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5 authors, including:

Kenneth Harrington McKeever
Rutgers, The State University of New Jersey

Henry John-Alder
Rutgers, The State University of New Jersey

William Brechue
A.T. Still University of Health Sciences

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Relationship between body composition, blood volume and maximal oxygen uptake

C. F. KEARNS, K. H. McKEEVER*, H. JOHN-ALDER[†], T. ABE[‡] and W. F. BRECHUE[§]

Equine Science Center, Rutgers, The State University of New Jersey, 08901, USA; [†]Department of Animal Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ, 08901, USA; [‡]Department of Exercise and Sport Science, Tokyo Metropolitan University, 1-1 Minamiohsawa, Hachioji, Tokyo 192-0397, Japan; [§]Department of Kinesiology, Indiana University Bloomington, Indiana 47405, USA

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Summary

It has long been known that body mass and, more specifically, lean body mass are strongly correlated with maximal oxygen uptake ($V_{O_{2max}}$) in man and animals. However, there are no data to date describing this phenomenon in the horse. The purpose of this paper is to examine the relationship between body composition and $V_{O_{2max}}$ in the horse. Twenty-three healthy and unfit Standardbred mares performed an incremental exercise test (GXT) to measure $V_{O_{2max}}$. Rump fat thickness (RTH), a measure of fat covering, was measured using B-mode ultrasound. Plasma volume, total blood volume and red cell volume were determined, using the Evan's Blue dye dilution technique and packed cell volume. $V_{O_{2max}}$ was correlated with body mass (r = 0.541; P<0.01) and exercise haematocrit (exHCT; r = 0.407; P<0.05) but not RTH or the other haematological variables. To eliminate the influence of body mass on the individual variables, a regression analysis was performed on the mass-residuals of $V_{O_{2max}}$, RTH, plasma volume and exHCT. The residuals of $V_{O_{2max}}$ were correlated negatively with the residuals of RTH (r = -0.687; P = 0.0003) and positively with the residuals of exHCT (r = 0.422; P = 0.045) but not plasma volume. $V_{O_{2max}}$ could be predicted from a linear combination of the residuals of RTH and exHCT (r = 0.767; P<0.0001). These data indicate that $V_{O_{2max}}$ in the horse is significantly related to fat-free mass (FFM), independent of body mass. Red blood cells from the splenic reserve constitute an important factor in the horse's ability to achieve a high $V_{O_{2max}}$. Therefore, lean body mass may be a more appropriate basis for assessing metabolic function in the athletic horse.

Introduction

The horse is an 'elite athlete' because it has an aerobic capacity that is 2 or 3 times greater than species of similar body size (i.e. steers; Jones *et al.* 1989). Racing breeds, such as the Thoroughbred and Standardbred, can achieve a relatively high $V_{O_{2max}}$ of 153 ml O_2/kg min (Rose *et al.* 1988) and 140 ml O_2/kg min (Tyler *et al.* 1996) respectively. Maximal whole body oxygen uptake ($V_{O_{2max}}$) is defined as the highest rate at which oxygen can be utilised by the body. It is related to chronic physical activity, body composition and relative health of the individual animal (Pollock and Wilmore 1990).

Two important determinants of $V_{O_{2max}}$ are body size and blood volume. Body size determines the quantity of active tissue while blood volume is important in the regulation and maintenance of cardiac output and the delivery of oxygen to that metabolic tissue and has been shown to be proportional to $V_{O_{2max}}$ in the comparative literature (Taylor *et al.* 1982). In man, it has been established that the amount of fat-free mass (FFM) is strongly related to an individual's ability to work at maximal aerobic intensities (Buskirk and Taylor 1957). Furthermore, in man, the relationship between FFM and performance has been well established (Pollock and Wilmore 1990). This relationship has also been observed in the equine athlete, with the most successful pacers and trotters having the largest amount of FFM and lowest percent body fat (Kearns *et al.* 2002). Interestingly, the most successful endurance horses have also been shown to have the best body condition scores (a measure of equine body composition; Lawrence *et al.* 1992). While the relation between FFM and performance has been shown in the horse, no published research has examined the relationship between FFM and aerobic capacity in this animal. It is well recognised that blood volume plays an important role in aerobic capacity of the horse (Persson 1967); however, no published data exist regarding the influence of body composition on blood volume in horses. Therefore, the purpose of our work was to examine the relationship between body composition, blood volume and $V_{O_{2max}}$ in horse.

Materials and methods

Animals

Twenty-three healthy, untrained, Standardbred mares, age 3–10 years, were evaluated. The mares were unfit, but accustomed to the laboratory and running on the treadmill prior to the start of the experiment. During the trial the horses were housed on pasture. Each mare was fed approximately 6 kg/day of alfalfa and grass hay and approximately 3 kg/day of a commercially available grain ration (split into 2 feedings). Water was provided *ad libitum*. The Rutgers University Institutional Animal Care Review Board approved all methods and procedures used in this experiment.

Maximal aerobic capacity test ($V_{O_{2max}}$)

This test was designed to measure maximal oxygen uptake and several indices of exercise performance. Prior to the test, the horses

*Author to whom correspondence should be addressed.

were weighed and were walked onto the treadmill. The horses then stood quietly for approximately 10–15 min equilibration period, during which 15 min standing calorimetry data were obtained.

During the incremental exercise tests the animals ran on a high speed horse treadmill (Sato I)[1] at a fixed 6% grade (4º) and wore the indirect open-flow calorimeter apparatus (Oxymax-XL)[2] used to measure oxygen uptake. The tests started at an initial speed of 4 m/s for one min. Speed was then increased to 6 m/s followed by incremental 1 m/s increases every 60 s until the horses reached fatigue. Fatigue was defined as the point where the horse could not keep up with the treadmill despite humane encouragement. After this, the treadmill was stopped and 10 min of postexercise calorimetry data were collected. Oxygen uptake was measured continuously during the test and recorded at 10 sec intervals using an open flow calorimetry system calibrated using previously reported methods (Hinchcliff *et al.* 1996; McKeever and Malinowski 1997).

Rump fat measurement

Rump fat thickness (RTH) was measured using B-mode ultrasonography (Aloka SSD-500)[3]. The site used for the measurement was determined by placing the probe over the rump at approximately 5 cm lateral from the midline at the center of the pelvic bone (Westervelt *et al.* 1976). The region was scanned and the position of maximal fat thickness was used as the measured site. The calculated average coefficient of variation (CV) based on 6 animals for this rump fat thickness determination was 3.6 ± 0.7%.

Percent fat and fat-free mass measurement

Percent fat was estimated from the equations of Kane *et al.* (1987), where: % fat = 2.47 + 5.47 (rump fat in cm). Fat mass was determined by multiplying % fat and total body mass. Fat-free mass (FFM) was calculated by subtracting fat mass from total body mass.

Blood chemistry

Blood samples (20 ml) were obtained during the tests at rest and during the last 10 s of each increment of the test to measure haematocrit (HCT). Blood samples were placed into prechilled tubes containing EDTA (Vacutainer)[4] and were placed immediately on ice. Haematocrit and plasma protein concentration were measured in duplicate using the microhaematocrit technique and refractometry.

TABLE 1: Physiological characteristics of the horses

Variable	Mean ± s.e.
Body mass (kg)	514.3 ± 12.3
Rump fat thickness (cm)	3.30 ± 0.02
Body fat (%)	22.3 ± 1.1
Fat-free mass (kg)	397.8 ± 8.0
Fat mass (kg)	116.5 ± 8.0
Absolute VO_{2max} (l/min)	58.5 ± 1.4
Relative VO_{2max} (ml/kg/min)	114.4 ± 2.6
HCT at rest (%)	37.3 ± 0.8
HCT at VO_{2max} (%)	54.9 ± 0.7
Plasma volume (l)	26.4 ± 1.4
Red Cell volume (l)	26.5 ± 1.5
Blood volume at rest (l)	39.7 ± 2.0
Splenic blood volume (l)	13.1 ± 1.0

Plasma volume

Resting plasma volume was measured using a modified Evans blue dye dilution method (McKeever *et al.* 1987). All measures were made while the horses were standing quietly in their respective stalls. Calculations of blood volume and red cell volume (RCV) were made using previously published methods (Persson 1967).

Statistical analysis

Results are expressed as means ± s.e. of the estimate. Correlation coefficients were derived using the Pearson product moment (Sigma Stat 2.0)[5]. To eliminate the influence of body mass on the individual variables, mass-independent residuals of VO_{2max}, RTH, plasma volume and exercise HCT were generated by regressing each of the primary variables on body mass. All data were log-transformed for these regressions. Subsequently, correlations involving the mass-independent residuals of each of the primary variables were analysed in linear and multiple regression models.

Results

Physiological characteristics of the horses

Physiological characteristics of the horses are presented in Table 1. The table summarises body composition, oxygen uptake and blood volume parameters for the horses of the present study.

TABLE 2: Correlation analysis. Pearson correlation coefficients/Prob > | R | under Ho; rho = 0

	Abs VO_2	Body mass	RTH	PV	RHCT	EXHCT	BVR
Abs VO_2	1.000	0.542	-0.153	0.055	0.124	0.410	0.081
	0.0	<0.01	0.49	0.80	0.57	0.05	0.71
Body mass		1.000	0.572	0.466	-0.081	0.149	0.471
		0.0	<0.01	0.02	0.71	0.50	0.02
RTH			1.000	0.447	0.014	-0.040	0.477
			0.0	0.03	0.95	0.86	0.02
PV				1.000	-0.444	0.091	0.983
				0.0	0.03	0.68	0.0001
RHCT					1.000	0.384	-0.277
					0.0	0.07	0.20
EXHCT						1.000	0.175
						0.0	0.43
BVR							1.000
							0.0

RTH = Rump fat thickness; PV = plasma volume; RHCT = haematocrit at rest; EXHCT = exercise haematocrit; BVR = blood volume at rest.

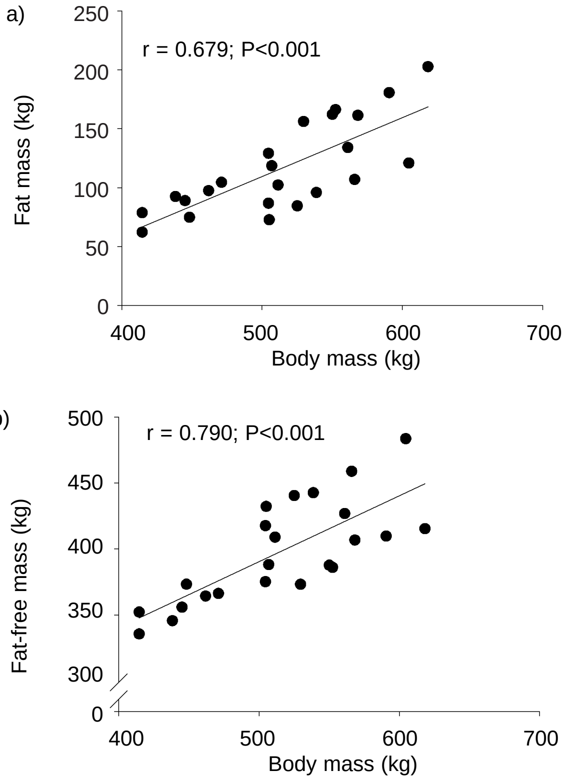

Fig 1: Scatter diagrams and linear regression lines relating a) fat mass to body mass and b) fat-free mass to body mass.

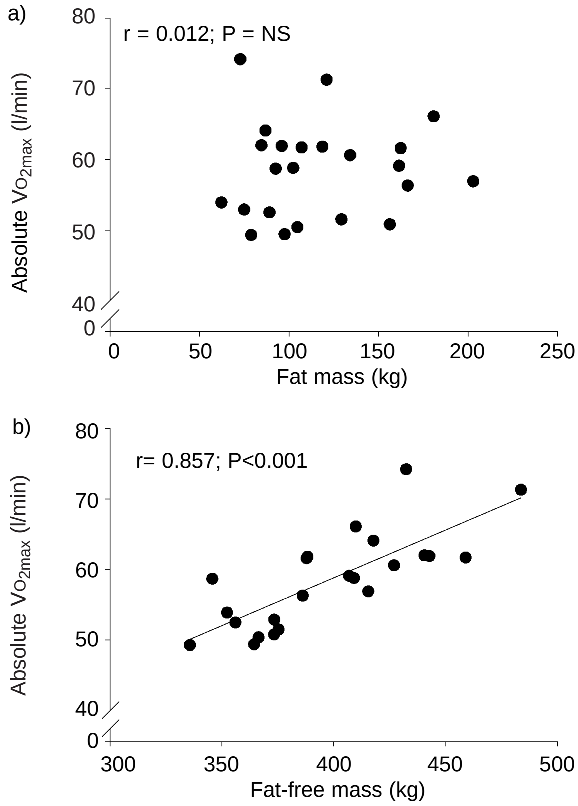

Fig 2: Scatter diagrams and linear regression lines relating a) absolute VO_{2max} to fat mass and b) absolute VO_{2max} to fat-free mass.

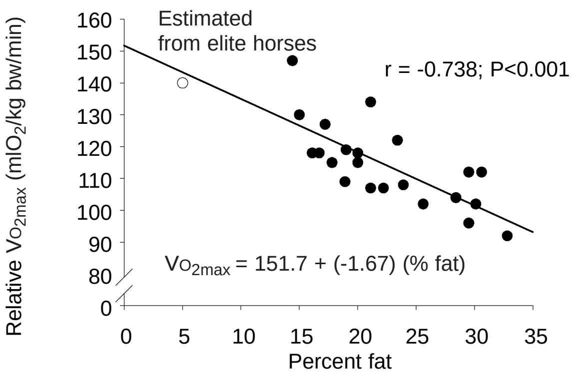

Fig 3: Scatter diagram and linear regression line relating relative VO_{2max} to % fat. The general equation to predict VO_{2max} in Standardbred horses is: VO_{2max} =151.7 + (-1.67) (% fat).

Regression analysis of calculated measures of body composition

Plots of individual values for body mass against fat mass and FFM are illustrated in Figure 1. Significant relationships between body mass and both fat mass (r = 0.679; P<0.001; Fig 1a) and FFM (r = 0.790; P<0.001; Fig 1b) were found. Plots of individual values for absolute VO_{2max} (l/min) against fat mass and FFM are presented in Figure 2. There was no correlation between absolute VO_{2max} and fat mass (r = 0.012; P = 0.957; Fig 2a) but there was a strong relationship between absolute VO_{2max} and FFM (r = 0.857; P<0.001; Fig 2b). Plots of individual values for relative VO_{2max} (ml/kg min) against % fat are contained in Figure 3. A strong negative correlation was found between those variables (r = -0.738; P<0.001).

Regression analysis of measures of blood volumes and body composition

Resting plasma volume was correlated with fat mass (r = 0.511; P = 0.012) and body mass (r = 0.466; P = 0.025) but not FFM (r = 0.200; P = 0.361; Fig 4a). Total blood volume at rest was correlated with fat mass (r = 0.535; P = 0.008) and body mass (r = 0.471; P = 0.023) but not FFM (r = 0.183; P = 0.404; Fig 4b). Neither plasma volume nor blood volume at rest was correlated with absolute VO_{2max} (Table 2).

Allometric correlation

VO_{2max} was significantly correlated with body mass (P<0.001) and exercise haematocrit (exHCT; P<0.05) but not RTH or the other haematological variables. To eliminate the influence of body mass on the individual variables, a regression analysis was performed on the mass-residuals of VO_{2max}, RTH, plasma volume and exHCT. The residuals of VO_{2max} were correlated negatively with the residuals of RTH (r = -0.687; P = 0.0003; Fig 5a) and positively correlated with the residuals of exHCT (r = 0.422; P = 0.045; Fig 5b) but not plasma volume (Fig 6). VO_{2max} could be predicted from a linear combination of the mass-independent residuals of RTH and exHCT (r = 0.767; P<0.0001).

Discussion

Allometry

Since all the physiological measures of the current study, such as FFM and blood volume, scale with body mass, care must be taken with simple correlative analysis that associates them with VO_{2max}. Both VO_{2max} and FFM scale with increasing body mass,

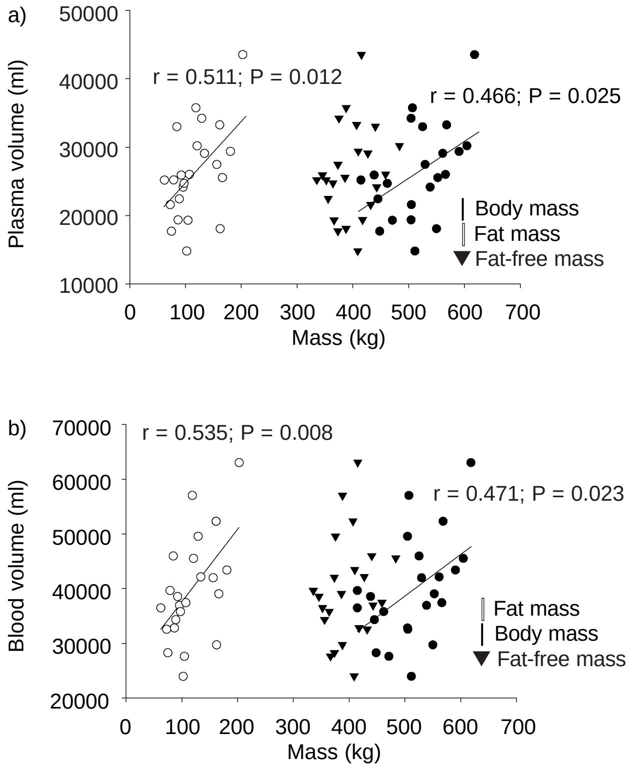

Fig 4: Scatter diagrams and linear regression lines relating a) plasma volume to body mass, fat mass and fat-free mass and b) total blood volume (at $\dot{V}O_{2max}$) to body mass, fat mass and fat-free mass.

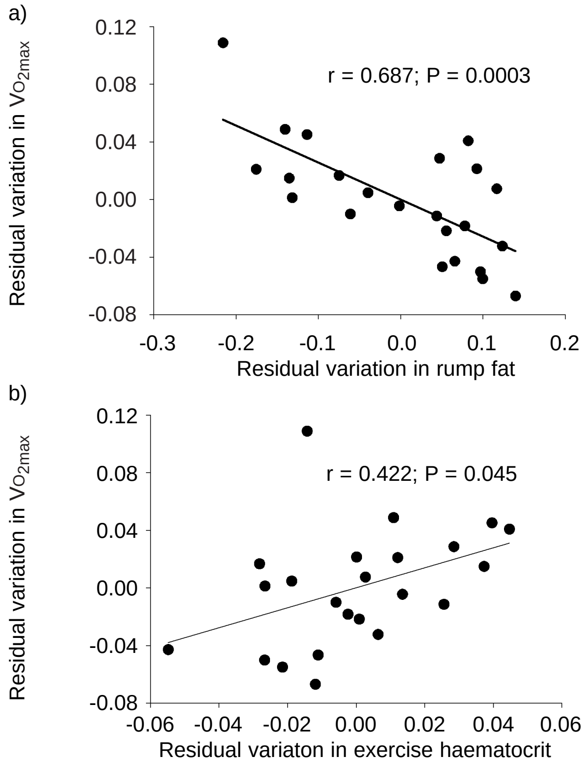

Fig 5: Scatter diagrams and linear regression lines relating A: the residual variation of absolute $\dot{V}O_{2max}$ to the residual variation of rump fat thickness and B: the residual variation of absolute $\dot{V}O_{2max}$ to the residual variation of exercise haematocrit.

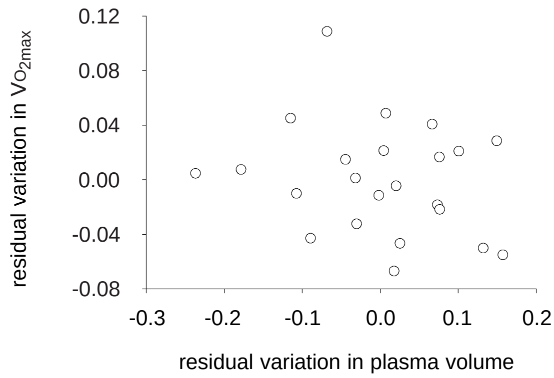

Fig 6: Scatter diagram relating the residual variation of absolute $\dot{V}O_{2max}$ to the residual variation of plasma volume

and one runs the risk, therefore, of making the nonsensical argument that body mass scales with body mass unless body mass is taken into account. The danger is that the real physiological relationship between the variables of interest to the investigator can become obscured by mass dependent interactions. To eliminate the influence of body mass on the individual variables, the residual variation of the physiological variables with regard to body mass were analysed. For the purpose of this statistical analysis, only the directly measured variables were considered and none of their derived associates. For example, rump fat thickness (RTH) was considered but not fat mass or FFM, because the latter measures are calculated from a regression equation associated with RTH and body mass. The analysis of the residuals indicated that $\dot{V}O_{2max}$ was correlated negatively to RTH and positively to exercise HCT (exHCT). The linear combination of RTH and exHCT were also related strongly to $\dot{V}O_{2max}$. Interestingly, plasma volume was not related. The inverse relation of $\dot{V}O_{2max}$ with RTH suggests the hypothesis that lower fat mass, and hence more FFM, independent of body mass are important determinants of aerobic capacity. Our data are in agreement with those of Buskirk and Taylor (1957) who found that FFM (r = 0.85), but not fat mass, was most strongly related to absolute $\dot{V}O_{2max}$ in man. This seems logical, since the majority of the energy consumed during locomotion is consumed by the working muscle (Taylor *et al.* 1982). Furthermore, the association of exHCT and $\dot{V}O_{2max}$, but not plasma volume, are also reasonable findings since it has been shown previously that total blood volume varies with body mass in horses while total haemoglobin varies with degree of adaptation in horses (Persson 1967).

Body masses and $\dot{V}O_{2max}$

Both fat mass and FFM demonstrate a strong linear relationship, increasing as a function of body mass in horses of the present study and this is in agreement with data from humans (Brechue and Abe 2002; Forbes 1987, 1992), chickens (Cartwright 1991) and cattle (Owens *et al.* 1995). It is not well understood why fat mass increases with increasing FFM; however, it is believed that this phenomenon may be a protective mechanism (Cartwright 1991; Owens *et al.* 1995). To this end, it has been speculated that fat deposition may accompany extreme muscle mass accumulation in order to maintain energy balance needs by retaining a larger proportion of essential fats than normal untrained populations (Owens *et al.* 1995; Brechue and Abe 2002). It has also been speculated that fat mass accumulation may be linked to insulin and insulin-like growth factors since both are linked to skeletal muscle enlargement and fat storage (Cartwright 1991; Brechue and Abe 2002).

Excess fat is detrimental to work because increased fat increases the load carried and this increases the oxygen cost at submaximal work loads (Buskirk and Talyor 1957). Data from the present study support the concept that the exceptionally high $\dot{V}O_{2max}$ seen in horses is related to body mass and, more importantly, to muscle mass. Horses have a larger percent of their body mass invested in muscle compared to other species. For example, domestic livestock of the meat industry, like steers, carry 30–40% of their live weight as muscle whereas racing breeds of horses carry 53–57% of their live weight as muscle and have a greater portion of that muscle mass distributed in the locomotor muscles of the hindlimbs (Gunn 1987). Furthermore, horse muscle has a higher total volume of mitochondria per unit body mass, nearly 3 times as large, compared to animals of a similar body mass such as the steer (Kayar *et al.* 1989, 1994). The dog, another 'elite athlete', has demonstrated similar superior mitochondria volume to weight matched pigmy goats (Vock *et al.* 1996). Given that across a wide range of species, there is a very close match between mitochondrial density and $\dot{V}O_{2max}$ per body mass (Taylor and Wiebel 1991), the large proportional amount of muscle may explain the large $\dot{V}O_{2max}$ per body mass seen in the horse.

Regression analysis of the percent body fat data reveals an interesting relationship. Horses of the present study had an average measured percent fat of 22.3% and an average measured $\dot{V}O_{2max}$ of 114.4 ml/kg/min. Interestingly, in a study of elite racing Standardbreds, it was found that they had an average measured percent fat of 8% but, more importantly, the most successful racers were even lower, averaging about 5% (Kearns *et al.* 2002). Using the current regression equation of the present study, the predicted $\dot{V}O_{2max}$ for those horses would be 138.4 and 143.4 ml/kg min, respectively. These values fall around the highest reported value for racing Standardbreds of 140.0 ml/kg min (Tyler *et al.* 1996), who, if we use the same regression formula, would be predicted to have a percent fat of around 7%. The regression equation applies equally as well to Thoroughbred horses. Thoroughbred body fat has been recorded as low as 1% (Gunn 1987) and the estimated $\dot{V}O_{2max}$ derived from the regression equation would be 150.1 ml/kg/min, which is very similar to the highest reported Thoroughbred $\dot{V}O_{2max}$ of 153.0 ml/kg/min (Rose *et al.* 1988). These data strongly support the notion that muscle mass, independent of body mass, is an important determinant of $\dot{V}O_{2max}$ in athletic horses.

Blood volumes and $\dot{V}O_{2max}$

With as much as 30–50% of total muscle mass active at $\dot{V}O_{2max}$ (Rowell 1986; Saltin and Gollnick 1983), the locomotor muscles can demand approximately 85% of maximal cardiac output during exercise. The resultant increase in muscle blood flow is a major stress on the finite cardiac output. Horses respond to this challenge by mobilising their reserve of red cell rich splenic blood into the central cardiovascular system (Persson 1967). The extra volume allows the horses to increase cardiac output by nearly 10 times over rest, whereas in man who do not mobilise splenic red cells, the greatest increase $\dot{V}O_{2max}$ is associated with a 7-fold increase in cardiac output above resting values (Saltin 1969). There is debate in the literature as to which component of splenic blood is more important to the development of the high $\dot{V}O_{2max}$ seen in horses. Some evidence suggests that the overall increase in circulating blood volume is important in determining venous return and Starling mechanisms of the heart and, therefore, elevation of aerobic performance in horses (Persson 1967; Hopper *et al.* 1991; Wagner *et al.* 1995; Hinchcliff *et al.* 1996; Knight *et al.* 1999). There is also evidence to suggest that the increase in circulating red cells and HCT, independent of volume, are more important to $\dot{V}O_{2max}$ (Person 1969; Wagner *et al.* 1995; McKeever *et al.* 1999). Haematocrit or, more precisely, the haemoglobin molecules contained within, are a primary determinant of arterial O_2 carrying capacity and O_2 delivery (Stevenson *et al.* 1994). Maximal O_2 delivery is tightly coupled with high levels of $\dot{V}O_{2max}$ (Saltin and Strange 1992). Such is the case in endurance-trained human subjects where superior aerobic capacities have been shown to be associated with higher haemoglobin content when comparisons are made with untrained-controls (Stevenson *et al.* 1994).

Data from the present study have demonstrated that blood and plasma volume are not independent of body mass in horses but increase as a function of body mass, while exHCT is independent and positively correlated with $\dot{V}O_{2max}$. Supporting the hypothesis that red cell volume may be more important in the relationship between $\dot{V}O_{2max}$ and blood volume are studies that directly manipulate red cell volume. Interestingly, administration of recombinant human erythropoietin resulted in an 11.6% increase in $\dot{V}O_{2max}$ despite a decrease in plasma volume and no change in total blood volume (McKeever *et al.* 1999). Apparently, the increased $\dot{V}O_{2max}$ was primarily related to the increases in both HCT and haemoglobin concentrations (McKeever *et al.* 1999). In a study by Wagner *et al.* (1995), splenectomy reduced $\dot{V}O_{2max}$ by 31% and these authors and concluded that the increase in haemoglobin concentration from splenic contraction is not essential for achieving a high $\dot{V}O_{2max}$ in horses. However, infusion with 11.7 l of low HCT (42%) blood returned $\dot{V}O_{2max}$ to approximately 94% of presurgical values (Wagner *et al.* 1995). Even though the authors infused low HCT blood, they still infused red cells. The addition of these new red cells allowed the horses to run longer and achieve a higher $\dot{V}O_{2max}$ (Wagner *et al.* 1995). Taken together, the data of Wagner *et al.* (1995) and the present data would seem to support the hypothesis that, in terms of splenic blood, the increase in red cell volume and HCT are more important than volume increase *per se* during exercise in horses.

Plasma volume, however, has been shown to correlate with $\dot{V}O_{2max}$ in man (Hagberg *et al.* 1998; Stevenson *et al.* 1994) and horses (Persson 1967). But, a closer inspection of the data of Hagberg *et al.* (1998) and Stevenson *et al.* (1994) shows that neither the blood nor plasma volume data of the trained runners or the untrained control human subjects by themselves were correlated to $\dot{V}O_{2max}$. It is only when both groups are considered together, that a significant correlation is seen. Similarly, Persson (1967) used horses of differing ages and training status to derive an overall significant correlation with work capacity. In 3-year-old horses of that study, there was a significant relationship between work capacity and total haemoglobin/bwt but not in total blood volume/bwt and work capacity (Persson 1967). Neither measure of blood volume was correlated to work capacity in the group of 4-year-old mares and geldings studied (Persson 1967). The fact that only untrained mares were considered in the present study may offer another explanation. These data are derived from only one such population of horses; perhaps if the data were included with data from elite or highly trained horses of similar ages, a different relationship would emerge. This hypothesis is supported by recent data where changes in $\dot{V}O_{2max}$ were proportional to changes in plasma volume following 8 week treatment with clenbuterol (2.4 mg/kg *per os*), exercise (20 min/day at 50% $\dot{V}O_{2max}$) or a combination of both (Kearns and McKeever 2002).

In conclusion, rump fat thickness, a measure of whole body fat cover, and exHCT, an indicator of arterial O_2 carrying capacity, are both independent of body mass and predictive of

$\dot{V}O_{2max}$ in horses of the present study. Horses may be able to achieve a larger $\dot{V}O_{2max}$ when compared to animals of similar body size because of their large proportional amount of skeletal muscle and large volume of red blood cells. These physiological adaptations are well suited for locomotion.

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Manufacturer's addresses

[1]Equine Dynamics, Inc., Lexington, Kentucky, USA.
[2]Columbus Instruments, Inc., Columbus, Ohio, USA.
[3]Aloka, Tokyo, Japan.
[4]Becton Dickinson, Inc, Franklin Lakes, New Jersey, USA.
[5]Jandel Scientific, San Rafael, California, USA.

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