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TRAINING-INDUCED ALTERATIONS OF THE FATTY ACID PROFILE OF RABBIT MUSCLES

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The present study was designed to investigate whether meat-type rabbits are able to perform treadmill running as a daily routine exercise, and if so, whether the exercise induces specific proportional changes in the fatty acid composition of their muscles. After a four-week training period 8-week-old rabbits were slaughtered and the total activity of plasma lactate dehydrogenase was measured, showing a significant difference between the exercised and control groups (429 ± 126 IU/l vs. 639 ± 203 IU/l). Furthermore the fatty acid composition of *m. longissimus dorsi* (MLD) and *m. vastus lateralis* (MVL) was determined by means of gas chromatography. Exercise increased the proportions of oleic acid (C18:1 n-9) in both MLD and MVL as compared to the control group. However, the level of stearic (C18:0) and arachidonic (C20:4 n-6) acids significantly decreased in the MVL after the exercise. Changes in the fatty acid profile resulting from the physically loaded condition were of the same tendency in both muscles, adding that the MVL might have been exposed to the exercise more intensively; alterations there occurred in a more pronounced manner. Based on the inference that the composition of membrane structure was also affected, these alterations may have important consequences on meat quality.

Key words: Exercise, muscle fatty acid composition, rabbit

Exercise is a widely applied method to follow metabolic responses induced. It has well-defined effects, especially on muscle metabolism. Not only the metabolism of specific metabolites can be modified, but the composition of muscle tissue can also be influenced. Several data have been published on training-induced physiological changes in humans (Brouns and van der Vusse, 1998; Helge et al., 2001), horses (Gottlieb et al., 1989), dogs, goats (McClelland et al., 1995) and rabbits (Meng and Pierce, 1990; Frimen et al., 1998). However, the way and extent in which the fatty acid composition of muscles can be influenced have been less intensively investigated. Although Andersson et al. (1998) and Helge et al. (2001)

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detected changes in the phospholipid fatty acid composition of human muscles and Helge et al. (1999) in that of rat muscles, no such investigation has been performed in rabbits.

During physical exercise mainly carbohydrates and fats serve as fuel sources for skeletal muscles. When increasing the activity and length of exercise after the depletion of the carbohydrate pools a more progressive fatty acid oxidation can occur, this in turn results in a higher endurance capacity (Brouns and van der Vusse, 1998). Regular training increases the rate of lipid utilisation as metabolisable energy source. Consequently the rate of fat utilisation depends on training status, body condition and on the intensity and endurance of exercise. Plasma triacylglycerols (TG), free fatty acids (FFA) and TG of muscle origin are the most important substrates to meet the energy demands of physical load (Turcotte, 1999). Chronic exercise of moderate intensity leads to a higher concentration of FFA in the blood plasma. FFA are important energy sources for muscles under physical load and are mostly metabolised via oxidation. This seems to be characteristic of a moderate load level, as high-intensity short-term exercise is paired with a decreased rate of fatty acid utilisation in muscles (Romijn et al., 1993). It has to be added that from fatty acids mainly middle and long chain ones are used in moderate intensity exercise (Sidossis et al., 1997). Furthermore, muscle TG can meet about 20–50% of total energy requirement. Besides TG hydrolysis, however, synthesis can also be very effective in the muscle. For this reason a very intensive muscle TG turnover is supposed during, and also after, heavy and prolonged exercise. Data in the literature show that the fatty acid composition of muscle triacylglycerols is not affected by physical load (Helge et al., 2001). In contrast, results are also published about the change of phospholipid fatty acid composition due to physical load (Andersson et al., 1998; Helge et al., 2001).

The present experiment was conducted to investigate whether middle and long chain fatty acids are actively used substrates during moderate-level treadmill exercise of rabbits. Furthermore, the proportional changes of fatty acids were measured to detect the specific utilisation order in a physically loaded condition.

The aim of this experimental approach was to detect possible changes in fatty acid composition in whole muscle homogenates of rabbits. From this aspect two muscles were chosen for analysis, which were supposed to be involved in the specific exercise bout of rabbit; *m. longissimus dorsi* (MLD) and *m. vastus lateralis* (MVL).

Materials and methods

Pannon White rabbits were kept in a closed building in cages, and fed *ad libitum* with a commercial pelleted diet (DE 10.3 MJ/kg, crude protein 16%, crude fat 3.4%, crude fibre 15.5%); the fatty acid composition of the diet is shown in Table 1. Production traits (body weight gain and daily feed intake) were measured during the total trial period.

Table 1

Fatty acid composition of the diet (w% of total)

Fatty acid	% of total
C14:0	0.91
C16:0	23.03
C16:1 (n-7)	0.24
C17:0	0.28
C18:0	4.10
C18:1 (n-9)	20.20
C18:2 (n-6)	44.60
C18:3 (n-3)	5.83
C20:4 (n-6)	trace

At the age of four weeks two groups of 6 individuals were selected randomly. The animals of the first group were not treated (control), whereas the rabbits of the second group were exposed to treadmill running twice daily, until exhaustion, during the total trial period. The treadmill was electrically driven and rabbits had to run on an endless belt of leather at a speed of 3 to 6 m/sec. The endurance and length of exercise did not show changes during the investigation; the track length was calculated from the belt speed and the duration of the exercise bout, and rabbits covered a distance of approximately 0.6–0.8 km a day. To avoid possible injuries, the speed of the treadmill could be controlled. Training was continued between 4 and 8 weeks of age in the exercised group. After the 8th week animals were slaughtered.

To follow the effects of moderate-level aerobic exercise also in a conventional way, basal total activity of lactate dehydrogenase was measured in both groups, once, at the end point of the trial. Therefore, blood samples were collected, treated with anticoagulant (K-EDTA), and blood plasma lactate dehydrogenase was measured enzymatically, on an automated equipment (Hitachi 917 Analyzer®), calibrated to rabbit blood samples.

Muscle samples were taken from the left *m. longissimus dorsi* (MLD) (always from the same location, i.e. between the 2nd to the 3rd lumbar vertebrae) and from the *m. vastus lateralis* (MVL). Samples were homogenised with chloroform : methanol (2:1) and the fat content was extracted. Prior to gas chroma-

tography, fatty acids were methyl-esterified with methanol, benzene and sulphuric acid (75:25:4). Fatty acid methyl esters were dissolved in n-hexane and were separated and analysed by a Chrom 5 equipment (Chrom 5 Lab., Prague). The chromatograph was equipped with a dual flame ionisation detector and 1.8 m × 3 mm (i.d.) column containing 100/120 ChromosorbW AW coated with 10% SP 2330. Results were given always in percentage (m/m) of total fatty acids. Statistical analysis was carried out with independent samples *t*-test, at the significance level of 0.05, using SPSS 10 for Windows (1999). Two-tailed, and when verifiable, one-tailed significance was taken into account (Snedecor and Cochran, 1989). The trial with the parameters above was carried out in two repetitions, and was approved by the Animal Experimentation Ethics Committee of the University of Kaposvár.

Results

In regard of the measured production traits no differences were found between the experimental groups. Resulting from the homogeneity of the feed the daily energy intake could be handled as identical in the treated and control groups.

Lactate dehydrogenase showed measurable differences between groups. Average values and SD for trained and untrained rabbits were 429 ± 126 IU/l and 639 ± 203 IU/l, respectively, and were found to be significantly different ($P < 0.028$).

Investigating the fatty acid composition detectable alterations were measured; data obtained from the analysis are shown in Table 2.

Table 2

Fatty acid composition of the MLD and MVL of trained and control rabbits (w% of total)

Fatty acid	Sample			
	MLD Trained mean ± SD	MLD Control mean ± SD	MVL Trained mean ± SD	MVL Control mean ± SD
C14:0	1.80 ± 0.39	1.66 ± 0.23	1.45 ± 0.25	1.30 ± 0.24
C15:0	0.41 ± 0.06	0.45 ± 0.11	0.46 ± 0.05	0.48 ± 0.04
C16:0	27.32 ± 0.82	26.97 ± 0.78	26.84 ± 1.47	25.97 ± 1.35
C16:1 (n-7)	2.50 ± 0.81	2.20 ± 0.39	2.31 ± 0.47	2.03 ± 0.50
C17:0	0.43 ± 0.04	0.59 ± 0.13	0.49 ± 0.06	0.57 ± 0.05
C18:0	7.85 ± 0.51	8.38 ± 1.04	8.41 ± 0.71[a]	9.49 ± 1.71[b]
C18:1 (n-9)	21.75 ± 1.82[a]	20.05 ± 0.49[b]	19.60 ± 1.23[a]	16.83 ± 1.47[b]
C18:2 (n-6)	23.97 ± 1.24	25.08 ± 1.13	27.61 ± 1.85	28.19 ± 1.04
C18:3 (n-3)	2.47 ± 0.49	2.50 ± 0.68	2.20 ± 0.35	2.12 ± 0.26
C20:4 (n-6)	5.36 ± 1.10	5.86 ± 0.93	5.73 ± 0.90[a]	7.13 ± 0.77[b]

Significance of differences; a, b: $P < 0.05$; MLD = *m. longissimus dorsi*; MVL = *m. vastus lateralis*

In MLD, the proportion of palmitic and palmitoleic acid (C16:0) tended to increase after exercise; this increase, however, did not reach the level of significance. The proportion of stearic acid (C18:0) was also affected by the moderate-level load, although this decrease was only slightly significant ($P < 0.06$). The proportion of oleic acid (C18:1 n-9) in the exercised animals was definitely increased ($P < 0.05$). Regarding the polyunsaturated fatty acids, linoleic acid (C18:2 n-6) was not significantly affected by this type of physical load: only a slight but not significant decrease was observed in the proportion of this fatty acid. A lower level of linolenic (C18:3 n-3) acid was measured in the MLD of the trained rabbits. The proportion of arachidonic acid (C20:4 n-6) showed a decrease, however this was not statistically significant.

Regarding the results in the MVL of the exercised group, only a slight increase was measured in the proportion of palmitic and palmitoleic acids. After the exercise the proportion of stearic acid decreased while that of oleic acid increased, both changes reaching the statistically significant level. The linoleic acid proportion showed a slight decrease in the exercised group. For arachidonic acid the decrease was larger than that seen in the MLD, and it reached the level of significance.

In the proportion of the saturated fatty acids of shorter carbon chain length, i.e. myristic acid (C14:0), pentadecanoic acid (C15:0) and margaric acid (C17:0), no systematic alterations could be measured in either muscle tested. Although margaric acid tended to show a decrease, significance was not proven.

Discussion

The differences obtained for lactate dehydrogenase may be due to the aerobic exercise performed in a regular manner. These findings are supported by data of the literature (Meng and Pierce, 1990) and our own trial experience that rabbits perform a typical aerobic exercise that can be characterised as moderate or medium level. The above results may be attributable to the specific experimental design with the main goal of training, where the differences between the experimental groups were the aerobic exercise and the related stress; the latter condition was possibly minimised.

However, proportional modifications were measured not only in this parameter but also in muscle fatty acid composition. The possible alterations of the fatty acid profile in MLD and MVL were expected to be definitely similar; differences were expected in regard of the same fatty acids, but with more pronounced alterations in MVL, as the latter might be involved in the exercise bout more intensively than MLD. In addition, the fibre type composition of the muscles analysed is not greatly different, but certain differences are present. Although both can be characterised as fast twitch muscles (Lutz et al., 1978;

Hamalainen and Pette, 1993), MVL also contains numerous 'fast twitch fatigue-resistant' fibres. Furthermore, exercise-induced changes in the fatty acid profile were published to occur independently of dietary fatty acid composition (Helge et al., 1999) and, what is more important, irrespectively of the fibre type distribution of the muscles (Andersson et al., 2000).

The results obtained for palmitic acid both in MLD and MVL are in accordance with the findings of McClelland et al. (1995), who described an activated palmitic acid metabolism during aerobic exercise. The elevated plasma concentration of C16:0 in a non-esterified form may indicate a higher turnover of palmitic acid. Andersson et al. (1998) detected a lower presence of palmitic acid in muscle phospholipids after physical training and an unaltered proportion in muscle triacylglycerols, which also suggests an active turnover of this fatty acid during exercise. In contrast, stearic acid was not considered in related publications as a fatty acid responsive to exercise-induced stimuli. Based on the results the authors suppose that stearic acid itself fails to indicate exercise-induced changes sensitively, but analysis of this fatty acid in relation to oleic acid data suggests their proportional alterations in a strong correlation.

Of the monoenoic fatty acids, oleic acid may sensitively show exercise-induced alterations. Andersson et al. (1998) showed a higher amount of this fatty acid in trained muscle phospholipids, whereas triacylglycerols were not affected. Helge et al. (2001) detected changes of the same type in humans after a four-week-long training period, but not in rats after an exercise trial of the same length. McClelland et al. (1995) determined oleic acid as the one making the greatest contribution to plasma non-esterified fatty acids after exercise. From that viewpoint, its higher proportion in the muscle fatty acid profile of exercised rabbits suggests an active oleic acid mobilisation and utilisation during physical load. The significant elevation of oleic acid in these samples (exercised MLD and MVL) might be of great importance, indicating the priority of this fatty acid as energy source during exercise. Considering the findings of Sidossis et al. (1998), proportional modifications of oleic acid can be handled as an indicator of exercise. It might be also of interest that the lower proportion of stearic acid was always paired with an elevated proportion of oleic acid, as in synthetic biochemical pathways these two metabolites are strongly related. These results are also supported by the findings of Husvéth et al. (1982) showing reverse changes of these two fatty acids in the blood and liver of dairy cows.

Regarding linoleic acid, numerous publications cited the above-mentioned differences due to exercise, but their results are equivocal, i.e. in rats an increase was detected (Helge et al., 1999), while in humans a decrease was shown (Andersson et al., 1998). However, the membrane fatty acid composition in mammals strongly depends on numerous important factors including body condition, so the specific differences should be handled carefully (Couture and Hulbert, 1995). In this study only a minor increase occurred for linolenic acid.

The proportion of arachidonic acid (C20:4 n-6) was more strongly affected by the training in MVL; findings may be caused by the higher exposure to load of this muscle. The results are in accordance with the findings of Helge et al. (1999). The somewhat lower proportion of arachidonic acid may indicate the activation of phospholipase A_2, suggesting membrane structure changes in the muscle cell.

Based on data of the literature and the findings of the present experiment, authors suppose the alterations to be located both in the membrane structures (mainly phospholipids) and muscle triacylglycerols of the muscle cells. However, further investigations are needed to clarify the extent of changes in the different lipid fractions.

Conclusions

After a trial period of four weeks of moderate load level in rabbits, defined changes occurred in the muscle fatty acid profile in this experiment. These changes were of the same tendency in both muscles analysed (MLD and MVL), irrespectively of the muscle type. The relatively slight changes might be partly attributed to the muscle type itself, as MLD and MVL both contain a relatively high proportion of glycolytic fibres with a higher glycolytic capacity and a related lower fatty acid turnover. Therefore, further investigation of a red type muscle of the hind leg, e.g. *m. soleus* could be rewarding. In addition, it was clarified that muscles of the hind leg were more strongly involved in the treadmill exercise. Future investigations will study the alterations of the triacylglycerol and phospholipid fractions, on the basis of the fact that membrane characteristics strongly influence technological parameters (e.g. dripping loss) of the meat.

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