Doctorate (Ph.D.) dissertation theses

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EFFECTS OF PHYSICAL ACTIVITY AND DIETARY FATS ON THE MUSCLE FATTY ACID COMPOSITION OF RABBITS

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1. Background of research, objectives

As a consequence of the continuous improvement of nutritional sciences, in the investigation of products of animal origin, physiological and biochemical aspects become more important. Since more than half of the total fatty acid sources are of animal origin, their composition is a highly interesting factor, with special attention on the essentiality of some fatty acids. Muscular fatty acid composition is highly variant; accordingly, in the current approach the effects of elevated physical activity and nutrition were investigated.

Elevated physical activity and electrical stimulation are known conditions influencing muscle composition, though these are generally applied with other aims than the present ones. The effects of exercise on skeletal muscle membrane lipids are partly understood, however, *in vivo* transcutaneous electrical miostimulation has not yet been analysed from this aspect. Though the incorporation of dietary lipids into different tissue lipids is a known fact, their remodeling, and its kinetics have only less been investigated.

Accordingly, two exercise methodologies (characteristically aerobic and anaerobic) and saturated or unsaturated dietary fatty acid complementation was used. The main goals of our experimental work were to investigate the followings:

- The effects of regular, low-intensity aerobic treadmill exercise on the fatty acid profile of rabbit skeletal muscles, as determined from complex lipids, and from fractionated phospholipids (structural lipids) and triglycerides (storage lipids). Exercise effects were, furthermore, investigated on the erythrocyte membrane fatty acid composition.

- The investigation of the detailed effects of the above exercise protocol onto serum metabolite concentrations and enzyme activities, by means of repeated blood samplings, with special attention on the changes of fat and carbohydrate metabolism.

- Examination of the effects of regular transcutaneous electrical miostimulation (anaerobic, localized, programmed muscle load) on the fatty acid composition of rabbit skeletal muscle complex lipids, phospholipids and triglycerides.

- Investigation of the metabolic effects of electrical miostimulation, as determined from serum clinical-chemical parameters, obtained from serial blood samplings along the treatment of growing rabbits.

- The follow-up of the incorporation of dietary (fully herbal) saturated and unsaturated fatty acids, and that of the remodeling process after feed changes, in skeletal muscle complex lipids.

2. Materials and methods

Pannon White male, weanling (at the age of 4 weeks) rabbits were involved in the studies. Number of animals, sampling characteristics and specified trial aims are summarized in the table below.

treatment	n	age (wk)	samples	aim of study
treadmill	6 (x2)	4-8	m.longissimus dorsi, m. vastus lateralis	muscle complex lipid and erythrocyte membrane fatty acid composition, plasma LDH
control	6 (x2)			
treadmill	8	4-8	serum, m. quadriceps femoris	serum metabolites & enzymes with weekly samplings, muscle PL and TG fatty acid composition
control	8			
TENS	10 SAT + 10 UNSAT	4-8	m.longissimus dorsi	muscle complex lipid fatty acid profile and oxidative stability
control	10 SAT + 10 UNSAT			
TENS	8	4-8	serum, m. longissimus dorsi	serum metabolites & enzymes with weekly samplings, muscle PL and TG fatty acid composition
control	8			
incorporation and remodeling of dietary fats	30 SAT	8-9-12	m.longissimus dorsi	muscle complex lipid fatty acid profile and oxidative stability, plasma lipids
	30 UNSAT			

2.1. Aerobic, treadmill exercise studies

Treadmill treatment was performed twice a day for four weeks, and 24 hours after the last exercise session the animals were slaughtered. The delayed sampling was based on the fact that resting, and not acute parameters were aimed to record. In the first setting *m. longissimus dorsi* (m.l.d.) and *m. vastus lateralis* (m.v.l.) complex lipids, while in a further study *m. quadriceps femoris* (m.q.f.) PL and TG fractions were selectively analysed, for fatty acid composition.

Blood samples were taken weekly in an analogous exercise trial. Serum clinical-chemical parameters recorded were the followings: total protein, albumin, creatinine, urea, triglyceride, total and HDL-cholesterol, free fatty acid, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, γ-glutamil transaminase, creatine kinase, lactate dehydrogenase and cortisol. Erythrocyte membrane was analysed at the end of a four-week trial, from the total blood amount.

2.2. Anaerobic miostimulation

Transcutaneous miostimulation, similarly to the treadmill settings, was performed twice a day, with the following settings: 2 electrodes/animal, 20 min/session, 20 ms impulse length, 10 mA, 30 Hz impulse frequency, in 28-day trials. Adhesive electrodes were fixed on the left side of m.l.d., at the 1^{st} ("-") and 4^{th} ("+") lumbar vertebrae. Muscle samples were dissected from the treated area, in the first approach for complex lipid, while in a further study for phospholipid (PL) and triglyceride (TG) fatty acid composition determination. In the blood sample analysis, for the sake of comparison, analogous settings with the treadmill experiments were applied.

2.3. Investigation of the incorporation and remodeling of dietary fatty acids

In this experiments a saturated (SAT, hydroganated oil) and an unsaturated (UNSAT, sunflower oil) diet was fed for basic four weeks, when in both groups the diets were changed. Muscle (m.l.d.) samples were taken at the diet change (8 wk of age), and furthermore 1 and 4 weeks after it. For a more precise investigation of the fat metabolism, blood samples were also taken (total lipid, total and HDL-cholesterol, free fatty acid).

2.4. Laboratory analyses

Fatty acid composition determination was always based on the *Folch*-extraction method. A low-pressure column chromatographic method was adapted to muscle sample PL separation, while eluted TGs were further purified on thin layer. Fatty acid analysis was performed by capillary gas-chormatography, from fatty acid methlye-esthers.

Blood serum samples were analysed on automatic analyzers with respective, authentic reagent kits.

2.5. Statistical analysis

Primary data of both the fatty acid and blood chemical analysis were compared by independent samples *t*-test, to test between-group differences. In the investigation of the dietary fatty acid incorporation multivariate ANOVA was used, with bodyweight as a covariant. SPSS 10.0 was used for the analysis.

3. RESULTS

3.1. Regular aerobic treadmill exercise

Based on the treadmill exercise experiments it can be stated that both m.l.d. and m.v.l. fatty acid profile showed similar responses to the regular load. This specific reaction, i.e. the proportional modification of different fatty acids (stearate, oleate and arachidonate) was found to be qualitatively independent of muscle type, since changes generally agreed in the two muscles. The two muscles investigated possess relatively high amounts of white glycolytic fibres, which may have also affect the above-mentioned - in part only slight - alterations. On the basis of the current results it can be added that the exposure to the load and the divergent fibre type distribution of different skeletal muscles induce alterations of differing severity, however, still in the proportion of identical fatty acids.

Marked proportional increases were described in the γ-linoleic and eicosaenoic acids due to regular exercise, in the PL fraction of the red m.q.f. Training mainly affected the proportions of highly unsaturated fatty acids: the sum of polienoic fatty acids tended to decrease, which was also reflected in the unsaturation index. On the contrary, in the triglycerides of the identical muscle only arachidic and nervonic acids were significantly affected by the regular load.

Along the exercise experiment general adaptation was detected in the trained rabbits, which was, at least in part, based on the lower serum LDH activity in the trained group. From the metabolites albumin, the transport molecule of free fatty acids, creatinine, referring to active muscle work and serum triglyceride concentration were provable higher in the loaded group. In contrast, neither body weight, nor feed intake was different between the treated and control groups. The aerobic exercise did not influence the fatty acid profile of erythrocyte membrane. However, the higher serum albumin concentration and the lower resting free fatty acid levels indicate a more intensive oxidative metabolism. In contrast, general enzymatic reaction remained quite low, though its tendencies also indicated the above-mentioned processes. The resting serum cortisol concentration of the treated and control groups was not different, suggesting that the above modifications were not induced by additive stress, but by the regular training.

3.2. Investigations on electrical miostimulation

Electrical miostimulation in its strongly localized form could primarily be characterized with anaerobic metabolism. Numerous proportional alterations were described in the m.l.d. complex lipid fatty acid profile when the electrical treatment was administered together with a highly unsaturated diet. Comparing these to those in the literature, similarities were found, however, the effects of this treatment on the muscle fatty acid profile was only analyzed before in *in vitro* trials. Interestingly, when feeding more saturated fatty acids (hydrogenated vegetable oil, SAT diet), probably also containing trans double bonds, an atypical intermediate metabolism was experienced. Though literature data suggest strong similarities between the metabolism of saturated and trans fatty acids, according to our current result, trans fatty acids seem to block fatty acid synthetic pathways effectively, meaning that these are less valuable as precursors for further fatty acids.

In the m.l.d. phospholipid fraction inverse proportional changes were detected by palmitic and pamitoleic acids, due to the regular miostimulation. Furthermore, the linoleic and γ-linolenic acid proportions decreased. Interestingly, as an effect of the treatment, mainly the proportions of fatty acids over the carbon chain length of 20 increased, especially those of high unsaturation (arachidonic acid, EPA, DHA). This may also stand behind the marked elevation of the unsaturation index. Moreover, in the estimated Δ5 desaturase activity a marked increase was found. The electrical treatment, therefore, unequivocally increased the membrane-lipid unsaturation.

Tendencies described in the m.l.d. triglyceride fatty acid profile show general agreement with those in the plasma FFA of other mammalian species (e.g. rat, dog), referring to a specific order in the TG fatty acids' mobilization, either from adipose tissue or from intramuscular TG pools.

According the results obtained from the repeated blood serum measurements, electrical stimulation seems to have metabolic consequences that are characteristic for a change in the substrate order. The enzymatic response to this type of treatment was less pronounced than that experienced by the metabolites. In the treated group markedly lower LDH activity was described, similarly to the treadmill-exercised rabbits. On the basis of the above concentration and activity changes it can be concluded that free fatty acids and serum triglycerides are preferred oxidizable substrates, even in case of muscle contractions induced exogenously.

3.3. Dietary fatty acid incorporation and remodeling

The fatty acid profile of m.l.d. complex lipids could effectively be modified feeding diets of highly differing unsaturation. In growing rabbits this could be reached within a four-week feeding period on a statistically provable level. In the rabbits preconditioned with either saturated or unsaturated fatty acid feeding, a strong "remodeling" took place following the feed change. In case of different fatty acids the incorporation process could be characterized with linear kinetics. The joint investigation of precursor-product fatty acids (and the estimated desaturase activities) clearly indicated the mammalian fatty acid synthetic pathways. Investigation of the stearate-oleate, linoleate-arachidonate and α-linolenate-EPA pairs indicate that the relative overload of a precursor fatty acid does not increase the proportion of the product fatty acid. In contrast, the relative lack of a precursor is sensitively reflected in the product proportion. By the stearate-oleate pair the increasing estimated Δ9 desaturase activity suggested the activated endogenous oleate sythesis.

Plasma total lipid concentration showed a likely age-associated increase in both treatments, as well as plasma triglyceride concentration. Moreover, the total cholesterol level was strongly, but only temporarily decreased by the change to the unsaturated diet, adding that an age-dependent increase of the total cholesterol is also known.

4. CONCLUSION

Muscular fatty acid profile is an important characteristic both from the viewpoint of physiology and nutrition. In the investigations of this current dissertation possible methods for the modification of muscular lipids were examined. Besides muscles, in all cases blood clinical-chemical parameters were analyzed. Muscle and serum data obtained both from the aerobic training and from the anaerobic miostimulation ensured a complex approach. This was highly helpful in the general understanding of the results. These experiments on physical load can clearly be defined as model-studies.

The effects of **moderate level, regular physical exercise** on muscle lipids are mainly known from experiments in the field of applied physiology. In this regard, rabbit is a proper model animal, when taking the high similarity of earlier results on other species (e.g. humans, rats) and those of the current trials into account. Alterations induced in our trials both in the muscle **complex lipids** and in the **fractionated lipid** fatty acid profile were comparable with results described in other mammalian species. Our results suggest that saturated or monoenoic fatty acids primarily originated in the muscle TG fraction, while polyunsaturated fatty acids refer mainly to the PL fraction. The relatively high power and financial demands of highly complicated analyses can be, therefore, in some way, predicted, with the application of more simple but still high-end (capillary gas-chromatography) methods.

The detailed investigation of the effects of **transcutaneous electrical miostimulation** was mainly based on its relative wide application area. Besides detecting the effects directly on the muscles, general adaptation was described by means of repeated serum samplings. On this basis regular miostimulation can be accepted as a special form of training. Both the fatty acid composition of muscular complex lipids and that of fractionated lipids indicate that regular miostimulation exerts effects similar to those experienced after the treadmill exercise, on the muscle fat composition. However, miostimulatiion does more strongly influence the muscle fatty acid profile than the treadmill training. From these mainly those of the structural lipids were pronounced; this fact clearly indicates the major differences existing in the fatty acid metabolism of the TG and PL fractions.

When applying physical load and **dietary fatty acid treatments** together, a more pronounced diet-effect has to be taken into account. Dietrary saturated or unsaturated fatty acid complementation affects the muscle complex lipid fatty acid profile so markedly, that the effects of the regular treadmill training were, in this case, not detectable.

In experimental settings when **hydrogenated vegetable oil is fed besides regular exercise treatment**, the presence of trans fatty acids may occur. Accepting the early results of *Privett et al. (1977)*, the intermediary metabolism of trans fatty acid isomers is, in some way, blocked, though their incorporation into tissue lipids is effective.

The effective lowering of additional **stress** during exercise protocols is of great importance, since catecholamine-stress is known to affect cardiac muscle PL fatty acid composition, e.g. in rats. In the present work in both the treadmill and the miostimulation experiments higher stress was avoided, which was proven by the low serum cortisol levels. Accordingly, not only compositional changes of tissue lipids, but also alterations in the CK and LDH activities can be merely attributed to the applied training protocols.

The training protocols investigated in the present work led to serious increases in the **oxidative stability** of muscles. Substrates possessing antioxidative characteristics (e.g. dietary vitamine E) are of great importance in this regard. However, increased muscle oxidative stability may be primarily caused by regular oxidative stimuli along the training protocol.

In countries of the European Union herbivore feeds are prohibited to contain components of animal origin. This, at least from our viewpoint, had a further, rather practical advantage: as plants do not contain arachidonic acid, the animal tissue linoleic acid - arachidonic acid relationship could effectively be characterized, since only the precursor fatty acid was present in the feed.

In dietary treatments the maximally detailed knowledge of **feed composition** is strictly necessary, including also micro-components. Dietary fatty acid profile can clearly indicate, if there is a “contamination” with components of animal origin. Mainly the presence of arachidonic acid can be informative in this regard. On the other hand, the oxidative stability of muscles was more strongly influenced by the vitamine E content of the diet. Since antioxidant substances in some cases likewise “covered” the pure effects of fatty acids, this factor has to be seriously taken into account in further experiments.

Based on the above facts it can be concluded that the applied experimental and analytical methodology was proper to fulfill the aims proposed.

5. New experimental results

1. The experimental results showed how the individual fatty acids of the divergent lipid fractions contribute to the energy supply of the sekeletal muscles under different types of physical load.

2. The regular aerobic training of rabbits induced proportional alterations of oleate, stearate and arachidonate in the fatty acid composition of *m. longissimus dorsi* and *m. vastus lateralis* full homogenates.

3. In the phospholipids of the *m. quadriceps femoris* the proportion of γ-linolenic acid, the n-3 / n-6 ratio and the unsaturation index decreased as a consequence of the 4-week aerobic training.

4. As a result of the regular electrical stimulation, in the membrane lipids of the *m. longissimus dorsi* the proportions of linoleic acid and α-linolenic acid decreased, those of eicosaenoic acid, arachidonic acid, EPA and DHA increased, while opposite changes were found for palmitate and palmitoleate; finally, the activity of Δ9 desaturase and the unsaturation increased.

5. The selectivity of the fatty acid oxidation during a strongly localized muscle load and aerobic training has been demonstrated in rabbits.

6. Scientific papers and lectures on the subject of the dissertation

6. 1. Articles in foreign languages

1. Szabó, A., Romvári, R., Fébel, H., Nagy, I., Szendrõ, Zs. (2001): Fatty acid composition of two different muscles in rabbits: Alterations in response to saturated or unsaturated dietary fatty acid complementation. *World Rabbit Sci. 9(4):155-158.*

2. Szabó, A., Romvári, R., Fébel, H., Bogner, P., Szendrõ, Zs. (2002): Training-induced alterations of the fatty acid profile of rabbit muscles. *Acta Vet. Hung. 50(3): 357-364.*

3. Szabó, A., Husvéth, F., Szendrõ, Zs., Repa, I., Romvári, R. (2003): Effects of transcutaneous electrical nerve stimulation on the fatty acid profile of rabbit *longissimus dorsi* muscle (preliminary report). *J. Anim. Phys. Anim. Nutr. 87(9-10): 309-314.*

4. Szabó, A., Romvári, R., Bogner, P., Hedvig Fébel, Szendrõ, Zs., (2003): Metabolic changes in meat type rabbits induced by regular submaximal aerobic exercise. *Acta Vet. Hung. 51(4): 503-512.*

5. Szabó, A., Mézes, M., Dalle Zotte, A., Szendrõ, Zs., Romvári, R. (2004): Changes of the fatty acid composition and malondialdehyde concentration in rabbit *longissimus dorsi* muscle after regular electrical stimulation. *Meat Sci. (accepted)*

6. Szabó, A., Fébel, H., Dalle Zotte, A., Mézes, M., Szendrõ, Zs., Romvári, R. (2004): Revesibility of the changes of rabbit muscle fatty acid profile. *Ital. J. Food Sci. (accepted)*

6.2. Articles in Hungarian

1. Szabó, A., Romvári, R., Fébel, H., Szendrõ, Zs. (2002): Két eltérõ izom zsírsavösszetétele, valamint annak változásai telített és telítetlen zsírsavkiegészítés hatására nyulakban. *Állattenyésztés és Takarmányozás. 51(6): 617-625.*

6.3. Full conference papers in proceedings

1. Szabó, A., Fébel, H., Romvári, R., Bogner, P. (2000): Examination of muscle and erythrocyte membrane lipid composition by means of gas chromatography on meat type rabbits. *Animal Science Days 2000, Eszék, szept. 21-23., pp. 77-78.*

2. Szabó, A., Fébel, H., Bogner, P., Szendrõ, Zs., Romvári, R. (2001): Investigation of fatty acid metabolism under physical load by means of NMR spectroscopy and gas chromatography on meat type rabbits. *12. Arbeitstagung über Haltung und Krankheiten der Kaninchen, Pelztiere und Heimtiere. Celle, 2001. május 10., pp. 176-182.*

3. Szabó A., Fébel H., Szendrõ Zs., Romvári R. (2001): Telített és telítetlen zsísavkiegészítés hatásának vizsgálata nyulak izom-zsírsavprofiljára. *13. Nyúltenyésztési Tudományos Nap, Kaposvár, 2001. május 23., pp. 41-45.*

4. Szabó A., Fébel H., Szendrõ Zs., Romvári R. (2002): Pannon fehér nyulak izom zsírsavösszetétele telített és telítetlen zsírsavkiegészítést követõen. *VIII. Ifjúsági Tudományos Fórum, Keszthely, 2002 márc. 28., CD kiadvány.*

5. Szabó, A., Husvéth, F., Szendrõ, Zs., Romvári, R. (2002): Krónikus elektromos stimuláció hatása húsnyulak hosszú hátizmának zsírsavprofiljára. *14. Nyúltenyésztési Tudományos Nap, Kaposvár, 2002 május 22., pp. 41-45.*

6. Szabó, A., Romvári, R., Fébel, H., Bogner, P., Szendrõ, Zs. (2002): Effects of physical training onto the muscle fatty acid profile of meat type rabbits. *9. Krmiva Konferencia, Opatija, 2002. május 29-31., pp. 174-177.*

7. Szabó. A., Romvári, R., Bogner, P., Fébel, H., Locsmándi L., Szendrõ Zs. (2003): Szérum metabolitok és enzimek vizsgálata növendék nyulakban, rendszeres fizikai terhelés mellett. *15. Nyúltenyésztési Tudományos Nap, Kaposvár, 2003 május 28., pp. 145-149.*