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METABOLIC CHANGES INDUCED BY REGULAR SUBMAXIMAL AEROBIC EXERCISE IN MEAT-TYPE RABBITS

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Pannon White growing rabbits (a group of 8) were exposed to treadmill exercise (3–9 m/s, 1.2–1.6 km/day) twice a day for 4 weeks, while additional 8 animals, kept inactive, were assigned as the control group. Weekly, 12 hours after exercise, venous blood was taken for serum metabolite and enzyme activity measurements. Total serum protein, albumin and creatinine levels significantly increased during the second half of the training, as compared to the control group. Triacylglycerol levels in the exercised group as compared to controls, however, were higher only after the first and the fourth weeks of the experiment. Resting non-esterified fatty acid (NEFA) concentration of the trained rabbits was lower at the end of the trial. On the other hand, there were no significant differences, as compared to the respective controls, in serum urea, total and HDL cholesterol levels. At the end of the exercise alkaline phosphatase activity was higher and total lactate dehydrogenase activity was lower in the trained rabbits. Serum alanine aminotransferase, aspartate aminotransferase and γ-glutamyl transpeptidase activities were not changed, while creatine kinase activity was slightly lower in the trained group. The serum cortisol concentration was not different in the trained and control rabbits.

Key words: Regular aerobic exercise, rabbit, serum metabolites, serum enzymes

Regular physical exercise has extensive metabolic effects, whose extent depends on both the intensity and the type of the exercise. In this aspect, moderate aerobic exercise appears to be of first importance (Turcotte, 1999). The metabolic consequences of a long-term training protocol include the changes in substrate metabolism (Geor et al., 2002), as the oxidation of skeletal muscle protein and fat measurably increases, while that of carbohydrates decreases (Dohm et al., 1981; Coggan et al., 1990; Pagan et al., 2002). The intense breakdown of both proteins and lipids leads to higher blood concentrations of their metabolites.

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During the onset of physical load, hepatic glucose output is elevated (Brooks and Donovan, 1983). Interestingly, the adaptation to long-term or regular exercise results in the reduction of the turnover rate of the whole body glucose pool, which can be partly explained by a strongly improved hypoglycaemic resistance due to training (Donovan and Sumida, 1997). This is, furthermore, coupled with a strong triacylglycerol (TAG) hydrolysis in the adipose tissue, resulting in a higher non-esterified fatty acid (NEFA) level in the blood. Therefore, the systematic adaptation to training can be better described on the basis of changes in lipid metabolism. One of the main energy sources of the skeletal muscles is NEFA, mobilised from adipose tissue and transported in albumin-bound form in the blood. Therefore, the availability of NEFA is solely limited by the transporting capacity of serum albumin (Spector et al., 1971). Lipoproteins are thought to be less important fuels for exercise, with a contribution to the total energy demand that is not more than 10% in humans (Ranallo and Rhodes, 1998). Protein derivatives (amino acids) are not primary fuels, but regular exercise causes considerable stress in the integrity and turnover of the muscle protein pool (Essen-Gustavsson and Jensen-Waern, 2002). The complex metabolic effects of a long-term adaptation to aerobic training are therefore prevalent and also characteristic of the workload. Moreover, this kind of metabolic adaptation is markedly different from the effects of short-term exercise.

Meng and Pierce (1990) and Szabó et al. (2002) have previously characterised a rabbit exercise of moderate intensity. In order to detect major metabolic alterations during a programmed exercise protocol that is characterised by relatively short but frequent and intensive sessions, the present study was aimed to determine symptomatic serum enzymes, protein and lipid metabolites.

Materials and methods

Animals and training protocol

Sixteen male Pannon White rabbits weaned at the 28th day of life were kept in a closed stable. Rabbits were fed a commercial pelleted diet (Table 1) and production traits (body weight, feed intake) were measured throughout the trial. Before the experiment began, the rabbits were randomly divided into two groups. The first group was kept without exercise (control group), while the rabbits of the second group were exposed to treadmill running twice daily, until exhaustion, for a period of 4 weeks. The speed of the treadmill belt was adjusted to the voluntary running speed of rabbits (3–9 m/s). The animals completed 1.2–1.6 km per day (without any pressuring influence). The experimental trial was approved by the Ethics Committee on Animal Experimentation of the University of Kaposvár.

Table 1

Chemical and fatty acid composition of the diet

Chemical composition		
Dry matter (DM)	(%)	88.0
Crude protein	% DM	16.0
Crude fat	% DM	3.9
Crude fiber	% DM	15.5
DE rabbit (calculated)	MJ/kg	10.3
Fatty acid composition		**% of total**
C8:0		0.52
C14:0		0.43
C16:0		20.72
C16:1 (n-7)		0.71
C17:0		0.21
C18:0		5.17
C18:1 (n-9)		22.07
C18:2 (n-6)		43.54
C18:3 (n-3)		5.45
C20:0		0.44
C20:1 (n-9)		0.74
C20:4 (n-6)		0.00
Σ saturated / Σ unsaturated		0.38

Blood sampling

Blood samples from the left ear vein were taken from all rabbits at the start (time-point 0 = 28th day of life), then weekly (time-points 1, 2, 3 and 4), 12 h after the exercise sessions. As the primary aim of the study was to characterise the chronic but not the acute effects of exercise, resting parameters were recorded. After withdrawal, the blood was immediately placed on ice, centrifuged and serum was transferred to the laboratory. Serum metabolites and enzyme activities were measured on a Konelab 20i® apparatus, using Konelab® Reagent Kits, except for NEFA, where a Randox® FA115 kit was used (Randox Laboratories LTD, UK). Total serum protein (TP), albumin (ALB), creatinine (CREA), urea, total and HDL cholesterol and triacylglycerol (TAG) concentrations, alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activities were determined weekly. In addition, at the end of trial non-esterified fatty acid (NEFA) and cortisol concentrations, aspartate aminotransferase (AST), γ-glutamyl transpeptidase (γ-GT), creatine kinase (CK) and basal total lactate dehydrogenase (LDH) activities were determined.

Statistical analysis

One-way analysis of variance with the least significant difference (LSD) 'post hoc' test was performed at the significance level of P = 0.05, using SPSS 10 for Windows (1999). Training-induced differences were correlated between groups on a week-by-week basis.

Results

During the trial period, no significant differences were detected in the production traits, body weight and feed intake (comparing the groups weekly). On the other hand, both the serum total protein and the serum albumin levels were elevated in parallel with age, although the increase for both was more rapid in the exercised than in the control group. As shown in Figs 1 and 2, from the third week of training the differences in blood proteins between the trained and control groups were significant. Significant differences in serum creatinine were also detected [except at the end of the second and fourth (P = 0.061) weeks]; at the beginning of the experiment the control values, while later those of the trained group were higher (Table 2). Serum urea content showed a likely age-associated increase, without significant differences between groups.

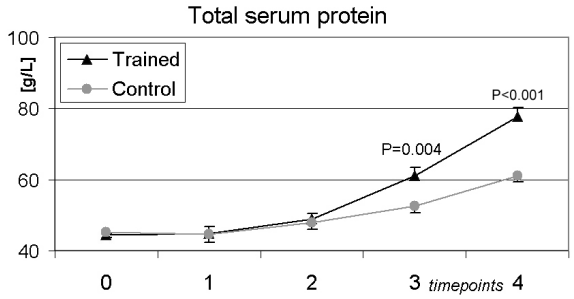

Fig. 1. Total serum protein content of the trained and control groups (mean ± SEM)

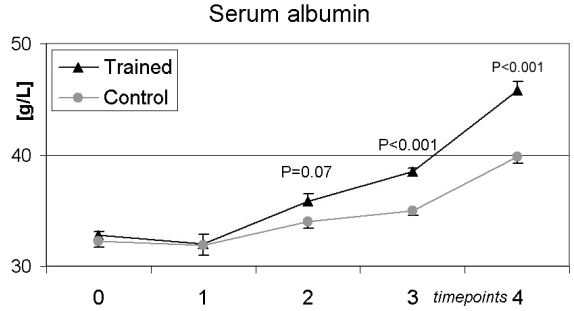

Fig. 2. Serum albumin content of the trained and control groups (mean ± SEM)

Table 2: Serum metabolites in the trained and control groups (mean ± SEM)

	Group	Time-points				
		0	1	2	3	4
Creatinine (μmol/L)	Trained	58.37 ± 0.14	40.00 ± 2.42[a]	40.33 ± 2.35	65.33 ± 5.62[a]	97.67 ± 3.48[*]
	Control	64.23 ± 8.00	54.62 ± 4.77[b]	46.25 ± 3.75	48.87 ± 2.16[b]	86.37 ± 4.97[*]
Urea (mmol/L)	Trained	2.97 ± 0.21	3.37 ± 0.09	3.68 ± 0.25	4.00 ± 0.21	4.40 ± 0.20
	Control	2.87 ± 0.10	3.44 ± 0.24	3.55 ± 0.18	3.86 ± 0.18	4.58 ± 0.30
Total cholesterol (mmol/L)	Trained	2.80 ± 0.10	1.90 ± 0.12	1.18 ± 0.09	2.08 ± 0.32	2.95 ± 0.14
	Control	2.70 ± 0.16	1.93 ± 0.13	1.34 ± 0.17	2.64 ± 0.23	3.20 ± 0.29
HDL cholesterol (mmol/L)	Trained	1.28 ± 0.05	0.38 ± 0.04	0.70 ± 0.07	0.95 ± 0.10	1.61 ± 0.13
	Control	1.39 ± 0.06	0.54 ± 0.03	0.40 ± 0.04	0.92 ± 0.10	1.57 ± 0.15
Triacylglycerol (mmol/L)	Trained	1.62 ± 0.08	2.00 ± 0.23[a]	1.54 ± 0.10	1.55 ± 0.09	1.92 ± 0.17[a]
	Control	1.57 ± 0.08	1.51 ± 0.13[b]	1.22 ± 0.07	1.44 ± 0.20	1.40 ± 0.09[b]
Non-esterified fatty acid (mmol/L)	Trained	–	–	–	–	0.19 ± 0.027[a]
	Control	–	–	–	–	0.27 ± 0.072[b]

Significance of differences in the given metabolite at the time-points marked: [a, b]$P < 0.05$; [*]$P = 0.061$

Table 3: Serum enzyme activities and cortisol concentration in the trained and control groups (mean ± SEM)

	Group	Time-points				
		0	1	2	3	4
ALP (IU/L)	Trained	545.40 ± 7.4	384.50 ± 46.1	411.00 ± 21.1	418.70 ± 46.6	697.80 ± 44.1[a]
	Control	516.60 ± 46.6	365.20 ± 21.9	419.20 ± 16.3	447.25 ± 38.8	495.00 ± 21.7[b]
ALT (IU/L)	Trained	29.13 ± 1.6	29.83 ± 3.21	37.00 ± 4.41	40.51 ± 5.21	46.25 ± 6.60
	Control	30.98 ± 1.97	35.00 ± 3.18	42.86 ± 4.02	42.33 ± 9.08	49.29 ± 6.35
AST (IU/L)	Trained	–	–	–	–	43.50 ± 4.53
	Control	–	–	–	–	44.14 ± 6.33
γ-GT (IU/L)	Trained	–	–	–	–	13.33 ± 4.53
	Control	–	–	–	–	11.25 ± 1.06
CK (IU/L)	Trained	–	–	–	–	1349.20 ± 235.8[*]
	Control	–	–	–	–	1993.40 ± 136.3[*]
LDH (IU/L)	Trained	–	–	–	–	394.20 ± 15.5[a]
	Control	–	–	–	–	734.70 ± 136.3[b]
Cortisol (nmol/L)	Trained	–	–	–	–	34.10 ± 1.47
	Control	–	–	–	–	39.05 ± 15.63

Significance of differences in the given enzyme at the time-point marked: [a, b]$P < 0.05$; [*]$P = 0.07$

Serum total cholesterol concentrations distinctly decreased during the first two weeks of the trial in both groups, then a significant increase was seen during the last two weeks (Table 2). However, significant differences between the two groups were not established. Similar changes associated with age were also found for HDL cholesterol, again without significant differences between the groups in either period of the trial. Triacylglycerol concentrations between the trained and the control groups, however, differed with statistical significance in the first and the fourth weeks; the former group having the higher values. At the end of trial, the resting NEFA concentration was significantly lower in the trained group (Table 2).

When comparing the trained and the control groups, the ALT activity showed no significant difference during the entire trial period (Table 3), nor did AST, γ-GT and CK activity, as measured at the end of the trial. After the fourth week the ALP activity, however, was clearly higher in the trained group than in the control. On the other hand, LDH activity at the end of the trial was significantly lower in exercised rabbits than in controls. No difference was found in the serum cortisol concentration between the groups at the end of trial (Table 3).

Discussion

The acute and chronic metabolic effects of exercise can be considerably different. In general, the acute effects are characterised by mild dehydration, rapid depletion of fuel sources and elevated concentrations of degradation products in the blood. According to our goal (i.e. to describe the long-term effects of exercise), the present study was designed to potentially eliminate interference by short-term metabolic changes.

Marked elevations in the total protein concentration upon training were shown here, and also reported by Ohira et al. (1977), although as an acute measure in the latter study. In contrast, Andrews et al. (1995) reported higher TP concentration in endurance-trained horses. The same was described for serum albumin, which also agrees with our present results. In Figs 1 and 2, the time course of the adaptation of both TP and ALB are clearly recognised: the latter showed a difference, as compared to controls, as early as at the end of the second week, while TP only a week later.

Besides being the major protein fraction in the serum, albumin also serves as the carrier of NEFA that is hydrolysed in and mobilised from the adipose tissue and which is one of the main fuels for the skeletal muscle under load (Turcotte, 1999), and whose importance can further increase with training (Dyck et al., 2000). Therefore, the increased NEFA transport in the blood requires elevated albumin levels [since the affinity of the NEFA-binding sites to albumin appears to be constant (Spector et al., 1971)].

The changes in serum creatinine concentrations suggested an adaptation process of the creatine pathway. Although the regular load decreased the CREA concentration after one week, this reduction was later compensated. From this point on, higher CREA concentrations were measured in the exercised rabbits, although at the end the difference was less significant ($P = 0.061$; Table 2). The higher serum CREA level in the trained group may partly be related to the elevated creatine concentration of muscles due to regular training: aerobic exercise bouts, such as those applied in the present trial, have been reported to increase the resting level of creatine (Putman et al., 1998). The biochemical basis of this might relate to the fact that repeated bouts of high-intensity exercise reduce the importance of glycogenolytic pathways in fuel metabolism.

Urea concentration of the serum was not influenced by the exercise protocol, indicating that in both groups a similar, age-dependent increase was only measured. This is in accordance with the previous finding (Krzywanek et al., 1996) that short bouts of exercise, even of high intensity, do not affect serum urea concentration.

The applied training protocol exerted noticeably less effect on serum lipids than described for the above-mentioned metabolites. The total cholesterol content manifestly decreased in the first two weeks of the trial, which was, however, followed by an increase up to the original levels. This tendency, i.e. a measurable decrease of the total cholesterol level, might be attributed to the diet change at the weaning, as no exercise effect could be proven. According to Hodson et al. (2001), an increase in the level of unsaturated fatty acids in the dietary fatty acid profile can in fact lower plasma total cholesterol. The saturated to unsaturated fatty acid ratio of rabbit milk is normally 4:1, while that of the current fully herbal rabbit feed was 1:2.64 (Table 1). The second, increasing phase (i.e. after the second week) might be the result of changes that are age related. Although the effects of exercise could not be established with high confidence, the trained group still showed slightly lower values during the three weeks of the increasing phase. For HDL cholesterol, the time course showed a minimum one week after weaning, which was followed by a slight but continuous increase. After all, the exercise protocol applied here seemed to have no particular effect on the serum HDL cholesterol concentration, which is surprising, when taking into account that serum HDL cholesterol is often elevated as a consequence of low-intensity, aerobic training in human studies (Sunami et al., 1999). The fact that rabbits are typical 'LDL animals' might, at least in part, explain this paradox.

Resting serum TAG concentrations in the exercised rabbits always exceeded those in the control ones, although considerable differences were only seen after the first and the fourth weeks of training. These findings seem to reflect the result of rather complex metabolic changes. In humans as well as in endurance-trained animals, no exercise has been performed during postprandial lipaemia, when blood TAG reaches high levels. As rabbits take up feed almost

continuously all day, their postprandial state is excessively expanded. Therefore, the role of serum TAG as fuel for exercise may be more important (when compared to other species), which might also explain our findings with serum TAG and seems to be supported by the results of Mackie et al. (1980), showing that chylomicron TAG are essential muscle fuel in rats and other rodents even in the post-exercise state.

It may seem somewhat contradictory that serum albumin increased as a consequence of regular exercise, while NEFA showed a decreased resting level. However, it is known that NEFA primarily peaks during exercise, reaching multiple resting values; at the same time this high level is transient, especially in trained subjects whose NEFA removal is strongly augmented (Klein et al., 1994). Analysing post-exercise, resting NEFA levels, Gastmann et al. (1998) described lower serum concentrations after a regular training programme than before. Moreover, the strongly decreased resting NEFA is accepted as a marker of the endurance-trained stage in athletes (Gastmann et al., 1998). Wolfe et al. (1990) explained this low resting concentration by a highly activated NEFA re-esterification during recovery in trained individuals, while Wigernaes et al. (2001) attributed it to the adaptation to regular exercise and to a consequently increased oxidative capacity of muscles. Since the NEFA concentration greatly and quickly fluctuates, measuring the resting value only provides limited information.

Furthermore, besides serum metabolites also symptomatic changes in enzyme activities were shown either in the literature or in our former study (Szabó et al., 2002).

The highly increased serum ALP activity of the exercised group is supported by the results of Mena et al. (1996) as an acute exercise effect. Furthermore, Brommer et al. (2001) reported chronic changes in the ALP activity of young horses exposed to a regular training protocol. The ALT activity of the control rabbits slightly exceeded that of the trained group. In a study on the effects of a single exercise bout, Metivier and Gauthier (1985) described significantly higher ALT activity values for the immediate post-exercise period in men. In contrast, our results indicate that the resting serum ALT activity is slightly decreased during a long-term exercise, which is in accordance with the findings of Koutedakis et al. (1993) that describe time-dependent changes of serum enzymes by showing a temporary elevation of serum enzymes in the post-exercise period. This again suggests that the chronic and acute effects may be different.

At the end of the exercise trial, the activities of both AST and γ-GT were basically identical in the trained and control groups, which agrees with the findings of Koutedakis et al. (1993) and Haralambie (1976) that prolonged training did not alter these enzyme activities. It is interesting to note that the level of serum CK activity was also less symptomatic for the exercise training. In contrast, a marked decrease of serum total LDH activity upon training suggests the occurrence of considerable changes in substrate metabolism (Szabó et al., 2002),

which also indicate that, besides anaerobic glycolysis, aerobic processes also became more dominant. In addition, LDH does not only indicate a lowered glycogenolytic activity of the skeletal muscle (Holloszy and Coyle, 1984), but also an activated lactate clearance (Donovan and Pagliasotti, 1990), both of which may have been affected by the regular physical load.

Summing up the above results, we conclude that submaximal aerobic exercise of rabbits decisively influences plasma metabolite concentrations, while changes in enzymatic activities are less adapted to this type of training. Moreover, all these changes were solely attributed to the regular exercise, since additional stress was not proven.

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