscle inorganic phosphate, adenosine triphosphate (ATP), creatine phosphate (CP), glucose-6-phosphate (G-6-P), lactate and glycogen levels at rest and after treadmill exercise in normo- and hypophosphatemic pigs. Data are given as mean± SE.

Muscle parameter	Time of muscle sampling	Group 1	Group 1-H	Group 2
		Normophosphatemic (n=5)	Hypophosphatemic (n=5)	Normophosphatemic (n=5)
Inorganic phosphate (mmol/kg)	Rest	–	46.6±1.4	50.4±2.5
	After exercise	–	46.4±2.4	54.4±3.7
G-6-P (mmol/kg)	Rest	10.4±3.5	7.3±1.6	6.3±1.4
	After exercise	14.4±3.4[ab]	2.9±0.6[a]	8.4±1.4[ab]
ATP (mmol/kg)	Rest	22.2±0.5	23.1±0.3	23.5±0.9
	After exercise	20.3±1.0	20.3±1.0	24.3±0.7
CP (mmol/kg)	Rest	69.1±9.4	55.4±4.3	55.7±2.3
	After exercise	69.0±6.3	54.1±4.7	49.6±5.6
Lactate (mmol/kg)	Rest	54.1±9.2	37.1±4.5	32.4±1.7
	After exercise	68.5±5.3[ab]	33.7±4.3	45.2±3.6[a]
Glycogen (mmol/kg)	Rest	438±17	454±7	375±30
	After exercise	330±5[a]	330±26[a]	240±36[a]

n = no. of animals.
a = significant difference as compared with resting value.
b = significant difference as compared with group 1-H.

in all groups after the exercise test and the mean decrease was 108 mmol/kg for group 1, 124 mmol/kg for group 1-H and 135 mmol/kg for group 2. No changes were seen in ATP, CP or inorganic phosphate levels with exercise.

In the normophosphatemic groups 1 and 2, G-6-P levels increased significantly during exercise by 4.0 and 2.1 mmol/kg, respectively, whereas in group 1-H, G-6-P levels decreased significantly by 4.4 mmol/kg. Lactate levels increased significantly during exercise by 14.4 mmol/kg in group 1 and 12.8 mmol/kg in group 2. No significant change was seen in lactate level in group 1-H.

Discussion

Hypophosphatemia was induced in the pigs by giving phosphate binding antacids. This model was used since an earlier study showed that low serum phosphate levels can be obtained already after 3 weeks with this treatment *(Håglin et al.* 1988). That study also showed that a suspension of aluminium phosphate did not induce similar alterations in the measured parameters as seen with a suspension of aluminium hydroxide. Pigs are fast growing animals and in this study they gained a mean of 15 kg during the 3 weeks they were fed antacids and developed hypophosphatemia. A control group of pigs that

did not differ in weight from the hypophosphatemic pigs was therefore also studied in order to exclude any effect of body weight on the parameters studied before and after the exercise test.

For practical reasons and in order to get resting samples under similar conditions the muscle biopsies were taken under anaesthesia several days before the actual exercise test. The results showed that the muscle parameters did not differ between the groups before the exercise test. The metabolic response to exercise was similar in both groups of normophosphatemic pigs even if the weight of the pigs differed. When the pigs performed moderate physical exercise on the treadmill we found elevated G-6-P and lactate levels in muscle in both groups of normophosphatemic pigs but in hypophosphatemic pigs the G-6-P level was suppressed and the lactate level unchanged. During this type of treadmill exercise glycogenolysis was important for energy utilization as seen from the lowered glycogen levels after exercise. Glycogenolysis did not seem to be altered by low serum phosphate levels as glycogen breakdown was similar in all groups of pigs. There were only small increases in blood and muscle lactate levels after exercise indicating that most of the energy from glycogen breakdown was obtained through oxidative metabolism of pyruvate. Glucose from blood is another substrate that is of importance for energy production during moderate exercise. The low G-6-P level in the hypophosphatemic pigs therefore indicates that the 1st step of glycolysis in which glucose is phosphorylated may be affected by hypophosphatemia. Glycolysis enhances utilization of phosphate and several studies indicate that marked shifts of phosphate occur from the extracellular fluid to the intramuscular compartment. When normophosphatemic dogs are hyperventilated together with glucose administration, the level of G-6-P increases in the muscle in parallell with a decrease in serum phosphate level *(Brautbar et al.* 1983b). Hind limb preparations from diet-induced hypophosphatemic rats show that glucose uptake is related to serum phosphate levels *(Davis et al.* 1979). In 2 human patients, severe hypophosphatemia developed when they were treated with insulin in connection with hyperglycemia *(Bohannon* 1989). Furthermore, chronic hypophosphatemia of different organs in humans is associated with insulin resistance and glucose intolerance *(DeFronzo & Lang* 1980). Results from the above mentioned studies and from the present study thus all agree with the hypothesis that phosphorylation of glucose is dependent on extracellular phosphate levels. If hypophosphatemia limits the phosphorylation of glucose this would also affect glycogen synthesis. A study on rats has shown that ATP and CP levels were unaltered but G-6-P, glycogen levels and inorganic phosphate content were lowered after 8 and 12 weeks of dietary phosphate restriction as compared with a control group *(Brautbar & Massry* 1984). The limited availability of phosphate for phosphorylation of glucose after exercise may be related to a decrease in total ATP turnover rate due to low serum levels influencing inorganic phosphate levels. ATP, CP and inorganic phosphate levels had not changed in the biopsies taken after exercise in this study. However, one can not exclude the possibility that the levels were different immediately after exercise as it was only possible to take the biopsies a few min after exercise had stopped. It is well known from studies on humans that replenishment of phosphate stores is a quick process whereas G-6-P and lactate levels stay increased for several minutes after exercise *(Essén & Kaijser* 1978). Other explanations

for the low G-6-P levels after exercise in the hypophosphatemic pigs could be a faster degradation of G-6-P or inhibition of the enzyme hexokinase.

It was of interest to note that the concentration of aluminium in serum from the hypophosphatemic pigs was higher than in normophosphatemic animals due to antacid treatment. It has been shown that increased aluminium levels may inhibit the activity of hexokinase in homogenates of various tissues from the guinea pig *(Harrison et al.* 1972). The enzyme hexokinase transfers the phosphate from ATP to glucose and is thereby responsible for the initiating step in glycolysis. Decreased glycolysis in rat brain was found to be due to an inhibition of hexokinase by aluminium *(Lai & Blass* 1984). The question is if the low levels of G-6-P after exercise in this study also may be related to an inhibition of hexokinase by the low, but significant, increase in serum levels of aluminium.

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Sammanfattning

Effekt av hypofosfatemi på muskelmetabolism efter arbete hos gris.

Fem grisar, med en medelvikt på 51 ± 5 kg utförde ett arbetsprov på en rullande matta (1,8 m/sec) under 10 min. Hypofosfatemi inducerades därefter med tillägg av aluminiumhydroxid (flytande antacida) till ett normalt foder. Efter 3 veckor då grisarnas medelvikt var 65 ± 9 kg upprepades arbetsprovet. Fem andra grisar med en medelvikt på 72 ± 4 kg utförde samma arbetsprov.

Muskelbiopsier (M. biceps) och blodprov togs från alla grisar 3-5 dagar före och omedelb art efter arbetsprovet. Hypofosfatemigrisarna hade signifikant lägre koncentrationer av serumfosfat och högre koncentrationer av serumaluminium än normofosfatemigrisarna. Hos alla grisar minskade glykogenhalten signifikant i muskeln (108-135 mmol/kg) med arbete medan koncentrationerna av adenosin trifosfat, kreatin fosfat och oorganiskt fosfat var oförändrade. I muskeln hos normofosfatemiska grisar ökade koncentrationerna av glukos-6-fosfat med 2-4 mmol/kg och laktat med 12,8-14,4 mmol/kg i samband med arbete. Däremot uppstod en signifikant sänkning av glukos-6-fosfat (4,4 mmol/kg) i muskel från hypofosfatemiska grisar och laktatkoncentrationen var oförändrad. Resultaten indikerar att lågt serumfosfat har inverkan på muskelmetabolism och glykolys i samband med fysiskt arbete.

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