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Evaluation basal lipid metabolism components and adiposity in trained Arabian horses for Endurance and Racing

Renata Farinelli de Siqueira[1*] Bianca Ozi Silva[2]
Mônica Lente Fernandes[2] Wilson Roberto Fernandes[2]

[1]Departamento de Clínica de Grandes Animais, Centro de Ciências Rurais,Universidade Federal de Santa Maria (UFSM), Av. Roraima 1000, 97105-900, Santa Maria, RS, Brasil. *Corresponding author. E-mail: refarinelli@gmail.com.
[2]Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo (FMVZ-USP), São Paulo, SP, Brasil.

ABSTRACT: Endurance and flat race are entirely different efforts and thus require completely different organism adaptations to achieve the performance. This study aimed to examine the difference in basal lipid metabolism in Arabian horses undergoing different training regimens for endurance and race and possible gender differences. The study enrolled sixty male and female' Arabian horses; thirty were trained for race, and thirty were trained for 80 km endurance races. The analyses included body condition score, body weight, body mass index, % body fat, and blood collected to determine triglycerides, total cholesterol, low-density proteins, high-density proteins, and non-esterified fatty acids. Ultrasonography was used to measure the thickness of the subcutaneous fat layer in the longissimus dorsi muscle, as well as the thickness of the rump fat and the cross-section of the gluteus medius muscle. This study showed that lipid profile is more influenced by type of exercise than by gender, being more efficient on endurance horses.
Key words: energetic metabolism, fatty acid oxidation, high-intensity exercise, horses.

Avaliação de componentes do metabolismo lipídico basal e adiposidade de cavalos Árabes treinados para corrida e para Enduro

RESUMO: Enduro e corrida são esportes completamente diferentes e, portanto, requerem adaptações diferentes do organismo para alcançar o desempenho físico. Este estudo teve como objetivo examinar a diferença no metabolismo lipídico basal em cavalos Árabes submetidos a diferentes regimes de treinamento, para resistência e para corrida e possível diferença de gênero. Sessenta cavalos árabes machos e fêmeas foram estudados, trinta treinados para corrida e trinta treinados para corridas de enduro de 80 km. As análises realizadas foram escore de condição corporal, peso corporal, índice de massa corporal, porcentagem de gordura corporal e sangue coletado para determinação de triglicerídeos, colesterol total, proteínas de baixa densidade, proteínas de alta densidade e ácidos graxos não esterificados. A ultrassonografia da espessura da camada de gordura subcutânea foi realizada sobre o músculo *longissimus dorsi*, espessura da gordura da garupa e seção transversal do músculo glúteo médio. Árabes de corrida apresentaram maiores valores de todos os parâmetros lipídicos estudados e área de secção transversa do músculo glúteo médio. Não houve diferença quanto ao gênero quando estudamos machos e fêmeas praticantes do mesmo esporte, com exceção da região dos músculos glúteos. Os resultados deste estudo mostram que o perfil lipídico é mais influenciado pelo tipo de exercício do que pelo gênero, sendo mais eficiente nos cavalos de enduro.
Palavras-chave: metabolismo energético, oxidação de ácidos graxos, exercício intenso, cavalos.

INTRODUCTION

Arabian horses are commonly believed to be one of the oldest and most influential horse breeds in the world. They possess stamina and many structural and functional adaptations for athletic performance.

Breeding evaluations are typically based on type and proper conformation, and in some countries (i.e., Polish, Middle Eastern countries, France), the selection is also made on racetracks (ROPKA-MOLIK et al., 2019) where young horses are introduced to flat race training. Once the flat racing career is completed, most horses are used for breeding or endurance competitions. Selected horses, typically 5 to 7 years of age, are prepared for moderate distances of up to 80 kilometers (NAGY et al., 2014). These two types of effort are completely different physiologically and thus require entirely different organism adaptations to achieve the set task (NAGY et al., 2014).

Although Brazil is among the greatest breeders of Arabian horses worldwide, there is no Arabian horse flat racing tradition. Only a few years ago, some stud farms sent some horses to the hippodrome in the city of São Paulo, where they were submitted to the same training model used for Thoroughbred horses and raced the exact distances matches. Understanding how exercise affects lipid metabolism can be useful in tailoring a specific training program for this type of horse.

Arabian horses are renowned for their capacity to maintain high-speed efforts over extended distances. Compared to other racehorse breeds, the muscle tissue of Arabian horses exhibits significant structural differences, primarily characterized by a

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prevalence of oxidative fiber type I. Its low glycogen content and high triglyceride storage capability distinguish this fiber type. In contrast, breeds such as Thoroughbred horses show a higher proportion of fiber type II A, as observed in studies by LÓPEZ-RIVERO et al. (1992 and 2014). The distribution of type 1 fibers in horses varies across muscle groups, with an average proportion of 44.9%. Notably, Arabians do not exhibit a predominant presence of type I fibers, especially in the longissimus dorsi muscle, where the proportions are 16.9% type I, 29.8% type 2A, and 53.2% type 2X (HYYTIÄINEN, 2014). This distribution indicates a higher proportion of type I fibers in Arabians compared to Quarter Horses or Thoroughbreds.

Fat metabolism plays a vital role in providing energy during prolonged exercise, as using fat stores can delay the depletion of glycogen reserves. The variation in fiber type distribution results in different performance traits; however, the specific mechanisms of fat metabolism in Arabian horses during race training remain poorly understood. Understanding the factors that can improve aerobic lipidic power in horses is vital for effective training programs in athletic horses.

This study examines the difference in basal lipid metabolism in Arabian horses undergoing different training regimens for endurance and flat races.

MATERIALS AND METHODS

Animals

Sixty Arabian horses were enrolled in this project; thirty trained for flat races (aged 6 ± 1.6 years old), and thirty trained for 80 km endurance races (aged 6 ± 2.1years old).

The racing group consisted of 15 mares, 10 stallions, and 5 geldings, and the horses were housed in São Paulo Jockey Club (23°32′56″ S, 46°38′20″ W). They were fed with coast cross hay [*Cynodondactylon* (L.) Pers.], alfalfa hay (*Medicago sativa*) and commercial concentrate (Royal Horse) containing guaranteed levels of the following nutrients: crude protein 140 g/kg (14%) (min); ethereal extract 70 g/kg (7%) (min); fibrous matter 100 g/kg (10%) (max). An equivalent of 1,0% body weight was provided three times a day.

The endurance group comprised 15 mares, 2 stallions, and 13 geldings and were housed in two training centers in Bragança Paulista (22°57′8″ S, 46°32′33″ W). The horses were fed with coast cross hay [*Cynodondactylon* (L.) Pers.], and commercial concentrate (Equitage, Guabi) containing guaranteed levels of the following nutrients: crude protein 120 g/kg (12%) (min); ethereal extract 90 g/kg (9%) (min); fibrous matter 100 g/kg (10%) (max). An amount equivalent of 1,0% body weight was provided twice a day.

All horses had been exercising regularly and training for specific practice for at least 1 year before enrolling. According to the Fédération Equestre Internationale(FEI), endurance events require a graduation that varies with age and the number of competitions in each category. A horse graded for an 80 km race can be considered an athlete with average experience. In turf, however, there is no compulsory graduation, but 1 year of regular training and competitions also gives the athlete average experience, as it is a high-intensity and short-lived sport.

Each racehorse usually exercised for 6 out of every 7 days, with a workout regimen consisting of 1 day of fast galloping (13–18 ms−1 for 1000–1200 m) and 5 days of slow work (6 to 8 ms−1 for 1500–2000 m), including warm up and cool down for 2 h from 5:00 to 7:00 am. Endurance training usually begins at 7:30 – 8:00 a.m. It consists of 3 alternating days of 3 hours (on average) of track riding (warm-up, trot, canter, and cool down), and every 15 days, a gallop trained in submaximal heart rate was performed on a sand track.

Blood analysis

Blood samples of all horses were taken in the morning after the horse had been trained(after 24 hours), from the jugular vein (at rest and before receiving the first meal) through the vacuum system and tube without anticoagulant for the analysis of triglycerides, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and non-esterified fatty acids (NEFA) performed on the Randox Rx Daytona automatic biochemical analyzer (Randox®, UK) using kits TR 2823, CH 3810, CH 2656, CH2666 and FA155, respectively.

Body measurements

All horses were weighed on digital scales and had their body condition score (BCS) evaluated in a triple-blind fashion according to the areas suggested by HENNEKE et al. (1983): neck, withers, shoulders, side, loin, and tail, ranging from 1 to 9.

The height of the withers was measured with the horse in pedestation, and each animal with the fore and hind limbs perpendicular to a flat floor was located. The BMI (body mass index) was calculated according to CARTER et al. (2009) [weight (kg)/height at the withers (m^2)]. The % body fat was calculated according to the formula established by KANE et al. (1987) [2.47 + (5.47*rump fat)]. Fat mass was determined by multiplying % body fat and total body mass.

Statistical analysis

Data were analyzed using a computerized statistical program (Minitab® 19 Statistical Software, Lean Six Sigma, Philadelphia, PA, USA) and were presented as mean ± SD. The distributions of the evaluated traits were used to assess according to the Kolmogorov–Smirnov test. The influence of exercise and gender was evaluated through analysis of two-way ANOVA (type of exercise and sex as independent variables), followed by a Tukey test. Significance was set at $P < 0.05$ in all cases.

RESULTS AND DISCUSSION

The results revealed significant differences between groups in body composition and fat metabolism components. Arabian racehorses showed higher body weight, BCS, fat layer LD, RFT, %body fat, and FFM, and higher values of lipid metabolism components (TG, T-Chol, LDL, HDL, and NEFA) (Table 1). These results agreed with PICCIONE et al. (2014), who studied lipid metabolism in Thoroughbreds.

The cross-section (depth) of the *gluteus medius* muscle also showed a difference, being higher in racehorses. This result can be explained by the propulsive muscles, like the gluteus medius, responsible for the power required in high-performance exercises (KLEIN et al., 2020), such as turf.

Lipids are essential substrates for skeletal muscle metabolism, and the type of exercise and intensity influence their use in horse athletes. Generally, exercise increases the rate of lipolysis and release of adipose tissue from free fatty acids and glycerol into the blood. The free fatty acids are used by the muscles recruited at work. In conditioned horses, the rate of lipolysis may exceed the use of fatty acids, which, in this case, are re-esterified by the liver (BERGERO et al., 2005; ASSENZA et al., 2012; LE MOYEC et al., 2019). Initiating lipid oxidation might require greater adaptation, such as that obtained through training. This may explain why horses adapted to endurance training (in lower intensity) have lower lipid component values and less fat layer deposition in the subcutaneous tissues than racehorses.

In a previous study (SIQUEIRA et al., 2019), we compared Thoroughbred racehorses' body composition and fat layer deposition with Arabian racehorses. There was no difference among BCS, triglycerides, cholesterol, and NEFA. But Arabians showed a greater fat layer in longissimus dorsi muscle and RFT, evidencing a difference in metabolism between the two breeds.

Overall, a study that observed exercise-induced transient hyperlipidemia supports our plasma lipid metabolite findings in racehorses (PÖSÖ et al., 1989; LI et al., 2012). The authors determined that plasma triglyceride concentration significantly increased during exercise, and the increase correlated with the intensity of the training as well as the activity of lipolysis. In addition, a significant rise in NEFA was noted; one-third of the NEFA released in lipolysis during the high-intensity exercise was calculated to be oxidized. At the same time, the remainder was used for hepatic re-synthesis of triglycerides.

Table 1 - Media ± SD of body composition and lipid components in all horses (males and females) submitted to different training regimens, flat races, and endurance.

	Arabian RaceHorses	Arabian EnduranceHorses	------------------P-----------------
Body weight (kg)	420 ± 1.70^{A}	398 ± 1.91^{B}	< .001
BCS (0-9)	4.67 ± 0.39^{A}	4.12 ± 0.04^{B}	0.03
Fat layer LD (cm)	0.21 ± 0.01^{A}	0.14 ± 0.01^{B}	< .001
RFT (cm)	0.32 ± 0.03^{A}	0.17 ± 0.01^{B}	< .001
% Body Fat	10.00 ± 0.16^{A}	8.43 ± 0.04^{B}	0.002
FM (kg)	40.1 ± 1.64	10.1 ± 1.22	< .001
BMI (kg/m^2)	162 ± 3.76	164 ± 3.87	0.666
TG (mg/dL)	40.5 ± 1.48^{A}	36.3 ± 1.67^{B}	0.001
T-Chol (mg/dL)	98.2 ± 1.66^{A}	94.1 ± 0.81^{B}	0.034
LDL (mg/dL)	47.0 ± 1.22^{A}	30.6 ± 1.42^{B}	0.002
HDL (mg/dL	68.1 ± 1.77^{A}	41.9 ± 2.02^{B}	< .001
NEFA (mmol/L)	0.89 ± 0.02^{A}	0.37 ± 0.05^{B}	< .001
Depht GM (cm)	9.19 ± 0.32^{A}	6.52 ± 0.65^{B}	< .001

Different letters between columns indicate statistical difference ($P < 0.05$).BW = body weight; BCS = body condition score; BMI = body mass index; LD = *Longissimus dorsi* muscle; RFT = rump fat thickness; GM = *Gluteus medius* muscle; TG = triglycerides; T-Chol = total cholesterol; LDL = low-density protein; HDL = high-density protein; NEFA = non-esterified free fat acids.

Evidence suggests that the skeletal muscle phenotype in highly endurance-trained humans also closely mirrors a muscle phenotype seen in and is associated with obesity and insulin resistance (GOODPASTER et al., 2001). Insulin-resistant skeletal muscle is characterized by lower oxidative capacity and lower postabsorptive rates of fatty acid oxidation. Elevated concentrations of lipids within skeletal muscle have also been linked to impaired muscle oxidative capacity and lower rates of fatty acid oxidation by muscle. This raises the possibility that the association between lipid accumulation within muscle and insulin resistance is influenced by a lower lipid oxidation capacity as an energy substrate (GOODPASTER et al., 2001; KLEIN et al., 2020). This apparent inconsistency is aptly termed “The Athlete’s Paradox” (GOODPASTER et al., 2001). Further studies should be performed to evaluate the lipid metabolism within the equine muscles and to elicit the effects of different trainings in the musculature.

As all horses had blood collected 24 hours post-exercise, a dietary factor may have influenced the levels of triglycerides and free fatty acids in the present study. The concentration of Ether Extract in the diet of Racing Arabians is lower than in the diet of Endurance Arabians. Increased LPL activity induced by this higher fat concentration in the diet may cause lower serum TG values during rest (GEELEN, 1999). OLIVEIRA (2016) demonstrated that supplementation with a higher fat content increased FFA levels and decreased pre-, during, and post-exercise TG levels. The same study indicated a decrease in TG levels after 10 minutes of exercise and an increase in FFA even in the post-exercise period.

Gender differences in sports horses receive little attention due to similar athletic capacity and basic hematological parameters in stallions and mares. Mixed populations are analyzed frequently. Although there is dimorphism regarding numerous parameters, in the present study, the only differences found were that the females’ racehorses showed higher values of T-Chol, and males had greater gluteus medius muscle. The same did not occur with Endurance horses; the only parameter that changed between the sexes was the greater RFT in females (Table 2).

Table 2 - Media ± SD of body composition and lipid components in the same sport but analyzed by gender.

---Arabian Race Horses---			
	Males	Females	P
BW (kg)	423 ± 1.99	414 ± 1.35	0.104
BCS (0-9)	4.56 ± 0.24	4.67 ± 0.22	0.833
Fat layer LD (cm)	0.20 ± 0.01	0.20 ± 0.02	0.971
RFT (cm)	0.30 ± 0.18	0.33 ± 0.06	0.615
% Body Fat	10.05 ± 0.24	10.12 ± 0.18	0.824
FM (kg)	40.2 ± 1.76	39.5 ± 1.47	0.788
BMI (kg/m^2)	166 ± 2.86	164 ± 2.64	0.619
TG (mg/Dl)	40.5 ± 1.32	42.4 ± 1.63	0.489
T-Chol (mg/Dl)	92.1 ± 0.85^B	107.3 ± 1.36^A	< .001
LDL (mg/Dl)	46.4 ± 0.88	48.5 ± 0.92	0.664
HDL (mg/Dl	67.4 ± 1.86	68.8 ± 1.22	0.881
NEFA (mmol/L)	0.88 ± 1.01	0.90 ± 1.01	0.234
Depht GM (cm)	10.01 ± 1.12^A	8.09 ± 0.77^B	0.005
---Arabian Endurance Horses---			
	Males	Females	P
BW (kg)	408 ± 2.81	380 ± 1.88	0.359
BCS (0-9)	4.06 ± 0.22	4.17 ± 0.16	0.743
Fat layer LD (cm)	0.14 ± 0.01	0.15 ± 0.01	0.210
RFT (cm)	0.17 ± 0.02^B	0.31 ± 0.01^A	0.013
% Body Fat	9.44 ± 0.06	9.43 ± 0.03	0.912
FM (kg)	10.1 ± 0.61	10.0 ± 0.28	0.916
BMI (kg/m^2)	165 ± 3.64	164 ± 2.86	0.546
TG (mg/Dl)	34.9 ± 1.17	35.5 ± 1.55	0.684
T-Chol (mg/Dl)	92.1 ± 1.64	94.9 ± 1.42	0.123
LDL (mg/Dl)	30.2 ± 1.20	31.2 ± 1.32	0.817
HDL (mg/Dl	40.9 ± 1.44	42.1 ± 1.80	0.788
NEFA (mmol/L)	0.36 ± 0.04	0.38 ± 0.02	0.838
Depht GM (cm)	6.93 ± 1.02	6.16 ± 0.70	0.598

Different letters between columns indicate statistical difference (P < 0.05).BW = body weight; BCS = body condition score; BMI = body mass index; LD = *Longissimus dorsi* muscle; RFT = rump fat thickness; GM = *Gluteus medius* muscle; TG = triglycerides; T-Chol = total cholesterol; LDL = low-density protein; HDL = high-density protein; NEFA = non-esterified free fat acids.

Human endurance athletes reported during exercise that utilization of plasma-derived fatty acids is more significant in trained men than in trained women, and although women have less sensitivity to catecholamines, they have greater sensitivity and concentration of hormone-sensitive lipase in relation to epinephrine (PARAVIDINO et al., 2007). CYWINSKA et al. (2011) found differences in catecholamine and cortisol levels in males and females of Thoroughbred horses. During exercise, catecholamines promote the release of NEFA into plasma, mainly from the hydrolysis of triacylglycerols in adipose tissue. Plasma concentration of NEFA reflects the balance between fatty acid mobilization and muscle use as energy fuel. A limitation of our study is that we did not evaluate these hormones.

To show the effect of exercise and the lack of effect of gender on the lipid parameters, table 3 shows the differences in body composition and the components of lipid metabolism in males and females trained for different sports separately. Racehorses presented greater body composition (except for body weight) and higher values of lipid metabolism components than Endurance horses.

Exercise and training programs significantly vary horses' lipid profiles and lipid utilization. Racehorses perform at higher speeds and are less able to use lipids as their primary fuel source.

Genetics significantly influences the lipid composition of Arabian horses, playing a pivotal role in their capacity to utilize lipids as an energy source during exercise. Recent studies underscore genetics'

Table 3 – Media ± SD of body composition and lipid components of the same gender but submitted to different training regimens, flat race, and endurance.

Males			
	Arabian Race Horses	Arabian Endurance Horses	P
BW (kg)	423 ± 1.99	408 ± 2.81	0.246
BCS (0-9)	4.67 ± 0.18^{A}	4.17 ± 0.15^{B}	0.017
Fat layer LD (cm)	0.20 ± 0.01^{A}	0.14 ± 0.01^{B}	0.008
RFT (cm)	0.30 ± 0.18^{A}	0.17 ± 0.02^{B}	0.013
% Body Fat	10.05 ± 0.24^{A}	9.44 ± 0.06^{B}	0.025
FM (kg)	40.2 ± 1.76	10.1 ± 0.61	< .001
BMI (kg/m^2)	166 ± 2.86^{B}	165 ± 3.64^{A}	0.832
TG (mg/Dl)	40.5 ± 1.32^{A}	34.9 ± 1.17^{B}	< .001
T-Chol (mg/Dl)	92.1 ± 0.85^{B}	92.1 ± 1.64	0.974
LDL (mg/Dl)	46.4 ± 0.88^{A}	30.2 ± 1.20^{B}	< .001
HDL (mg/Dl	67.4 ± 1.86^{A}	40.9 ± 1.44^{B}	< .001
NEFA (mmol/L)	0.88 ± 1.01^{A}	0.36 ± 0.04^{B}	< .001
Depht GM (cm)	10.01 ± 1.12^{A}	6.93 ± 1.02^{B}	< .001
Females			
	ArabianRace Horses	Arabian Endurance Horses	P
BW (kg)	414 ± 1.24	380 ± 1.88	0.231
BCS (0-9)	4.56 ± 0.84^{A}	4.06 ± 0.71^{B}	0.009
Fat layer LD (cm)	0.20 ± 0.02^{A}	0.15 ± 0.01^{B}	0.002
RFT (cm)	0.33 ± 0.06	0.31 ± 0.01	0.842
% Body Fat	10.12 ± 0.18^{A}	9.43 ± 0.03^{B}	0.024
FM (kg)	39.5 ± 1.47	10.0 ± 0.28	< .001
BMI (kg/m^2)	164 ± 2.64	164 ± 2.86	< .001
TG (mg/Dl)	42.4 ± 1.63^{A}	35.5 ± 1.55^{B}	< .001
T-Chol (mg/Dl)	107.3 ± 1.36^{A}	94.9 ± 1.42^{B}	< .001
LDL (mg/Dl)	48.5 ± 0.92^{A}	31.2 ± 1.32^{B}	< .001
HDL (mg/Dl	68.8 ± 1.22^{A}	42.1 ± 1.80^{B}	< .001
NEFA (mmol/L)	0.90 ± 1.01^{A}	0.38 ± 0.02^{B}	< .001
Depht GM (cm)	8.09 ± 0.77^{A}	6.16 ± 0.70^{B}	< .001

Different letters between columns indicate statistical difference (P < 0.05).BW = body weight; BCS = body condition score; BMI = body mass index; LD = *Longissimus dorsi* muscle; RFT = rump fat thickness; GM = *Gluteus medius* muscle; TG = triglycerides; T-Chol = total cholesterol; LDL = low-density protein; HDL = high-density protein; NEFA = non-esterified free fat acids.

crucial role in shaping Arabian horses' lipid profile (ROPKA-MOLIK, 2019; MYCKA et al., 2023). Investigations have revealed that in the muscles of Arabian horses subjected to prolonged exercise, there is an induced expression of genes associated with fatty acid degradation and the metabolism of fatty acids for energy production. Moreover, research has pinpointed specific genes linked to lipid metabolism that exhibit differential expression in Arabian horses undergoing training for flat racing (ROPKA-MOLIK, 2019; COSGROVE et al., 2020). These findings underscore the substantial impact of genetics on lipid metabolism in Arabian horses, particularly in the context of their exercise routines. In Brazil, horses of lineage for endurance or conformation still compete in turf racing events. To adapt a training program to extract the best athletic performance, future studies in genetics and metabolomics of the various Arabian horse lineages in the country are also necessary.

CONCLUSION

This study showed that the type of training regimen influences the lipid profile more than gender and is more efficient on endurance horses.

Further studies are needed to understand the effect of different workloads on horse lipid metabolism during exercise and in different categories to understand the peculiarities of the Arabian racehorse and its ability to use lipids as an energy source more profoundly.

AUTHORS' CONTRIBUTIONS

Renata Farinelli de Siqueira, Bianca Ozi Silva and Mônica Lente Fernandes: blood samples, ultrasonography, analysis and writing. Wilson Roberto Fernandes: writing review.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

The animal study protocol was approved by the Ethics and Animal Welfare Committee of the University of São Paulo (CEUA FMVZ-USP, number 2174300918).

DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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