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THE INFLUENCE OF PHYSICAL TRAINING ON THE pH OF SKELETAL MUSCLE IN PIGS*

By

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LINDBERG, P., N. LANNEK and L. BLOMGREN: *The influence of physical training on the pH of skeletal muscle in pigs.* Acta vet. scand. 1973, 14, 359—365. — Treadmill training of pigs was shown to retard the pH-drop of longissimus dorsi biopsy samples. Training similarly had an inhibiting effect on the post-mortem pH, ATP and glycogen, and on fluid loss (expressible juice) of the gracilis muscle.

physical training; pH of muscle.

Muscular degeneration (MD) in pigs was described by *Ludvigsen* (1953) as an acute disease in hogs, which are transported to the slaughterhouse. The symptoms are lethargy, hyperpyrexia, dyspnoe, cyanosis, muscular twitches and weakness. Post-mortem changes are mainly paleness and juiciness of skeletal muscles, which are easily separated into bundles. There is a fast pH-drop in the muscles after the pig has been killed, and this is caused by accumulation of lactic acid.

This original observation was followed by a number of published reports (cf. *Briskey* 1964), in which the main interest was focused on the post-mortem changes of the skeletal muscles (pale, soft, exudative). Only recently the disease character of the entity, as first reported by *Ludvigsen*, has received support by important observations on live pigs (*Muyllé et al.* 1968, *Oyaert* 1971).

It is known that some swine breeds are more easily affected than other breeds. We have reported that physical training of pigs by repeated running on a treadmill will increase their resistance to the stress syndrome (*Lannek et al.* 1967, 1971, *Lan-*

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nek 1967, Rülcker 1968). It was found that physical exercise in conventional, i. e. untrained pigs leads to a low level of adenosine triphosphate (ATP) in the muscles. This, by a feed back mechanism, will promote the glycogen breakdown and an excessive accumulation of lactic acid. Trained pigs were shown to have significantly higher muscle ATP after physical stress.

The present paper reports effects of training on pH of the longissimus dorsi and gracilis muscles.

MATERIAL AND METHODS

Pigs weighing about 25 kg were obtained from herds, which are free from manifest enzootic pneumonia and atrophic rhinitis. They were of Swedish Landrace or Landrace - Large White cross strains. Females and castrated males were about equally represented. The pigs were divided into groups comprising 9—11 animals. Each group was kept in a pen measuring 12 m². The pens were in the open air, only provided with a sheltering roof above a wooden platform for sleeping. A commercial allmash meal and water were given ad lib. in separate troughs.

Physical training and short time stress were performed on a treadmill at 2.5 m/sec. as described previously (Rülcker 1968). Pigs in experiment 100 were trained 2 days a week (Tuesday and Friday) and pigs in experiment 102, 105 and 106 3 days a week (Monday, Wednesday and Friday). Training was started shortly after the pigs had arrived, and continued until samples were taken. The pigs then weighed 85—100 kg. Trained as well as untrained pigs would reach this body-weight after about 14 weeks. Thus, training did not seem to cause any significant delay of body growth. Feed consumption was not controlled. No interfering diseases were observed during the experiments.

Biopsies of the longissimus dorsi were performed with a biopsy needle (Bergström 1962), inner diameter 4 mm, about 10 cm in front of the iliac crest and 5 cm from the medial line. This was done within 5 min. after the end of stressing on the treadmill. Muscle samples, weighing 100—200 mg, were quickly wiped free from blood on a filter paper and placed in 10 ml of paraffin oil, which had been prewarmed to 40°C, at a depth of about 2 cm. The specimens were kept at this temperature on a water bath for 45 min. They were then taken out and blotted on a filter paper. Adhering oil was dissolved by washing in ethyl ether, and

the samples were finally dried on a filter paper. The tissue was homogenized in 20 vol. of 0.02 % monoiodo acetate in a glass homogenizer.

pH_1 was measured by a pH-meter (Radiometer type 25).

pH_0 was similarly estimated in samples, which were immediately removed from the paraffin oil medium and thus without keeping on the water bath for 45 min.

ATP was extracted with 10 vol. of 0.1 M glycine buffer, pH 11, by homogenization in a Potter-Elvehjem homogenizer on boiling water for one min. (Kalbhen & Koch 1967). ATP was determined fluorometrically with luciferase (Strehler 1962).

Muscle glycogen was determined according to Hultman (1967).

RESULTS

The experimental error of the pH_1 determination was calculated from double sampling in 15 untrained pigs. After stressing on the treadmill two biopsy samples were taken from each pig, one from the left and one from the right longissimus dorsi. The experimental error (i. e. standard error of the differences) was 0.057 pH units.

The effect of stress on the pH_1 was determined in biopsy samples from 10 unstressed and 62 stressed pigs. All pigs were untrained. pH_1 of the unstressed group was 6.227 ± 0.178 (mean ± 1 s) and that of the stressed group 6.098 ± 0.179 . The mean difference, 0.129 units, is significant ($P < 0.05$).

The influence of temperature after sampling on the pH_1 was estimated in biopsy samples of 10 stressed pigs. A sample from each pig was divided into two halves, one was kept in paraffin oil at 40°C and the other one at 43°C for 45 min. Mean pH_1 at 40°C was 6.167 and at 43°C 5.856. The mean difference 0.311 (standard error of mean difference 0.027) is significant ($P < 0.001$).

Results of pH_1 determinations of biopsy samples of the longissimus dorsi in experiments 102, 105 and 106 are shown in Table 1. The experiments were run in the autumn 1970, autumn 1971 and winter 1972, respectively.

Results in experiment 100 are shown in Table 2. The pigs were killed by using a bolt pistol immediately after they had been stressed, and they were then exsanguinated by slitting their throats. A hind leg including the ham was cut off the carcass, and

Table 1. pH_i of longissimus dorsi biopsy samples. Values are mean ± 1 s and number of pigs (within brackets). Trained pigs are different from untrained pigs ($P < 0.01$, analysis of variance).

	Trained pigs	Untrained pigs
Experiment 102	6.158 \pm 0.126 (9)	6.061 \pm 0.145 (10)
„ 105	6.141 \pm 0.138 (9)	6.070 \pm 0.135 (11)
„ 106	6.362 \pm 0.090 (9)	6.282 \pm 0.087 (9)

Table 2. Estimations on the gracilis muscle of stressed pigs (experiment 100). The pigs were killed and exsanguinated, and a hind leg, cut off the carcass, was kept at 17°C for 45 min., when sampling was done.

	pH_i^a	ATP ($\mu\text{M/g}$ wet tissue) ^a	Fluid loss (ml) ^a	Glycogen (mg/g wet tissue) ^a
Trained pigs	6.288 \pm 0.251 (10) ^b	1.021 \pm 0.396 (10)	0.540 \pm 0.837 (10)	3.994 \pm 2.164 (9)
Untrained pigs	5.907 \pm 0.313 (50)	0.624 \pm 0.330 (50)	1.808 \pm 1.641 (50)	1.758 \pm 1.399 (48)

^a difference trained pigs vs untrained pigs significant ($P < 0.01$, Wilcoxon's test).

^b values are mean ± 1 s and number of pigs (within brackets).

kept at about 17°C for 45 min. Samples were then taken from a deep part of the gracilis muscle, i.e. a surface layer about 1 cm thick was not used.

The pH_0 to pH_i drop in biopsy samples at 40°C was 0.542 \pm 0.203 units (mean ± 1 s) in untrained, stressed pigs ($n = 22$), and 0.493 \pm 0.214 in trained, stressed pigs ($n = 9$). The difference is not significant ($P > 0.05$).

DISCUSSION

Exercise on the treadmill at 2.5 m/sec. for 3 min. ("stress") was easily performed by the trained pigs. Most of the untrained pigs showed fatigue, however, by stumbling movements and a tendency to kneel on their hind legs. The effect of treadmill exercise in untrained animals was to depress the pH_i of longissimus dorsi biopsy samples by 0.129 units as compared to non-exercised controls. Rülcker (1968) reported a depressing effect of 0.2–0.4 units in the gracilis muscle of untrained pigs. His higher figures are probably explained by two factors. He avoided stress in the control animals by shooting them while they were

unworried and resting in the pens. This was not possible in our experiments, as we had to restrain all animals, even those which had not run on the treadmill, in order to obtain the biopsy samples. Restraining was done by using a nose twitch. This causes fear and physical activity in the animals, when they try to get free. Further, in his experiments, the samples to be used for pH-measuring, remained left in the ham for 45 min. until they were cut out for homogenization. They were thus exposed to a temperature sloping from 43°C to 40°C in stressed, untrained pigs. The hams of unstressed controls had a temperature curve running about 2° lower. With our procedure samples from exercised as well as from non-exercised pigs were kept at a constant temperature of 40°C.

The effect of elevating the temperature of the biopsy samples from 40°C to 43°C during 0—45 min. was a depression of pH_1 by 0.311 units, i. e. approx. 0.1 units per temperature degree. Physical activity of pigs before slaughter, at an amount corresponding to the treadmill exercise in our experiments, would then be expected to lower pH_1 by a sum of the terms 0.129 and 0.2, i. e. 0.3—0.4 units.

It is seen from Tables 1 and 2 that trained pigs have a higher pH_1 than untrained ones. The difference is more pronounced if the pigs are killed and the muscle sample remains in the ham until it is cut out for pH estimation at 45 min. (Table 2). This is probably because untrained pigs have a higher ham temperature than trained ones (*Rülcker*). When biopsy samples are compared, difference in temperature exists only before the sample is taken out of the muscle, i.e. until 0 min. The pH_0 to pH_1 -drop in untrained, stressed animals was not significantly faster than in trained, stressed animals. This indicates that the lower pH_1 in biopsy samples of untrained pigs was a result of chemical events taking place before the muscle sample was cut out, or at least before it was placed in the paraffin oil.

The results shown in Table 2 further demonstrate that training inhibits breakdown of ATP and glycogen and the loss of fluid. This is in accordance with earlier reports (*Rülcker et al.* 1967, *Rülcker* 1968). The mechanism by which training protects to the development of PSE-changes was supposed to be a more effective oxidative phosphorylation with inhibition of anaerobic glycolysis (cf. *Chance* 1965). This assumption is supported by recent observations (*Eikelenboom* 1972) indicating that stress-

susceptible pigs have reduced ability to synthesize ATP. The development of lactic acidosis due to exercise is similarly inhibited in trained pigs (*Lannek et al.*, to be publ.).

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SAMMANFATTNING

Inverkan av fysisk träning på pH i skelettmuskulaturen hos grisar.

Träning av grisar på ergometer minskade pH-fallet i biopsier från *M. longissimus dorsi*. Träningen hade vidare en inhiberande verkan på det post-mortala pH, ATP och glykogen samt på utpressbar vätska i *M. gracilis*.

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