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ORIGINAL ARTICLE

Evaluation of energetic metabolism of horses in long-distance exercise: Accutrend® Plus versus laboratory

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Abstract The use of human equipment in veterinary medicine is very common; therefore, it is essential that it is tested and validated. In this study, a comparison was made between blood levels of glucose, lactate, triglycerides, and cholesterol obtained by the portable device Accutrend® Plus and those found in laboratory methods considered standard for the measurement of parameters of 11 Arabian (and Arabian crossed) horses which completed prolonged endurance effort. Collection was made in five stages: during rest, after 66 km, at the end of the race of 160 km, and 2 and 15 h after the race. Three venous blood samples were collected, the first being used immediately for the measurement of the parameters on the portable device and the other two—with sodium fluoride and without anticoagulant—were packed and immediately sent to a reference laboratory. Statistical analysis consisted of the Student's *t* test, Pearson correlation, and graphic representation of Bland and Altman (1986) to compare the two measurement methods and Tukey's test for evaluating the effect of variation in relation to the effort according to the standard laboratory method for parameter measurement. Agreement and correlation were not found between the values of glucose, lactate, triglycerides, and blood cholesterol measured by the portable device Accutrend® Plus and the referenced laboratory methods for these parameters in horses undergoing the endurance test, which reinforces the need for more research to definitely validate or invalidate the use of this technology for the species.

Keywords Horse · Biochemistry · Portable · Endurance

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Introduction

Various physiological factors are responsible for the superior athletic performance of horses: high aerobic capacity; large stocks of intramuscular energy substrates, especially glycogen; high volume of mitochondria in the muscle; ability to increase capacity to carry oxygen at the start of the exercise by means of splenic contraction; efficiency in locomotion; and efficient thermoregulation (Hinchcliff and Geor 2004).

Among the prolonged effort, the endurance race is one of the most challenging competitions of equestrian sport and has become more demanding for horses in relation to speed, leading to an increase in metabolic, musculoskeletal, and cardiovascular work (Fraitpoint et al. 2012). The energy demanded for this exercise is preferably obtained through the aerobic metabolic pathway, the main substrates being carbohydrates and fatty acids (Pösö et al. 2004).

The carbohydrate in glucose form is the main energy source for mammalian cells (Kaneko 2008). The anaerobic glycolysis converts glucose into energy and generates pyruvate, which can be converted into lactate. The tissue that represents the major source of lactate is skeletal muscle, which releases it into the blood plasma so that, soon after, it can be

metabolized by the liver (Stockham and Scott 2008). As for lipid substrates, these can be said to have numerous functions in the animal body, being cholesterol and triglycerides the most studied lipoproteins in veterinary medicine (Stockham and Scott 2008). They are released from liver as very low-density lipoproteins (VLDL) and may have been synthesized in response to raise delivery of non-esterified fatty acids (Pösö et al. 2004). These variables may be utilized to identify insulin resistance in obese horses (Frank et al. 2006) while other researchers found out that horses differ from other athletic animals in that there is an increase in the circulating triglycerides (TG) during exercise in the racehorse (Pösö et al. 1989).

The laboratory determination of glucose, lactate, triglycerides, and blood cholesterol involves enzymatic, colorimetric, and/or photometric spectrum methods (Bruss and Lipids and ketones 2008; Kaneko 2008; Stockham and Scott 2008). The practicality of portable devices for this type of measurement enabled these to be widely used not only in human medicine but also in veterinary medicine; as for the latter, it is important to prove its efficacy in animals, which has been done by several authors (Franchini et al. 2004; Aleixo et al. 2006; Bluwol et al. 2007; Santos et al. 2008). Although, Teixeira-Neto et al. (2011) tested the measurements of glucose, lactate, triglycerides, and cholesterol through the portable device Accutrend® Plus and concluded that the same was ineffective in the equine clinic, these authors developed the study with a group of horses at rest. Therefore, the present study aimed to evaluate the accuracy of the same portable equipment in equines before, during endurance effort of 160 km, and at recovery period.

Material and methods

The protocol of this study was approved by the Ethics Committee of Animal Use (CEUA) of the Institute of Biological Sciences of the University of Brasilia under number 47819/2012.

Eleven horses (male and female), aged between 10 and 16 years, with a mean body mass of 390±31.4 kg, of Arabian breed or Arabian crossbreed, completed the endurance test of 160-km distance at an average speed of 17 km/h in moderate climate conditions. On the night prior to take off (rest), within the 66 and 160 km (end of ride), and 2 and 15 h after the exercise, jugular vein puncture took place immediately after the official veterinary examination, and the blood samples was divided into five times (T_0 to T_4), respectively. Three blood samples were collected, and the first one, in a 3-mL syringe, was used immediately for the measurement of glucose, lactate, triglycerides, and cholesterol in the device Accutrend® Plus as recommended by the manufacturer. The following samples were placed in a negative pressure tube (Vacuette®), being one without anticoagulant for serum biochemical tests and the other with sodium fluoride for glucose and blood lactate tests. These samples were properly packed in a container with water and ice and processed in less than 4 h according to laboratory determination (Sante Laboratorio, Brasilia-DF).

The samples stored in tubes containing sodium fluoride were used to determine the concentration of lactate and blood glucose through the device YSI 2300 STAT Plus. Enzymatic kits (Liquiform/Labtest®) were used for laboratory determination of total cholesterol and triglycerides from serum samples, in the semi-automatic biochemical analyser Bio200/2000.

For the comparison between laboratory tests results and those obtained from the Accutrend® Plus, tatistical methods were used such as Student's t test with paired samples with a significance level of 5 %, the Pearson correlation test, for the purpose of observing the existence of any distribution pattern between the results of the two devices and the research method in agreement with Bland and Altman (1986).

The effect of the effort on the biochemical variables studied were statistically evaluated by the variance analysis of repeated measures (rmANOVA), followed by Tukey's test when necessary, with a significance level of 5 %, by the computerized statistical program GraphPad InSTAT (version 5.0).

Results and discussion

The mean (standard deviation) of the values of glucose, lactate, cholesterol, and triglyceride in the blood are shown in Table 1. Differences were observed ($p<0.05$) between the parameters measured by the methods, at all times (Student's t test).

In relation to glucose values, a significant difference was identified ($p<0.001$ for T_0 to T_3 and $p<0.01$ in T_4) between the values found by the portable device and the laboratory method, corroborating Hollis et al. (2008), who stated that portable glucose meters used for humans did not agree with the laboratory standards when used on horses due to the difference in glucose distribution in the plasma and in the erythrocytes of both species. The same authors found positive agreement between glucose values obtained by human glucometer and laboratory methods simply using blood plasma of animals measured by the portable device. It reveals that the practicality of immediate measurement was lost, which would have been the main advantage of this equipment, time and greater chances of error to the values obtained are added (Hackett and McCue 2010).

According to Reusch and Wess (2000), glucose values are overestimated when the haematocrit is below 30 % and underestimated when haematocrit is above 55 %. However, the mean haematocrit values (±standard deviations) found in T_0 to T_4, in the present study were 35.20±5.28 %; 45.74±3.83 %; 46.07±6.81 %; 43.02±6.07 %, and 38.21±3.81 %,

Table 1 Mean [standard deviation] values of blood glucose, lactate, triglycerides, and cholesterol, measured by laboratory methods and portable device Accutrend Plus®, in horses (n=11) during an endurance race 160 km long, Brasília-DF, 2012

Parameteres	Methods	T_0	T_1	T_2	T_3	T_4
Glucose (mg/dL)	Accutrend	98,55 [17,9]***	125,09 [21,27]***	146,91 [20,88]***	153,64 [51,44]***	137,73 [43,18]**
	Laboratory	66,23 [7,06]	72,26 [10,25]	67,49 [20,69]	75,02 [12,16]	80,22 [5,97]
Lactate (mmol/L)	Accutrend	1,60 [0,44]***	2,35 [0,35]***	3,28 [0,78]***	2,73 [0,46]***	2,93 [1,97]*
	Laboratory	0,47 [0,13]	1,14 [0,17]	1,63 [0,40]	0,93 [0,22]	0,74 [0,14]
Triglycerides (mg/dL)	Accutrend	–	133,50 [87,70]*	178,91 [140,86]**	114,67 [62,61]*	–
	Laboratory	32,09 [5,50]	47,36 [14,81]	57,27 [23,68]	34,45 [7,67]	34,64 [9,43]
Cholesterol (mg/dL)	Accutrend	152,88 [11,51]**	158,70 [4,83]***	161,18 [7,68]***	166,00 [9,12]***	163,45 [8,15]***
	Laboratory	106,55 [18,92]	106,00 [21,48]	114,73 [15,13]	117,18 [19,88]	103,55 [33,25]

T_0 refers to the pre-ride or baseline, T_1 after 66 km, T_2 at the end of the race (160 km), T_3 2 h later and T_4 15 h after the race

Statistical difference: *P<0.05; **P<0.01; ***P<0.001, Student's t test

respectively, revealing that none of the values were outside the range established by the referred to authors. Bluwol et al. (2007) demonstrated that the determination of glucose in total blood from portable devices were effective in dogs. Moreover, Aleixo et al. (2006) suggested a repeat blood glucose test in dogs, in order to verify the accuracy of the result, since factors such as haematocrit, dehydration, and sample size might change it.

Moreover, Frank et al. (2006) found in horses at rest values of glucose between 49.8 to 74.1 mg/dL. These authors measured glucose through a colorimetric assay with values similar to the YSI 2300 methodology. Regarding exercise, the elevations in glucose concentration (Table 1) during the stages of exercise have been related to the effect of catecholamines and glucagon on the liver (Simões et al. 1999), both increasing the release and reducing the

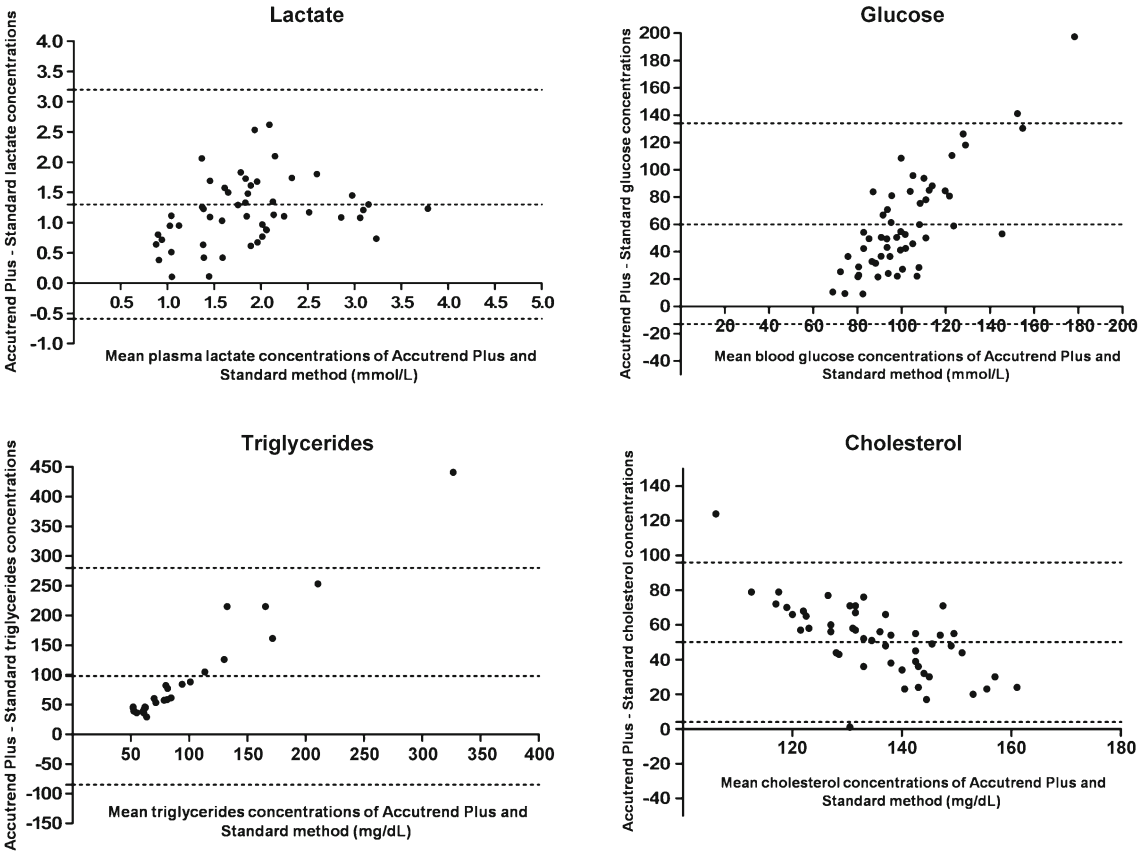

Fig. 1 Comparison of Accutrend Plus device and laboratory standard method blood concentrations of glucose, lactate, triglycerids and cholesterol by Bland and Altman graphical method (1986)

uptake of glucose. Additionally, adrenalin promotes a rapid and potent control of glycogenolysis during exercise, and in horses, this fact is directly related to the intensity of effort (Ferraz et al. 2008).

The plasma lactate showed a significant difference ($p<0.001$ for T_0 to T_3 and $p<0.05$ in T_4) between values found by the portable device and the laboratory. Teixeira-Neto et al. (2011) found differences in the glucose and lactate measurements by Accutrend® Plus compared to laboratory methods, of equines at rest. Franchini et al. (2004), while testing the portable lactimeter Accusport®, from the same manufacturer, in human athletes, found that it overestimated the lactate when values were below 5 mmol/L, and the reverse occurred when values were above 5 mmol/L. In this study, all values of lactate found in the horses were below 5 mmol/L and were also overestimated when compared to laboratory results. Although some studies on exercise in horses used Accusport® device to determine lactate (Ferraz et al. 2008; Lindner et al. 2009), similar rest values were found, comparing to the present study.

The reference values of triglycerides in equines at rest, between 4 and 44 mg/dL (Kaneko 2008), are outside the range of the portable device reading (70–600 mg/dL); therefore, the statistical analysis could not be performed at times T_0 and T_4 (indicated by the word "LOW" on the display), corroborating the results of Teixeira-Neto et al. (2011), with total blood obtained from equines at rest. The difference between the values found by the two methods of measurement in other times were significant ($p<0.05$ at T_1 and T_3, $p<0.01$ at T_2); however, Naylor and Durward-Akhurst (2012) compared the Accutrend® Plus with the laboratory method in measuring triglycerides in equines treated at a teaching hospital and found no significant differences between the methods.

The cholesterol results measured by Accutrend® Plus differed significantly ($p<0.01$ at T_0, $p<0.001$ from T_1 to T_4) from those found by laboratory methods and revealed no correlation through Pearson's method. In a study with humans, the portable device Accutrend® GCT revealed cholesterol values below those found by laboratory methods, with low agreement, however, with a strong correlation to Pearson's test (Hidalgo et al. 2012), differing from the present study, in that all values measured by the portable device overestimated those found by the laboratory method.

It is important to report that Frank et al. (2006) determined the plasma triglyceride and cholesterol concentrations through enzymatic colorimetric reagents and the automated discrete analyser. They found triglycerides and cholesterol values quite different values (11.0–22.6 and 73.0–88.1 mg/dL, respectively) when compared with our values.

Bland and Altman (1986) suggested a method of graphical representation when comparing two techniques, usually a well established and a new one. This method was considered the best for comparing two methods that should measure the same amount, as it is considered that differences are acceptable within a clinical point of view (Hirakata and Camey 2009). Figure 1 represents the values obtained from glucose, lactate, cholesterol, and triglycerides according to this method. As can be seen, none of the graphs have as a mean of difference between techniques a value equal or close to zero and their limits of agreement represent a very wide range due to the discrepancy of values, thus not indicating positive agreement between the two methods.

Conclusion

The use of equipment developed for humans by veterinary medicine must always be accompanied by studies that validate its efficacy in the target species. Several authors have tested the accuracy of the portable devices of the type *point-of-care* in measuring blood parameters in humans and domestic animals, but few studies were found in relation to equines, which reinforces the need for more research to definitely validate or invalidate the use of this technology for this species.

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