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LIPID MOBILIZATION IN RAINBOW TROUT

(*ONCORHYNCHUS MYKISS*)

Servane Bernard

Thèse soumise à

l'Ecole des études supérieures et de la recherche

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LIPID MOBILIZATION IN RAINBOW TROUT

(*ONCORHYNCHUS MYKISS*)

SUMMARY

Even though lipids are thought to be a major metabolic fuel in fish during aerobic activity, little is known about their mobilization. Therefore, the goal of my thesis was to quantify lipid mobilization in fish. By analogy with mammals, I hypothesized that when fish are subjected to submaximal exercise, they mobilize their triacylglycerol (TAG) reserves above resting levels, which would result in: 1) increases in fatty acid (NEFA) and glycerol appearance rates (also termed fluxes), and 2) a decrease in the percent of NEFA reesterified by the TAG:FA substrate cycle. To test this hypothesis, I measured NEFA and glycerol rates of appearance, and I calculated the intracellular TAG:FA cycling rate in rainbow trout swimming for 1 h at either 1.0 or 1.5 bl s^{-1}, and 4 days at 1.0 bl s^{-1}. This study is the first to measure glycerol appearance rate and TAG:FA cycling in fish. In rainbow trout, the average values of NEFA and glycerol appearance rate were 5.33 ± 0.34 and 4.09 ± 0.16 µmol kg^{-1} min^{-1}, respectively; and the relative intracellular TAG:FA cycling rate was 68.0 ± 1.5 % . Regardless of swimming duration or speed, neither glycerol and NEFA appearance rates, nor the TAG:FA cycling rate changed when the fish were exercised. These results clearly show that, contrary to what I had hypothesized, rainbow trout do not mobilize their TAG reserves beyond resting levels during low intensity exercise (Chapter 2).

From the absence of change in the intracellular TAG:FA cycling rate with exercise, I conjected that the TAG:FA cycle was a true futile cycle in fish. In mammals, the fraction of the fatty acids reesterified in the TAG:FA cycle depends upon the availability of fatty acids for the enzymatic reactions (Wolfe *et al.*, 1990). Therefore, I formulated the hypothesis that in fish, a reduction in the availability of the fatty acids caused by an inhibition of lipolysis, would not result in a decrease in the percentage of fatty acids reesterified. To test this hypothesis, I studied the effect of an infusion of 10 mg of acipimox, an antilipolytic drug, on the glycerol and fatty acid appearance rates and intracellular TAG:FA cycling in rainbow trout. No significant effect of acipimox on either NEFA or glycerol kinetics was observed, with the exception of a decrease in NEFA concentration and palmitate appearance rate. These ambiguous results appear to be mainly due to the high inter-individual variability in the magnitude and response time of the fish to the acipimox treatment. Since no significant effect of acipimox on glycerol and fatty acid appearance rates was observed, it was not possible to determine whether the TAG:FA cycle plays any role in regulating lipid metabolism in rainbow trout. This question needs to be further investigated.

This thesis demonstrates that during submaximal exercise fish do not mobilize their triacylglycerol reserves above their resting levels. This result contrasts with what was expected by analogy to the mammalian situation or from body composition studies in exercising fish.

RESUME

Bien que les lipides soient considérés comme un carburant métabolique important lors de la nage prolongée chez les poissons, leur mobilisation est peu connue chez ces animaux. Le but de ma thèse était donc de quantifier la mobilisation des lipides chez les poissons. Par analogie à ce qui se produit chez les mammifères, j'ai émis l'hypothèse selon laquelle les poissons, lorsqu'ils sont soumis à un exercice sous-maximal, mobilisent leurs réserves de triacylglycérol (TAG) au delà des niveaux de repos. Ainsi, je prévoyais qu'une mobilisation accrue des réserves lipidiques résulterait en une augmentation des taux d'apparition (ou flux) du glycérol et des acides gras (AG), ainsi qu'en une diminution du pourcentage d'acides gras réestérifiés par le cycle de substrat TAG:AG. Pour tester cette hypothèse, j'ai mesuré les flux du glycérol et des acides gras, ainsi que le taux de recyclage intracellulaire TAG:AG chez la truite arc-en-ciel soumise à un exercice d'une heure à 1.0 ou 1.5 lgc s^{-1}, ou de quatre jours à 1.0 lgc s^{-1} (longueur de corps par seconde). Le taux d'apparition du glycérol et le recyclage intracellulaire TAG:AG n'avaient jamais été déterminés auparavant chez les poissons. Chez la truite arc-en-ciel, les valeurs moyennes des flux du glycérol et des acides gras étaient respectivement de 5.33 ± 0.34 et 4.09 ± 0.16 µmol kg^{-1} min^{-1}, et le taux relatif de recyclage intracellulaire TAG-AG était de 68.0 ± 1.5 %. Ces valeurs n'ont pas changé au cours de l'exercice, quelques fussent la vitesse et la durée de l'exercice. Contrairement à l'hypothèse que j'avais émise, ces résultats montrent que, durant un exercice de faible intensité, la truite arc-en-ciel ne mobilise pas ses réserves de TAG au delà des niveaux de repos.

En raison de l'absence de changement du recyclage intracellulaire TAG:AG lors de l'exercice, j'ai suggéré que le cycle TAG:AG était un véritable cycle futile chez les poissons. Chez les mammifères, le pourcentage d'acides gras réestérifiés par le cyle TAG:AG dépend de leur disponibilité pour les réactions enzymatiques (Wolfe *et al.*, 1990). En conséquence, j'ai émis l'hypothèse selon laquelle, chez les poissons, une réduction de la disponibilité des acides gras, par inhibition de la lipolyse, n'entraîne pas de diminution du pourcentage d'acides gras réestérifiés par le recyclage TAG:AG. Pour tester cette hypothèse, j'ai étudié les effets de l'acipimox, une drogue anti-lipolytique, sur le taux d'apparition du glycérol et des acides gras, et sur le taux de recyclage intracellulaire TAG:AG chez la truite arc-en-ciel. Aucun effet significatif de l'acipimox sur la cinétique du glycérol ou des acides gras n'a été observé, à part une diminution de la concentration des acides gras et du taux d'apparition du palmitate. Ces résultats ambigües seraient expliqués par la variabilité importante de l'amplitude et du temps de réponse des poissons à l'administration d'acipimox. Comme les flux du glycérol et des acides gras n'ont pas été significativement affectés par l'acipimox, il n'a pas été possible de déterminer si le cycle TAG:AG joue un rôle dans la régulation du métabolisme des lipides chez la truite arc-en-ciel. Des études supplémentaires s'avèrent donc nécessaires pour déterminer si le cycle TAG:AG est un véritable cycle futile chez les poissons.

Cette thèse démontre que l'exercice sous-maximal chez les poissons n'entraîne pas la mobilisation des réserves de TAG au delà des valeurs de repos. Ce résultat contraste avec ce qui avait été prévu par analogie à ce qui se produit chez les mammifères ainsi qu'à partir des études de composition corporelle menées chez les poissons soumis à un exercice aérobie.

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CHAPTER 1

GENERAL INTRODUCTION

Animals mobilize specific metabolic fuels to sustain their energy needs. The relative contribution of carbohydrates, lipids or proteins depends upon the type of food they eat (Walton and Cowey, 1982), their level of activity (rest *vs.* exercise; untrained *vs.* trained) (Weber, 1992) and their life history pattern (Sheridan, 1994). For instance, aerobic exercise in postabsortive mammals is primarily sustained by carbohydrate and lipid oxidation, whereas protein catabolism provides less than 2% of their total energy (Weber, 1992). However, anaerobic exercise causes a shift in fuel utilization towards the sole use of carbohydrate sources (Hochachka and Somero, 1984), a pattern also observed in fish (Driedzic and Hochachka, 1978).

Fuel preference of fish at rest and during aerobic exercise is not well defined. However, it is generally accepted that, contrary to mammals, fish rely to a much greater extent on protein and lipid metabolism, whereas the contribution of carbohydrate catabolism to total energy use is very low (Walton and Cowey, 1982). In the following sections, I will summarize the present state of our knowledge about the use of the different oxidative fuels in fish.

Metabolic fuel preference during aerobic activity in fish

Proteins

The increased reliance of fish on protein catabolism to sustain aerobic exercise is particularly evident during the last stages of long migrations, when lipid and carbohydrate reserves become depleted (Driedzic and Hochachka, 1978; Mommsen *et al.*, 1980). Also, under normal nutritional conditions, proteins constitute a large proportion of their daily caloric intake (Wilson, 1989). Therefore, it is not unreasonable to think that the routine metabolism of fish may be mainly supported by amino acids, particularly after feeding (Moyes and West, 1995). Unfortunately, the evidence dealing with the importance of protein in energy metabolism during submaximal activity is contradictory. Body composition studies on coho salmon (*Oncorhynchus kisutch*) show that exercise causes an increase in their relative use of proteins (Krueger *et al.*, 1968). Other studies on non-salmonid species report an increase in their ammonia quotient (ratio of ammonia excretion to oxygen consumption) (Kutty, 1972). Van den Thillart (1986) used indirect calorimetry to measure the contribution of protein, lipid, and carbohydrate oxidation to total energy expenditure in rainbow trout. This author found that protein oxidation accounted for over 80% of their metabolic rate at rest and during exercise. In the same species, Lauff and Wood (1996) found that only 30% of resting metabolic rate was sustained by protein oxidation. Moreover, these authors found that the absolute contribution of proteins did not increase with exercise, but that all the additional energy needed for locomotion was provided by carbohydrates and lipids.

Carbohydrates

Lauff and Wood (1996) found that in juvenile rainbow trout, carbohydrate oxidation accounted for 23% of the oxygen consumption at rest and increased with aerobic exercise. This finding contrasts with traditional thinking that the contribution of carbohydrates to resting energy metabolism is extremely low in fish (Van den Thillart, 1986; Walton and Cowey, 1982), as indicated by the low carbohydrate content of fish diets (Halver, 1972). The high carbohydrate use reported by Lauff and Wood (1996; 1997) during exercise is consistent with the fact that fish red muscle is principally recruited during prolonged swimming (Driedzic and Hochachka, 1978), and has a high capacity for carbohydrate oxidation (Moyes and West, 1995). However, during prolonged periods of aerobic swimming, the supply of carbohydrates to red muscle could be limited (Moyes and West, 1995). Glycogen reserves in the muscle are usually not large enough to supply the high energy demand that occurs during prolonged exercise (Moyes and West, 1995), and recent measurements of circulatory glucose flux show that the use of hepatic glucose does not increase during aerobic exercise in rainbow trout (*Oncorhynchus mykiss*) (D. Shanghavi, personal communication). Lactate also constitutes a possible source of carbohydrate fuel for the working oxidative muscle (Moyes and West, 1995). At the highest intensities of aerobic exercise, fish recruit both oxidative red muscle and glycolytic white muscle (Hudson, 1973; Johnston and Moon, 1980). Therefore, it has also been hypothesized that lactate produced from white muscle glycogen could be transported to the red muscles for oxidation (Moyes and West, 1995). However, Weber (1991) showed that in rainbow trout the transfer of lactate from white to red muscle was of no significance in supplying energy to working muscles.

Lipids

The potentially large contribution of lipids to the aerobic energy metabolism of fish is widely accepted (Driedzic and Hochachka, 1978; Henderson and Tocher, 1987; Mommsen *et al.*, 1980; Walton and Cowey, 1982). However, recent indirect calorimetry studies report widely different degrees of participation of lipid oxidation to metabolic rate. In resting rainbow trout, according to Van den Thillart (1986), lipid oxidation accounts only for 20 % of oxygen consumption, while Lauff and Wood (1996; 1997) measured that 47 to 66 % of oxygen consumption could be attributed to lipid oxidation. Lauff and Wood (1996; 1997) also found that it increased with aerobic exercise. With the exception of these indirect calorimetry studies, the contribution of lipids to fish energy metabolism has not been measured often. During migration, which is a period of sustained exercise combined with starvation and gonadal development, sockeye salmon (*Oncorhynchus nerka*) lose more than 60% of their muscle, and 90% of their internal organ lipid content (Idler and Bitners, 1960). Krueger *et al.* (1968) also reported that prolonged swimming caused a large decrease in total body lipid content of coho salmon (*Oncorhynchus kisutch*) and calculated that 16% and 45 % of their caloric losses came from lipids at high and low swimming speeds, respectively. *In vitro* measurements of lipid oxidation rate and of β-oxidation enzyme activities show high capacities of red muscle mitochondria to oxidize lipids (Henderson and Tocher, 1987; Moyes *et al.*, 1989; Moyes *et al.*, 1992). Taken together the above data suggest that an adequate supply of fatty acids to muscle mitochondria is necessary to meet the energy needs of swimming. The mobilization of lipid reserves should provide these fatty acids.

Mobilization of the lipid reserves

Lipid reserves in fish

In agreement with the idea that lipids constitute an important metabolic fuel in teleost fish, this fuel is stored in large amounts in their bodies, mainly as triacylglycerol (triglycerides). Lipids are even said to represent the largest energy reserve in fish (Moyes and West, 1995). However, contrary to mammals that store most of their lipids in adipose tissue (Hochachka and Somero, 1984), fish reserves are distributed in three main depots. Even though the importance of each of these depots can differ between fish species (Weber and Zwingelstein, 1995), mesenteric fat (also called adipose tissue) is generally considered as the most important lipid reserve, followed by hepatic and intramuscular fat (Sheridan, 1988). The lipids stored in these depots come either from *in situ* lipid synthesis or from intestinal absorption of dietary lipids. Dietary lipids are absorbed by the intestinal cells as free fatty acids, reesterified in these cells and transported to the liver as chylomicra, which are composed primarily of triacylglycerol. The liver re-distributes these lipids via the circulatory system, bound to albumin-like proteins or as lipoproteins (chylomicra, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL)) to the adipose tissue and muscle (Sheridan, 1988). Both red and white muscle possess lipid reserves, but red muscle can contain twice as much (Driedzic and Hochachka, 1978; Sheridan, 1994). The intramuscular lipid reserves of red and white muscle also differ in that the lipid depots of white muscle are distributed between the muscle fibers, while the red muscle lipids are essentially intracellular (Sheridan, 1994).

Mobilization of lipids: liplolysis and reesterification

In fish, lipid substrates mobilized from fat reserves, are thought to be transported to the working muscles primarily as non-esterified fatty acids (NEFA) (Farkas, 1969). However, it is important to keep in mind that numerous other circulatory lipids (triacylglycerol, phospholipids, and cholesterol) that are transported in chylomicra, VLDL, LDL, and HDL can also supply energy for muscular contraction (Sheridan, 1988; Weber and Zwingelstein, 1995).

In mammals, lipid mobilization of the triacylglycerol reserves of the adipose tissue occurs by lipolysis. During lipolysis, triacylglycerol is hydrolyzed into glycerol and NEFA by the successive actions of triacylglycerol, and monoacylglycerol lipases before being released into the bloodstream (Allen, 1976). Therefore, the stimulation of lipolysis results in an increase in glycerol and NEFA fluxes (Weber *et al.*, 1993; Wolfe *et al.*, 1990). Contrary to fatty acids, glycerol released by lipolysis cannot be metabolized by the adipose tissue which lacks glycerokinase activity (see below) (Weber *et al.*, 1993; Wolfe *et al.*, 1990). Therefore, the rate of glycerol release in the circulation is a measure of the lipolytic rate (Wolfe *et al.*, 1990).

The mechanisms by which lipids are mobilized from fish lipid reserves are ill understood (Henderson and Tocher, 1987). Quantitative measurements of the lipolytic rate or of the circulatory NEFA appearance rate have never been performed in fish with the exception of rainbow trout where NEFA flux was measured during hypoxia and acute changes in temperature (Haman *et al.*, 1997). Lipolysis of long chain triacylglycerol (triacylglycerol where the acyl chains are composed of 14 to 24 carbons) has been observed in the three main lipid depots of fish: muscle (Bilinski and Lau, 1969; Sheridan *et al.*, 1985), liver (Sheridan *et al.*, 1985) and adipose tissue (Sheridan and Allen, 1984). However, only lipase from trout liver and adipose tissue has been isolated and partially purified (Harmon *et al.*, 1991; Sheridan and Allen, 1984), (for review see Sheridan, 1988 and 1994).

Mobilization of lipid reserves has been studied much more thoroughly in mammals where the lipolytic rate is always higher than necessary to supply sufficient energy (Weber, 1992). Not all the fatty acids released by lipolysis enter the circulation as an important fraction is reesterified into triacylglycerol (Newsholme and Crabtree, 1976). During reesterification the fatty acids are acted upon by fatty acyl-CoA synthetase and are linked with glycerol-3 phosphate, a product of glycolysis (Lehninger *et al.*, 1993). The reesterification of one triacylglycerol requires the energy of at least 7 phosphate bonds (Elia *et al.*, 1987). Because lipolysis and reesterification are opposed and simultaneous non-equilibrium reactions, and are catalyzed by different enzymes, the overall process fits the description of a substrate cycle: the triacylglycerol: fatty acid cycle or TAG:FA cycling. TAG:FA cycling can be both intracellular (fatty acids are reesterified in the lipolytic tissue without entering the circulation) and extracellular (fatty acids travel via the circulation to their site of reesterification) (Newsholme and Crabtree, 1976; Wolfe *et al.*, 1990). The TAG:FA cycle has been shown to amplify the response of the NEFA flux to hormones regulating lipolysis (Campbell *et al.*, 1992; Newsholme and Crabtree, 1976; Wolfe *et al.*, 1990).

Regulation of lipid mobilization

In mammals, triacylglycerol lipase is a hormone-sensitive lipase that is controlled via a signal transduction mechanism similar to that which controls glycogen phosphorylase in liver and muscle tissues. This signal transduction mechanism constitutes a phosphorylation-dephosphorylation cascade where the first step is allosterically regulated by cAMP (Lehninger *et al.*, 1993). Like mammalian lipase, fish hepatic and adipose triacylglycerol lipases are activated by cAMP mediated phosphorylation (Harmon *et al.*, 1993; Michelsen *et al.*, 1994; Migliorini *et al.*, 1992). However, the sensitivity of fish lipase to numerous hormones (*e.g* norepinephrine, ACTH, insulin and glucagon) has been demonstrated only on isolated hepatocytes from rainbow trout . *In vitro* treatment of trout adipose tissue by ACTH and norepinephrine failed to stimulate lipolysis. The lipolytic role of several hormones (*i.e.* prolactin and growth hormone) was also studied *in vivo*, but stimulation of lipolysis did not occur in every case (for review see Sheridan, 1988 and 1994).

In mammals, regulation of lipid mobilization also occurs at the level of reesterification. When triacylglycerol reserves are mobilized, simultaneous to the increase in the lipolytic rate there is a decrease in the fraction of fatty acids reesterified in the TAG:FA cycle. This results in increasing the availability of fatty acids for oxidation (Campbell *et al.*, 1992; Newsholme and Crabtree, 1976; Wolfe *et al.*, 1990). The enzymatic pathway of fatty acid reesterification is regulated more by substrate availability than by hormones (Wolfe *et al.*, 1990). Therefore, the oxidation rate of fatty acids, as well as the rate of blood flow through the lipid depot can passively affect the reesterification rate (and consequently lipid mobilization) by modifying the availability of the fatty acids (Edens *et al.*, 1990; Wolfe *et al.*, 1990). In fish, the role of the TAG:FA cycle in lipid mobilization has never been investigated.

Goals of investigation

Lipids are thought to be a major metabolic fuel in fish during aerobic activity. But little is known concerning their mobilization. Therefore, the aim of this thesis was to quantify lipid mobilization in fish. By analogy with mammals, I hypothesized that, when fish are subjected to submaximal exercise, they mobilize their triacylglycerol (TAG) reserves above resting levels, which would result in 1) increases in fatty acid (NEFA) and glycerol fluxes, and 2) a decrease in the percent of NEFA reesterified by the TAG:FA substrate cycle. This hypothesis was tested by measuring *in vivo* turnover rates of fatty acids and glycerol in exercising fish, and by calculating the intracellular TAG:FA cycling rate. Metabolite fluxes were determined by using a new continuous infusion technique (Haman and Weber, 1996; Haman *et al.*, 1997) on rainbow trout (a common model in fish physiology) swimming either for 1 hour at 1.0 and 1.5 bl s^{-1} (body length per second) or for 4 days at 1.0 bl s^{-1} (Chapter 2). This study is the first to measure glycerol flux and TAG:FA cycling in fish.

In Chapter 2, the intracellular TAG:FA cycle did not change with exercise, regardless of swimming duration or speed. This result indicates that TAG:FA cycling could be a true futile cycle in fish. Since in mammals the fraction of the fatty acids reesterified in the TAG:FA cycle depends on the availability of the fatty acids for the enzymatic reactions (Wolfe *et al.*, 1990), I attempted to test the hypothesis that a reduction in the availability of the fatty acids caused by an inhibition of lipolysis would not lead to a decrease in the percentage of fatty acids that were reesterified. However, I did not succeed in inhibiting lipolysis by using acipimox, an antilipolytic drug, of which I studied the effects on the plasma glycerol and fatty acid turnover rates, and the intracellular TAG:FA cycling rate in rainbow trout (Chapter 3).

In Chapter 4, the general conclusions of my thesis are presented. I summarize my findings on the mobilization of lipid reserves in fish, and suggest avenues for future work.

CHAPTER 2

FATTY ACID AND GLYCEROL KINETICS OF RAINBOW TROUT. EFFECT OF ENDURANCE SWIMMING.

Introduction

It is generally accepted that nonesterified fatty acids (NEFA) derived from lipid stores in muscle, liver and mesenteric adipose tissue represent an important metabolic fuel for endurance swimming in teleosts (Driedzic and Hochachka, 1978; Henderson and Tocher, 1987; Lauff and Wood, 1997; Mommsen *et al.*, 1980; Walton and Cowey, 1982). However, the evidence showing that fish mobilize this energy reserve during exercise is only indirect, and little is known of the actual process of triacylglycerol breakdown in ectotherms (Henderson and Tocher, 1987; Moyes and West, 1995). Early studies showed that fat stores of salmon decrease during natural migration (Idler and Bitners, 1960) and after swimming for 24 h in the laboratory (Krueger *et al.*, 1968). It is also known that many fish tissues contain active lipolytic enzymes (Sheridan, 1988). In contrast, the mammalian response is much better documented because thorough investigations of lipid kinetics have been carried out in this group of vertebrates (Van der Vusse and Reneman, 1996). Results from *in vivo* experiments clearly show that prolonged exercise causes the stimulation of triacylglycerol hydrolysis in mammals which leads to a major increase in the rate of appearance (R_a) of NEFA and glycerol in their circulation (Shaw *et al.*, 1975; Weber *et al.*, 1993; Wolfe *et al.*, 1990). The effects of exercise on NEFA and glycerol kinetics of fish are unknown and their glycerol fluxes have never been measured *in vivo*, even at rest.

Upon hydrolysis, each triacylglycerol yields 3 NEFA and 1 glycerol, but the ratios of R_a NEFA / R_a glycerol measured in mammals are always below 3. This is because a fraction of the fatty acids released through lipolysis is reesterified *in situ*, whereas the glycerol cannot be directly metabolized because glycerokinase activity is absent in adipocytes (Weber *et al.*, 1993; Wolfe *et al.*, 1990). The simultaneous occurrence of lipolysis and reesterification is the basis of the triacylglycerol: fatty acid cycle (TAG:FA cycle), a substrate cycle known to play an important role in regulating NEFA availability for working muscles in humans (Wolfe *et al.*, 1990). When triacylglycerol reserves are mobilized, lipolytic rate is stimulated while the fraction of total fatty acids released that is reesterified decreases, thereby providing more fatty acids for oxidation in muscles (Campbell *et al.*, 1992; Newsholme and Crabtree, 1976).

The goal of this study was to test the hypothesis that submaximal swimming causes the mobilization of fish triacylglycerol reserves, resulting in an increase in the rates of appearance of NEFA and glycerol: a response similar to mammals exercising at equivalent intensities. In addition, the role of the TAG:FA cycle in regulating fatty acid availability during exercise was assessed. Also by analogy to exercising mammals, a decrease in the percentage of fatty acids reesterified in the TAG:FA cycle was expected. To start, rainbow trout were exercised for one hour at 1 body length per second, but no significant increase in lipolytic rate was measured under these conditions. Therefore, additional experiments were carried out at higher speed or during 4 days of sustained swimming.

Materials and Methods

Animals

Rainbow trout, *Oncorhynchus mykiss* (Walbaum) of both sexes (451-823g) were purchased from Linwood Acres Trout Farm (Campbellcroft, Ontario, Canada) and held in a 1300 l flow-through tank at 13°C. They were kept in dechloraminated, well oxygenated water under a 12h:12h L:D photoperiod. Animals were acclimated to these conditions for at least 1 month before initiation of the experiments and they were fed Purina trout chow 3 times per week until satiation.

Catheterization

The surgery consisted of a double cannulation of the dorsal aorta under ethyl-N-aminobenzoate sulphonic acid anaesthesia (MS-222) as described previously (Haman and Weber, 1996). For the first series of experiments (1h swimming at 1 or 1.5 bl s^{-1}), heparin (Organon Teknica) was used as anticoagulant at 10 units/ml of Cortland saline (Wolf, 1963). However, heparin could potentially stimulate lipolysis and it was substituted with sodium citrate (12.9 mmol l^{-1}) for the second series of experiments (swimming for 4 days at 1 bl s^{-1}) to check for this possibility. After surgery, animals were allowed to recover for 36 h in a swim tunnel with a weak water current (11 cm s^{-1}), just strong enough to ensure adequate oxygenation. Under these conditions, the animals were not swimming, but they were resting on the floor of the swim tunnel.

Swim tunnel

Measurements were carried out in a modified Blazka-type swim tunnel (Beamish, 1978). It consisted of a polyvinylchloride tube (1.5 m in length and 0.2 m in diameter) immersed in a 550 l flow-through tank. Two 'honey comb' grids were placed inside the tube to delimit a 28 l swimming chamber (90 cm long) and to ensure laminar flow. The upper part of this chamber was closed by a transparent lid with a longitudinal slit allowing access to the catheters. Water flow was powered by an electrical trolling motor (Mini Kota, 17Lbs Thrust) connected to an adjustable power supply (Harrison 6433B DC, Hewlett Packard) to control water velocity. This swim tunnel was adequate to exercise fish up to 79 cm s^{-1}. Velocity was calibrated by videotaping the repeated release of a dye suspension through the chamber and by counting film frames. For each experiment, water velocity was corrected according to Webb (1974) and Nelson *et al.* (1996) to take into account the acceleration of water around individual animals of different sizes. A metal grid was placed at the downstream end of the swimming chamber and connected to a 12V power supply. It was manually activated on a few rare occasions when the fish was leaning against it during measurements. The upstream end of the swimming chamber was kept darker than the downstream section and the animals would typically swim in the upstream portion to avoid bright light.

Continuous infusions of isotopes

Labeled metabolites were infused as described previously (Haman and Weber, 1996; Haman *et al.*, 1997). The infusate was prepared daily with 2- ^{3}H glycerol

(Amersham 37.0 Gbq/mmol) and 1-^{14}C palmitate (Amersham 1.85-2.2 Gbq/mmol). Trout plasma was collected from donor individuals of the same batch of fish and used as a source of lipid-binding proteins. The 1-^{14}C palmitate was supplied commercially in toluene, dried under nitrogen and resuspended in ethanol to obtain a solution of 1 μCi/μl. A subsample of this solution was mixed with 400 μl of plasma and well agitated before adding 2-^{3}H-glycerol dissolved in Cortland saline. While the fish was at rest, an infusion of the isotope mixture was started with a calibrated syringe pump (Harvard apparatus, South Natick, MA) at 1ml/h. Infusion rates ranged from 469,000 to 1,040,000 DPM kg^{-1} min^{-1} for each isotope.

Exercise protocols and blood sampling

In a first series of experiments, glycerol and fatty acid kinetics were determined before, during, and after 1 h of swimming at 1 bl s^{-1} (n=6 animals) or at 1.5 bl s^{-1} (n=6). Isotope infusion was started 1 h before swimming and it was continued for 30 min after the end of exercise to monitor recovery. For every fish, a total of 9 blood samples of 0.5 ml each were drawn throughout the infusion. In a second series of experiments, glycerol and fatty acid kinetics were measured before and during 4 days of continuous swimming at 1 bl s^{-1} (n=6). On day 1 of the experiment, the first isotope infusion was performed just before starting exercise to quantify resting kinetics. Every 24 h after the beginning of exercise, an additional 1 h infusion was performed to monitor the effects of endurance swimming. A 0.8 ml blood sample was taken at the end of each infusion. Before starting the last infusion (day 4), an additional blood sample was taken to measure residual activity from the 4 previous infusions (1 at rest and 3 during exercise). However, the residual specific activities of glycerol and palmitate were so low in these samples that no correction was made in the calculations. In both series of experiments, hematocrit was measured on each blood sample. Hematocrit did not decrease significantly throughout the experiments and all values were above 20%.

Preparation of blood samples

Immediately after sampling, the blood was centrifuged and the plasma was separated. Twenty five percent of the plasma was stored at - 20°C and used later to measure total NEFA concentration with an analytical test-kit (NEFA C, Wako Chemicals, Osaka, Japan). The remaining seventy five percent of the plasma was mixed with 25 ml chloroform:methanol (Folch, 2:1) (Folch *et al.*, 1957) immediately after separation to avoid the adsorption of glycerol on the walls of the tubes. Glass tubes were used at this step because preliminary experiments showed that glycerol adsorption is much lower on glass than on plastic. The Folch mixture was kept at -20°C for a maximum of 24 h before it was filtered to remove proteins, washed with 7.5 ml distilled water and centrifuged. The aqueous and organic phases were separated and re-extracted with methanol:water (40:30) and chloroform, respectively. After centrifugation, each tube contained an aqueous and an organic phase which were separated before pooling both aqueous phases and both organic phases. Each pooled phase was then dried with a rotating evaporator (Büchi RE 121 Rotavapor). The aqueous phase was resuspended in ethanol:water (1:1) and the organic phase in hexane:isopropanol (3:2).

Glycerol

Glycerol concentration was determined on a volume of aqueous phase corresponding to 100 µl of plasma. This volume was dried under nitrogen and resuspended in hydrazine buffer before measuring concentration on a Beckman DU 640 spectrophotometer at 340 nm as described previously (Weber *et al.*, 1993). At this step of the analysis, total tritium activity of the aqueous phase was only found in glycerol and glucose and it was counted on the equivalent of 5 µl of plasma. Percent activity in glycerol was obtained by separating glucose from glycerol using thin layer chromatography as follows. A subsample of the aqueous phase was evaporated and concentrated in ethanol:water before spotting it on a silica gel plate (60 F_{254}, Merck, Germany). The plate was then developed with chloroform:methanol (40:24), and the glucose and glycerol fractions were scraped in separate scintillation vials. They were resuspended in 3 ml of ethanol:water (1:1) and counted in ACS-II scintillation fluid (Amersham, Canada) on a Tri-Carb 2500 counter (Packard, Canada). At least 80% of the ^{3}H activity of the spotted sample was recovered in the glucose and glycerol fractions.

Fatty acids

Neutral lipids, NEFA and phospholipids present in the organic phase were separated by filtration on Supelclean solid phase extraction tubes (LC-NH2, Sigma, St Louis). The neutral lipids were eluted with chloroform:isopropanol (2:1), the NEFA with isopropyl ether:acetic acid (98:2), and the phospholipids with methanol. A subsample of each fraction was counted to determine the distribution of ^{14}C and ^{3}H activity in plasma lipids.

Because no ^{14}C is incorporated into fatty acids other than palmitate, the activity found in the NEFA fraction was equal to palmitate activity. The remainder of the NEFA fraction was used to determine the relative distribution of individual fatty acids using gas chromatography as described previously (McClelland *et al.*, 1995; Tserng *et al.*, 1981).

Calculations and statistics.

Palmitate concentration was calculated by multiplying NEFA concentration by the fractional contribution of palmitate to total NEFA. Glycerol and palmitate rates of appearance (R_a) were computed with the steady state equation of Steele (1959):

$$R_a\ (\mu\text{mol kg}^{-1}\text{min}^{-1}) = \frac{\text{rate of the isotope infusion (DPM kg}^{-1}\text{ min}^{-1})}{\text{steady state specific activity (DPM }\mu\text{mol}^{-1})}$$

NEFA rate of appearance was determined by dividing palmitate rate of appearance by the fractional contribution of palmitate to total NEFA.

The triacylglycerol: fatty acid cycle (= TAG:FA cycle or fatty acid reesterification) consist of the intracellular TAG:FA cycle, in which fatty acids are reesterified in the lipolytic tissue without entering the circulation, and the extracellular TAG:FA cycle, in which fatty acids travel via the circulation to their sites of reesterification (Newsholme and Crabtree, 1976; Wolfe *et al.*, 1990). In this study, only the intracellular TAG:FA cycle was assessed. Absolute and relative rates of intracellular triacylglycerol:fatty acid cycling were calculated as follows (Wolfe *et al.*, 1990):

$$\text{TAG:FA cycling rate} = (3 \times R_a \text{ glycerol}) - R_a \text{ NEFA}$$

$$\text{\% TAG:FA cycling rate} = (\text{TAG:FA cycling rate}) / (3 \times R_a \text{ glycerol})$$

The student's t-test was used to determine the effects of swimming speed (1.0 vs 1.5 bl s^{-1}) and anticoagulant (heparin vs sodium citrate). In all other cases, statistical differences were assessed using two-way analysis of variance (ANOVA) or Friedman's test (when the assumptions of normality or homoscedasticity were not met) with time and fish as the main factors. When significant changes were detected by ANOVA, Dunnett's test was used to determine which specific means were different from the resting value. Percentages were transformed to the arcsine of their square root before analysis and all values given are means ± SEM.

Results

Swimming for 1 h at 1 bl s^{-1} had no significant effect on the concentrations and fluxes of glycerol, palmitate and total fatty acids ($p>0.05$). Therefore, values for all these parameters of lipid metabolism were averaged throughout the experiments (Table 2.1). The rate of intracellular fatty acid reesterification calculated from R_a glycerol and R_a NEFA was high: 76.72 ± 1.20 % of total NEFA released through lipolysis and it remained constant throughout the experiments (Table 2.1).

Increasing exercise intensity to 1.5 bl s^{-1} had no significant effect on glycerol, palmitate or fatty acid concentration throughout the experiments ($p>0.05$) (Fig. 2.1A, 2.2A and 2.3A). Mean glycerol and NEFA concentrations were respectively 0.20 ± 0.01 μmol l^{-1} and 0.63 ± 0.03 mmol l^{-1}. Glycerol and palmitate specific activities also did not change with exercise ($p>0.05$), but were highly variable (Fig. 2.1B and 2.2B). This variability reflects significant differences between the fish ($p<0.001$), due to different infusion rates. Glycerol, palmitate and NEFA rate of appearance remained constant between rest, exercise, and recovery ($p>0.05$) (Fig. 2.1C, 2.2C and 2.3C). Mean glycerol and NEFA flux were respectively 3.47 ± 0.24 μmol kg^{-1} min^{-1} and 3.73 ± 0.28 μmol kg^{-1} min^{-1}. The reesterification rate of fatty acids calculated from these flux did not vary significantly between rest, exercise, and recovery (6.73 ± 0.59 μmol kg^{-1} min^{-1} or 61.63 ± 1.96 %).

Table. 2.1. Plasma glycerol, palmitate and total fatty acid (NEFA) concentration and rates of appearance (R_a) in rainbow trout. Glycerol and palmitate specific activity (SA), as well as absolute and relative rate of reesterification are presented. Only mean values (n = 6) ± SEM for the entire experiment are given because one hour exercise at 1.0 bl s^{-1} had no effect ($p>0.05$).

[glycerol] (mmol l^{-1})	0.38 ± 0.03
glycerol SA x 10^{-5} (disints min^{-1} μmol^{-1})	1.26 ± 0.06
R_a glycerol (μmol kg^{-1} min^{-1})	8.09 ± 0.67
[palmitate] (μmol l^{-1})	124.14 ± 4.70
palmitate SA x 10^{-5} (disints min^{-1} μmol^{-1})	8.18 ± 0.3
R_a palmitate (μmol kg^{-1} min^{-1})	1.09 ± 0.07
[NEFA] (mmol l^{-1})	0.57 ± 0.03
R_a NEFA (μmol kg^{-1} min^{-1})	4.85 ± 0.24
reesterification (μmol kg^{-1} min^{-1})	9.49 ± 2.08
percent of resterification (%)	76.72 ± 1.20

Fig. 2.1. Plasma concentration (A), specific activity (B) and rate of appearance (C) of glycerol (R_a glycerol) in rainbow trout during rest, exercise at 1.5 bl s^{-1} and recovery. Values are means ± SEM (n = 6).

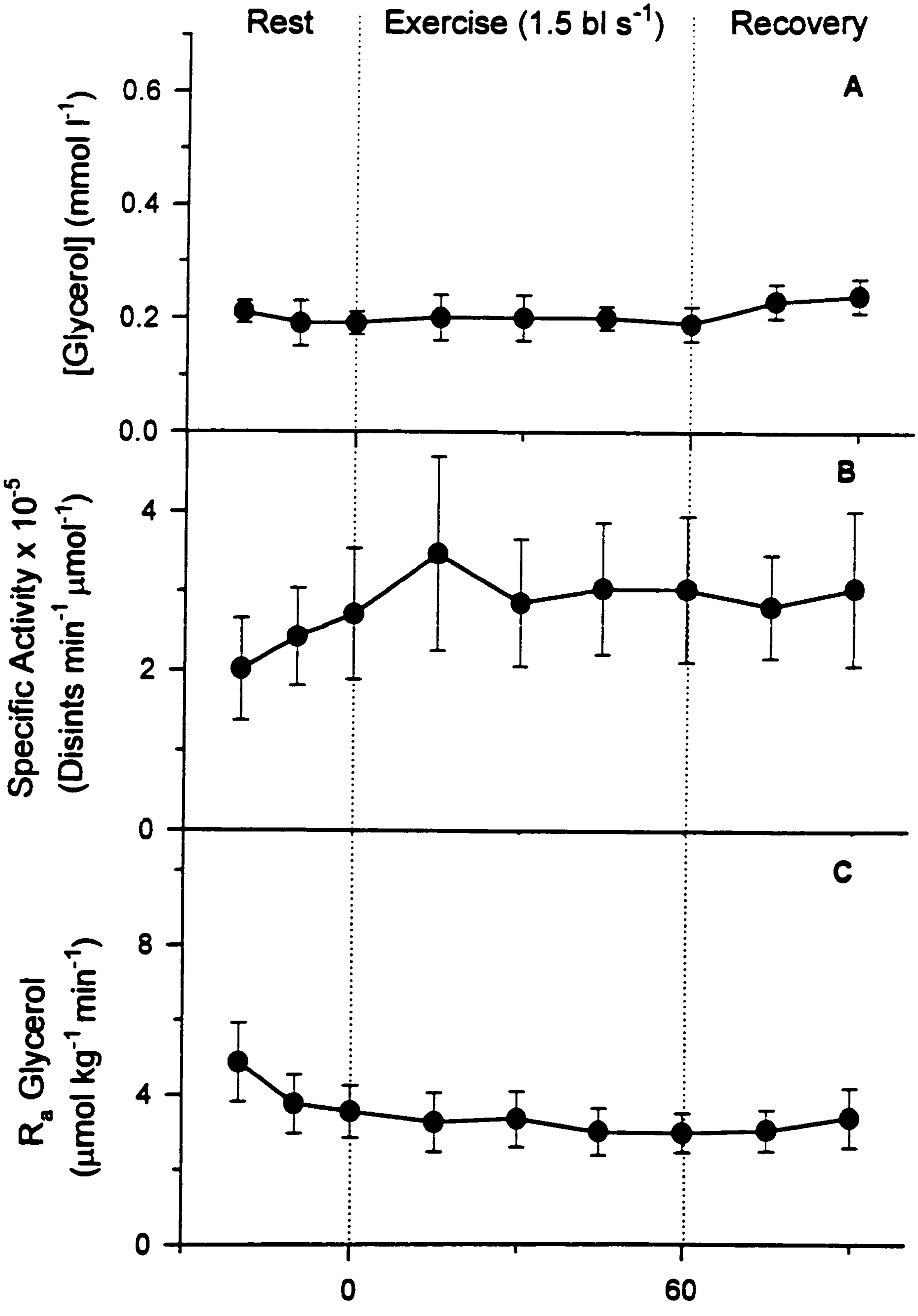

Rest
Exercise (1.5 bl s-1)
Recovery
A
[Glycerol] (mmol l-1)
0.6
0.4
0.2
0.0
B
Specific Activity x 10-5 (Disints min-1 µmol-1)
4
2
0
C
Ra Glycerol (µmol kg-1 min-1)
8
4
0
0
60
Time (min)

Fig. 2.2. Plasma concentration (A), specific activity (B) and rate of appearance (C) of palmitate (R_a palmitate) in rainbow trout during rest, exercise at 1.5 bl s^{-1} and recovery. Values are means ± SEM ($n = 6$).

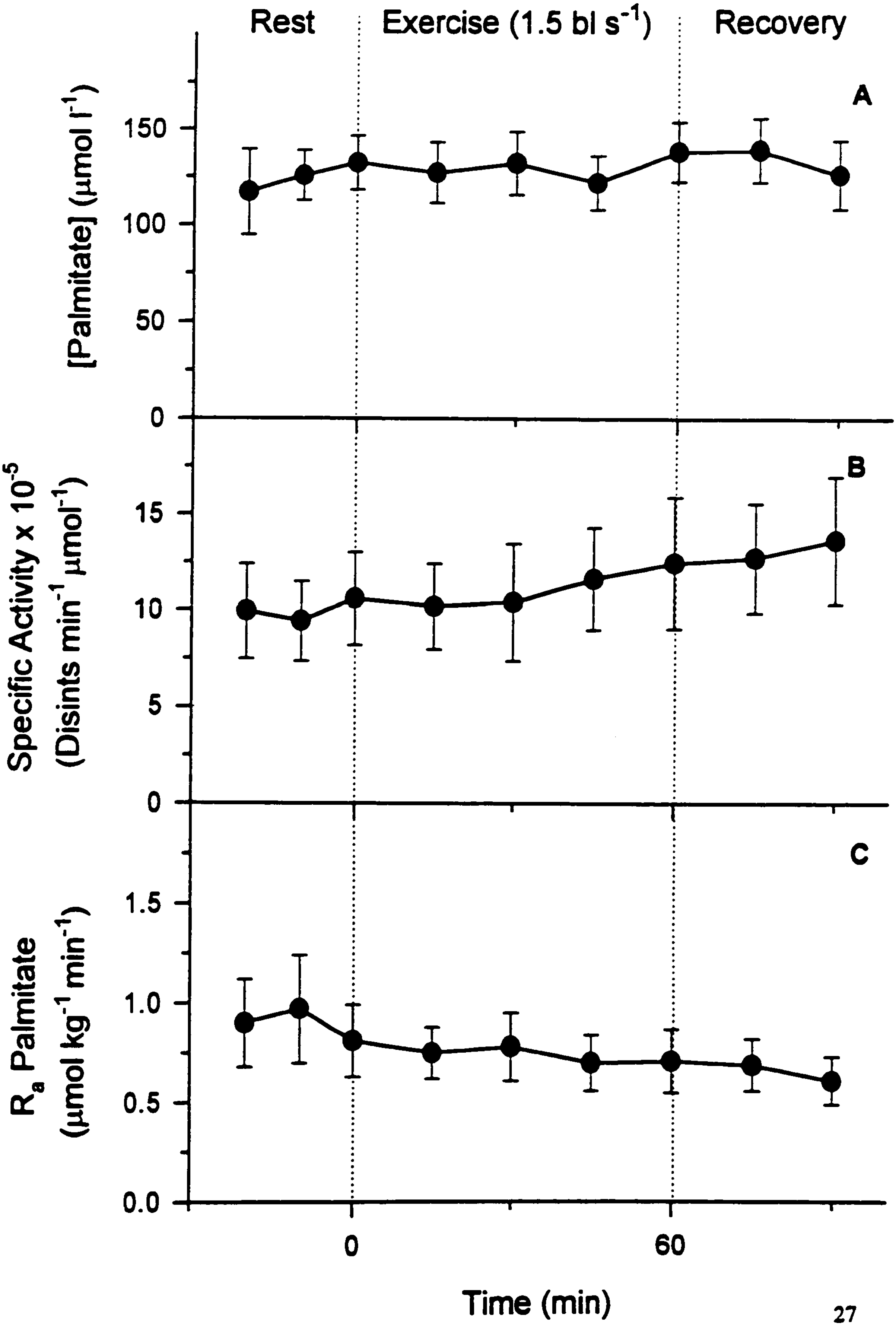
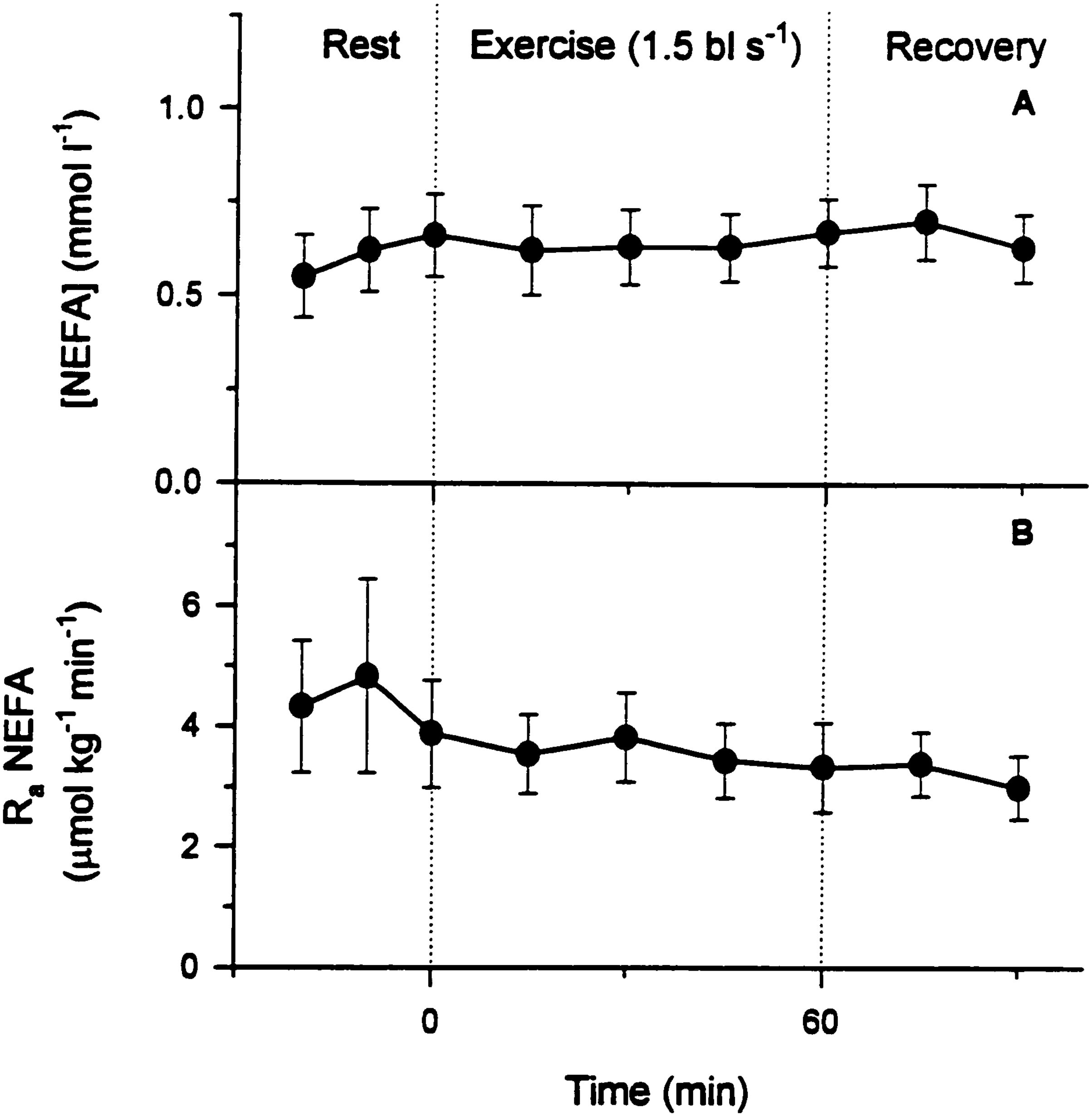

Rest
Exercise (1.5 bl s^{-1})
Recovery
A
[Palmitate] (µmol l^{-1})
150
100
50
0
B
Specific Activity x 10^{-5}
(Disints min^{-1} µmol^{-1})
15
10
5
0
C
R$_a$ Palmitate
(µmol kg^{-1} min^{-1})
1.5
1.0
0.5
0.0
0
60
Time (min)

Fig. 2.3. Plasma concentration (A) and rate of appearance (B) of fatty acids (R_a NEFA) in rainbow trout during rest, exercise at 1.5 bl s^{-1} and recovery.

Values are means ± SEM (n = 6).


Rest
Exercise (1.5 bl s^{-1})
Recovery
A
[NEFA] (mmol l^{-1})
1.0
0.5
0.0
B
R_a NEFA
(µmol kg^{-1} min^{-1})
6
4
2
0
0
60
Time (min)

Some differences exist between the results obtained at 1.0 bl s^{-1} and at 1.5 bl s^{-1}. Glycerol concentration, glycerol, palmitate and NEFA rate of appearance are significantly lower at 1.5 bl.s^{-1} ($p<0.001$). For glycerol, this difference already exists at rest ($p<0.001$), but this is not the case for palmitate and NEFA, which differs between the two speeds only during exercise and recovery ($p<0.05$). Absolute and relative reesterification rates were also significantly different between the two speeds, even at rest ($p<0.001$).

Similarly to what was found in the two first series of experiments, endurance exercise lasting four days had no significant effect on glycerol, palmitate or NEFA kinetics ($p>0.05$) (Fig. 2.4 to 2.6). Calculated reesterification rate was 7.66 ± 0.92 µmol kg^{-1} min^{-1} or 63.84 ± 2.82 % and did not change between rest, exercise, and recovery ($p>0.05$).

The distribution of ^3H and ^{14}C activity in different plasma metabolites was quantified using ion exchange chromatography. Because the results obtained during 1 hour exercise at 1.0 and 1.5 bl.s^{-1} are the same, only data collected at 1.5 bl s^{-1} are presented (Fig. 2.7). Throughout the experiments, the percentage of ^3H activity in glycerol decreased as tritiated glycerol was incorporated into neutral lipids ($p<0.05$). In contrast, the percentage of tritium in the plasma glucose and phospholipids did not change throughout the experiments ($p>0.05$) (Fig 2.7A). A similar pattern was observed for ^{14}C-palmitate. An incorporation of ^{14}C-palmitate in the neutral lipids was accompanied by a decrease in the percentage of ^{14}C activity in the plasma NEFA ($p < 0.05$), whereas no ^{14}C activity was incorporated into the phospholipids (Fig 2.7B).

The distribution of ^{3}H and ^{14}C activity in different plasma metabolites observed during prolonged exercise (4 days at 1.0 bl s^{-1}) (Fig 2.8), is quite different from that described above. Tritiated glycerol and ^{14}C-palmitate were incorporated in small amounts into the neutral lipids, and in very large amounts into the phospholipids ($p<0.05$). This incorporation was also accompanied with a decrease in ^{3}H activity in glycerol and in ^{14}C activity in NEFA ($p<0.05$).

Table 2.2 presents both resting concentrations and rates of appearance of glycerol and NEFA measured when heparin and sodium citrate were used. Except for glycerol concentration, values found for the other parameters were not affected by the anticoagulant chosen ($p<0.05$).

Fig. 2.4. Plasma concentration (A) and rate of appearance (B) of glycerol (R_a glycerol) in rainbow trout at rest (time = 0) and for 4 days of exercise at 1.0 bl s^{-1}.

Values are means ± SEM (n = 6).

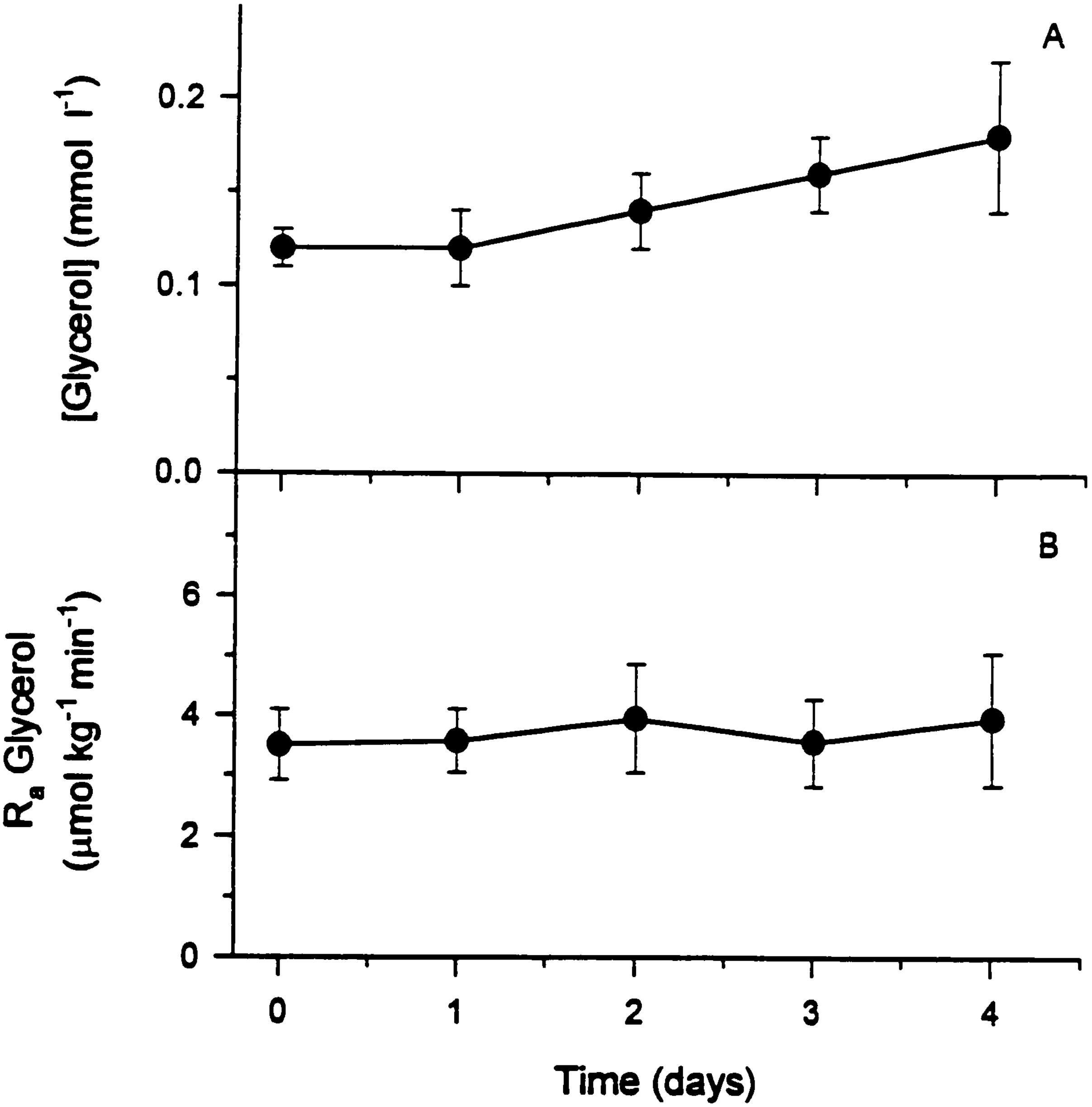

A
[Glycerol] (mmol l^{-1})
0.2
0.1
0.0
B
R$_a$ Glycerol
(µmol kg^{-1} min^{-1})
6
4
2
0
0
1
2
3
4
Time (days)

Fig. 2.5. Plasma concentration (A) and rate of appearance (B) of palmitate (R_a palmitate) in rainbow trout at rest (time = 0) and for 4 days of exercise at 1.0 bl s^{-1}. Values are means ± SEM (n = 6).

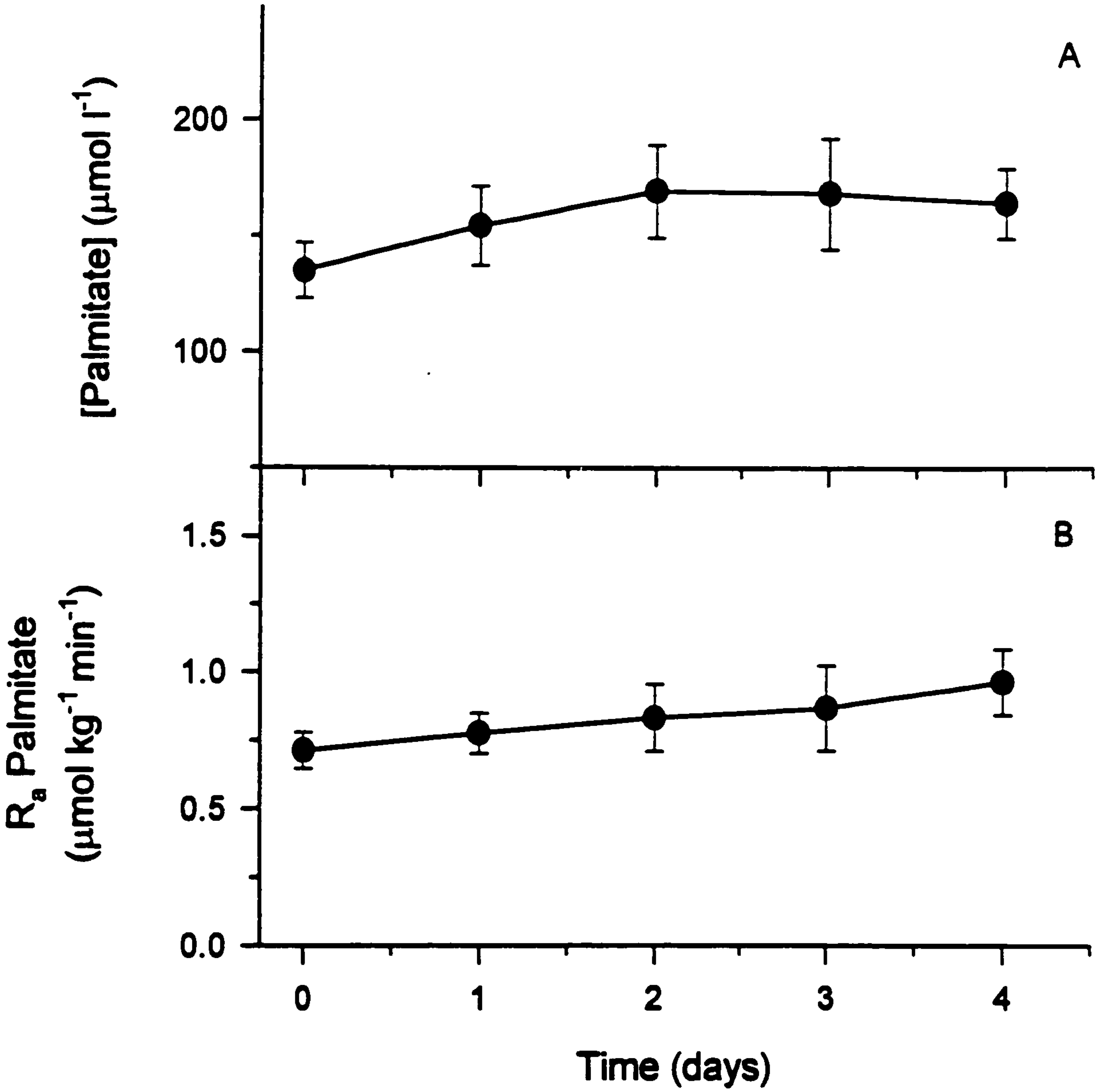

A
[Palmitate] (μmol l^{-1})
200
100
B
R$_a$ Palmitate (μmol kg^{-1} min^{-1})
1.5
1.0
0.5
0.0
0
1
2
3
4
Time (days)

Fig. 2.6. Plasma concentration (A) and rate of appearance (B) of fatty acids (R_a NEFA) in rainbow trout at rest (time = 0) and for 4 days of exercise at 1.0 bl s^{-1}. Values are means ± SEM (n = 6).

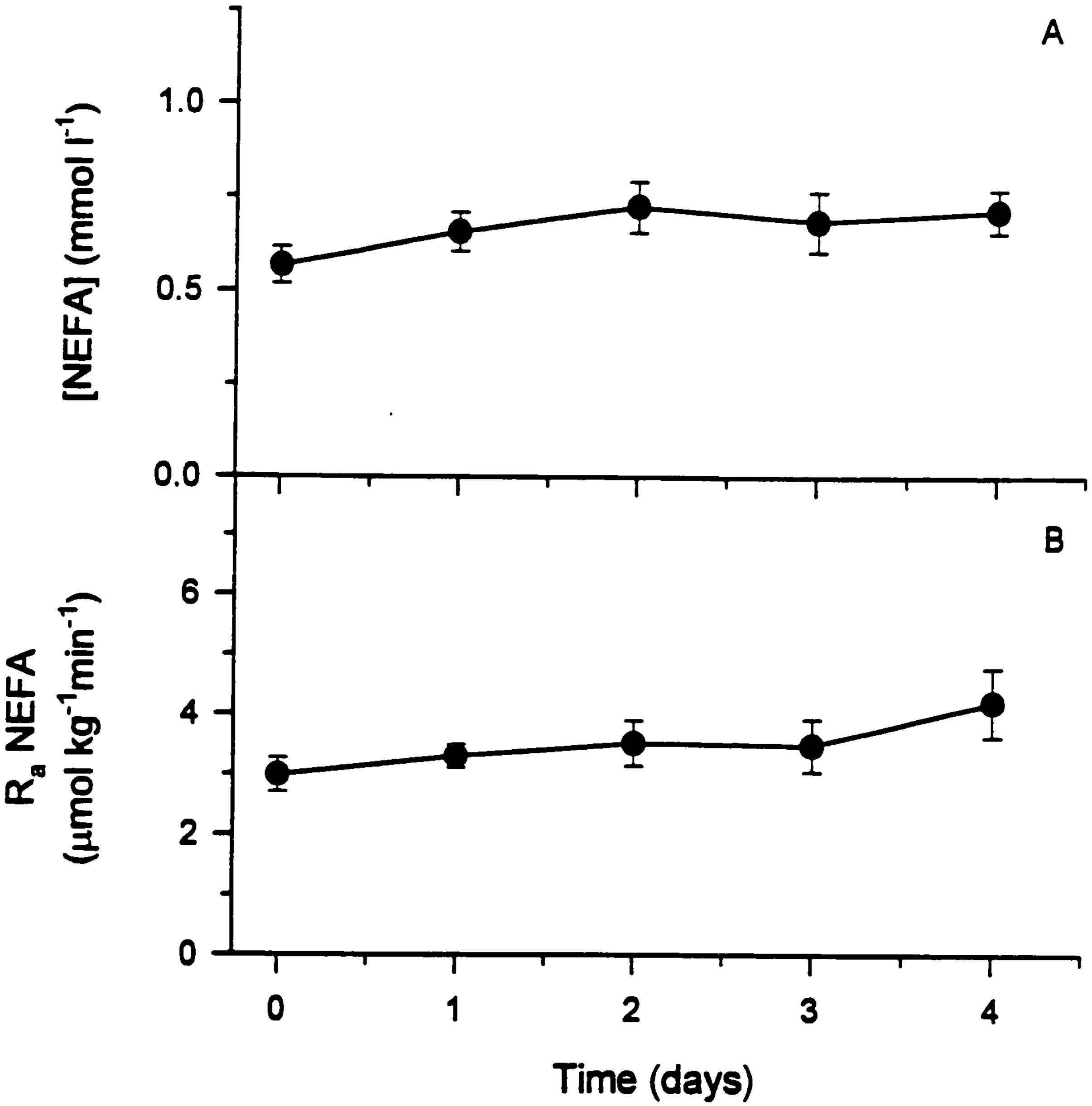

A
[NEFA] (mmol l^{-1})
1.0
0.5
0.0
B
R_a NEFA
(µmol kg^{-1}min^{-1})
6
4
2
0
0
1
2
3
4
Time (days)

Fig. 2.7. (A) Distribution of ^{3}H activity in glucose, glycerol, neutral lipids and phospholipids during rest, one hour exercise at 1.5 bl s^{-1} and recovery.

(B) Distribution of ^{14}C activity in fatty acids (NEFA), neutral lipids and phospholipids during rest, one hour exercise at 1.5 bl s^{-1} and recovery. (Values are mean percentage of the total ^{3}H or ^{14}C activity, n = 6). * indicates a significant difference from the resting value.

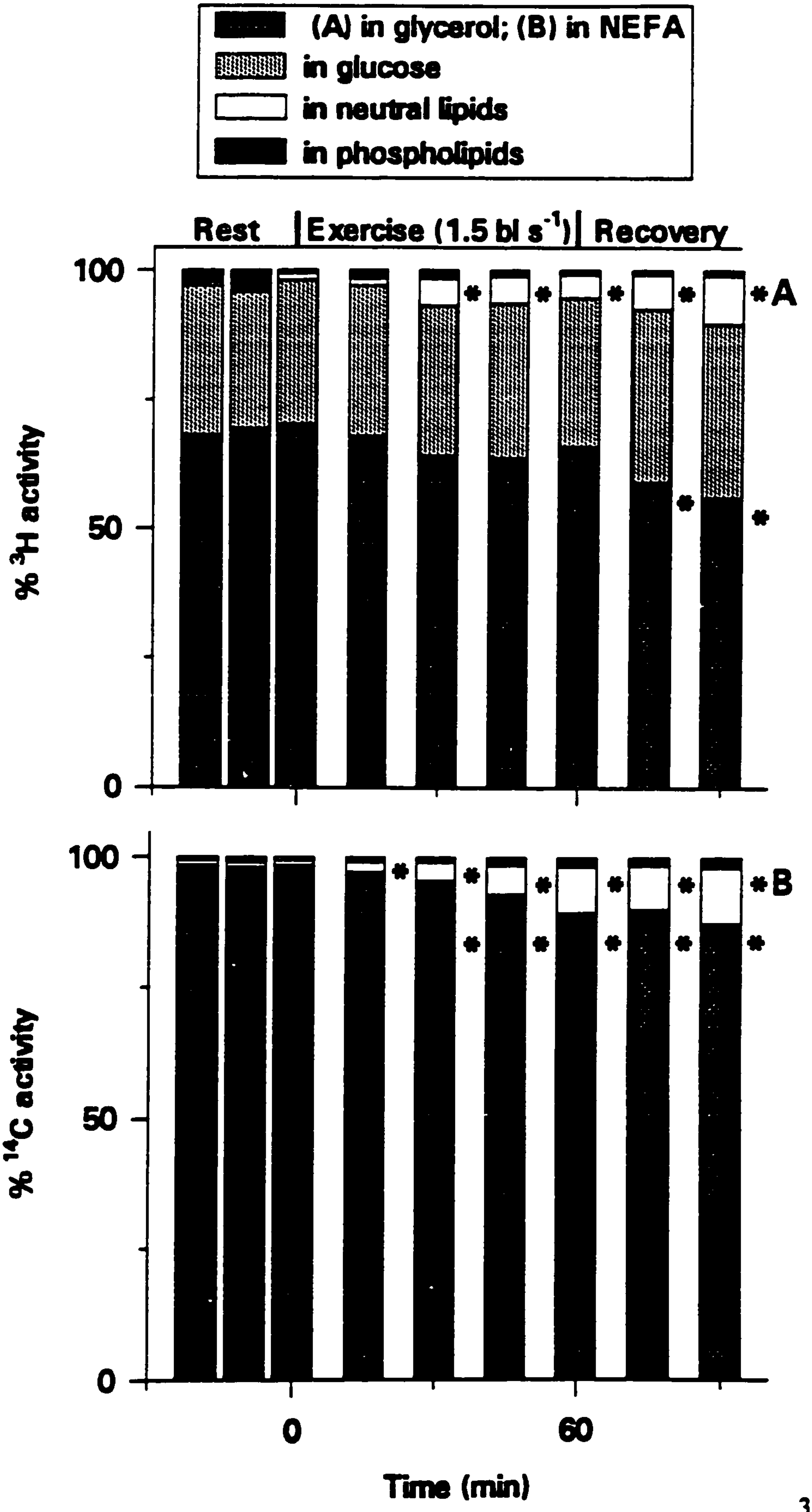

(A) in glycerol; (B) in NEFA
in glucose
in neutral lipids
in phospholipids
Rest
Exercise (1.5 bl s^{-1})
Recovery
100
50
0
% ^{3}H activity
*
A
100
50
0
% ^{14}C activity
B
0
60
Time (min)

Fig. 2.8. (**A**) Distribution of ^{3}H activity in glucose, glycerol, neutral lipids and phospholipids at rest and during 4 days of exercise at 1.0 bl s^{-1}.

(**B**) Distribution of ^{14}C activity in fatty acids (NEFA), neutral lipids and phospholipids at rest and during 4 days of exercise at 1.0 bl s^{-1}. (Values are mean percentage of the total ^{3}H or ^{14}C activity, n = 6). * indicates a significant difference from the resting value.

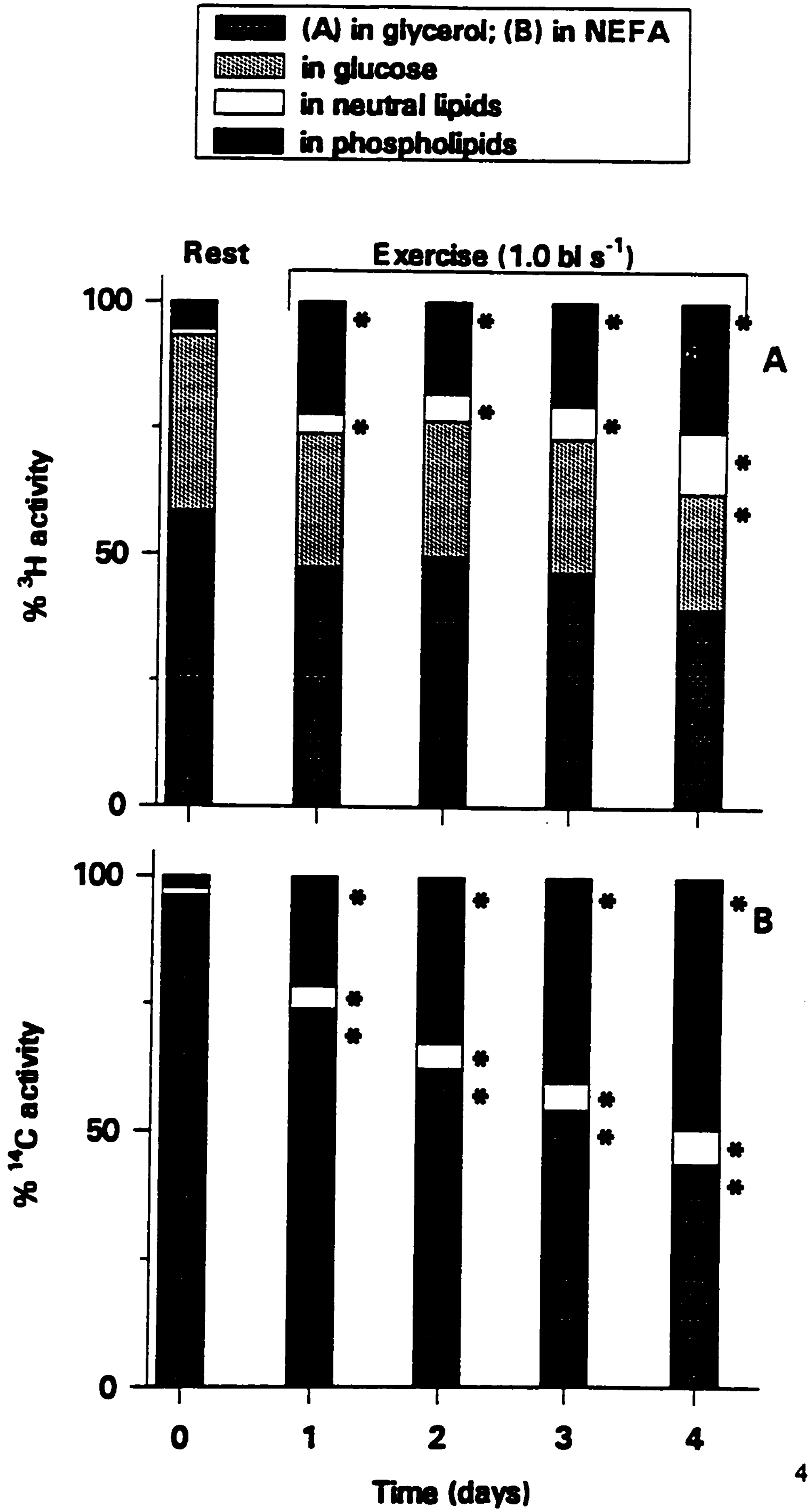

(A) in glycerol; (B) in NEFA
in glucose
in neutral lipids
in phospholipids
Rest
Exercise (1.0 bl s^{-1})
A
B
% ^{3}H activity
% ^{14}C activity
100
50
0
0
1
2
3
4
Time (days)
*

Table. 2.2. Concentration and rate of appearance (R_a) of glycerol and NEFA in rainbow trout when heparin or sodium citrate were used as anticoagulants. Values are means ± SEM, (n = 12) for heparin group, (n = 6) for sodium citrate. * indicates a significant difference between anticoagulants.

	[glycerol] (mmol l^{-1})	R_a glycerol (μmol kg^{-1} min^{-1})	[NEFA] (mmol l^{-1})	R_a NEFA (μmol kg^{-1} min^{-1})
heparin	0.25 ± 0.13	5.90 ± 0.48	0.62 ±0.42	4.39 ± 0.39
sodium citrate	0.12 ± 0.01*	3.51 ±0.59	0.58 ± 0.05	2.99 ± 0.29

Discussion

In mammals, exercise causes the mobilization of lipid reserves with a maximal response occurring at a low work intensity of 40-60 % VO_2max (Weber *et al.*, 1996; Wolfe *et al.*, 1990). This mobilization results in a 2 to 4 fold increase of R_a glycerol and R_a NEFA (Fig. 2.9; Table 2.3). In contrast, I did not observe any change either in R_a glycerol or R_a NEFA in rainbow trout subjected to one hour of submaximal exercise at 1.0 and 1.5 bl s^{-1} (Table 2.1; Fig 2.1 to 2.3). For the purpose of comparison, these exercise intensities were estimated to correspond approximately to 30 and 50 % VO_2max, or 50 and 70 % U_{crit}, respectively (where U_{crit} is the maximum sustainable swimming speed) (Beamish, 1978; Kiceniuk and Jones, 1977; Webb, 1971). Because rainbow trout normally spend a majority of their time swimming, one hour of exercise may have been too short to trigger the mobilization of triacylglycerol reserves. Therefore, I extended exercise duration to four days at 1 bl s^{-1}, an average speed naturally used by migrating salmon (Beamish, 1978; Quinn, 1988). During these long-term experiments each fish swam an average distance of about 140 km. Even after such prolonged aerobic exercise, there was no change of R_a glycerol or of R_a NEFA (Fig. 2.4 to 2.6), showing clearly that, during submaximal exercise, rainbow trout do not mobilize their triacylglycerol reserves above resting levels.

Fig 2.9. Glycerol (A) and NEFA (B) rate of appearance (R_a), presented as a percentage of the resting value, during one hour of exercise at 1.5 bl s^{-1} for trout (values are means ± SEM; n = 6) and at 40% VO_2max for dog, human and goat. Original values and references are presented in Table 2.3.

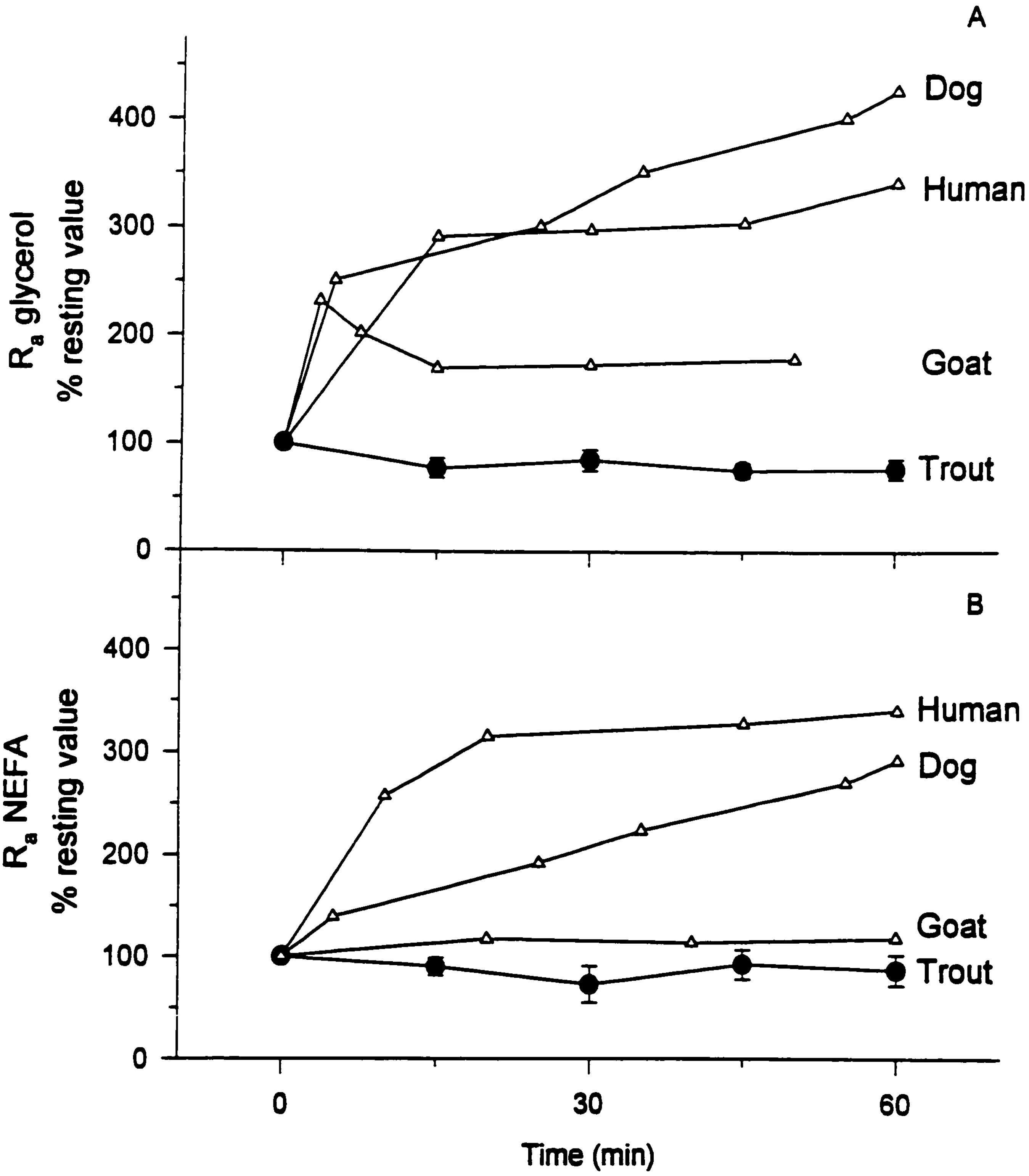

A
R_a glycerol
% resting value
0
100
200
300
400
Dog
Human
Goat
Trout
B
R_a NEFA
% resting value
0
100
200
300
400
Human
Dog
Goat
Trout
0
30
60
Time (min)

Table 2.3. Glycerol and NEFA rate of appearance(R_a) at rest and after 45 to 60 min of exercise for four species: dog, human, goat and trout. Exercise intensity was 40% VO_2max for the mammalian species and 75% Ucrit for the trout. Values are means ± SEM. When resting and exercising values were significantly different , $p < 0.05$ is indicated, otherwise NS for non-significant.

		R_a glycerol (μmol kg^{-1} min^{-1})			R_a NEFA (μmol kg^{-1} min^{-1})		
Species	**References**	**Rest**	**45 to 60 min**	**p**	**Rest**	**45 to 60 min**	**p**
Dog	Shaw *et al.* (1975)	4.4 ± 1.6	17.7 ± 0.1	$p < 0.05$	15.5 ± 2.2	42.2 ± 6.6	$p < 0.05$
Human	Wolfe *et al.* (1990)	2.1 ± 0.5	6.4 ± 0.4	$p < 0.05$	5.3 ± 1.3	17.5 ± 1.2	$p < 0.05$
Goat	Weber *et al.* (1993)[a] Weber *et al.* (1996)[b]	3.9 ± 0.2[a]	6.9 ± 1.1[a]	NS	20.1 ± 1.2[b]	23.8 ± 3.8[b]	NS
Trout		4.06 ± 0.49	2.98 ± 0.52	NS	4.35 ± 0.67	3.34 ± 0.74	NS

If the trout had been relying on their intramuscular lipid depots instead of circulatory fatty acids, it is conceivable that it would not have caused an increase in R_a NEFA because the fatty acids would have been oxidized without appearing in the bloodstream (Wolfe *et al.*, 1990; Moyes and West, 1995). However, this scenario is very unlikely because the mobilization of triacylglycerol stored in muscles would have resulted in an increase of R_a glycerol since trout muscle, which lacks glycerokinase activity, cannot metabolize glycerol (Newsholme and Taylor, 1969).

From R_a NEFA and R_a glycerol measurements, I calculated the intracellular TAG:FA cycling rate of trout, and showed that on average about 68 % of the fatty acids were reesterified. Fish and mammals are similar in the sense that their relative reesterification rates are high (> 60%). However, these two groups of vertebrates are different because most of the reesterification occurs intracellularly in fish, but extracellularly in mammals (Wolfe *et al.*, 1990). The enzymatic reactions of reesterification depend on the availability of substrate (fatty acids) (Wolfe *et al.*, 1990). Therefore, if the fatty acids released by lipolysis were leaving the tissue (liver, adipose tissue) at a slower rate in fish compared to mammals, this could explain the higher rate of the intracellular TAG:FA cycling observed in fish. This slower exit rate of fatty acids could be due to a lower blood flow through the tissue, which would also affect the availability of lipid-binding proteins.

Intracellular reesterification is an enzymatic process that requires the energy of seven to eight phosphate bonds (ATP→ ADP) per triacylglycerol formed. Six phosphate bonds are required to activate the fatty acids while one is needed to convert glucose or glycerol into glycerol phosphate. One extra phosphate bond is required if the latter reaction is achieved by the intermediate conversion of glycerol to glucose (Elia *et al.*, 1987). Since the hydrolysis of one mole of ATP releases 7.3 kcal (Lehninger *et al.*, 1993), the cost of the intracellular cycling of one mole of triacylglycerol varies between 51.1 and 58.4 kcal. Therefore, in trout used in this study the estimated energetic cost of intracellular TAG:FA cycling at rest would have been 4.4 to 5.1 % of their metabolic rate or 1.8 ~ 2 μmol O_2 kg^{-1} min^{-1}.

In rainbow trout, the absolute and the relative intracellular TAG:FA cycling did not change with exercise. This contrasts with what I had expected by analogy to mammals, where, as observed by Weber *et al.* (1993) and Wolfe *et al.* (1990) in goats and humans, the relative rate of TAG:FA cycling decreases during exercise.

The decrease in TAG:FA cycling observed in mammals, occurs simultaneously to the increase in lipolysis caused by exercise, and thus amplifies the increase in the availability of fatty acids for oxidation. Consequently, the TAG:FA cycle has been shown to serve as a regulator of mammalian lipid metabolism. Because the intracellular TAG:FA cycling rate that I measured in trout remained constant, I suggest that this cycle does not play a role in the regulation of lipid metabolism in fish, and that it could be a true futile cycle. However, I cannot speculate on the effects of exercise on *extracellular* reesterification as it was not measured in this study.

In mammals, the increased oxidation rate of fatty acids observed during exercise reduces the availability of the fatty acids to be reesterified. This yields a decrease in the relative reesterification rate (Edens *et al.*, 1990; Wolfe *et al.*, 1990). Therefore, the constant rate of intracellular reesterification in exercising fish also indicates that fatty acid oxidation might not increase with exercise. This interpretation is partly in agreement with Lauff and Wood's (1996) findings obtained using indirect calorimetry on exercising rainbow trout. In agreement with my result, these authors found that over the duration of 3 days of exercise at one speed (55% U_{crit}), fish did not change their rates of lipid oxidation. However, in disagreement with my result, the constant rate of lipid oxidation observed by Lauff and Wood (1996) changed when swimming speed changed, since at 55 and 80% U_{crit} the lipid oxidation rates were respectively 24 % and 75 % higher than at rest. It is possible that I did not detect an increase in oxidation rates between rest and exercise if this increase had only affected extracellular TAG:FA cycling which I did not measure.

Despite the high rate of intracellular TAG:FA cycling, R_a NEFA appears to be at all times well in excess of the rate of fatty acid oxidation. This indicates that a large fraction of the circulating fatty acids may be reesterified by extracellular TAG:FA cycling. Assuming that 1) the resting oxygen consumption of the fish was 40 μmol O_2 kg^{-1} min^{-1}

(Haman *et al.*, 1997), 2) an average fatty acid consists of a chain of 18 carbons and its oxidation requires 26 oxygen molecules, and 3) energy needs are entirely supported by fatty acid oxidation, than, less than 40% of the circulating fatty acids (R_a NEFA) can be oxidized. Therefore, considering that all the circulatory fatty acids that are not oxidized are reesterified, extracellular TAG:FA cycling can be estimated to be 60% of R_aNEFA (fatty acids entering the circulation) or 20% of the total fatty acids released by lipolysis. Considering this apparent importance of the extracellular TAG:FA cycling in fish, it would be of interest to quantify this cycle and to measure possible changes during exercise.

Since rainbow trout oxidize less than 40% of their circulating fatty acids (R_a NEFA) at rest, why do they have such a large NEFA turnover rate? The answer to this question is unknown. Because fish are ectotherms, changes in environmental temperature can alter their cell membrane fluidity (Bell *et al.*, 1986) and diffusion processes (Desaulniers *et al.*, 1996; Sidell and Hazel, 1987). A rapid reorganisation of membrane phospholipids or an increase in the lipid content of the cytoplasm would counteract these alterations in the properties of the cell membrane (Bell *et al.*, 1986; Desaulniers *et al.*, 1996; Sidell and Hazel, 1987). It can be hypothesized that the high NEFA flux observed in trout could be part of this compensatory mechanism.

This study provides the first determination of *in vivo* glycerol kinetics in fish. The rate of appearance of glycerol is commonly used as a measure of lipolytic rate in mammals. This assumption is well supported in mammals because 1) glycerol is only released into the bloodstream by lipolysis, 2) all the glycerol released by lipolysis enters the bloodstream because adipose tissue cannot metabolize it (Brooks *et al.*, 1982) and 3) partial lipolysis of triacylglycerol is unlikely (Brooks *et al.*, 1982). Can R_a glycerol also be used as an index of the lipolytic rate in fish?

In fish, as in mammals, lipolysis seems to be the only pathway for glycerol production, therefore, glycerol appearing in the bloodstream comes solely from triacylglycerol hydrolysis. Glycerol metabolism requires the presence of glycerokinase which catalyzes the phosphorylation of glycerol into glycerol-3-phosphate (Lech, 1970). If this enzyme was very active in lipid reserves, than the glycerol derived from lipolysis could be metabolized without appearing in the bloodstream. Under these conditions, the lipolitic rate calculated from R_a glycerol would be underestimated. In rainbow trout, measurement of glycerokinase activity in adipose tissue is not available. Newsholme and Taylor (1969) provide evidence showing that rainbow trout muscle lacks glycerokinase activity. Because the liver is an important site of gluconeogenesis and triacylglycerol synthesis, it is expected to possess glycerokinase activity. In rainbow trout, the hepatic activity of glycerokinase seems similar or slightly lower to that of the gluconeogenic enzymes (Lech, 1970; Suarez and Mommsen, 1987). Therefore, it is possible that a fraction of the glycerol released by hepatic lipolysis was metabolized before it entered the bloodstream, and thus the lipolytic rate could have been underestimated.

Despite the lack of evidence that R_a glycerol is an index of the lipolytic rate in fish, an underestimation of lipolytic rate by measurement of R_a glycerol seems unlikely. Because three fatty acids and one glycerol are released following the hydrolysis of one molecule of triacylglycerol, the theoretical ratio of R_a NEFA to R_a glycerol is equal to three. Therefore, if R_a glycerol (rate of appearance of glycerol in the blood) was an underestimation of the lipolytic rate (rate of glycerol production by lipolysis), than the R_a NEFA / R_a glycerol ratio measured would be higher than three (Leibel and Hirsch, 1985). This is not the case in this study where the average R_a NEFA / R_a glycerol is equal to 0.97 ± 0.04 (n=18).

In exercising rainbow trout, an incorporation of ^{3}H-glycerol and ^{14}C-palmitate into plasma neutral lipids and phospholipids was also observed. When the animals were swimming for one hour at either 1.0 or 1.5 bl s^{-1}, a small fraction of the infused ^{3}H and ^{14}C activity was incorporated in neutral lipids (Fig 2.7). However, because the specific activity of neutral lipids could not be determined, the effect of exercise on this incorporation could not be distinguished from progressive labeled incorporation that might have occured at the same rate in resting animals infused for 2.5 hours. Contrary to what happened during 1 hour swimming, during more prolonged exercise (4 days at 1.0 bl s^{-1}) ^{3}H and ^{14}C activity was primarly incorporated into the phospholipids and to a smaller extent into the neutral lipids (Fig. 2.8). Labeled glycerol and palmitate present in the plasma after an infusion can either be degraded or incorporated into hepatic lipids which are then released into the plasma. In sea bass (*Dicentrarchus labrax*), Zwingelstein (personnal communication) found that the incorporation of glycerol into hepatic lipids was at a maximum only after 24 to 48 hours following an injection of ^{3}H-glycerol. Because the incorporation of activity in the hepatic lipids is a long process, it may not have been as large in the short-term as in the long-term swimming experiments. Furthermore, during the long-term swimming experiments, the incorporation of activity in hepatic lipids could have been amplified by the large pool of labeled metabolites that was accumulating due to the repetitive infusions.

Heparin was used as an anticoagulant in the first series of experiments (swimming for 1 hour at 1.0 or 1.5 bl s^{-1}). However, because this compound can stimulate lipolysis, it was substituted with sodium citrate for the second series of experiments (swimming for 4 days at 1 bl s^{-1}) to check for this possibility. When the resting values were compared between heparin and sodium citrate-treated fish, no significant differences were observed, except for a small increase in glycerol concentration (Table 2.2). R_a glycerol, R_a NEFA and NEFA concentration were not elevated by heparin at the doses used in the present study. Therefore, I recommend the continued use of heparin in studies on lipid kinetics in fish, because it is a much more potent anticoagulant than sodium citrate.

This study shows that, contrary to mammals, rainbow trout do not mobilize their triacylglycerol reserves above resting levels during prolonged exercise. This conclusion is based on the absence of change of the glycerol and fatty acid kinetics of the trout even after 4 days of endurance swimming. This study also provides the first measurement of intracellular TAG:FA cycling rate in fish and shows that in rainbow trout, 68.0 ± 1.5 % of the fatty acids released by lipolysis are reesterified intracellularly. Therefore, in fish, as in mammals, a large fraction of the fatty acids released by lipolysis is reesterified by this cycle. However, contrary to mammals, the intracellular TAG:FA cycling rate did not decrease during exercise. This result suggests that the TAG:FA cycle does not play a role in the regulation of lipid metabolism in fish, and, therefore, that it could be a true futile cycle in fish.

CHAPTER 3

ANTI-LIPOLYTIC ACTION OF ACIPIMOX ON GLYCEROL AND FATTY ACID KINETICS IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

Introduction

In mammals, lipolysis and simultaneous reesterification continuously breakdown and synthesize triacylglycerol stored in the adipose tissue. These processes constitute the triacylglycerol:fatty acid substrate cycle (TAG:FA cycling). The TAG:FA cycle reesterifies an important fraction of the fatty acids released by lipolysis either in the adipose tissue itself (intracellular TAG:FA cycling) or elsewhere, including the liver or the muscles (extracellular TAG:FA cycling) (Newsholme and Crabtree, 1976). In mammals, TAG:FA cycling has been found to be of great importance in amplifying the response of substrate flux to a given change in a regulator (*e.g.* hormone) (Wolfe *et al.*, 1990). When triacylglycerol reserves are mobilized above the resting levels (*e.g.* during prolonged exercise), parallel to the increase in the lipolytic rate, there is a decrease in the fraction of fatty acids reesterified. This results in an increase in the availability of fatty acids for oxidation (Campbell *et al.*, 1992; Newsholme and Crabtree, 1976; Wolfe *et al.*, 1990).

TAG:FA cycling appears to play no role in the regulation of fatty acid metabolism in fish, contrary to mammals (Chapter 2). In resting rainbow trout, the intracellular TAG:FA cycling represents over 60 % of the fatty acids released by lipolysis. This cycling rate does not decrease during prolonged exercise (Chapter 2). Although the extracellular TAG:FA cycling is not known in fish, this result suggests that the TAG:FA cycling could be a true futile cycle in fish.

Wolfe (1990) indicated that in mammals the percentage of fatty acids reesterified is not under the control of an active regulatory process, but rather depends on the availability of fatty acids. Therefore, considering that the TAG:FA cycle is a true futile cycle in rainbow trout, I would expect that a reduction in the availability of fatty acids caused by an inhibition of lipolysis, would not lead to a decrease in the percentage of fatty acids reesterified. I attempted to test this hypothesis by studying the effect of acipimox, an antilipolytic drug used in the treatment of hypertriglyceridemia in humans (Christie *et al.*, 1996; Fuccella *et al.*, 1980), on the plasma glycerol and fatty acid appearance rates and intracellular TAG:FA cycling in rainbow trout.

Materials and Methods

Animals

Rainbow trout, *Oncorhynchus mykiss* (Walbaum) of both sexes (601-877g) were purchased from Linwood Acres Trout Farm (Campbellcroft, Ontario, Canada) and held in a 1300 l flow-through tank at 13°C. They were kept in dechloraminated, well oxygenated water under a 12h:12h L:D photoperiod. Animals were acclimated to these conditions for at least 1 month before initiation of the experiments and they were fed Purina trout chow 3 times per week until satiation.

Catheterization

The surgery consisted of a double cannulation of the dorsal aorta under ethyl-N-aminobenzoate sulphonic acid anaesthesia (MS-222) as described previously (Haman and Weber, 1996). Once the catheters were in place (and any time it was necessary thereafter in the experiments), they were flushed with a solution of sodium citrate (12.9 mmol l^{-1}) to prevent coagulation. After surgery, each animal was placed in an opaque Plexiglass box (60 x 16 x 18 cm) irrigated with dechloraminated and well oxygenated water. The box was tightly sealed with an opaque lid through which a small opening had been drilled to allow access to the catheters. Animals were allowed to recover 24 hours after surgery before the start of the experiment.

Continuous Infusions of Isotopes

Labeled metabolites were infused as described previously (Haman and Weber, 1996; Haman *et al.*, 1997). The infusate was prepared daily with 2- ^{3}H glycerol (Amersham, 37.0 Gbq/mmol) and 1-^{14}C palmitate (Amersham, 1.85-2.2 Gbq/mmol) as indicated in Chapter 2. The isotope mixture was continuously infused at the rate of 1ml h^{-1} before, during, and after the anti-lipolytic drug treatment with a calibrated syringe pump (Harvard apparatus, South Natick, MA). Infusion rates ranged from 491,000 to 991,000 DPM kg^{-1} min^{-1} for each isotope.

Anti-Lipolytic Treatment

A solution of acipimox (5-methylpyrazine-carboxilic acid 4-oxide, Farmitalia Carlo Erba, Milan, Italy), an anti-lipolytic drug analogue to nicotinic acid (Fuccella *et al.*, 1980), was prepared daily in Cortland fish saline at the concentration of 1 g l^{-1}. One hour after the start of the infusion of the labeled metabolites, the acipimox solution was infused for one hour at the rate of 1ml h^{-1}. Thus, each fish received a dose of 10 mg of acipimox, regardless of their weight.

Blood Sampling

Throughout the experiment, 6 blood samples of 0.8 ml each were taken. Basal glycerol and fatty acid kinetics were determined from 2 blood samples which were drawn 40 and 60 min, respectively, after the start of the infusion of the labeled metabolites. Four other blood samples were taken 120, 180, 210 and 240 min after the start of the

^{3}H-glycerol and ^{14}C-palmitate infusion. At these times, the acipimox treatment was already completed. Hematocrit, which was measured on each blood sample, did not vary significantly throughout the experiments and all values were above 20% (average value throughout the experiment: 25 ±1.10 %).

Preparation and Analysis of Blood Samples

The preparation and the analysis of the blood samples to determine glycerol and NEFA concentrations, as well as glycerol and palmitate specific activities, were conducted as described in Chapter 2.

Metabolic Rate Measurements.

Oxygen consumption was measured throughout the experiments as described previously (Haman et *al.*, in press). Briefly, the external water supply to the box was stopped for periods of 10 min and the same water was recycled within the 16 liter closed system. During each 10 min period, the O_2 concentration was recorded each minute using a calibrated oxygen electrode (Oxyguard, Handy MK III, Valox ltd). After each measurement, saturated PO_2 conditions were re-established by resuming the external water supply and by flushing the recycled water from the system. The equation of Steffensen (1989) for closed system respirometry was used to calculate the rate of oxygen consumption.

Calculations and statistics.

Palmitate concentration, rates of appearance of glycerol, palmitate and NEFA, as well as intracellular rates of fatty acid reesterification (absolute and relative), were calculated as described in Chapter 2.

Statistical differences were assessed using a two-way analysis of variance (ANOVA) or a Friedman's test (when the assumptions of normality or homoscedasticity were not met) with time and fish as the main factors. When significant changes were detected by ANOVA, Dunnett's test was used to determine which specific means were different from the resting value. Paired t-test was used to compare the results obtained before and after the acipimox treatment. Percentages were transformed to the arcsine of their square root before analysis and all values given are means ± SEM (n=7).

Results

The acipimox treatment had no effect on the metabolic rate, which remained constant at 32.4 ± 5.4 μmol O_2 kg^{-1} min^{-1} ($p>0.05$). Glycerol, palmitate and fatty acid concentrations did not change significantly throughout the experiments ($p>0.05$) (Fig.3.1 to 3.3 A). Mean glycerol and NEFA concentrations were 0.19 ± 0.01 mmol l^{-1} and 0.55 ± 0.03 mmol l^{-1}, respectively. Glycerol and palmitate specific activities also did not change with acipimox treatment ($p>0.05$) (Fig.3.1B and 3.2B). The high variability of glycerol and palmitate specific activity after 240 min of infusion of the labeled metabolites was due to a large increase in the specific activity of one of the fish. Glycerol rate of appearance (R_a glycerol) remained constant throughout the experiments ($p>0.05$), at an average value of 3.60 ± 0.25 μmol kg^{-1} min^{-1} (Fig. 3.1C). In contrast, palmitate appearance rate decreased significantly with acipimox treatment from 0.75 ± 0.13 to 0.45 ± 0.08 μmol kg^{-1} min^{-1} ($p<0.05$) (Fig. 3.2 C). Mean NEFA rate of appearance (R_a NEFA) had a tendency to decline, from 3.63 ± 0.62 to 2.13 ± 0.37 μmol kg^{-1} min^{-1}, however, this change was not significant ($p>0.05$) (Fig. 3.3 C). Since neither R_a glycerol nor R_a NEFA changed significantly with the acipimox treatment, the absolute and relative rates of fatty acid reesterification calculated from these results, remained constant throughout the experiment at an average value of 7.86 ± 0.63 μmol kg^{-1}min^{-1} and 70.05 ± 1.97 %, respectively ($p>0.05$).

Fig. 3.1. Plasma concentration (A), specific activity (B) and appearance rate (R_a) (C) of glycerol in rainbow trout before, during, and after the infusion of 10 mg of acipimox. Values are means ± SEM (n = 7).

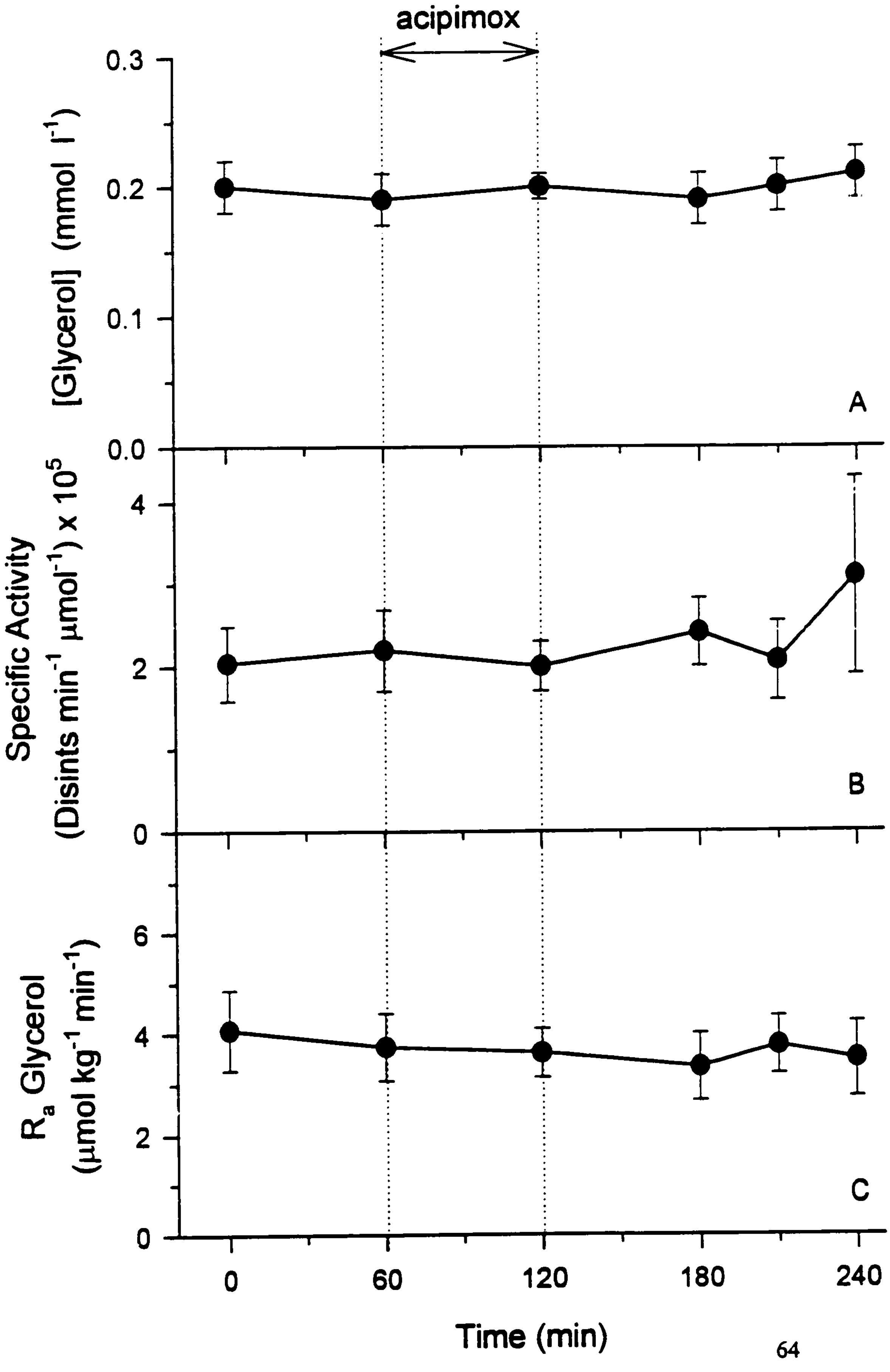

acipimox
[Glycerol] (mmol l^{-1})
0.3
0.2
0.1
0.0
A
Specific Activity
(Disints min^{-1} µmol^{-1}) x 10^5
4
2
0
B
R$_a$ Glycerol
(µmol kg^{-1} min^{-1})
6
4
2
0
C
0
60
120
180
240
Time (min)

Fig. 3.2. Plasma concentration (A), specific activity (B) and appearance rate (R_a) (C) of palmitate in rainbow trout before, during, and after the infusion of 10 mg of acipimox. Values are means ± SEM (n = 7).

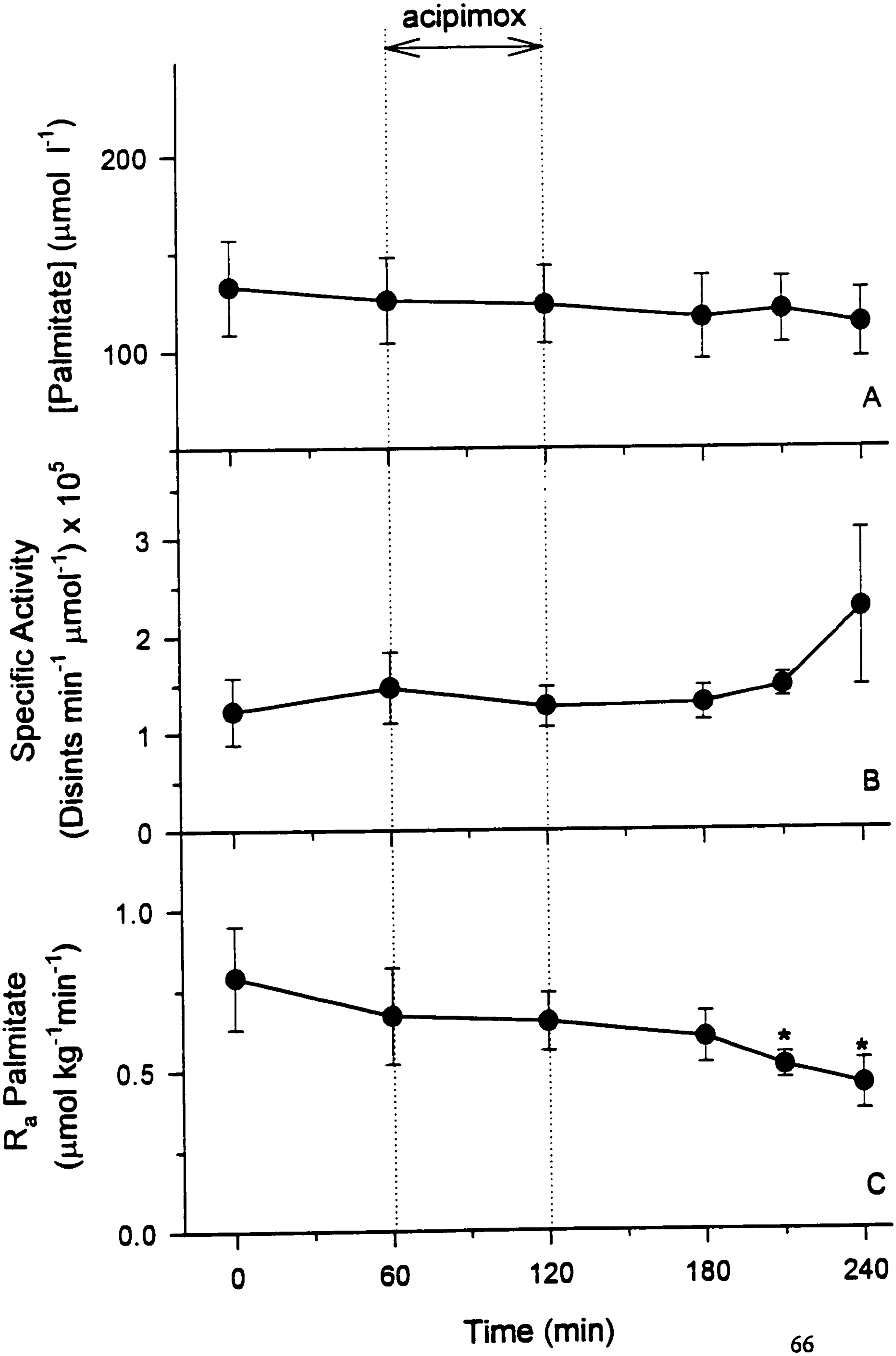
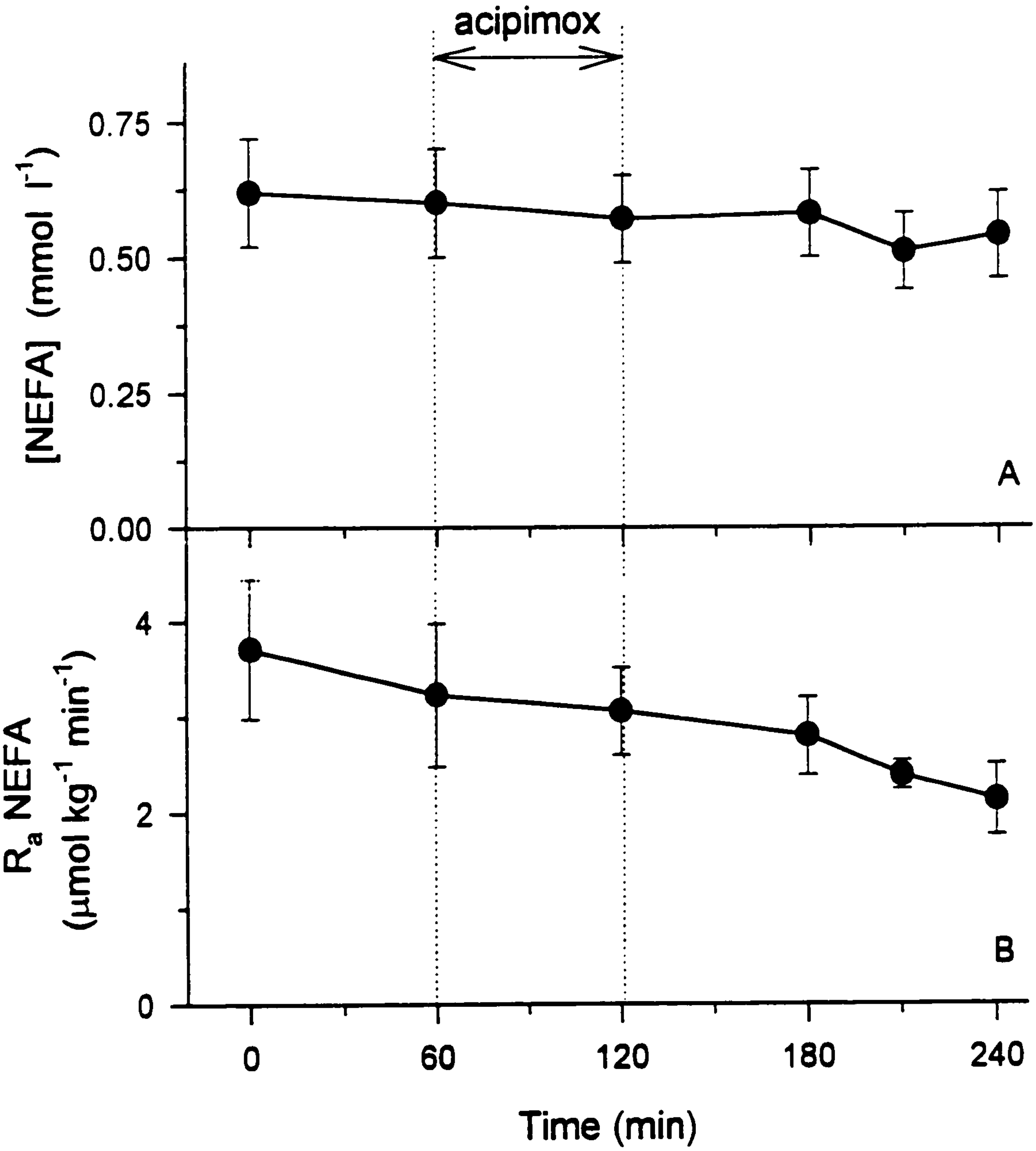

acipimox
[Palmitate] (μmol l-1)
200
100
A
Specific Activity
(Disints min-1 μmol-1) x 10^5
3
2
1
0
B
Ra Palmitate
(μmol kg-1min-1)
1.0
0.5
0.0
*
*
C
0
60
120
180
240
Time (min)

Fig. 3.3. Plasma concentration (A) and appearance rate (R_a) (B) of fatty acids (NEFA) in rainbow trout before, during, and after the infusion of 10 mg of acipimox. Values are means ± SEM (n = 7).


acipimox
[NEFA] (mmol l^{-1})
0.75
0.50
0.25
0.00
A
R_a NEFA (μmol kg^{-1} min^{-1})
4
2
0
B
0
60
120
180
240
Time (min)

It is important to note that one of the 7 fish studied presented an increase in concentration and rate of appearance of both glycerol and NEFA, which was an unexpected response to the antilipolytic drug treatment (Table 3.1.). If this fish was excluded from the analysis, a significant decrease in R_a NEFA, would be observed. However, since no experimental or analytical problem was noticed during this particular study, the data obtained from this fish were included in the analysis. Examination of the individual fish data indicated also that concentration and appearance rates of glycerol and NEFA tended to decrease with a variable amplitude and at different times for each fish. In order to reduce the influence of this variability on the overall results, control values (before acipimox administration) were compared with the lowest value obtained following the acipimox infusion (Fig. 3.4 A and B). This comparison revealed a significantly lower NEFA concentration after acipimox treatment. However, the absence of a significant effect of acipimox on glycerol concentration, R_a glycerol and R_a NEFA was confirmed ($p>0.05$) (Fig 3.4). It is also interesting to note that, even though the absolute reesterification rate of fatty acids seemed to be lower after acipimox treatment, percent of reesterification remained the same before and after acipimox administration ($p>0.05$) (Table 3.2).

The distribution of ^{3}H and ^{14}C activity in the different plasma metabolites was quantified using ion exchange chromatography. Throughout the experiments, the percentage of ^{3}H activity in glycerol decreased as tritiated glycerol was incorporated into the neutral lipids ($p<0.05$). A small incorporation of tritiated glycerol into glucose also occurred, but this was only significant at 120 min after the start of the labeled metabolite infusion (Fig. 3.5A). In contrast, the percentage of tritium in the plasma phospholipids did not change throughout the experiments ($p>0.05$). A similar pattern was observed for the distribution of the ^{14}C-palmitate among the NEFA, the neutral lipids and the phospholipids (Fig. 3.5B). A large incorporation of ^{14}C- palmitate into the neutral lipids was accompanied by a decrease of the percentage of ^{14}C activity in the plasma NEFA ($p<0.05$). However, a small but significant incorporation of ^{14}C palmitate into the phospholipids was also observed ($p<0.05$).

Table 3.1. Glycerol and fatty acid (NEFA) appearance rate (R_a) found for fish #4 before and after acipimox treatment (120 min after the end of the treatment). The average values of glycerol and fatty acid appearance rate measured for the 6 other fish, in the same conditions, are also presented for comparison.

		before acipimox treatment	after acipimox treatment
R_a glycerol (μmol kg^{-1} min^{-1})	fish # 4	1.50	5.563
	all the other fish	4.45 ± 0.66	3.16 ± 0.30
R_a NEFA (μmol kg^{-1} min^{-1})	fish # 4	1.05	2.81
	all the other fish	4.15 ±0.75	2.02 ± 0.17

Fig. 3.4. Plasma glycerol and NEFA concentration (A) and appearance rate (R_a) (B) in rainbow trout before (control) and after acipimox administration in rainbow trout. The values presented as after acipimox treatment are the lowest obtained.

Values are mean ± SEM (n=7).

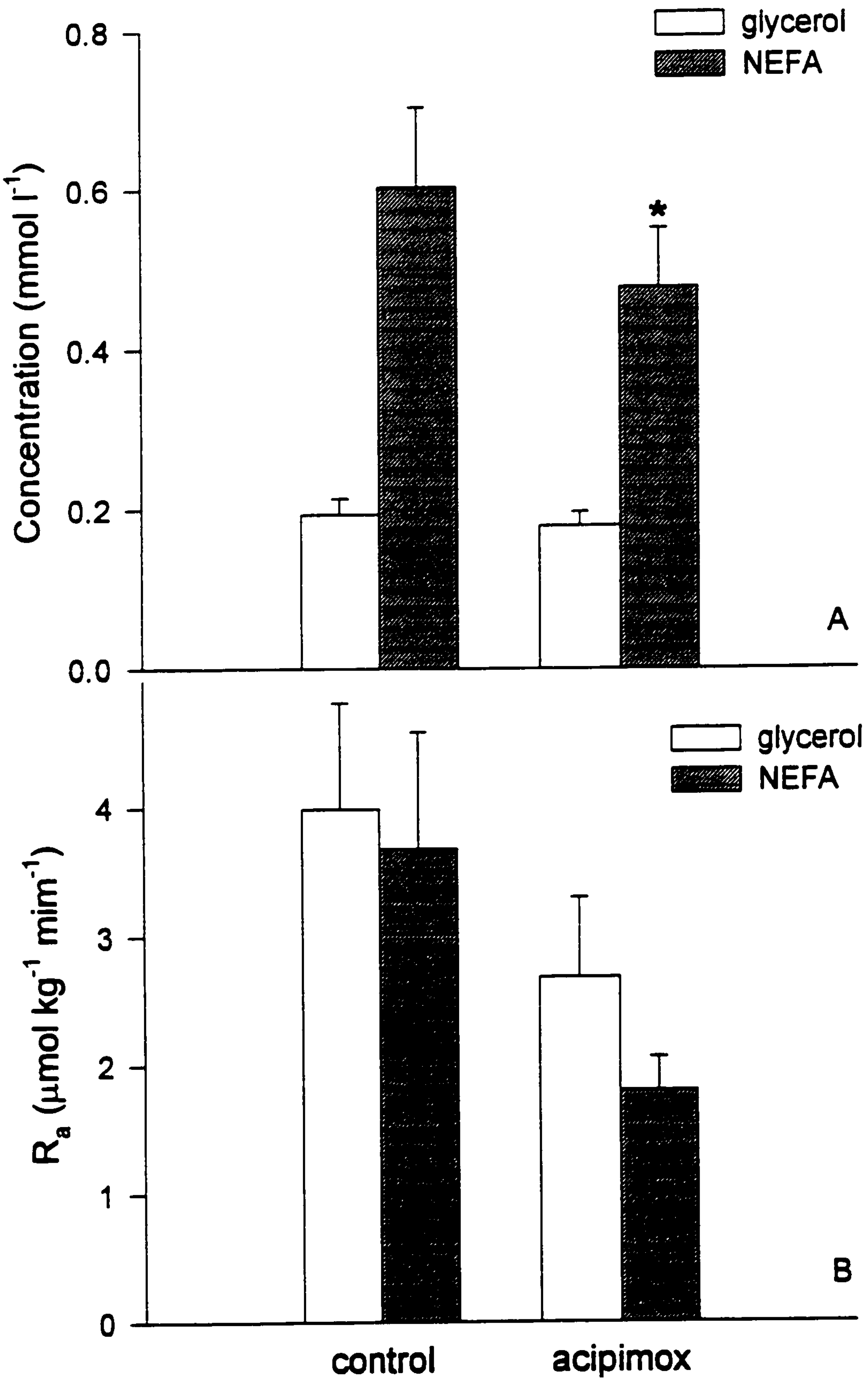
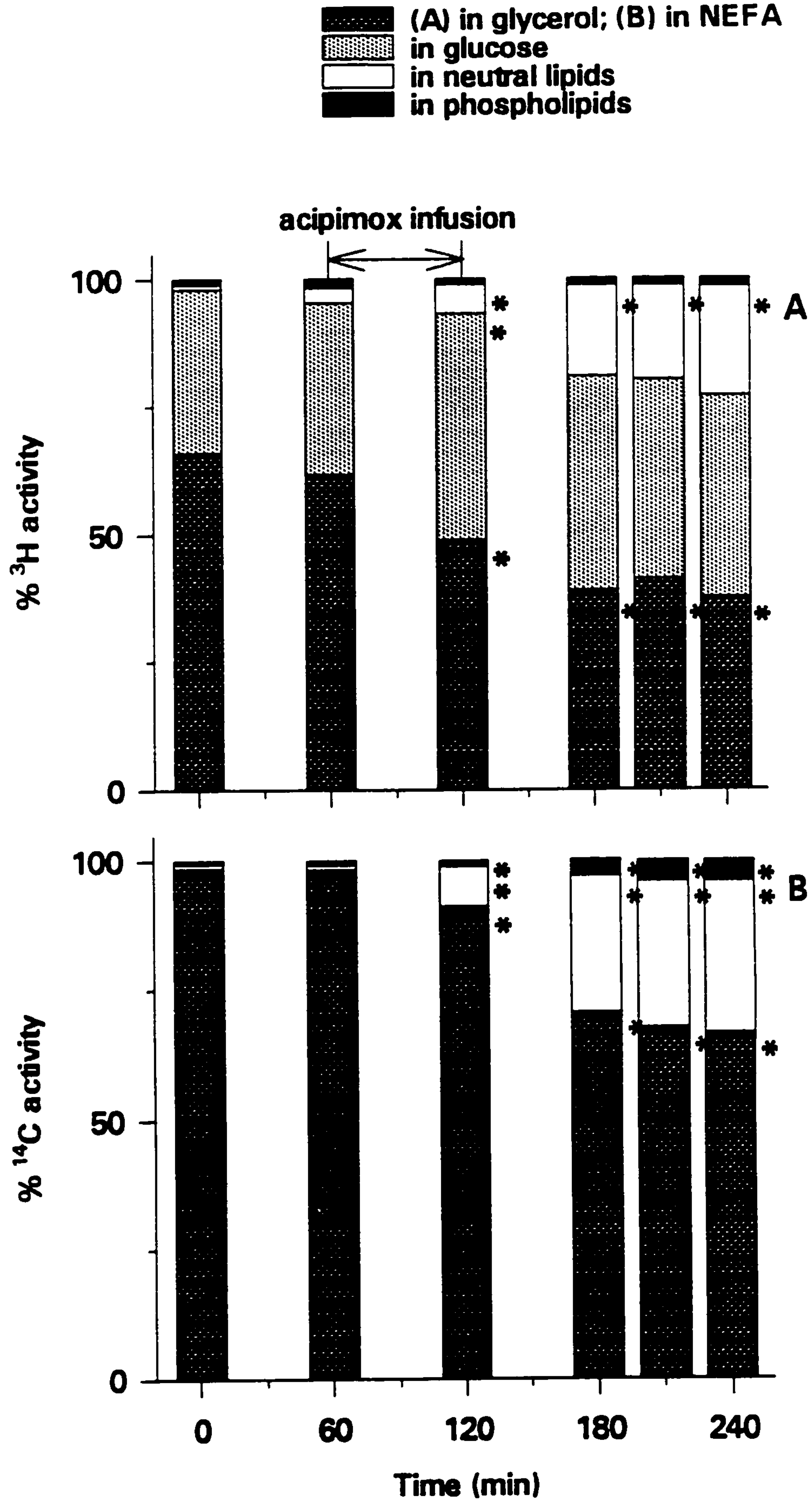

glycerol
NEFA
0.8
0.6
0.4
0.2
0.0
Concentration (mmol l^{-1})
*
A
glycerol
NEFA
4
3
2
1
0
R$_a$ (µmol kg^{-1} mim^{-1})
B
control
acipimox

Table. 3.2. Absolute and relative rates of intracellular reesterification before (control) and after acipimox administration in rainbow trout. The absolute reesterification rate (μmol kg^{-1} min^{-1}) after acipimox treatment was calculated by averaging the lowest value obtained for each fish. The relative reesterification rates (%) for both control and treatment were calculated from the absolute rates. Values are mean ± SEM (n=7).

	control	acipimox
Reesterification (μmol kg^{-1} min^{-1})	8.28 ± 1.72	6.08 ± 1.61
Percent of reesterification (%)	68.7 ± 4.3	69.0 ± 3.6

Fig. 3.5. (A) Distribution of ^{3}H activity in glucose, glycerol, neutral lipids and phospholipids before, during and after the infusion of 10 mg of acipimox. (B) Distribution of ^{14}C activity in fatty acids (NEFA), neutral lipids and phospholipids before, during, and after the infusion of 10 mg of acipimox. Values are mean percentages of the total ^{3}H or ^{14}C activity, n = 7. * indicates a significant difference from the resting value.


(A) in glycerol; (B) in NEFA
in glucose
in neutral lipids
in phospholipids
acipimox infusion
100
50
0
% ^{3}H activity
A
100
50
0
% ^{14}C activity
B
0
60
120
180
240
Time (min)

Discussion

The goal of this study was to determine whether a reduced availability of fatty acids influenced intracellular TAG:FA cycling in rainbow trout. The drug acipimox was selected over nicotinic acid to inhibit lipolysis, because acipimox is 20 times more potent and has a more prolonged effect in humans (Fuccella *et al.*, 1980). The absorption of 250 mg of acipimox has been shown to suppress almost completely the increase in plasma FFA and glycerol concentration normally induced by endurance exercise (Gautier *et al.*, 1993). Other studies on humans showed that acipimox elicited decreases in plasma NEFA concentration of 48 to 63% dependent upon the administrated dose (Fuccella *et al.*, 1980; Gautier *et al.*, 1994; Nuutila *et al.*, 1994). The present study is the first to investigate the effect of acipimox in fish. In rainbow trout, the infusion of 10 mg of acipimox reduced plasma NEFA concentration by 20% (Fig 3.4), but had no effect on glycerol concentration. In humans, Saloranta *et al.* (1994) showed that acipimox also significantly decreased the NEFA flux and a similar pattern was observed here for palmitate (Fig 3.2 C). However, despite a gradual decline in the mean NEFA and glycerol appearance rates, no significant effect of the acipimox treatment could be demonstrated. These results indicate that, in rainbow trout, acipimox (10 mg) infused in the arterial circulation had no significant effect on glycerol and fatty acid kinetics.

Considering this ambiguous effect of acipimox, one wonders if the acipimox mechanism of action was not impaired in rainbow trout. Christie *et al.* (1996)

demonstrated in isolated rat adipocytes that the antilipolytic properties of acipimox were due to its capacity to reduce intracellular cAMP concentrations through the inhibition of adenylate cyclase. Subsequent to a suppression in intracellular cAMP levels, there is a decrease of the activity of cAMP-dependent protein kinase, which resulted in a reduced activation of hormone sensitive lipase. In fish, the presence of triacylglycerol lipase has been demonstrated by Harmon *et al.* (1991) and Sheridan and Allen (1984); these authors isolated and partially purified this enzyme from rainbow trout liver and adipose tissue. Fish triacylglycerol lipase has been shown to be activated by a cAMP-mediated phosphorylation (Harmon *et al.*, 1993; Michelsen *et al.*, 1994; Migliorini *et al.*, 1992). Therefore, there is apparently no theoretical reason why the acipimox mechanism of action should be impaired in fish.

The possibility that the lack of an acipimox effect on glycerol and fatty acid kinetics was due to an insufficient dose of acipimox was also considered. Assuming that 1) acipimox diffuses into total body water (70% of body weight in a teleost fish (Holmes and Donaldson, 1969)), 2) that acipimox is neither metabolized nor excreted in fish, then, its plasma concentration at the end of the acipimox infusion can be estimated at 20.5 ± 1.1 $\mu g\ ml^{-1}$. In humans, concentrations 20 to 100 times lower were sufficient to reduce NEFA concentration by more than 45 % (Fuccella *et al.*, 1980). Considering that the relatively high doses of acipimox used in fish had no effect on glycerol and fatty acid kinetics, the likelihood that acipimox is ineffective in fish can not be completely excluded. However, it is also possible that rapid excretion or metabolism of the acipimox in rainbow trout had reduced the amount of acipimox reaching the lipid depots. This possibility has been rejected in humans because acipimox excretion takes several hours, and because no significant metabolism of acipimox was detected (Fuccella *et al.*, 1980). Unfortunately, no similar information is available for fish.

The ambiguous results obtained here in rainbow trout appear more likely due to the high variability in the timing, duration and amplitude of the response of individual fish to acipimox. Saturating the system (receptors, G_i proteins, adenylate cyclase, or any further step in the signal transduction pathway) by using higher doses of acipimox might allow one to obtain a more uniform inhibition of the lipolysis in fish, and to reduce the effect of a rapid degradation or excretion of the drug in fish. Also, longer infusions of acipimox could be used to prolong the inhibitory effect of acipimox. This could reduce the variability of the response to the drug. Moreover, particular care should be taken in the interpretation of results of metabolic studies using acipimox, since its ability to decrease intracellular cAMP levels could also affect gluconeogenesis and glycogenolysis (Lee *et al.*, 1996; Saloranta *et al.*, 1994).

By inhibiting gluconeogenesis, acipimox may have increased the pool of the labeled glycerol available for reesterification and, therefore, explain the large incorporation of tritiated glycerol into neutral lipids. However, tritiated glycerol was also incorporated into the neutral lipids when rainbow trout were subjected to one hour of exercise at 1.5 bl s^{-1} (Chapter 2). Therefore, this incorporation may not result from an effect of acipimox, and may have also occurred in non-treated animals infused for several hours. This is supported by the observation that ^{14}C-palmitate was also incorporated into neutral lipids during both exercise and acipimox treatment. Therefore, the difference in the amount of labeled metabolite incorporated into neutral lipids between the two experiments could just be due to the different durations of the infusions (2.5 vs. 4 h).

Since no significant effect of acipimox on glycerol and fatty acid turnover rate was observed, no conclusion can be drawn about the futility of the TAG:FA cycling in rainbow trout. However, it is interesting to note that the absolute intracellular reesterification rate of the fatty acids tended to be lower after the acipimox treatment, while the corresponding percentage of reesterification remained constant (Table 3.2). Because this response would be expected if the TAG:FA cycling was a true futile cycle, it indicates that this cycle should be investigated further to determine whether it plays any role in regulating NEFA availability.

CHAPTER 4

GENERAL CONCLUSION

Contrary to what I had hypothesized, rainbow trout do not mobilize their triacylglycerol reserves above resting levels during prolonged exercise. This conclusion is based on measurements of NEFA and glycerol kinetics in rainbow trout swimming for 1 hour at 1.0 or 1.5 bl s^{-1}, or for 4 days at 1.0 bl s^{-1}. At rest, R_a glycerol (measured for the first time in this study) and R_a NEFA were 5.55 ± 0.44 and 4.17 ± 0.34 μmol kg^{-1} min^{-1}, respectively. These fluxes did not change with exercise. This lack of response strongly contrasts with the 2-4 fold increase observed in mammals exercising at similar intensities.

From the measured values of R_a NEFA and R_a glycerol, I calculated the rate of fatty acid reesterification and found that at rest, 68.0 ± 1.5 % of the fatty acids released by lipolysis were reesterified into triacylglycerol intracellularly. This shows that in fish, like in mammals, the TAG:FA substrate cycle is very active. Furthermore, even though the extracellular TAG:FA cycling rate was not directly measured in this study, my results suggest that a large fraction of the circulating fatty acids could have been reesterified extracellularly.

The intracellular TAG:FA cycling did not change with prolonged exercise in rainbow trout. This result suggests that, contrary to what is observed in mammals, the TAG:FA cycle does not play a role in the regulation of lipid metabolism, and therefore, this cycle could be a true futile cycle in fish.

This question was investigated by studying the effect of acipimox, an antilipolytic drug which had never been used in fish before, on R_a NEFA, R_a glycerol and the intracellular TAG:FA cycling rate in resting rainbow trout. However, it was not possible to determine if the TAG:FA cycle is playing a role in the regulation of lipid metabolism in rainbow trout. Acipimox had no significant effect on glycerol and fatty acid kinetics and appeared to be a much poorer inhibitor of lipolysis in fish than it is in mammals. The reasons for this lack of response to acipimox in fish are unknown, but high rates of degradation or excretion of the drug, or simply an ineffectiveness of acipimox in fish, could count among these reasons.

Certainly, fish lipid mobilization and its control is a rich field for further investigation. The determination of the role of the TAG:FA cycle in fish lipid metabolism is of particular interest. In order to have a complete view of the importance of this cycle, the quantification of the extracellular TAG:FA cycle appears necessary. This requires the simultaneous measurement of R_a glycerol, R_a NEFA, as well as the rate of fatty acid oxidation. Also, the futility of the TAG:FA cycle in fish could be further investigated following a protocol similar to the one used in this study, but with higher doses of acipimox or another more potent inhibitor of lipolysis.

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IMAGE EVALUATION
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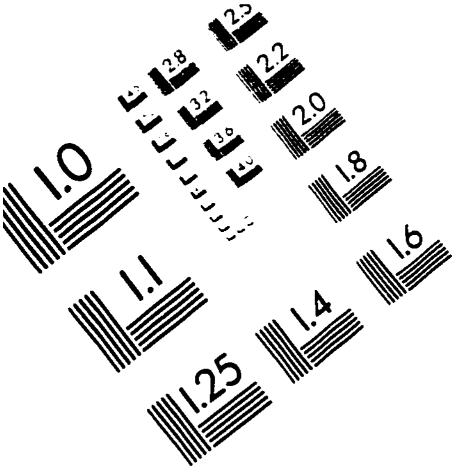
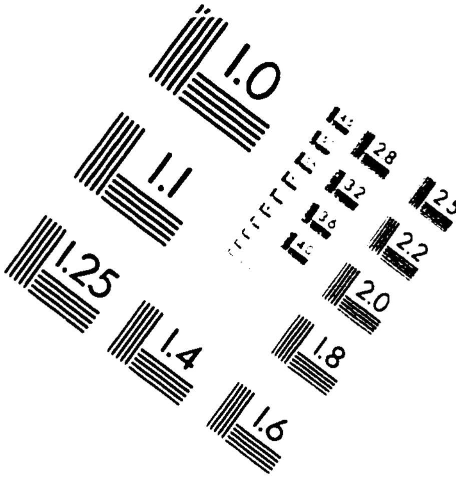
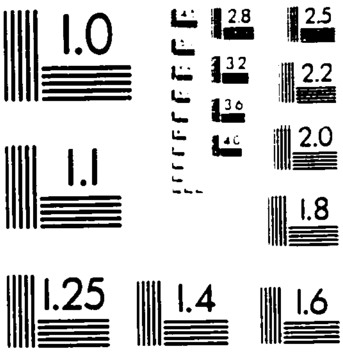

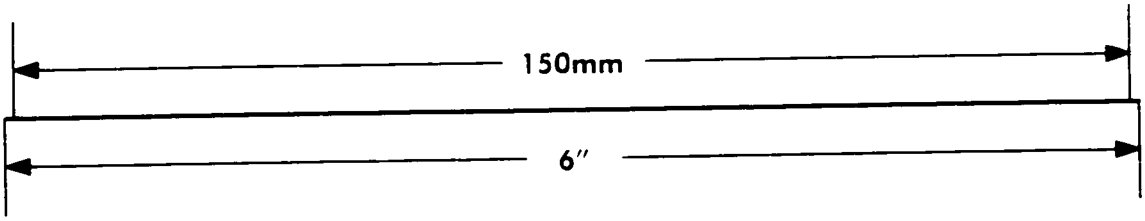

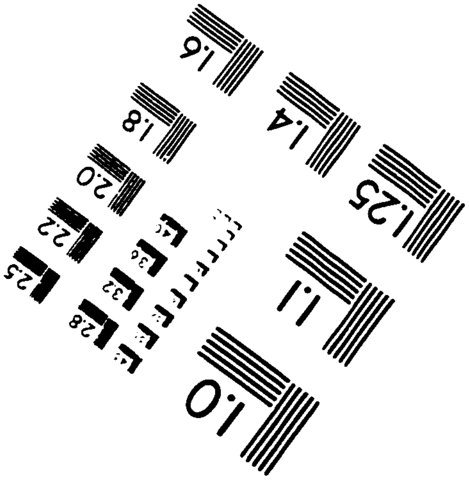
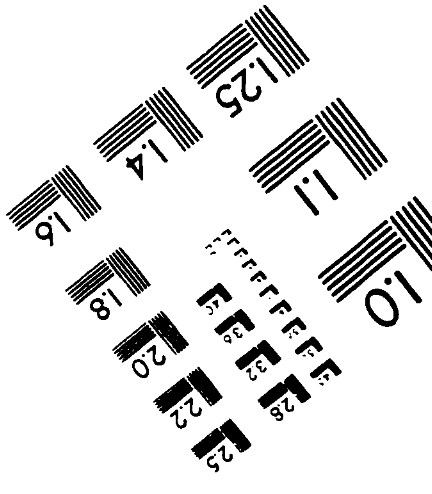

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