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Original Research

# Lipid and Lipoprotein Profiles Modification in Athletic Horses After Repeated Jumping Events

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ABSTRACT

The aim of this study was to evaluate the effect of physical exercise on serum nonesterified fatty acids (NEFAs), triglycerides, total cholesterol (total chol), high-density lipoproteins (HDLs), low-density lipoproteins (LDLs), and very low–density lipoprotein (VLDL) fraction levels in Jumper horses. Horses took part to a show jumping competition represented by repeated events. Blood sampling was performed before the first day of competition (T0), within 10 minutes from the end of each competition ($T1_{POST}$, $T2_{POST}$, $T3_{POST}$), and in the day after competition (R1). Statistical analysis showed a significant effect of exercise ($P < .001$) on all studied parameters. Nonesterified fatty acids showed higher values at $T1_{POST}$, $T2_{POST}$, and $T3_{POST}$ than T0. Higher serum triglycerides, VLDLs, and HDLs levels were found at $T1_{POST}$, $T2_{POST}$, $T3_{POST}$, and R1 than T0. Total cholesterol and LDLs values showed a significant decrease at $T1_{POST}$, $T2_{POST}$, $T3_{POST}$, and R1 than T0. The observed changes in lipid and lipoprotein parameters could represent the metabolic response to physical exercise during the 3 days of competition that led to an increase of NEFAs and triglycerides levels after the physical exercise which act as energy support, whereas during the recovery period, the energy production was assured by oxidation of serum triglycerides. The increase in serum HDLs, decrease in total chol, and LDLs point out the positive effects of physical exercise on the lipoproteins profile in trained horses suggesting the reverse cholesterol transport from peripheral tissues to the liver for new biochemical processes in trained Jumper horses.



## 1. Introduction

In horses, exercise and training are known to have considerable effects on animal metabolism [1]. Similarly to other stressors, it needs adequate responses to reestablish homeostatic equilibrium. The physiological processes induced by physical exercise result in changes of the levels of several blood variables including lipid parameters [2–6]. Lipids play a crucial role in mammals' metabolism; in fact, these biological molecules function as storage form of energy (triglycerides), vital component of cell membranes, and as precursor of all steroid hormones (cholesterol). Lipids are insoluble in plasma and are transported bound to carrier proteins called lipoproteins including high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low–density lipoprotein (VLDL). During exercise, lipid metabolism is subjected to several variations due to hormonal changes characterizing physical exercise. Particularly, the catecholamines lead to an increase of plasma-free fatty acids concentrations by the activation of the hormone-sensitive lipase in adipose tissue [7–9]. The lipid reserves should increase in muscle as well as lipoprotein lipase concentration that promotes the triglycerides

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degradation in muscular and adipose tissues [5]. The effect of occurrence, frequency, and intensity of physical activity on lipid profile and, particularly, on serum cholesterol and triglycerides concentration has been previously studied in equine species [10,11]. However, the response of the lipid profile to an exercise session or training program is different depending on the type of exercise undertaken, its intensity, frequency, and duration [12]. Within the Olympic equestrian disciplines, the effects of exercise on several blood parameters related to energy utilization are still not well established in show jumping. To improve the knowledge about the ability of Jumper horses to use lipids as energy source, the aim of the present study was to evaluate the serum lipids and lipoproteins values in well-trained Jumper horse during 3-day events.

## 2. Materials and Methods

### 2.1. Animals

The study was carried out on 14 well-trained Italian Saddle horses (seven geldings and seven females, 9–11 years old, mean body weight 500 ± 20 kg). The body weight of each horse was obtained by direct weighting by means of a weighting platform (PS3000HD Heavy Duty Floor Scale, Breckwell, UK). All horses were managed equally and housed in individual boxes under natural photoperiod (mean temperature 25°C ± 6°C, relative humidity 67% ± 3%). The horses were fed with standard rations constituted by hay (first cut meadow hay, sun cured, and late cut) and by a mixture of cereals (oats and barley, 50% each), according to INRA (Institut National de la Richerche Agronomique) specifications [13].

The percentage composition of the mixture was dry matter 87% and moisture 13%. The dry matter contained 9.11% digestible protein, 13.05% crude protein, 20.7% crude fiber, and 3.42% crude lipid, as well as 0.80 Forage Horse Units/kg. The ration was administered three times a day: 8:00 AM, 12:00 PM, and 5:00 PM. The feeding times were respected also during the competition days. Water was available ad libitum.

### 2.2. Show Jumping Course

Each horse enrolled in the present study was transported to the competition site the day before the first competition to avoid stress. All horses enrolled in the present study were trained in the same center and have the same level of training and the same experience for show Jumping competition. Horses took part to a show jumping competition organized in 3 days. Animals competed at 11.00 AM at each day of competition. Each race session was preceded by 20 minutes warm-up consisting in: walk, trot, and gallop with six jumps (height: from 100 to 140 cm). During the first day, horses competed with the following technical specifications: total length, 550 m; obstacles height, 140 cm; and total efforts, 13 (seven verticals, six oxer, and one triple combination). During the second day, horses competed with the following technical specifications: total length, 600 m; obstacles height, 145 cm; mixed competition including efforts, 15 (eight verticals, seven oxer, one double combination, and one triple combination). During the third day, horses competed with the following technical specifications: total length, 600 m; obstacles height, 145 cm; total efforts, 14 (eight verticals, six oxer, one double combination, and one triple combination). animals competed.

### 2.3. Blood Sampling and Analysis

The same operator performed blood sampling from each animal, by jugular venipuncture in vacutainer tubes containing cloth activator agent (Terumo Co, Tokyo, Japan) to perform serum analysis. Blood sampling was performed before the first day of competition (T0) to obtain baseline levels of each studied parameters, within 10 minutes from the end of each race ($T1_{POST}$, $T2_{POST}$, and $T3_{POST}$), and 24hours after T3 (R1).

Immediately after collection, blood samples were placed in refrigerated bags and transported to the laboratory for the analysis. The tubes were left to room temperature for 20 minutes, and then, they were centrifuged at 3,000 rpm for 10 minutes. The obtained sera were stored at −20°C until analysis. The samples were not hemolized, and they were analyzed by means of an automated analyzer UV Spectrophotometer (model Slim SEAC, Florence, Italy) using commercially available kits (Biosystem S.A., Bacelona, Spain), to assess the concentration of nonesterified fatty acids (NEFAs), triglycerides, total cholesterol (total chol), HDLs, and LDLs. Very low–density lipoprotein fraction was estimated as one fifth of the concentration of triglycerides [14].

The study was approved by the president of the Veterinary Commission for the competition and by the owners of all horses. All treatments, housing, and animal care reported below were carried out in accordance with the standards recommended by the EU Directive 2010/63/EU for animal experiments.

#### 2.3.1. Statistical Analysis

All data were tested for normality of distribution using the Shapiro–Wilks test. All data were normally distributed ($P > .05$), and the statistical analysis was performed. One-way repeated measures analysis of variance (ANOVA), followed by Bonferroni post hoc comparison test, was applied to determine statistical effect of exercise on serum NEFAs, triglycerides, total chol, HDLs, LDLs, and VLDLs values. $P$ values $< .05$ were considered statistically significant.

Data were analyzed using the software Statistica 7 (Statsoft Inc).

## 3. Results

All results are expressed as mean ± standard error of the mean. The trend and the related statistical significances found for the parameters studied in Jumper horses throughout the monitoring period are reported in Fig. 1. The application of one-way ANOVA revealed a statistically significant effect of exercise ($P < .001$) on serum NEFAs, triglycerides, VLDLs, total chol, HDLs, and LDLs values. In particular, higher values of serum NEFAs were found at $T1_{POST}$, $T2_{POST}$, and $T3_{POST}$ than T0. Significant higher serum