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## **Lipid Utilization Pathways Induced by Early Training in Standardbred Trotters and Thoroughbreds**

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### **Abstract**

Controversial results on lipid utilization as an energy source during training in horses are found in the literature. The objective of this study was to assess blood lipid profile during different training programs in horses. Seventeen Standardbred horses ( $400 \pm 50$  kg) and 17 Thoroughbred horses ( $380 \pm 15$  kg) followed different training programs. Blood lipid profile, including triglycerides (TGs), total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL), was analyzed, and very-low-density lipoprotein (VLDL) concentration was calculated. Data were analyzed using Student t test, and linear regressions were done. Cholesterol and LDL decreased during training programs in Standardbred trotters ( $P = .0001$  and  $P = .0053$ , respectively), and VLDL was found to be close to the significance level ( $P = .053$ ). Blood lipid profile, including TGs ( $P = .0014$ ), cholesterol ( $P = .0057$ ), HDL ( $P = .0128$ ), LDL ( $P = .0091$ ), and VLDL ( $P = .0014$ ), varied throughout the training program in Thoroughbred horses. Negative slope of blood lipids and positive slope of TG linear regression in Standardbred trotters were significant for all parameters ( $P \leq .05$ ), whereas cholesterol and LDL regression showed poor P and  $r^2$  values and HDL P value was slightly above the significance level ( $P = .069$ ) in Thoroughbred horses. TGs and VLDL showed a positive linear trend in Thoroughbred horses ( $P = .002$ ). Exercise and different training programs lead to significant variations of lipid profile and lipid utilization in horses. Lipid utilization as an energy source improved with training in Standardbred trotters, whereas this was not the case in Thoroughbred horses. Further studies on the effect of training programs using different conditions and horse breeds would be necessary to understand lipid utilization as an energy source in athletic horses.

### **1. Introduction**

Lipids are important energy substrates for metabolism in skeletal muscle. The contribution of oxidative metabolism in contracting muscular fibers depends on different factors such as exercise duration and intensity, as well as hormonal, dietary, nutritional, and training status [1]. Lipids are water-insoluble substances and are therefore bound to lipoproteins when transported into the blood or tissues. The effect of exercise training on blood lipids, triglycerides (TGs), free fatty acids, cholesterol, very-low-density lipoproteins (VLDLs), low-density lipoproteins (LDLs), and high-density lipoproteins (HDLs) is equivocal [2]. Several animal and human studies suggest that cholesterol and VLDL are not to be considered a major source of aerobic energy during exercise [1]. Therefore, one would not expect their plasma concentration to change with exercise. Nevertheless, changes do occur: neither dramatic nor unequivocal, but interesting changes can be observed. According to the literature, when isolating VLDL from total serum TG, some authors found a more significant muscle uptake of circulating VLDL during exercise in humans and rodents [3] and [4]. Moreover, it was shown that the turnover rate of VLDL is significantly higher during exercise than at rest [5]. In humans, several studies were carried out on exercise-induced decrease of TG because lipid metabolism is related to the occurrence of atherosclerotic plaques and cardiovascular diseases [6] and [7]. Genetic-based or acquired alterations of lipid and lipoprotein

metabolism, such as hyperlipemia syndrome, have been described in horses [8], but neither the occurrence of these pathologies nor the improvement in the knowledge on effort physiology was able to increase the interest on lipid profile in sport horses. Over the past decade, our group has been working on the trend of different blood parameters in athletic horses to understand the effect of exercise, mainly focusing on traditional training [9], [10], [11], [12], [13], [14], [15] and [16]. Understanding the factors that can improve the aerobic lipidic power in horses is of utmost importance for effective training programs in athletic horses. For this purpose, we performed a longitudinal research to assess the effect of training and of different workloads on lipid profile in Standardbred trotters and Thoroughbred horses during a 3-month preracing season training period.

## 2. Materials and Methods

### 2.1. Animals

Seventeen Standardbred horses (10 males and 7 females) and 17 Thoroughbred horses (10 males and 7 females) were enrolled in the present study. The Standardbred horses were aged 3-4 years (mean body weight:  $400 \pm 50$  kg) and Thoroughbred horses were aged 2 years (mean body weight:  $380 \pm 15$  kg).

### 2.2. Training Programs

Standardbred horses were in a standard training program on a 1,000-m track (La Favorita racetrack, Palermo, Sicily, Italy). Thoroughbred horses were in a standard training program on a 1,200-m track (Mediterraneo racetrack, Siracusa, Sicily, Italy). Eighty-day training programs (Table 1) involved training for 6 days a week and 1 day of rest. Training and general animal care were performed by professional staff who were not associated with the research team.

### 2.3. Diets

All horses were stabled in individual boxes at natural indoor temperature ( $18^{\circ}\text{C}$ - $20^{\circ}\text{C}$ ) and subjected to the same feeding schedule. The horses were fed standard rations, calculated to fulfil all the main nutritional requirements according to INRA (Institut National de la Recherche Agronomique, France) specifications [17]. The diet of Standardbred horses included hay (first-cut meadow hay, sun-cured hay, late-cut hay; 5 kg/horse  $\times$  day as fed), oats (approximately 3.5 kg/horse  $\times$  day as fed), barley (approximately 1 kg/horse  $\times$  day as fed), and a mixed-feed "oat balancer" (1 kg/horse  $\times$  day as fed). The ration was divided into two meals per day, one fed at 8 am and the other at 5 pm. The diet of Thoroughbred horses included hay (first-cut meadow hay, sun-cured hay, late-cut hay; 5 kg/horse  $\times$  day as fed), oats (4 kg/horse  $\times$  day as fed), and a mixed-feed "oat balancer" (1 kg/horse  $\times$  day as fed). The daily ration was divided into three meals per day, which were fed at 8 am, 12 am, and 5 pm. Diet composition is presented in Table 2. Water was available ad libitum.

### 2.4. Body Condition Score

Body condition score (BCS) was measured at the beginning (T0) and at the end (T80) of the experimental period using a 9-point scale proposed by Henneke et al. [18], in which 1 represents extremely emaciated, 5 represents ideal BCS, and 9 represents extremely fat.

### 2.5. Blood Sampling and Analysis

Blood samples were collected to evaluate TGs, total cholesterol, HDL, LDL, and VLDL concentrations. Blood samples were drawn at 6 am every 20 days, starting from T0. Blood was collected by jugular vein puncture and stored in Vacutainer tubes (BD, Franklin Lakes NJ, USA) with no additive. The serum concentrations of TGs, total cholesterol, HDL, and LDL were assessed using an ultraviolet spectrophotometer and then VLDL concentration was calculated. All procedures presented in this study complied with current regulations covering animal experimentation in Italy.

### 2.6. Statistical Analysis

Data were analyzed using SPSS software package (SPSS 17.0, Chicago, IL). Kolmogorov-Smirnov test was applied to determine the data distribution. Student t test for paired data was used to compare the values of all parameters at T0 and T80. Linear regression analysis was used to study

the trend of mean values measured at T0, T20, T40, T60, and T80, both for Standardbred and Thoroughbred horses. Results are presented as mean  $\pm$  standard deviation. Statistical differences were considered for P values  $<.05$ .

### 3. Results

The mean BCS was found to be  $5 \pm 1$  for Standardbred trotters and  $4 \pm 1$  for Thoroughbred horses, without variations throughout the experimental period. Lipid metabolism parameters are shown in Table 3 and Table 4. Linear regression equations are summarized in Fig. 1 and Fig. 2.

All lipid metabolism parameters presented a normal distribution. Decreased cholesterol (P = .0001) and LDL (P = .0053) concentrations were found during early training period in Standardbred trotters. Similarly, VLDL concentration was found to be close to the significance level (P = .0530). No significant differences were found for TG (P = .0829) and HDL (P = .298) in Standardbred horses. Lower cholesterol (P = .0057), HDL (P = .0128), and LDL (P = .0091) concentrations were observed at T80 than at T0 in Thoroughbred horses. Higher TG (P = .0014) and VLDL (P = .0014) concentrations were found at T80 than at T0 in Thoroughbred horses. Lipid profile linear regressions in Standardbred trotters were significant for all parameters ( Fig. 1), whereas cholesterol and LDL regressions showed poor P and r<sup>2</sup> values and HDL P value was slightly above the significance level (P = .069) in Thoroughbred horses ( Fig. 2). The slope was negative for all regressions, except TG, in Standardbred trotters, whereas TG and VLDL showed a positive trend in Thoroughbred horses.

### 4. Discussion

Based on our results, serum TGs and total cholesterol concentrations were within normal physiological ranges in both Standardbred and Thoroughbred horses [19]. The same is true for lipoprotein fractions HDL and LDL. Exhaustive monographs are not available for TGs and total cholesterol; instead, only reports of research performed on small horse groups are available, with results that are not always unequivocal. In fact, in a study conducted on Thoroughbreds and Shetland ponies [20], it was found that HDL is the main lipoprotein fraction present in blood serum, followed by LDL and then VLDL. In a recent article on Turkman horses [21], the ratios were different, with a higher concentration of LDL compared with HDL. A more comprehensive study on lipoprotein fractions, and in particular on the effect of training on these fractions, is lacking in horses. On the contrary, similar studies are available concerning human athletes [7], [22], [23], [24], [25], [26] and [27]. All studies conducted in humans agree on the beneficial effect of aerobic exercise, performed on a regular basis, on lipid profile, and on the positive impact of moderate regular physical activity on the prevention of cardiovascular diseases [23], [24], [25], [26] and [27]. It is important to point out that the lipid transport in horses is not the same as in humans, as a large fraction of lipids, and of cholesterol in particular, is taken up by HDLs [28]. However, it is well known that in horses too, lipids are a primary energy source and are involved in all kinds of exercise, both short and long lasting [29] and [30]. In our study, Standardbred trotters started the training season in a traditional way [31]. From this point of view, the working schedule is considered adequate for trotting horses, which are in fact middle-distance runners, capable of taking up energy from both aerobic and anaerobic pathways. For athletes competing in 1,600-m races, as in our case, the training season starts with aerobic strengthening sessions (4 d/wk long-lasting exercise), with a lactic acid metabolism solicitation interposed (2 d/wk so-called jog days and fast days) in a 4-2 scheme, as traditionally recommended [31]. Nevertheless, the specific power work, more recently recommended by French authors [32], is lacking; this step is in fact based on an individual functional evaluation that is, in our situation, still lacking in the trotting horse or that is performed for research purposes [33]. Standardbred trotters are then mainly submitted to aerobic training, as confirmed by the increase in the V<sub>2</sub> values, as measured for the same horses during the experimental period considered in the present study and published previously [10]. The same

training method is likely responsible for the blood serum decrease of catecholamine, glucocorticoids, and insulin concentrations, with downregulation of lipolysis, decreased mobilization of nonesterified fatty acids (NEFAs), and a better of energy from the same NEFAs by muscle fibers after training [29]. A lesser quantity of NEFA is then transferred to the liver, thus involving a lower VLDL [6] and LDL [34] synthesis. In fact, training, in absence of changes in body weight and body composition, reduces fasting plasma VLDL-TG concentration by suppressing hepatic VLDL-TG secretion rate in humans [6] and [35] and in others trained animals [36]. The same pathway could likely lead to a lower TG synthesis in horses and to a lowering of TGs and HDL levels in blood serum. Our results are in accordance with this hypothesis because there is a progressive lowering of the lipid metabolism parameters induced by the training phase, as was shown in humans [6]. We concluded that our results are evidence for a well-conducted aerobic training in adapted horses: all the trends shown by regressions are negative, and all the lipid metabolism concentrations are lower at the end compared with the beginning of the training period. The ability to use lipids as an energy source was increased by the adopted training schedule. Some differences were found in our study, concerning the effect of training in Thoroughbreds. Our group consisted of 2-year-old horses that were at their first training experience. For this group, again, the training schedule was traditional, but somehow inspired by the evidence that aerobic exercise is the basis for the training schedule of rookie gallopers [29]. Workloads performed at speeds lower than 600 m/min (trot or canter) on long distances are thus recommended for these horses in the first month of training [29]. Our horses followed a similar schedule, but since the beginning, they also underwent sessions on 2,000-m tracks at a speed of 550-600 m/min, which is considered as anaerobic work for experienced horses. Rookie gallopers are normally not individually tested for their working capacity at the beginning of the training period; therefore, this kind of exercise could be considered maximal or supramaximal. A similar effort in horses that were never trained before could induce a metabolic response with increased blood serum concentrations of catecholamine and glucocorticoids. The increase of some lipid metabolism parameters that was found in our study, and in particular TGs and VLDL, is likely dependent on this effect. In fact, the induced changes in Thoroughbred horses that were found in the present study can be interpreted as a stress response to the beginning of the training. The aerobic training, in fact, leads to a solicitation and to an activation of type I and IIA muscle fibers [30]. As a result, the ability to use lipids as energy substrate increases and, as a metabolic response, the lipolysis is enhanced. However, because of the “natural” lower ability of the Thoroughbred horses to use lipids as energy substrate, as well as the low percentage of type I and IIA fibers, the released lipids cannot be used in a proper way, as the ability to burn lipids by the muscle fibers is still low. This can explain the positive slopes of the linear regression equations of TGs and VLDL of Thoroughbred horses and the final higher concentration of these two parameters compared with initial values. If we consider the performance indicators observed in our previous study [10], Thoroughbred horses had lower V2 and V4 at the beginning of the season compared with Standardbred horses, and at the end of the considered period, the V4 was lower in Standardbred than in Thoroughbred horses. The effect of training was far more important on V4 in Thoroughbreds, confirming the ability of these horses to perform at higher speeds and their lower ability to increase their potential at low speeds when lipids are the main fuel source. In fact, the oxidative potential of the muscle in Thoroughbreds increases only after 9 months of aerobic training [29]. For this reason, the lipolytic stress response induces an increase in concentration of NEFAs, but the same NEFAs are not safely used. This occurs owing to an inadequate oxidative capacity and higher blood lactate concentrations, which are induced by the maximal exercise, that inhibit their utilization [29] and [30]. As a consequence of a lack of adequate oxidative capacity, we can state that one-third of the released NEFAs are oxidized and two-thirds are used for TG synthesis [37]. It is also likely that the VLDL synthesis can have, under these circumstances, a protective effect against the possible toxic effect of NEFAs on tissue [6]: they are promptly re-esterified to TGs. The trend is not clear for the other studied parameters, but the final concentrations that were lower at the end of the training period for the other lipoproteins and for

total cholesterol, together with the previously discussed results, can be seen as the effect of the anaerobic training in horses more adapted to supramaximal exercise. Some articles report that most of the hypotriglyceridemic effect associated with training should be attributed to the last bout of exercise rather than considering it to be the result of metabolic adaptations to exercise training [38]. The different response that we found in Thoroughbreds compared with Standardbred trotters stands for the hypothesis of an important effect of training schedule on lipid profile. This is particularly true for rookie horses: in this horse category, training plans must be well established and adapted to the different individuals. These metabolic differences between Thoroughbred and Standardbred horses reflect the known breed variation in muscle fiber types, as shown in other breeds [39]. If the evaluation of workload is not adapted to the working capacity—determined by effort tests and by the calculation of important parameters such as V<sub>2</sub>, V<sub>4</sub>, and V<sub>200</sub>—errors can occur, and the plan can lead to a shortening of the horse's racing career or poor performances.

## 5. Conclusion

The results of this study show that lipid profile and lipid utilization are influenced by exercise in athletic horses. Further studies are needed to understand the effect of different workloads on horse lipid metabolism, in different breeds and categories, and to understand in a deeper way, the peculiarities of the athletic horse and its ability to use lipids as an energy source.

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Table 1.

Weekly training program protocol for all horses

<b>Gait</b>	<b>Duration (Minutes)</b>	<b>Speed (m/Minutes)</b>
Standardbreds		
First and fourth day		
Walk	10	100
Slow trot	25	350
Walk	10	100
Second and fifth day		
Walk	15	100
Slow trot	25	350
Walk	10	100
Third day		
Walk	15	100
Trot	6	670
Walk	15	100
Sixth day		
Walk	15	100
Trot	Simulation race 1,600 m	
Walk	15	100
Thoroughbreds		
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

Table 2.  
Chemical composition of diet

Content	Thoroughbreds Standardbreds	
	%	
Dry matter (DM)	86	87
Moisture (%)	14	13
	% of DM	
Horse digestible protein (MADC) <sup>†</sup>	7.4	9.1
Crude protein	11.4	12.1
Crude fiber	23.5	20.7
NDF <sup>b</sup>	33.8	32.5
ADF <sup>c</sup>	28.5	26.2
ADL <sup>d</sup>	8.1	7.8
Ether extract	2.5	3.4
	UFC <sup>e</sup> /kg DM	
Net energy	0.77	0.8

<sup>a</sup>Matières azotées digestibles chez le cheval. <sup>b</sup>Neutral-detergent fiber. <sup>c</sup>Acid-detergent fiber. <sup>d</sup>Acid-detergent lignin. <sup>e</sup>Unité fourragère cheval (horse feed unit).

Table 3.  
Blood lipid profile (mean ± SD) in Standardbred trotters during early training (days 0-80 from beginning of training; n = 17)

Parameters	Days From Beginning of Training Period				
	0	20	40	60	80
Triglycerides (mg/dL)	49.21 ± 15.81	42.95 ± 14.41	43.11 ± 10.54	42.10 ± 13.95	40.30 ± 12.90
Total cholesterol (mg/dL)	130.90 ± 16.09	127.60 ± 18.71	122.10 ± 16.78	119.90 ± 16.88	113.40 ± 10.98
HDL (mg/dL)	48.89 ± 13.24	49.35 ± 7.52	47.83 ± 5.84	47.35 ± 7.25	45.75 ± 5.56
LDL (mg/dL)	72.15 ± 14.68	69.52 ± 14.04	65.68 ± 13.36	64.16 ± 14.18	59.56 ± 7.99
VLDL (mg/dL)	9.68 ± 3.28	8.58 ± 2.88	8.46 ± 2.19	8.25 ± 2.87	8.07 ± 2.68

HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoproteins.

Table 4.  
Blood lipid profile (mean ± SD) in Thoroughbred horses during early training (days 0-80 from beginning of training; n = 17)

Parameters	Days From Beginning of Training Period				
	0	20	40	60	80
Triglycerides (mg/dL)	41.36 ± 14.13	43.77 ± 13.58	50.46 ± 10.04	54.36 ± 10.04	56.97 ± 11.20
Total cholesterol (mg/dL)	103.40 ± 25.09	126.50 ± 39.34	97.61 ± 17.50	111.10 ± 33.96	85.05 ± 7.07
HDL (mg/dL)	49.88 ± 6.05	47.14 ± 6.04	48.10 ± 5.88	47.45 ± 4.93	44.13 ± 6.64
LDL (mg/dL)	45.26 ± 21.13	70.63 ± 35.42	39.43 ± 15.17	52.84 ± 30.36	30.71 ± 8.45
VLDL (mg/dL)	8.27 ± 2.82	8.75 ± 2.71	10.09 ± 2.86	10.87 ± 2.00	11.39 ± 2.24

Fig. 1.

Linear regressions of different lipid profile parameters in Standardbred trotters during early training.

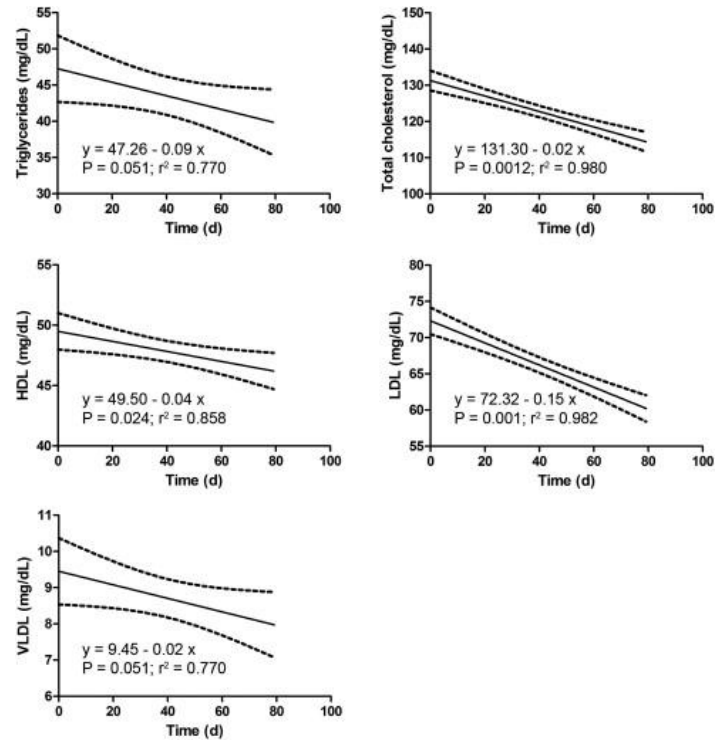


Fig. 2.

Linear regressions of different lipid profile parameters in Thoroughbreds during early training.

