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Adaptation of Common Carp (*Cyprinus carpio L.*) to Regular Swimming Exercise II. Metabolism

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Abstract

Altogether 24 (12 control and 12 treated) growing (initial bodyweight: 50.4 ± 20.1 g) common carps (*Cyprinus carpio L.*) were subjected to a regular training protocol for 35 days, with daily 30 min strenuous exercise bouts aiming to describe blood serum clinicochemical alterations. Samples were taken 4 times (0 as control and 3 time points during training, every 9th day) from all fish. In the nitrogenous serum compounds the training protocol increased albumin and oxidized glutathione concentration at time point 3, while neither total protein, nor creatinine reacted to swimming. From the lipid metabolites triacylglycerol provided higher values at time point 3. Total and HDL cholesterol concentrations were unaltered, meanwhile the HDL percentage in the total cholesterol fraction provided an age-associated increase and a significantly lower final value in the trained group. Within enzymes aspartate and Alanine Aminotransferase (AST and ALT) provided markedly higher activity values at time point 3. Gamma-GT, pseudo cholinesterase, alkaline phosphatase and lactate dehydrogenase (LDH) showed no significant changes induced by regular swimming. In the serum ions training exerted temporary hypokalemic and hypocalcemic effect (time point 2), while sodium, inorganic phosphate and magnesium were unaffected by exercise. Both groups showed an age-associated increase in the serum sodium concentrations. It was assumed that even longer term regular submaximal, but exhaustive exercise exerts only mild effect on the substrate metabolism of carp, a slowly swimming (with burst-like movements) benthic feeder.

Keywords: Common carp; Swimming exercise; Metabolic adaptation; Serum compounds

Introduction

The number of fish species exceeds 32400 [1] and most of them swim by undulations of the whole body [2]. Fish locomotion is powered by at most three (but mostly only two) morphologically differing myotomal muscle fibre types. Red oxidative muscles provide generally maximally 10% of the whole body musculature and are responsible for continuous cruise swimming (characteristic of river-living species), while white muscle fibres provide ca. 50% of body mass, being myoglobin-poor and thus powering primarily short term, burst-type locomotion [3], latter being characteristic for the benthic feeder species or escaping situations. The metabolic consequences of sustained or intermittent strenuous activity include the alteration of substrate metabolism, strictly bound to muscle fiber types as the oxidation of skeletal muscle protein and fat measurably increases, while that of carbohydrates decreases [4]. The intensive breakdown of both proteins and lipids leads to higher blood concentrations of their breakdown metabolites (free amino acids and non-esterified fatty acids, NEFA) although in fish, due to the high white muscle proportions blood glucose and lactate levels provide increases [5]. Lactate and glucose accumulation followed by hepatic glycogen depletion is largely distinct among fish species. It is proved that in situ glycogenic removal of lactate dominates in fish [6], at least slow swimming benthic species, such as common carp. Besides this, generally determinant, anaerobic fermentative pathway muscle utilizes ATP replenished from the hydrolysis of phoshagens [5]. However, the prolonged onset of exercise (mostly characteristic for marine or migratory species) leads to the complete oxidation of carbohydrates, and later to that of fats and amino acids to fuel ATP replenishment within the muscle cell. Ultimately, aerobic metabolism turns to dominate, indicating a radical shift to lipid metabolism [5]. The blood lipid metabolism of fish differs from the homoeothermic vertebrates, as NEFA oxidation is not stimulated by exercise in some marine fish, e.g. rainbow trout [7], due to the lack of glycerokinase activity, which is however not true for freshwater species [8]. Meanwhile NEFA are less utilized, it was published for rainbow trout that lipolytic rate is not altered by strenuous exercise in muscles [7]. Moreover, authors found that trouts do not mobilize triacylglycerol stores to exceed the resting blood plasma niveau to fuel working muscles (via NEFA), even during 4 days of continuous swimming. This was partly explained by the very intensive in situ NEFA re-esterification.

The complex piscine metabolic adaptation to long-term aerobic training is less studied, in particular in freshwater species providing short, but high-intensity swimming bursts [9]. Moreover, this kind of metabolic adaptation is markedly different from the effects of short-term exercise. In order to detect major metabolic alterations during a programmed exercise protocol that is characterized by relatively short and intensive sessions, the present study was aimed to determine symptomatic serum enzymes, protein and lipid metabolites in a cyprinid species (common carp) less studied from this aspect.

Materials and Methods

Training settings, handling of fish, sampling

One-summer-old common carps (average weight: 50.4 ± 20.1 g, n=12 trained and 12 control) were introduced into a 500 l fish tank

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and overwintered in the Fish Laboratory of the Kaposvár University (Hungary). During the overwintering period fish could acclimate to laboratory conditions. During the conditioning and the experimental period a commercial feed (Aller Aqua, proximate and fatty acid profile is shown in Table 1) was fed ad libitum. In the experiment carps were sub-divvied for two groups, experimental (trained) and control and were kept in a single tank sub-divvied into two identical parts. The swimming facility was self-constructed, and installed to a recirculating system. It was a lengthwise halved plastic tube placed in a bigger trough. The ends of the tube were closed with a tightly woven mesh. Water velocity was adjusted by changing the level of the raceway and the volume of the influent water. Water velocity was measured with FP311 Global Water Flow Probe propeller-based current measuring instrument. Trained fish were exercised daily in a four-week period, 30 minutes every day, at constant velocity (0.6 m/s).

Blood sampling and analysis

Blood samples from the tail vein were taken (with 22 g needles) from all fish at the start (time point 0), then every 11th day (time points 0, 1, 2, 3), 12 hours after the exercise sessions. As the primary aim of the study was to characterize the chronic, but not the acute effects of exercise, resting parameters were recorded. After withdrawal into Eppendorf tubes the blood was immediately placed on ice, left to clot, centrifuged (1500 g/10 min) and serum was stored frozen (-70oC) until analysis. Clinical chemical analysis was performed on automated equipment (Hitachi 917) in a single analytical run. Serum oxidized glutathione concentration was measured spectrophotometrically [10].

Proximate composition		
Crude protein (%DM)	45	
Crude fat (% DM)	15	
Nitrogene-free extract (% DM)	21.9	
Crude ash (%DM)	6.9	
Crude fiber (%DM)	3.3	
Gross energy (MJ/kg)	20.8	
Fatty acid composition	**Total lipid**	**PL**
C14:0	6.45	0.92
C15:0	0.39	0.19
C16:0	18.40	22.09
C16:1 n7	7.45	1.39
C17:0	0.43	0.33
C17:1 n7	0.19	0.13
C18:0	3.23	3.93
C18:1 n9	15.60	15.56
C18:2 n6	19.21	38.82
C18:3 n6	0.24	0.06
C18:3 n3	3.40	3.29
C20:0	0.41	0.17
C20:1 n9	2.09	0.44
C20:2 n6	0.38	0.22
C20:3 n6	0.13	0.08
C20:3 n3	0.07	0.02
C20:4 n6	0.72	0.76
C20:5 n3	13.73	3.88
C22:0	0.22	0.24
C22:5 n3	1.49	0.71
C22:6 n3	5.70	6.74
Σ saturated	29.52	27.9
Σ monoenoic	25.33	17.5
Σ polyenoic	45.10	54.6
Σ n3	24.38	14.6
Σ n6	20.67	39.9
Σ n9	17.70	16.0
Σ n6 / Σ n3	0.85	2.73
C18:0 / 16:0	0.18	0.18
C18:1 n9 / C18:0	4.82	3.96
UI (unsaturation index)	189.17	172.4
average FA chain length	17.84	17.9

Table 1: Proximate composition and fatty acid profile of the feed.

Body weight

Body weight was measured at the start and end of treatment.

Statistical analysis

From the basic dataset outlier values [11] outliers were filtered and the remaining data were tested for normality (Shapiro-Wilk test). Between-group (trained vs. control, as marked by lowercase letters in Table 2) differences were analysed by independent samples t-test at the significance level of 0.05 at each time point, while within group (i.e. trained or control), time-dependent alterations (differences among time points) were analysed with one-way ANOVA with the Turkey post hoc test (marked by uppercase superscripts in Table 2) [12].

Ethical issues

The experiment was approved by the Animal Experimentation Ethics Committee of the University of Kaposvár, as allowed by the Somogy County Animal Health and Food Control Authority (allowance no.: 1151/006/SOM/2005).

Results

Body weight

Neither the initial (51.4 ± 26.5 vs. 49.7 ± 17.9 for control and trained, resp.), nor the final (59.8 ± 37.4 vs. 52.7 ± 20.8, resp.) bodyweight was different between groups.

Blood serum clinical-chemistry

The basic serum clinical chemical results are summarized in Table 2. Results were interpreted handling training and ageing separately. Within the nitrogenous serum compounds the training protocol led to a significant increase of the albumin concentration at time point 3, while neither total protein, nor creatinine did not indicate the effect of regular swimming. Age-associated alteration was not found for any of the above-mentioned metabolites. The serum oxidized glutathione concentration was higher at the final sampling in the trained fish (Figure 3). From the lipid metabolites triacylglycerol provided slightly higher values throughout the training period in the swimming fish, which was only significant at time point 3. In contrast, neither total, nor HDL cholesterol concentrations changed due to regular exercise. Their relation, i.e. the HDL percentage in the total cholesterol fraction provided an age-associated increase and a significantly lower final value in the trained group at time point 2 (Figure 2). The total cholesterol concentration of the trained fish provided a significant increase (ageing-associated) along the study; a similar trend was found for the HDL fraction, and also for its percentage in the total cholesterol.

From the enzymes analysed aspartate amino transferase (AST) provided nearly two-fold higher activity in the trained fish at time point 3, with minor differences during the preceding weeks. Similarly, alanine aminostransferase (ALT) serum activity was markedly higher at time point 3 in the exercised group, and a similar condition was found at time point 0, while in the intermittent period (time points

		Timepoints			
	Group	0	1	2	3
Nitrogenous metabolites					
Creatinine (μmol/L)	Trained	0.55 ± 1.51^{A}	0.36 ± 0.81^{AB}	1.75 ± 2.26^{B}	0.02 ± 0.00^{A}
	Control	3.00 ± 7.35^{A}	0.90 ± 1.73^{A}	1.00 ± 2.16^{A}	0.18 ± 0.60^{A}
Total protein (g/L)	Trained	27.2 ± 5.58^{A}	30.7 ± 8.34^{A}	29.5 ± 8.27^{A}	31.8 ± 3.57^{A}
	Control	27.0 ± 2.45^{A}	29.6 ± 9.97^{A}	33.9 ± 12.03^{A}	30.0 ± 3.10^{A}
Albumin (g/L)	Trained	16.2 ± 2.82^{A}	14.7 ± 7.50^{A}	17.4 ± 6.76^{A}	17.7 ± 4.58^{aA}
	Control	12.5 ± 4.37^{A}	13.0 ± 3.16^{A}	19.0 ± 8.71^{A}	12.4 ± 3.32^{bA}
Ox. glutathione (micromol/g prot.)	Trained	6.13 ± 0.94^{AB}	768 ± 2.21^{BC}	8.72 ± 2.1^{C}	5.05 ± 0.79^{aA}
	Control	6.72 ± 0.86^{A}	7.41 ± 1.94^{A}	7.37 ± 1.56^{A}	6.90 ± 0.83^{bA}
Lipid metabolites					
Cholesterol (mmol/L)	Trained	2.84 ± 0.66^{A}	3.02 ± 0.58^{AB}	3.10 ± 0.33^{AB}	3.47 ± 0.48^{B}
	Control	2.68 ± 0.43^{A}	2.90 ± 0.56^{A}	3.02 ± 0.57^{A}	3.22 ± 0.64^{A}
HDL cholesterol (mmol/L)	Trained	1.29 ± 0.09^{A}	1.80 ± 0.65^{B}	1.54 ± 0.41^{AB}	2.24 ± 0.15^{C}
	Control	1.31 ± 0.09^{A}	1.50 ± 0.28^{A}	1.77 ± 0.58^{AB}	2.25 ± 0.31^{B}
HDL% in total	Trained	47.3 ± 9.3^{A}	59.4 ± 17.4^{AB}	50.5 ± 17.3^{AB}	65.1 ± 4.9^{aB}
	Control	49.6 ± 6.7^{A}	53.8 ± 16.1^{AB}	61.1 ± 23.0^{AB}	71.0 ± 6.26^{bB}
Triacylglycerol (mmol/L)	Trained	1.88 ± 0.27^{A}	2.38 ± 0.22^{B}	2.38 ± 0.27^{B}	2.86 ± 0.33^{aC}
	Control	1.93 ± 0.17^{A}	2.15 ± 0.31^{A}	2.11 ± 0.54^{A}	2.32 ± 0.29^{bA}
Enzymes					
AST (IU/L)	Trained	123,7 ± 61.75^{AB}	116.9 ± 49.36^{AB}	95.8 ± 63.14^{A}	178.0 ± 66.69^{aB}
	Control	93.2 ± 51.36^{A}	126.4 ± 132.66^{A}	80.4 ± 23.88^{A}	98.9 ± 46.46^{bA}
ALT (IU/L)	Trained	2.27 ± 0.79^{aAB}	1.64 ± 1.57^{A}	1.83 ± 1.80^{A}	4.33 ± 3.60^{aB}
	Control	1.33 ± 0.82^{bA}	3.60 ± 4.77^{A}	2.80 ± 1.32^{A}	1.09 ± 1.04^{bA}
Gamma-GT (IU/L)	Trained	1.27 ± 1.49^{A}	2.36 ± 4.39^{A}	1.83 ± 1.19^{A}	1.33 ± 1.78^{A}
	Control	1.17 ± 0.983^{AB}	0.30 ± 0.48^{A}	2.40 ± 1.65^{B}	0.91 ± 1.38^{AB}
Alkaline phosphatase (IU/L)	Trained	101.5 ± 65.78^{A}	109.4 ± 73.45^{A}	117.0 ± 75.04^{A}	121.8 ± 87.61^{A}
	Control	108.2 ± 55.40^{A}	132.9 ± 59.26^{A}	137.1 ± 71.17^{A}	160.0 ± 96.09^{A}
Pseudo cholinesterase (IU/L)	Trained	48.1 ± 25.89^{A}	83.9 ± 46.30^{B}	37.1 ± 17.12^{A}	38.0 ± 24.50^{A}
	Control	30.1 ± 14.28^{A}	54.1 ± 57.26^{A}	53.5 ± 31.50^{A}	45.7 ± 42.86^{A}
LDH (IU/L)	Trained	678.9 ± 540.74^{A}	837.4 ± 721.81^{A}	436.0 ± 441.57^{A}	1012.3 ± 588.34^{A}
	Control	340.5 ± 211.15^{A}	450.2 ± 476.56^{A}	388.8 ± 166.89^{A}	704.5 ± 565.27^{A}
Ions					
Na (mmol/L)	Trained	138.6 ± 6.62^{A}	144.7 ± 9.68^{AB}	145,4 ± 6.64^{AB}	147.0 ± 3.46^{B}
	Control	135.5 ± 10.61^{A}	142.6 ± 9.22^{AB}	148.5 ± 3.75^{AB}	151.5 ± 8.72^{A}
K (mmol/L)	Trained	1.35.5 ± 0.84^{aA}	1.27 ± 0.87^{A}	1,34 ± 0.59^{aA}	1.07 ± 0.55^{A}
	Control	2.95 ± 0.59$^{b\,B}$	1.87 ± 0.61^{AB}	1.98 ± 0.67$^{b\,AB}$	0.80 ± 0.56^{A}
Cl (mmol/L)	Trained	113.5 ± 2.94^{aB}	103.1 ± 10.07^{A}	112.5 ± 7.69^{B}	110.8 ± 2.89^{B}
	Control	132.0 ± 12.85^{bB}	108.1 ± 8.31^{B}	109.9 ± 12.79^{B}	110.0 ± 4.00^{A}
Ca (mmol/L)	Trained	2.23 ± 0.17^{aA}	2.25 ± 0.19^{A}	2.35 ± 0.59^{aA}	2.44 ± 0.13^{A}
	Control	2.41 ± 0.16^{bA}	2.59 ± 0.70^{A}	2.99 ± 0.74^{bA}	2.53 ± 0.19^{A}
P (mmol/L)	Trained	3.16 ± 1.33^{A}	4.04 ± 1.02^{AB}	4.50 ± 1.32^{B}	3.68 ± 0.87^{AB}
	Control	3.35 ± 2.15^{A}	3.26 ± 1.25^{A}	3.80 ± 1.40^{A}	3.80 ± 1.90^{A}
Mg (mmol/L)	Trained	0.90 ± 0.23a^{A}	0.87 ± 0.22^{A}	0.87 ± 0.23^{A}	1.20 ± 0.25^{B}
	Control	0.70 ± 0.10^{bA}	0.81 ± 0.21^{A}	0.96 ± 0.24^{AB}	1.13 ± 0.24^{A}
Significance of differences in the given parameter at the time-point marked: $^{a,\,b}P<0.05$					
Significance of differences in the given parameter within the group between time-points: $^{A,\,B,\,C}P<0.05$					

Table 2: The basic serum clinical chemical results of the trained and control group.

1-2) no systematic inter-group difference was proven. Gamma-GT and pseudo cholinesterase provided no systematic change, neither as a result of training, nor as that of ageing. Alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) provided slightly higher activities in the trained fish throughout the study (without statistical significance).

In the serum ions training exerted temporary hypokalemic and hypocalcemic effect (time point 2), while sodium, inorganic phosphate and magnesium were unaffected by exercise. Though the experiment lasted only for 35 days, both groups showed an clear age-associated increase in the serum sodium concentrations (Figure 1).

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 quickly oxidizable fuel sources and elevated concentrations of degradation products in the blood. According to our goal (i.e. to describe the longer-term adaptation to exercise), the present study was designed to potentially eliminate interference by short-term metabolic changes. Thus, training events on the sampling days were omitted. As far as the authors are aware, there is a lack of information in the literature concerning the effects of training

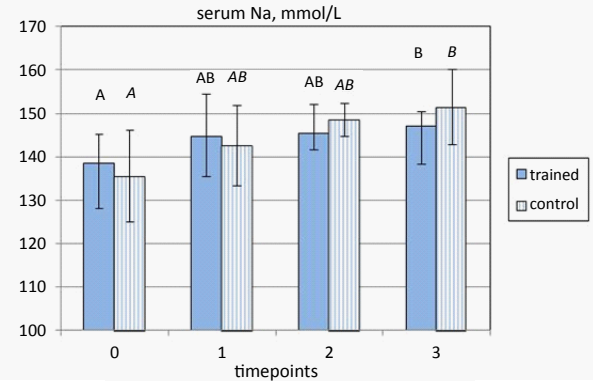

Figure 1: Ageing associated elevation of the serum sodium concentration of control and trained fish (capital letters indicate the differences between timepoints within groups).

on the metabolic adaptation of freshwater fish, in particular common carp. Clinical chemical follow-up studies on carp were so far conducted to elucidate the effects of anaesthesia [13] and transport [14]. Exercise induced clinico-chemical adaptation was analysed in golden fish (Salminus maxillosus), and in the lipid metabolism in rainbow trout [15], while marine teleost species were preferably studied [4]. Thus, this study part intended to describe metabolic adaptation as assessed by blood serum analysis in growing common carps exposed to increased physical activity in a controlled trial setting.

Nitrogenous metabolites

From within all nitrogenous metabolites analysed only albumin provided statistically higher concentration in the regularly trained fish, at the final sampling. Besides being the major protein fraction in the serum, albumin is the carrier molecule of non-esterified fatty acids (NEFA). These are in fish oxidizable fuels [5], thus, increased albumin concentration might refer to intesified storage lipid hydrolysis and transport in the serum. It has to be added that the number of NEFA binding sites of albumin appears to be constant, at least in mammals [16]. In contrast, it was early elucidated that NEFA oxidation is not stimulated by exercise in rainbow trout [7], and trout muscles are fully deficient in glycerokinase activity [8]. For freshwater fish, in particular carp this information is lacking, but the skeletal muscle glycerokinase activity of other freshwater fish species, such as plaice (Pleuronecte platessa) and flounder (Pleuronecte flesus) is low but detectable. It may thus be plausible that regular submaximal exercise did sligthly augment NEFA flux in the studied carps. Albumin is not the only NEFA binding protein in fish [17]. Analyzing the possible reasons of the elevated serum albumin levels a further factor may be considered: regular exercise leads to hypervolemia, coupled with increased albumin concentration to maintain osmolality. It is rather interesting that neither total protein (TP) nor creatinine responded to the submaximal exercise (and aging). Albeit relevant literature is scarce, transport-associated stress response was coupled with lower serum TP level in carps [14]. This is again consonant with the above findings, proving that protein catabolism was not present in the carps as an energy shuttle. As piscine kidneys excrete much of the nitrogenous waste as creatine, from which creatinine is formed [18], its training-unaffected concentration again refers to the fact that protein catabolism was not induced by the swimming treatment.

Lipid metabolites

The extent of NEFA in covering exercise energy requirements of fish have been analysed, and were found to be less important, as compared to lipoproteins (LP) [7]. Resting triacylglycerol turnover largely exceeds the requirements of endurance swimming in rainbow trout [15]. The contribution of esterified fatty acids (dominantly triacylglycerols of LPs) to fuel exercise metabolism of freshwater fish is still unknown, but seems to be important, based on our findings. In addition, in trout it was reported a largely increased lipoprotein lipase (LPL) activity after endurance swimming in the red muscles [19]. Interestingly, the strongly activated LPL activity was not accompanied by changes of serum lipopoprotein concentrations. During recovery from high-intensity exercise (such as the case of carps), white muscle (dominant in the carp fillet [20]) mitochondria oxidize lipids to fuel creatine phosphate and glycogen synthesis [21]. However, intramuscular triacylglycerol concentration is generally unaffected by exercise [22]. It seems thus that not stored, but circulating triacylglycerols serve as an important fuel source for fish muscles, which is not typical for homoeothermic vertebrates [23].

We found supportive results in the carps, in the serum triacylglycerols (time point 3, significant elevation), while total and HDL cholesterol fractions failed to respond quantitatively to exercise training. However, the relation of the two cholesterol fractions (HDL% in total) was significantly lowered by the exercise to the end of the training period (timepoint 2), while both groups showed an age associated increase in this parameter (Figure 2), as supported by other authors [24], but only humans (fish results were not available). Albeit less relevant for fish, we found an identical age-related increase in growing broiler chickens [25]. Rainbow trout resting triacylglycerol flux is not exceeded even during 4 days of continuous swimming [7]. Our exercise load was markedly lower that this, but it has to be mentioned that carps were so exhausted by the end of the sessions that they could be caught with hands. Taking the above results into account it seems that carps provide burst-like, intensive but short exercise bouts, of which the energy requirement may by primarily covered from intramuscular lipids [5]. and to a lesser part from intracellular fuels (both muscle and liver glycogene (see LDH)).

Enzymes

The typical hepatic enzymes ALT and AST reacted to the exercise with elevated serum activity values, at the final sampling. The markedly elevated activities regularly indicate hepatocellular damage. Similar

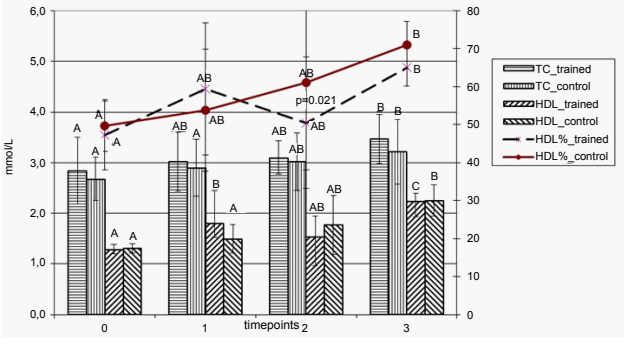

Figure 2: Total cholesterol, HDL cholesterol and the HDL% in the total cholesterol during the training period in the control and trained fish (capital letters indicate the differences between time points within groups).

results for AST were reported by other authors in carps, where serum AST activity was a direct correlate of transport associated stress [14]. Interestingly, ALT failed to respond to this condition in the cited report. Moreover, AST activity values ranged over the literture data during the entire study, even the initial sampling. Although it was not directly determined, exercise or capture associated stress may as well lead to hepatocellular damage and consequent ALT and AST activity increases in snapper (Chrysophrys auratus) [26].

Lactate dehydrogenase (LDH) was showing non-significantly higher activities troughout the study in the trained carps. We assume that the large standard deviation led to the lack of statistical significance between groups. We experienced that carps maintain an irregular, burst-type swimming even in constant speed water flow. It is thus supposed that glycolytic potential was only slightly increased while lactate is a known glycogenic substrate of fish muscles [6].

Alkaline phosphatase (ALP) in veretabrates is a diagnostic marker of hepatobiliary and bone disorders [27]. In our dataset a slight but not significant increasing tendency was found for both groups, without inter-group differences. It has to be added that isoenzymes of ALP were not analyzed separately, thus its origin (liver, bone, intestine) is not clear. Evaluated together with gamma-GT providing minimal and physiological fluctuations, it seems that hepatobiliary disorders can be excluded. On the other hand, neither strenuous nor mild exercise affects the serum activity of gamma-GT. The physiological role of gamma-GT is to break down extracellular glutathione and making its amino acids available to the cells [28]. Meanwhile the activity of gamma-GT was not significantly higher in the trained fish, we found significantly higher oxidized glutathione levels in this group (but only at the final sampling) (Figure 3). These results agree with other authors [29] in humans, describing largely increased oxidized glutathione (GSH) levels after exercise in humans. GSH is a potent antioxidant, preventing cellular membrane lipids which are targets of oxidative damage during strenous exercise [30]. Taking the gamma-GT results also into account we suppose a mild alteration of the glutathione redox status due to exercise in which GSH oxidation was augmented.

Serum ions

Sodium and chloride concentrations of freshwater fish range from 130 to 150 mmol/L [17]. Any deviations from this are associated with gill or renal diseases or water acidity or altered hardness. For chloride, we found slightly lower values, without tendentious alterations, while sodium was unaltered by training. In contrast, an age-dependent increase was found for serum sodium concentration in both groups (Figure 1). Earlier studies on birds [31,32] provided similar, age dependent sodium level increases, most probably referring to the relationship of this ion with the increasing dry matter content of the body along ageing. As far as the authors are aware, in freshwater fish this is the first instance to prove this alteration.

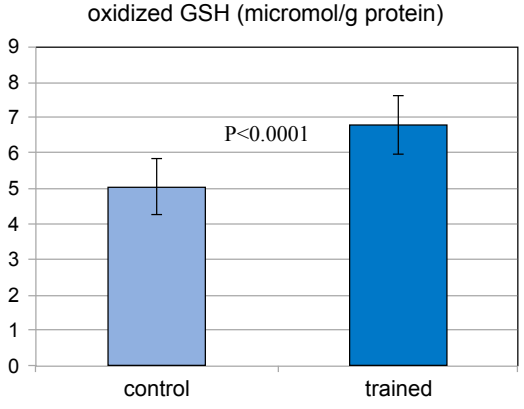

Figure 3: The serum oxidized glutathione concentration of the control and trained fish (means ± SD) at time point 3.

Capture procedure and consequent exhaustion related stress can increase serum sodium, potassium, chloride and calcium concentrations [27]. In our study in none of these ions were similar findings found (i. e. trained vs. control comparison), in contrast, potassium and calcium decreased (time point 2 and the initial value). The increased concentration of these ions is generally attributed to muscle cell membrane injuries and consequent leak. The opposite was found in our study (only magnesium provided initially (initial, zero point sampling) higher concentration in the exercised groups. Based on the less fluctuating ion concentrations and the slight training associated between group differences sarcolemma damage was not supposed in our study.

Conclusions

Summarized, in common carp characterized with burst-like swimming we found slightly elevated lipoprotein utilization (decreased HDL cholesterol% in the total cholesterol), severe hepatocellular damage (ALT, AST, most probably induced by the concomitant stress), well balanced serum ion concentrations providing mostly age-associated changes (sodium) after a 35-day training period. Based on the finally increased albumin concentration we supposed slight hipervolemia, while increased oxidized glutathione concentration referred to elevated antioxidant capacity. It was assumed that even longer term regular strenuous exercise exerts only mild effect on the substrate metabolism of carp, being a slowly swimming benthic feeder.

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References

1. (2013) Fish Base version 4.
2. Lighthill MJ (1969) Hydromechanics of aquatic animal propulsion. Annual Review of Fluid Mechanics 1: 413-446.
3. Bernal D, Donley JM, McGillivray DG, Aalbers SA, Syme DA, et al. (2010) Function of the medial red muscle during sustained swimming in common thresher sharks: contrast and convergence with thunniform swimmers. Comp Biochem Physiol A Mol Integr Physiol 155: 454-463.
4. Davison W (1997) The effects of exercise training on teleost fish, a review of recent literature. Comparative Biochemistry and Physiology A 117: 67-75.
5. Hochachka PW (1985) Fuels and pathways as designed systems for support of muscle work. J Exp Biol 115: 149-164.
6. Pagnotta A, Milligan CL (1991) The role of blood glucose in the restoration of muscle glycogen during recovery from exhaustive exercise in rainbow trout (Oncorhynchus mykiss) and winter flounder (Pseudopleuronectes americanus). J Exp Biol 161: 489-508.
7. Bernard SF, Reidy SP, Zwingelstein G, Weber JM (1999) Glycerol and fatty acid kinetics in rainbow trout: effects of endurance swimming. J Exp Biol 202: 279-288.
8. Newsholme EA, Taylor K (1969) Glycerol kinase activities in muscles from vertebrates and invertebrates. Biochem J 112: 465-474.
9. Hinterleitner S, Huber M, Lackner R, Wieser W (2011) Systemic and Enzymatic Responses to Endurance Training in Two Cyprinid Species with Different Life

Styles (Teleostei: Cyprinidae). Canadian Journal of Fisheries and Aquatic Sciences 49: 110-115.

10. Sedlak J, Lindsay RH (1968) Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissues with Ellman's reagent. Anal Biochem 25: 192-205.
11. Dean RB, Dixon WJ (1951) Simplified Statistics for Small Numbers of Observations. Anal Chem 23: 636-638.
12. SPSS for Windows 10.0 (1999) SPSS Inc Chicago, IL.
13. Velisek J, Svobodova Z, Piackova V, Groch L, Nepejchalova L (2005) Effects of clove oil anaesthesia on common carp (*Cyprinus carpio L*). Veterinary Medicine 50: 269-275.
14. Dobsiková R, Svobodova Z, Blahova J, Modra H, Velisek J (2009) The effect of transport on biochemical and haematological indices of common carp (*Cyprinus carpio L*). Czech Journal of Anim Science 54: 510-518.
15. Magnoni L, Vaillancourt E, Weber JM (2008) High resting triacylglycerol turnover of rainbow trout exceeds the energy requirements of endurance swimming. Am J Physiol Regul Integr Comp Physiol 295: 309-15.
16. Spector AA, Fletcher JE, Ashbrook JD (1971) Analysis of long-chain free fatty acid binding to bovine serum albumin by determination of stepwise equilibrium constants. Biochemistry 10: 3229-3232.
17. Sheridan MA (1988) Lipid dynamics in fish: aspects of absorption, transportation, deposition and mobilization. Comparative Biochemistry and Physiology 90: 679-690.
18. Thrall MA (2004) Veterinary haematology and clinical chemistry. Lippincott, Williams and Wilkins, Philadelphia, USA.
19. Magnoni L, Weber JM (2007) Endurance swimming activates trout lipoprotein lipase: serum lipids as a fuel for muscle. Journal of Experimental Biology 210: 4016-4023.
20. Varga D, Szabó A, Romvári R, Hancz Cs (2011) Preliminary study for macroscopic determination of the white to red muscle ratio, and its correlation with flesh quality parameters (in Hungarian). Acta Agraria Kaposvariensis 15: 85-90.
21. Moyes CD, Schulte PM, Hochachka PW (1992) Recovery metabolism of trout white muscle: role of mitochondria. Am J Physiol 262: 295-304.
22. Richards JG, Mercado AJ, Clayton CA, Heigenhauser GJF, Wood CM (2002) Substrate utilization during graded aerobic exercise in rainbow trout. J Exp Biol 205: 2067-2077.
23. Turcotte LP (1999) Role of fats in exercise: Types and quality. Clinics in Sports Medicine 18: 485-498.
24. Kipreos G, Tripolitsioti A, Stergioulas A (2010) The effects of anaerobic training in serum lipids and arachidonic acid metabolites. Biology of exercise 6.2: 5-12.
25. Szabó A, Milisits G (2007) Clinicochemical follow-up of broiler rearing – a five-week study. Acta Vet Hung 55: 451-462.
26. Wells RMG, McIntyre RH, Morgan AK, Davie PS (1986) Physiological stress responses in big gamefish after capture: Observations on serum chemistry and blood factors. Comparative Biochemistry and Physiology A 84: 565-571.
27. Bishop ML, Fody EP, Schoeff LE (2005) Clinical Chemistry. Lippincott Williams and Wilkins Philadelphia, USA.
28. Whitfield JB (2001) Gamma Glutamyl Transferase. Cri Rev Cli Lab Sci 38: 263-355.
29. Sastre J, Asensi M, Gasco E, Pallardo FV, Ferrero JA, et al. (1992) Exhaustive physical exercise causes oxidation of glutathione status in blood: prevention by antioxidant administration. Am J Physiol 263: 992-995.
30. Kerksick C, Willoughby D (2005) The Antioxidant Role of Glutathione and N-Acetyl- Cysteine Supplements and Exercise-Induced Oxidative Stress. J Int Soc Sports Nutr 2: 38-44.
31. Vasicek L, Shwendenwein I, Voill S (1991) Chemical blood analysis to establish standard values in turkeys of different ages. Deutsche Tierarztliche Wochenschrift 98: 126-129.
32. Szabó A, Mézes M, Horn P, Sütő Z, Romvári R (2005) Developmental dynamics of some blood biochemical parameters in the growing turkey (Meleagris gallopavo). Acta Vet Hung 53: 397-409.