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Trout plasma metabolites, blood factors and spleen contraction: responses to exercise*

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Fish react to handling and capture with a burst of exercise that affects them deeply. The present study examines the effect of such severe exercise and the time course of recovery on the hematology (including spleen response) and metabolism of a population of cultured rainbow trout. Exercise was induced by continuous chasing for 5 min when the trout showed signs of exhaustion. Such exercise led to spleen contraction and an increase in haematocrit values. Carbohydrates were mobilized and anaerobic glycolysis produced lactate without significant effect on lipid metabolism. The conclusion is reached that the respiratory properties of rainbow trout blood do not change following severe exercise, while muscle anaerobic metabolism is sligthly activated as deduced from the fast and short lactacidemia observed, which may have been related to a reduced stressing component, as the exercise was performed in the same environment in which the fish were reared.

Key words: Blood, Exercise, Metabolism, Recovery, Spleen, Stress, Trout.

The conditions created during fish culture are very different from those to which the fish are exposed in their natural environment. Fish react to handling and capture with a burst of severe exercise, which has a profound physiological effect on many teleost fish species (6, 19, 32).

Hematological responses to exercise in teleost fishes may involve hemoconcentration, i.e. an increase in the hematocrit level (Hct), red blood cell count (RBC) and blood hemoglobin concentration (*Hb*). The amplitude and time course of the change will depend on various factors: the species, the intensity and duration of the exercise, the method used to induce it, the environmental temperature, the nutritional condition, the animal's age, the

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nature of the population, and individual training (3, 27, 30). After exercise, the values return to rest values within 4 hours (14), after which an inversion of the response may be observed (8, 33). The spleen of teleost fishes contracts during severe exercise, supplying erythrocytes to the circulating blood, thus contributing to hemoconcentration (21, 35, 36). While the origin of these changes is diverse, their hematological consequences may be devoted to increasing the blood oxygen carrying capacity (14, 20, 22).

Muscular activity in fish affects metabolism similarly as in higher vertebrates: glycogen is mobilized, while pyruvate and lactate, which diffuse from muscle and lead to a prolonged increase in blood concentration, are produced (10, 19). Exhaustive, burst-type exercise is fuelled by anaerobic metabolism, which results in glycogen depletion and lactate accumulation in the white muscle, the blood compounds of energy metabolism being affected. Plasma lactate increases during exercise as well as within the first 2 hours after exercise, and returns to rest levels after 8-12 hours (10, 17, 30). Plasma glucose may not show any change (10, 19) or an increase within 30-60 min (11, 13, 17) or several hours after exercise (34). Data on how blood pyruvate changes in relation to exercise are very scarce, although it seems to change similarly as blood lactate (13, 29).

Many of the above mentioned data were obtained in experimental conditions with devices and installations very different from those used for fish culture. The present study examines the effect of severe exercise on both blood metabolic and respiratory (including spleen) responses in a population of cultured rainbow trout, and determines the significance of the magnitude of both responses, and follows the time course of the metabolic and hematological parameters during recovery. To ensure that the fish response was due solely to the induced exercise and not to any external stressing factor, the fish were chased in the tank where they were normally reared. This procedure induced a submaximal locomotion speed and a degree of stress response very similar to that produced in fish when they are manipulated during normal maintenance.

Materials and Methods

Animals and maintenance.– Female rainbow trout (*Oncorhynchus mykiss*) were obtained from a commercial trout farm. Groups of 10 fish were kept in laboratory conditions in rectangular tanks filled to 250 l for 2 months (February and March; photoperiod: 10L:14D; temperature: 15 ± 1 °C; feed ratio: 2 % body weight/day; and with a continuous supply of aerated dechlorinated tap water).

Experiments.– Trout (280-400 g) were starved for 40 h before experimentation. They were then exercised to exhaustion by vigorous chasing for 5 min in the tank in which they were kept. Fish were quickly netted before, immediately after exercise, and after resting for 30, 60, 120 or 240 min and were killed by a sharp blow to the head. Two ml of blood was immediately drawn from the caudal vein into heparinized syringes. One ml was used for hematological determinations and the other for obtaining plasma by centrifugation (10 min, 2,000 g). This was then stored frozen until needed for biochemical analysis. The spleen of the fish was excised and weighed.

Hematological determinations.– Hematocrit (*Hct*) values were obtained by centrifuging (10 min) with an ORTO radial centrifuge for microhematocrit tubes. Red blood cell counts (*RBC*) were estimated by using a Thoma chamber. Hemoglobin (*Hb*) concentrations were obtained by the cyanmetahemoglobin method (28) centrifuging (15 min, 800 g) to remove turbidity. These data were used to calculate the derived parameters: mean cell volume (*MCV*), mean cell hemoglobin (*MCH*) and mean cell hemoglobin concentration (*MCHC*).

Analysis.– Plasma concentrations of lactate (7), glucose (25) and free fatty acids (26) were determined following different methods and using commercial kits (Boehringer-Mannheim). Blood pyruvate concentration was measured enzymatically by spectrophotometric methods (2). All determinations were performed in duplicate.

Statistical analysis.– An ANOVA test ($p < 0.05$) and a multiple range test were carried out by a statistic program (SYSTAT) for PC computers.

Results

The spleen somatic index showed a significant decrease immediately after exercise and throughout the recovery period (4 h), the spleen contraction induced by exercise having been maintained (table I). After exercise there was a significant increase in *Hct* values. In addition, heterogeneity in the *RBC* data was demonstrated statistically by a multiple range test, the highest values being observed both immediately and 30 min after exercise. These

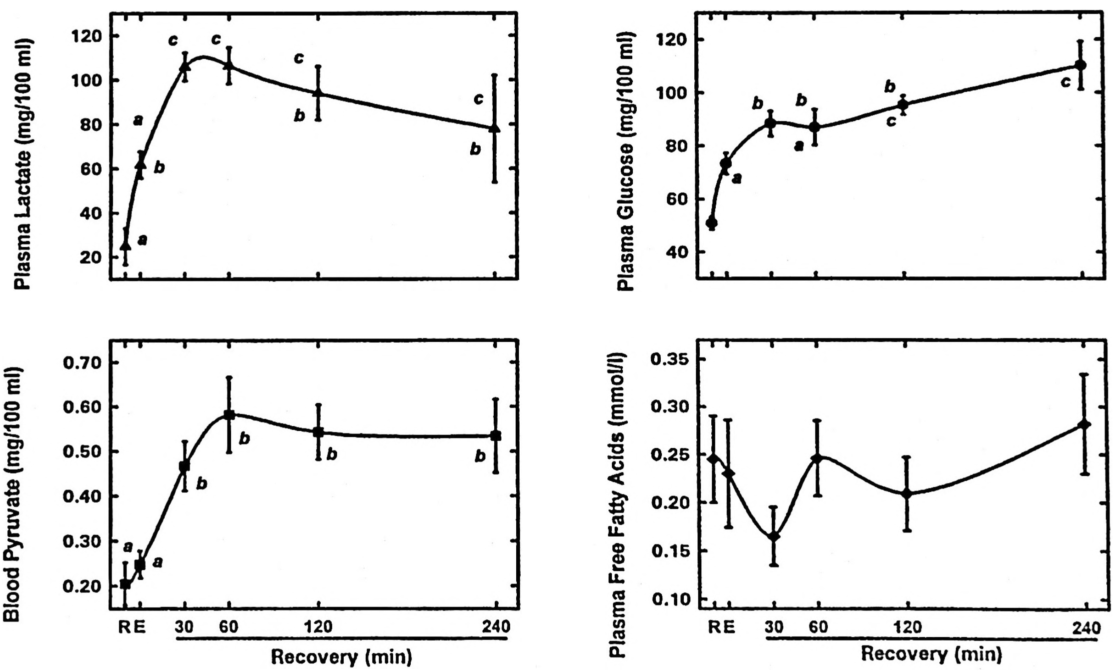

Fig. 1. *Variations in plasmatic and blood levels of several metabolites in rainbow trout (Oncorhynchus mykiss) after exhausting exercise.*
Points are the mean ± SE of the mean. a, b and c indicate the statistically homogenous groups.

Table I. *Variations in hematological parameters of rainbow trout* (Oncorhynchus mykiss) *with exhausting exercise.*
Values are the mean ± SD. Number of individuals in brackets. MCV = Mean Corpuscular Volume. MCHC = Mean Corpuscular Hemoglobin Concentration. MCH = Mean Corpuscular Hemoglobin.

At rest	After exercise	Recovery			
		30 min	60 min	210 min	240 min
SPLEEN-SOMATIC INDEX (% b. w.)					
0.158±0.069 (10)	0.101±0.021 (10)	0.089±0.023 (10)	0.093±0.046 (10)	0.099±0.053 (10)	0.095±0.029[a] (10)
HEMATOCRIT (%)					
30.95±5.74 (10)	37.70±3.95 (10)	35.35±4.77 (10)	33.30±3.42 (10)	34.48±3.93 (10)	32.40±3.45[b] (10)
HEMOGLOBIN (g/100 ml)					
5.99±0.85 (8)	6.55±1.03 (8)	6.78±0.91 (8)	6.48±0.92 (10)	6.63±0.99 (10)	6.06±0.50 (10)
RED BLOOD CELL (x 10^3/mm^3)					
1145.3±203.0 (10)	1310.3±169.3 (9)	1323.1±135.8 (10)	1196.6±218.1 (8)	1192.2±211.7 (10)	1091.7±159.5 (9)
MCV (μm^3)					
272.7±50.2 (10)	293.7±45.0 (9)	268.6±35.4 (10)	289.8±41.8 (8)	293.0±29.4 (10)	297.1±32.4 (9)
MCHC (%)					
18.32±2.68 (8)	17.06±2.17 (8)	19.21±1.67 (8)	19.33±2.53 (8)	19.52±4.40 (8)	18.84±1.40 (8)
MCH (pg)					
51.20±12.02 (8)	51.13±5.52 (7)	51.61±6.19 (8)	57.67±17.41 (6)	58.13±19.80 (8)	57.57±7.20 (7)

ANOVA: [a]$p < 0.008$; [b]$p < 0.017$.

values returned to their rest levels at the end of the 4 h recovery period (table I).

The variations observed in metabolite concentrations after exercise and during the 4 h recovery period are shown in fig. 1. Lactate concentrations rose after exercise and during the first hour of recovery, falling slowly thereafter. A similar pattern, but more delayed, showed the change in pyruvate levels, which rose after the first 30 min of recovery and remained high until the end of the recovery period examined. There was a significant increase in the plasmatic glucose level after exercise, a plateau being reached between 30 and 60 min before rising once again during the rest of the recovery period examined. Free fatty acid concentrations did not vary significantly.

Discussion

In the present study the hematocrit value increases with exercise, a similar pattern being followed by the red blood cell count. Similar results have been found using equivalent exercise intensities (12, 14, 21). At least two factors are probably involved in the variations of the hematocrit values during exercise (21): the contribution of splenic blood cells and erythrocyte swelling. The decrease in the splenic somatic index in rainbow trout after exercise and its evolution during the recovery period indicates that the spleen contracts during intense exercise. Some other species show strong spleen contraction with exercise (35, 36) such contraction having been correlated with the capacity for aerobic exercise (22). However, spleen contraction in trout seems to be more responsive to hypoxia than to exercise (21). In the present work, small but significant changes were observed in both spleen-somatic index and hematocrit level. The latter can be attributed to a slight change in the red blood cell count. The splenic contraction, therefore, must be incomplete and produces only a small hematological impact after exercise. During the recovery period, the blood cell count returned to initial values and led to a hematocrit reduction, that almost reached their rest values after 60 min of recovery. Other authors reported a stronger response and higher hematocrit values which were maintained for 2-4 h (14, 21, 29).

Intense exercise induced by chasing also had significant metabolic repercussions as indicated by variations in blood glucose, lactate and pyruvate concentrations. Plasma lactate increased immediately after exercise, peaked after 30 min recovery and then decreased slowly. During the intense swimming activity, the anaerobic metabolism built high concentrations of lactate in the large white muscle. In salmonids, 10-20 % of the total lactate produced is released into the blood, while the rest is slowly used by metabolic processes *in situ*, particularly by glycogenesis, the Cori cycle likely playing a minor role in lactate metabolism in fishes (10, 16, 19). Several authors have pointed to a peak being reached after 1-2 h of recovery (10, 17), and only in few cases after 30-60 min (29). Moreover, the values they observed at the peak after intense exercise are higher than those found in the present work.

Blood pyruvate concentration showed a similar pattern to that of plasmatic lactate. However, no trend towards a decrease to rest levels at the end of four hours of recovery was observed (fig. 1). A similar trend has been reported previously (13, 29). The pyruvate blood levels during exercise are mainly thought to reflect the concentration in the muscle (29), and as lactate-to-pyruvate ratio does not change significantly in trout muscle during fatiguing exercise lasting 10 to 30 min (5), the rise in blood pyruvate must reflect the activation of muscle glycolysis during exercise. Another possible origin of post-exercise pyruvate may be the amino acid catabolism or the replenishment of the muscle adenylate pool, including ATP (4).

In the present study, plasmatic glucose concentration rises with exercise in rainbow trout. The hyperglycaemic response, when observed immediately after exercise, has been associated with liver glycogen mobilization in exercised, or stressed, rainbow trout (17), while, in other cases, no glycogen decrease has been observed (13). The response can also be attributed to a gluconeogenic origin, as there is evidence that exercise increases the amino acid catabolism in muscle (MARÍN *et al.*, unpublished). The plasmatic glucose concentration continues to rise during the recovery period (4 hours after exercise). Its evolution (a rise followed by a plateau and then another increase) may be related to the activation pattern of the endocrine system. Intense exercise and stress in fish are accompanied by increases in plasma concentrations of at least two types of hormones: catecholamines and cortisol. The rise in catecholamines is rapid and short, whereas the cortisol response is slower and more prolonged (10). However, the metabolic consequences of these endocrine changes are not fully understood. Isolated trout hepatocytes increase glucose output due to glycogen breakdown as well as increased gluconeogenesis in response to added catecholamines (15, 24). An adrenaline injection produces hyperglycaemia through an increase in liver gluconeogenesis (18). On the other hand, the role of cortisol as a direct promoter of glycogen synthesis and hyperglycemia has been questioned (1). Nevertheless, recent results indicate that cortisol increase after exercise exerts a negative influence on lactate and glycogen recovery metabolism, partially due to a permissive cortisol effect on catecholamine action and to its inhibitory effect on muscle glycogen synthase (10), which may also partially explain the maintenance of hyperglycemia. The fact that glucose concentration continues rising during the recovery period while lactate concentration falls after its early peak value (between 30-60 min) might also be due to a shift in tissue substrate used after exercise (34).

Although lipids are the principal energy fuel sources for long-term sustained swimming, while muscle glycogen serves as immediate energy source for vigorous exercise (6, 19, 23), there is evidence of an increased fatty acid oxidation in trout white muscle during and after intense exercise (10, 31). If it were so in the present work, it was not reflected in any change in plasma FFA.

In conclusion, the burst swimming induced (by continuous chasing for 5 min) does not seem to involve any substantial change in the blood respiratory properties, perhaps because the exercise is largely anaerobic and independent of circulatory and respiratory systems (9), although they do play a role during recovery. The changes observed in lactate and pyruvate reflect the activation of muscle anaerobic metabolism, although their size indicates that such activation is slight. Perhaps the exercise induced has a reduced stressing component as it was carried out in the same environment (tank) where the fish was reared. On the other hand, the role of glucose cannot be minimized, as an increased blood concentration is observed both after exercise and during recovery. This suggets that the role of glucose is not so much to replenish the muscle glycogen stores (10) as to act as a metabolic fuel in other tissues involved during the recovery effort such as heart, brain, etc., whose metabolic changes during and after exercise are poorly known in fish.

P. MENDIOLA, M. D. HERNÁNDEZ, J. DE COSTA y S. ZAMORA. *Metabolitos del plasma, factores sanguíneos y contracción del bazo en la trucha arco iris: respuestas al ejercicio.* J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (2), 217-224, 1997.

Los peces reaccionan frente a la captura y la manipulación con una actividad física intensa (ejercicio intenso) que les afecta considerablemente. En el presente trabajo se estudia el efecto del ejercicio intenso y cómo se desarrolla la recuperación hematológica, incluída la respuesta del bazo, y metabólica en una población de truchas de piscifactoría. El ejercicio se provoca mediante persecución continua durante 5 minutos, tiempo suficiente para que las truchas se fatiguen y se produzca contracción del bazo y aumento del valor hematocrito. Hay movilización muscular de carbohidratos y, a través de la glucolisis anaerobia, producción de lactato, sin cambios en el metabolismo lipídico. Se concluye que las propiedades respiratorias de la sangre apenas se modifican debido al ejercicio intenso empleado, mientras que sí se activa parcialmente el metabolismo anaerobio muscular, según se deduce del incremento rápido y corto observado en la lacta-

cidemia, que podría deberse a que el ejercicio se desarrolló en el mismo tanque en que los animales vivían, con lo que se pudo haber reducido el componente de estrés de la respuesta metabólica.

Palabras clave: Bazo, Ejercicio, Estrés, Metabolismo, Recuperación, Sangre, Trucha.

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