Journal of Equine Veterinary Science 34 (2014) 1181–1187

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Serum Lipid Modification Related to Exercise and Polyunsaturated Fatty Acid Supplementation in Jumpers and Thoroughbred Horses

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ARTICLE INFO

Article history:
Received 24 March 2014
Received in revised form 16 June 2014
Accepted 9 July 2014
Available online 16 July 2014

Keywords:
Lipid metabolism
Horse
Polyunsaturated fatty acids
Exercise

ABSTRACT

The importance of polyunsaturated fatty acids (PUFAs) within the different biological functions of animals has been widely recognized. In this study, exercise and PUFAs supplementation effects on serum triglycerides, total cholesterol, and nonesterified fatty acids (NEFAs) concentration were evaluated in athletic horses. Two sport horse types (10 Italian saddle jumpers and 10 Thoroughbreds) were equally divided into two groups. Jumpers and Thoroughbred experimental groups (A_J and A_T) received 4-week PUFAs supplementation and control groups (B_J and B_T) received no dietary supplement. Before starting the PUFAs supplementation (T0) and at the end of the experimental period (T4), horses were subjected to simulated events. From each subject, blood samples were collected every 7 days at rest, before and after the first test ($T0_R$ and $T0_{PE}$), and before and after the second test ($T4_R$ and $T4_{PE}$). Higher triglycerides and NEFA concentrations at $T0_{PE}$ and $T4_{PE}$ than $T0_R$ and $T4_R$ in both groups were found as a result of exercise ($P < .005$), but lower triglycerides and NEFA concentrations at $T4_{PE}$ in group A_J than group B_J ($P < .05$) and in group A_T ($P < .005$) than group B_T were found as a result of PUFAs supplementation. Effects of PUFAs supplementation was highlighted by the statistically significant lower triglycerides and NEFA concentrations found at $T4_{PE}$ than $T0_{PE}$ in groups A_J ($P < .05$) and A_T (triglycerides: $P < .05$; NEFAs: $P < .0001$).

1. Introduction

Lipids play a crucial role in mammals' metabolism. These compounds contribute to the maintenance of cellular integrity and to the transmission and transduction of cellular signals. Moreover, they are important energy substrates for metabolism in skeletal muscle providing essential metabolic intermediates [1], and they are involved in all kinds of exercise, both short and long lasting in equine species [2,3].

During exercise, lipid metabolism is subjected to several variations because of hormonal changes associated with physical exercise [4]. The primary regulators are the catecholamines (norepinephrine and epinephrine), which, in addition to their effects on the cardiovascular and respiratory systems, induce metabolic changes in muscle, liver, and adipose tissue allowing the mobilization and utilization of energy substrates. The catecholamines increase the availability of fuels for energy transduction. These hormones activate hormone-sensitive lipase in adipose tissue, which results in an increase of plasma-free fatty acid concentrations [1,4]. The lipid reserves should increase in muscle as well as lipoprotein lipase concentration that promotes the triglycerides degradation in muscular and adipose tissues [5].

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http://dx.doi.org/10.1016/j.jevs.2014.07.005

In horse nutritional research, the investigation of dietary polyunsaturated fatty acids (PUFAs) has grown in popularity on modifying inflammation, hematological and clotting parameters [6–8]. Several studies showed an effect of PUFAs supplementation on oxidative metabolism in athletic horse [1,9,10]. Omega-3 and omega-6 fatty acids are two of the most biologically significant PUFA classes. These essential fatty acids offer a variety of health-related benefits in a wide variety of physiological and pathologic conditions [6–11]. Moreover, there is some evidence that fat supplementation improve aerobic and anaerobic metabolism in horse by the increasing capacity of the uptake and the oxidation of fatty acids in muscle with consequent rise of muscle glycogen content and utilization rate during exercise [12]. Studies carried out on humans and horse showed that the omega-3 fatty acids supply energy without provoking exercise-induced dismetabolic myopathies, stabilize cellular membranes improving the permeability and the exchange of substrates involved in energetic metabolism [13,14], and increase the deformability of red blood cells improving the oxygen transport at the peripheral level [15]. Horses need to consume both omega-3 and omega-6 fatty acids in a proper ratio. The ratio of these compounds is closely dependent on diet. Horse's natural diets contain substantial amount of omega-3 fatty acids because of intake of pasture grass. However, omega-3 to omega-6 fatty acids ratio changes quickly when horses no longer have access to pasture and are fed with the traditional equine diet that minimizes the supply of omega-3 fatty acids and over supplies omega-6 fatty acids.

Although fatty acids are often added to the diets of exercising horses, because of its energy density, few studies have reported their effect on lipid metabolism in athletic horse [9,10]. Therefore, the aims of this study were to make a contribution to the knowledge of dynamic metabolic processes taken place during physical exercise in athletic horse and to evaluate the effects of PUFA-supplementation diet on serum triglycerides, total cholesterol, and nonesterified fatty acids (NEFAs) concentration in jumper and Thoroughbred horses under specific training programs.

2. Materials and Methods

2.1. Experimental Procedure

Twenty regularly trained horses, 10 crossbred Italian saddle (IS) jumpers (7–10 years old; eight geldings and two males; mean body weight, 500 ± 30 kg) and 10 Thoroughbreds (2–5 years old; four geldings and six males; mean body weight, 390 ± 15 kg), were enrolled in this study with the informed owner consent.

Before starting the study, horses were subjected to clinical examination and routine hematology and biochemistry at rest conditions, to insure of their healthy status. All animals were housed in individual boxes (3.50 × 3.50 m) under natural spring photoperiod (sunrise at 6 AM, sunset at 6 PM) and 18°C–21°C indoor temperature. The diet consisted of hay (first cut meadow hay, sun cured, and late cut; 8 ± 1 kg/d; 6.9% crude protein on average) and mixed cereals (oats and barley, 50% each; 3.5 ± 0.5 kg/d), three times a day (at 7 AM, 12 AM, and 6 PM). Cereal mixture composition (dry matter basis) was 13.0% crude protein, 20.7% crude fiber, and 3.4% other extracts; the estimated net energy content was 0.8 UFC (Unitè Fouragire Cheval). Water was available ad libitum. Both IS jumper and Thoroughbred horses were randomly divided into two equal groups of five subjects each. The experimental groups A_J (IS jumpers) and A_T (Thoroughbreds) received the PUFAs supplement (Table 1) Omega Horse (NBF Lanes, Milan, Italy), 70 mL/d (total n-3 PUFAs, 22.5 g; eicosapentaenoic acid, 11.5 g; docosahexaenoic acid, 7.7 g) for 30 days. The control groups B_J (IS jumpers) and B_T (Thoroughbreds)

Table 1
Polyunsaturated fatty acids supplement Omega Horse composition.

Active Principle	Content %
α-Myristic acid (C12:0)	7.0
Palmitic acid (C13:0)	18.0
Palmitoleic acid (C16:1)	9.5
Stearidonic acid (C18:0)	4.5
Oleic acid (C18:1)	19.0
Linoleic acid (C18:2)	1.5
α-Linolenic acid (C18:3)	0.5
Eicosapentaenoic acid (C20:5)	18.0
Docosapentaenoic acid (C22:5)	4.0
Docosahexaenoic acid (C22:6)	12.0
Other unsaturated fatty acids	5.8
Vitamin E	0.2
Butylhydroxytoluene	0.015

Table 2
Specific training program of jumper and Thoroughbred horses.

Thoroughbreds			
Days of wk	Gait	Duration (min)	Speed (m/min)
First and fourth day	Walk	10	100
	Trot	20	200
	Canter	6	350
	Walk	10	100
Second and fifth day	Walk	15	100
	Trot	20	200
	Gallop	3	800
	Walk	10	100
Third and sixth day	Walk	15	100
	Trot	8	300
	Walk	15	100
	Walk	15	100
Jumpers			
Days of wk	Gait	Duration (min)	Obstacle Height (cm)
First and third day	Walk	5	
	Trot	30	
	Gallop	20	
	Obstacle		90
	Walk	5	
Second, fourth, and sixth day	Walk	5	
	Trot	25	
	Gallop	25	
	Walk	5	
Fifth day	Walk	5	
	Trot	30	
	Gallop	20	
	Obstacle		1.20
	Walk	5	

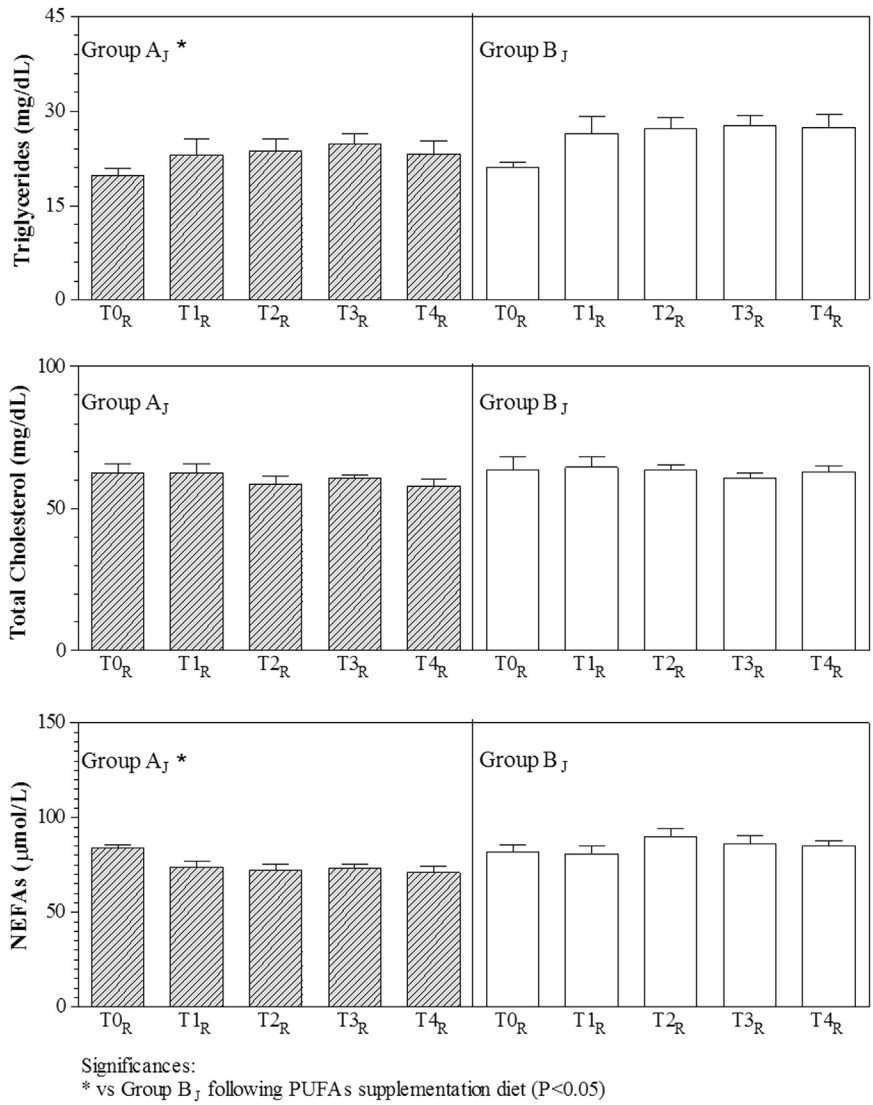

Fig. 1. Triglycerides, total cholesterol, and NEFA concentrations obtained at rest condition in experimental (A_J) and control (B_J) jumper horses. NEFAs, nonesterified fatty acids; PUFAs, polyunsaturated fatty acids.

received no dietary supplement during the experimental period. Before starting the dietary supplement administration (T0) and at the end of the experimental period (T4), horses were subjected to simulated events: IS jumpers performed a show jumping course (350 m length, 14 obstacles of 1.20 ± 0.10 m height); Thoroughbreds performed a 1700-m race (average speed, 800 m/min). During the experimental period (30 days), animals carried on with their specific training programs for jumpers and Thoroughbreds for 6 days a week with a day of rest (Table 2). From each subject, blood samples were collected by jugular puncture in nonheparinized tubes to test triglycerides, total cholesterol, and NEFAs. Blood collection was performed every 7 days at rest ($T0_R$-$T1_R$-$T2_R$-$T3_R$-$T4_R$), before and after the first test ($T0_R$ and $T0_{PE}$) (within 10 minutes from the end of exercise), and before and after the second test ($T4_R$ and $T4_{PE}$).

After standing at room temperature for 20 minutes, the Falcon tubes were centrifuged at 1,300*g* for 10 minutes and the obtained serum was stored at −25°C until analyzed.

Sera, not lipemic or hemolyzed, were analyzed with commercially available kits (triglycerides, glycerol phosphate oxidase and/or peroxidase; Biosystems, Reagents and Instruments, Barcelona, Spain; cholesterol, colorimetric enzymatic method cholesterol oxidase-peroxidase antiperoxidase; Giesse Diagnostics, Rome, Italy; NEFA determination in serum and plasma; Randox, Crumlin, UK) by means of an automated analyzer ultraviolet-visible spectrophotometer (model Slim SEAC, Florence, Italy).

All treatments, housing, and animal care reported previously were carried out in accordance with the standards recommended by the European Directive 2010/63/EU for animal experiments.

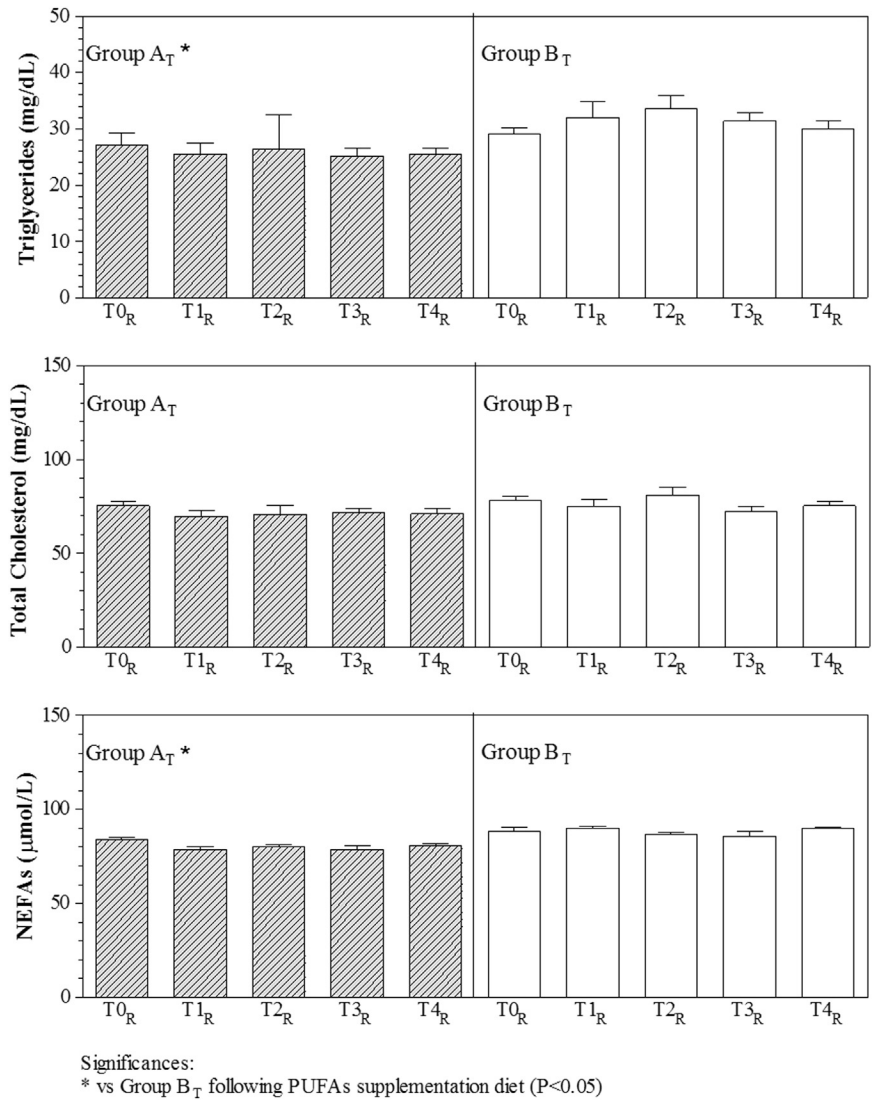

Fig. 2. Triglycerides, total cholesterol, and NEFA concentrations obtained at rest condition in experimental (A_T) and control (B_T) Thoroughbreds horses. NEFAs, nonesterified fatty acids; PUFAs, polyunsaturated fatty acids.

2.2. Statistical Analysis

Before statistical analysis, all data were tested for normality of distribution using the Kolmogorov–Smirnov test. $P < .05$ was considered statistically significant. All data were normally distributed, and statistical analysis was performed on mean values.

Two-way repeated measures analysis of variance (ANOVA) was applied to determine statistical significant effect of time and PUFAs supplementation on serum triglycerides, total cholesterol, and NEFA values obtained at rest ($T0_R$-$T1_R$-$T2_R$-$T3_R$-$T4_R$). The same statistical model analysis was applied on values of serum triglycerides, total cholesterol, and NEFAs obtained at $T0_R$, $T0_{PE}$, $T4_R$, and $T4_{PE}$ to evaluate statistical effect of exercise. Bonferroni multiple comparison test was applied for post hoc comparison. Paired *t* test was used to evaluate statistical significant effect of PUFAs supplementation on parameters obtained after exercise ($T0_{PE}$ vs. $T4_{PE}$). The data were analyzed using the STATISTICA 8 software (StatSoft, Inc).

3. Results

All the results were expressed as means ± standard deviation. The application of two-way ANOVA showed no statistical effect of time on triglycerides, total cholesterol, and NEFA values in experimental (A_J and A_T) and control (B_J and B_T) groups at rest condition ($P > .05$), whereas significant effect of PUFAs supplementation on serum triglycerides and NEFAs in groups A_J and A_T ($P < .05$) was found. In particular, significantly lower triglycerides and NEFAs concentration in groups A_J and A_T than B_J and B_T was found (Figs. 1 and 2).

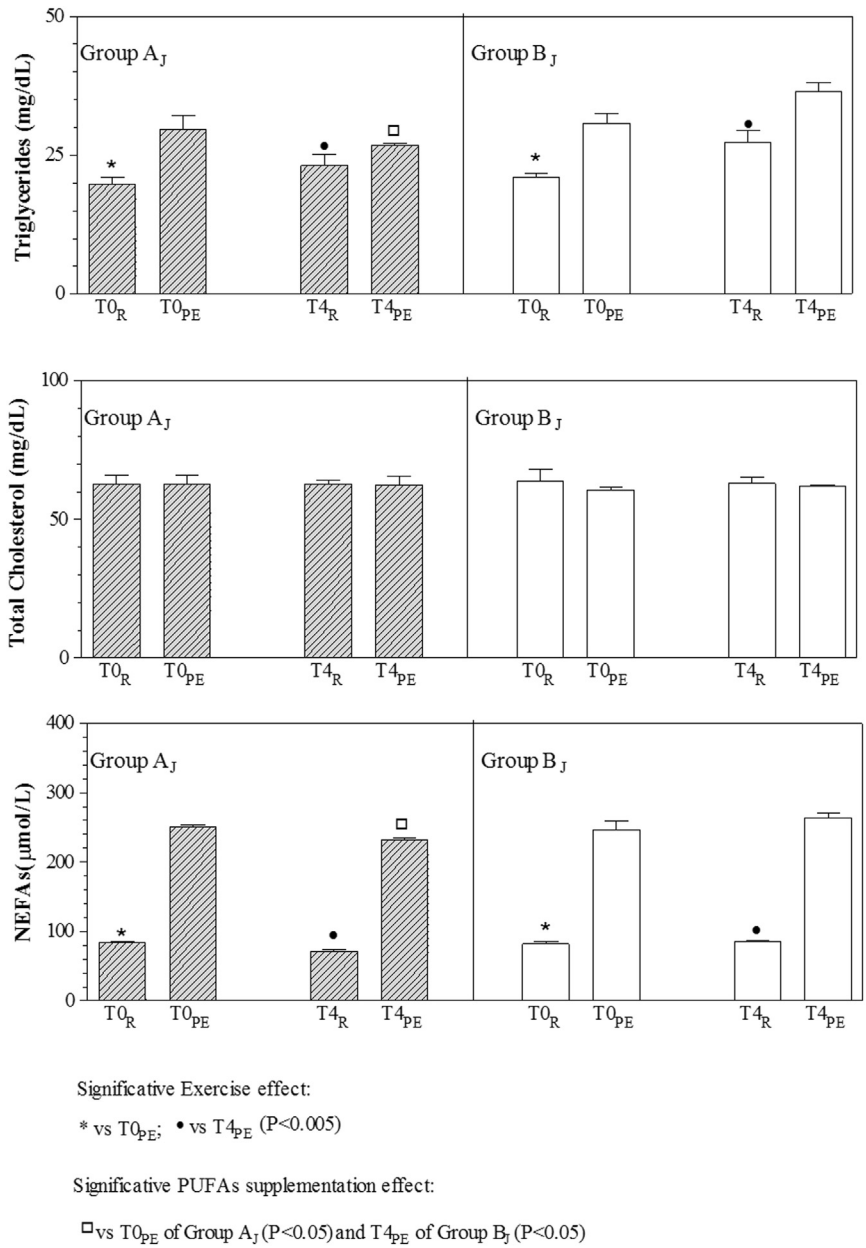

Fig. 3. Effect of exercise and PUFAs supplemented diet on triglycerides, total cholesterol, and NEFAs values obtained before ($T0_R$, $T4_R$) and after ($T0_{PE}$, $T4_{PE}$) the two simulated events in experimental (A_J) and control (B_J) jumper horses. NEFAs, nonesterified fatty acids; PUFAs, polyunsaturated fatty acids.

The same statistical model analysis showed a significant effect of exercise on serum triglycerides and NEFA values obtained at $T0_R$, $T0_{PE}$, $T4_R$, and $T4_{PE}$ in experimental (A_J and A_T) and control (B_J and B_T) groups. In particular, higher triglycerides and NEFA values at $T0_{PE}$ and $T4_{PE}$ than $T0_R$ and $T4_R$ ($P < .005$) were found both in jumper and Thoroughbred horses. Moreover, significant effect of PUFAs supplementation at $T4_{PE}$ in groups A_J ($P < .05$) and A_T ($P < .005$) than groups B_J and B_T was found (Figs. 3 and 4).

Paired *t* test showed statistical significant effect of PUFAs supplementation on triglycerides and NEFAs concentration obtained after exercise ($T0_{PE}$ vs. $T4_{PE}$) in groups A_J (triglycerides: $P < .05$; $t = 4.15$; NEFAs: $P < .05$; $t = 3.20$) and A_T (triglycerides: $P < .05$; $t = 2.80$; NEFAs: $P < .0001$; $t = 12.33$) (Figs. 3 and 4, respectively).

4. Discussion

Serum triglycerides and total cholesterol concentrations found in the present study were within normal physiological ranges in both jumper and Thoroughbred horses throughout experimental period [16]. Although little is known about NEFAs physiological ranges in horses, the

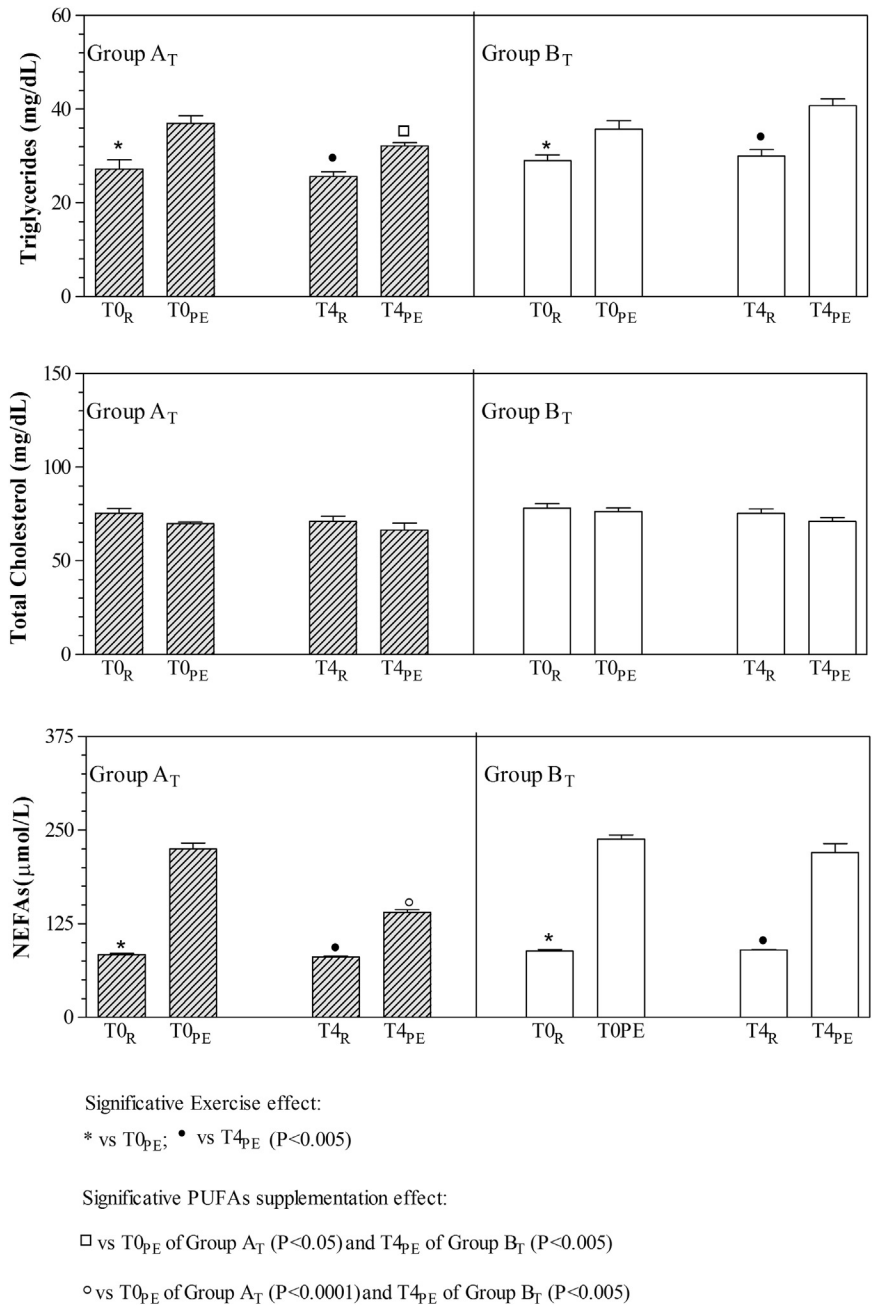

Fig. 4. Effect of exercise and PUFAs supplemented diet on triglycerides, total cholesterol, and NEFA values obtained before ($T0_R$, $T4_R$) and after ($T0_{PE}$, $T4_{PE}$) the two simulated events in experimental (A_T) and control (B_T) Thoroughbreds horses. NEFAs, nonesterified fatty acids; PUFAs, polyunsaturated fatty acids.

values obtained in the present study comply with findings of Piccione et al [14].

The analysis of our results showed a significant effect of exercise and PUFAs supplementation on triglycerides and NEFAs values both in jumper and Thoroughbred horses. In particular, significant higher triglycerides and NEFA concentrations at $T0_{PE}$ and $T4_{PE}$ than $T0_R$ and $T4_R$ in jumper and Thoroughbred horses were found. The increase of these parameters after the simulated events represents a metabolic response of horse to physical exercise and may be because of the known low ability of the jumper and Thoroughbred horses to use lipids as energy substrate. Therefore, the released lipids cannot be used in a proper way, as the ability to burn lipids by the muscle fibers is still low [17]. As a consequence, the levels of NEFAs increased and the surplus of unused NEFAs returned to the adipose tissue to be reesterified to triglycerides [5,18].

After simulated events, NEFAs returned to baseline levels throughout training period. These results suggest that daily training program induced a metabolic adaptation

to the stress induced by physical exercise [19]. At muscular level, training increases the energetic metabolism and the recovery ability improving the metabolization of NEFAs [14]. This metabolic adaptation seems to be increased by PUFA-supplementation diet. In fact, in our study, a significant decrease in triglycerides and NEFAs concentration was found in experimental groups compared with control groups during both training period and after the simulated events following omega-3 fatty acids supplementation.

Decreased serum triglycerides are the most consistently reported result of fish oil supplementation in humans [19], rats [20], and horse [1]. The exact mechanism responsible for this decrease has not yet been elucidated; however, many studies have shown that n-3 fatty acids downregulate enzymes associated with triglyceride synthesis [21]. Other researchers have reported that triglycerides decrease following fatty acid–supplementation diet could be attributed to increased lipoprotein lipase activity and a possible increase in fatty acid oxidation [22,23]. In horses, very low-density lipoprotein (VLDL) particles are the main transporters of triglycerides and contain 57% triglycerides and 15% phospholipids compared with low-density lipoprotein particles, which contain 5.5% triglycerides and 22% phospholipids [24]. Increasing the activity of lipoprotein lipase might increase the clearance of the triglyceride-rich VLDL particles from the bloodstream, resulting in decreased circulating triglycerides. Changes in the activity of these enzymes could suggest that horses subjected to a fat-supplementation diet have increased capacity for the uptake and oxidation of fatty acids in muscle. The activity of these enzymes was not measured in this study, so it is unknown as to whether triglycerides concentration was regulated by the activity of lipoprotein lipase or by decreased triglyceride synthesis. NEFAs values also showed a significant decrease in the experimental groups compared with control groups. Free fatty acids concentration represents the balance between fatty acid mobilization from adipose tissue and their use by muscle. Thus, the lower levels of serum NEFAs in experimental groups following PUFA-supplementation diet compared with control groups may be due either to decreased fatty acid mobilization or to an increased use of the free fatty acid for energy production [10].

5. Conclusions

The importance of PUFAs within the different biological functions of animals has been widely recognized. In particular, the study of metabolic response's change during exercise following PUFA-supplemented diet is a key point to better understand the relationship between omega-3 fatty acids and performance of the athletic horse. The results obtained in this study showed an effect of exercise and PUFA-supplemented diet on lipid profile in jumper and Thoroughbred horses. Further studies need to be carried out to better understand the effect of different workloads on lipid metabolism in different horse's breed and to clarify mechanism underlying the potentially protective effects of fatty acid–supplemented diet in athletic horse.

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