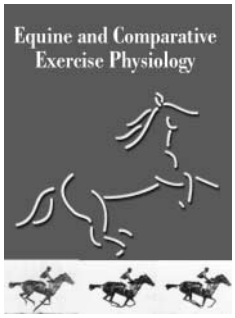
Equine and Comparative Exercise Physiology 1(1); 23–32 DOI: 10.1079/ECEP20031



Carbohydrate metabolism in exercising horses

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Submitted 23 November 2002: Accepted 3 March 2003 **Review Article**

Abstract

Carbohydrate and fat are the predominant sources of energy during exercise in mammals. Carbohydrates, such as muscle glycogen and plasma glucose, and fats from adipose tissue and intramuscular triglycerides are oxidized during exercise in amounts and proportions that vary depending on the exercise intensity, level of fitness and nutritional status. In horses, muscle glycogen, and to a lesser extent plasma glucose, are oxidized in substantial amounts during low-, moderate- and high-intensity exercise. Carbohydrate availability to skeletal muscle affects exercise performance in humans, however this relationship is not well outlined in horses. Glucose supplementation by intravenous administration during exercise in horses increases duration of moderate-intensity exercise. However, the effect of glucose supplementation by ingestion of a soluble carbohydrate-rich meal prior to exercise on athletic performance has not been established in horses. Low muscle glycogen concentrations prior to exercise in horses are associated with decreased time to exhaustion at moderate- and high-intensity exercise. Nutritional interventions intended to enhance muscle glycogen resynthesis have proved less successful in horses than in other species. Replenishment of muscle glycogen after strenuous exercise in horses is not complete until 48–72 h after exercise, whereas in humans and laboratory animals it is complete by 24 h. The slower rate of muscle glycogen replenishment after exercise in horses may be related to an inherent lower ability to digest starch and other sources of glucose, a lower ability to synthesize muscle glycogen, or both. The aim of this review is to describe the present understanding of carbohydrate metabolism in the exercising horse, its implications on nutrition and athletic performance, and to contrast it with that in other species.

Keywords: exercise; glycogen; glucose; starch; muscle

Introduction

The athletic horse has a large capacity to perform muscular work compared with many other mammals, including humans. Energy to perform work is obtained from oxidation of carbohydrate and fat and, to a minimal extent, protein. The energy requirements of athletic horses are met by ingestion of these nutrients. However, while metabolism and energy transduction in muscle fibres of mammalian species are similar, the energy requirements of different athletic species are met by ingestion of very different diets.

The horse is a herbivore that has adapted its gastrointestinal function for hindgut fermentation. Unlike ruminants, equids have a small simple stomach, followed by 60–70 ft (18–22 m) of small intestine where digestion and absorption of soluble carbohydrates, fat and protein occur. Microbial fermentation occurs in the caecum and large colon, which hold 80–100 l of liquid and house billions of bacteria and protozoa, with the breaking down of plant fibre releasing volatile fatty acids (VFAs), the most abundant being acetate, propionate and butyrate[1]. Energy intake of horses is primarily from carbohydrates in forages and grains. Fibre in forages is fermented in the hindgut and the VFAs produced used for synthesis of fatty acids, and from propionate, glucose. Starch and sugars in grains and molasses are digested primarily in the small intestine and absorbed as monosaccharides and eventually transformed to glucose or fat.

Glucose is stored as glycogen in liver and muscle. Glycogen is a branched polymer of glucose with a mixture of α-1,4 and α-1,6 linkages between glucose units. Muscle glycogen constitutes over 90% of the carbohydrates in the body (Table 1) and the amount of glycogen is muscle fibre type-dependent. Fast-twitch muscle fibres (Type-II) have greater glycogen content

Table 1 Fuel stores, distribution within the body and energy storage in a 450 kg horse[29]

Fuel	Tissue	Grams	Energy (kcal)
Triglycerides	Adipose tissue	40 000	360 000
Triglycerides	Muscle	1400–2800	12 600–25 200
Glycogen	Muscle	3150–4095	13 230–17 200
Glycogen	Liver	90–300	380–1260
Glucose	Plasma	27	110

than slow-twitch muscle fibres[2,3]. Fat is stored in both adipose tissue and in muscle, both at intracellular (myocyte) and extracellular sites. In contrast to carbohydrates, intramyocellular triglycerides make up only ≈5% of the fats in the body. Slow-twitch muscle fibres have greater lipid content than fast-twitch high oxidative muscle fibres, and the amount of lipid in fast-twitch low oxidative muscle fibres is negligible[4,5]. Intracellular triglycerides are seen within muscle fibres as lipid droplets in close proximity to mitochondria.

Differential storage of glycogen and intramyocellular triglycerides among muscle fibre types is related to inherent metabolic differences of the muscle fibres. Muscle fibre types are established based on differences in contractile properties and oxidative/glycolytic enzymatic profiles (slow-twitch, fast-twitch high oxidative and fast-twitch low oxidative), based on differences in pH sensitivity of the myofibrillar ATPase (Type-I, -IIA and -IIB) and based on myosin heavy-chain (MyHC) expression (Type-I, -IIA and -IIX). Type-I fibres have a MyHC isoform that hydrolyses ATP slowly, resulting in a slow cross-bridge cycle, together with a small cross-sectional area, a high number of capillaries, greater storage of lipids and a high oxidative capacity. However, their glycolytic capacity and glycogen content are lower than that of other fibre types. In contrast, Type-II fibres have MyHC isoforms that create fast cross-bridge cycling and therefore develop force rapidly. Type-IIX fibres are adapted for high power outputs for a limited time because they have a low oxidative capacity and limited oxygen availability (as reflected by their large cross-sectional area and relatively low capillary supply). Type-IIA fibres, however, have a considerable number of both capillaries and mitochondria, and rely on glycolytic and oxidative metabolism; they are therefore able to sustain high power outputs for longer than Type-IIX fibres. Hybrid Type-IIAX fibres are intermediate in their properties[6,7].

Glucose and fatty acids stored within and outside the muscle are used as fuels during exercise in horses, as well as in other athletic species. However, quantitatively the contributions of different fuels vary among different athletic species due to inherent metabolic and nutritional differences. Our aim is to describe the present understanding of carbohydrate metabolism in the exercising horse, its implications on nutrition and athletic performance, and to contrast it with that in other species.

Energy metabolism and fuel sources during exercise

During exercise, skeletal muscles perform mechanical work using chemical energy obtained from fat and carbohydrate oxidation. The relative contributions of fat and carbohydrate used to fuel exercise depend on exercise intensity, level of fitness, sex, menstrual phase in the case of women, environmental conditions, type of meal ingested and interval from ingestion to initiation of exercise.

All of these factors influence muscle and liver glycogen concentrations and circulating hormone status (insulin, glucagon, catecholamines) and will dictate the mixture of substrates utilized to fuel exercise. Many, but not all, of these factors have been evaluated in horses.

Intensity of exercise is generally described relative to the percentage of maximal oxygen consumption (% $\dot{V}O_{2max}$). Increasing exercise intensities shift the predominant substrate contribution to energy expenditure from fat to carbohydrate. In addition, high-intensity exercise increases reliance on intramyocellular stores of carbohydrate (glycogen granules) and fat (triglyceride droplets). Some of the mechanisms that limit fat oxidation with increasing exercise intensities include reduced non-esterified fatty acid mobilization from adipose tissues due to maintained lypolysis but reduced blood flow to adipose tissue[8], and decreased activity of carnitine palmitoyltransferase I, which is responsible for transport of long-chain fatty acids from the sarcoplasm to the mitochondria, due to decreases in intracellular pH[9]. The increase in muscle glycogen degradation as exercise intensity increases is related to activation of glycogen phosphorylase, which cleaves a single glucose molecule from glycogen. Increased activity of phosphorylase is in response to increased sarcoplasmic calcium concentration, associated with muscle contractions, and hormonal stimulation by adrenaline-mediated β-receptors and the intracellular second messenger 3′,5′-cyclic adenosine monophosphate[10].

Studies performed in trained male and female human athletes demonstrate that increasing exercise intensity from 25% $\dot{V}O_{2max}$ to 85% $\dot{V}O_{2max}$ alters the relative contributions of blood-borne glucose oxidation, muscle glycogenolysis, plasma non-esterified fatty acids (NEFAs) and intramyocellular triglyceride (IMTG) oxidation to energy expenditure[11,12]. At low exercise intensity (25% $\dot{V}O_{2max}$) oxidation of plasma NEFAs accounts for most of the energy requirements. At moderate exercise intensity (65% $\dot{V}O_{2max}$) the contribution from oxidation of muscle glycogen, blood-borne glucose and IMTGs is

greater than at lower exercise intensity. At moderate to high exercise intensity (85% $\dot{V}O_{2max}$) the contribution from muscle glycogenolysis increases exponentially and the relative contribution from the rest of the fuels decreases significantly[11,12]. In summary, greater energy fluxes required during high-intensity exercise are met by increased reliance on intramuscular substrate stores, predominantly from muscle glycogen.

Horse studies

Horses have a higher mass-specific aerobic capacity than humans. Thoroughbred horses have a maximal oxygen consumption ($\dot{V}O_{2max}$) of approximately 160 ml $O_2\,kg^{-1}\,min^{-1}$ and Olympic-calibre human athletes have a $\dot{V}O_{2max}$ of approximately 70–80 ml $O_2\,kg^{-1}\,min^{-1}$ (refs 13–15). Similar to pronghorn antelopes, which have a $\dot{V}O_{2max}$ of $\approx$300 ml $O_2\,kg^{-1}\,min^{-1}$ (ref. 16), the high $\dot{V}O_{2max}$ of horses is probably due to the pressure of natural selection on horses to become more athletic and aerobic animals, as well as selective breeding of those horses with greater athletic ability. Horses, when compared with humans, have a greater aerobic capacity because, relative to body mass, the former have a greater oxygen-carrying capacity, greater cardiac output and greater oxygen conductance at a capillary level[17]. Other reasons for the apparent greater mass-specific aerobic capacity in horses compared with humans are differences in body composition, the proportion of muscles engaged during running in quadrupeds versus bipeds and differences in muscle mitochondrial density. Muscle mass as a percentage of body weight ranges from $\approx$52% in Thoroughbred racehorses to $\approx$42% in average horses of other breeds, and in humans from $\approx$47% in elite road-cyclists to $\approx$40% as the average for male young adults[18, 19]. Not only is the muscle mass greater in horses than in humans but more importantly the proportion of muscles engaged during exercise in quadrupeds is much greater ($\approx$70–80%) than in bipeds ($\approx$30–40%). In addition, mitochondrial density of equine muscle – the volume of mitochondria per volume of muscle fibre – ranges from 6% to 8.5% depending on muscle group and fibre type, whereas that of humans ranges from 2% to 6%[20,21]. In summary, horses have a high aerobic capacity due to cardiovascular, muscular and metabolic adaptations and to a greater mass of active skeletal muscle relative to body weight.

At the same exercise intensity relative to $\dot{V}O_{2max}$, the mass-specific rates of oxygen consumption ($\dot{V}O_2$) and the energy expenditure are approximately two-fold higher in horses than in humans; in addition higher energy fluxes in horses are associated with greater contribution from carbohydrate oxidation to energy expenditure. Other athletic (i.e. dogs) and non-athletic (i.e. goats) mammals have a similar two-fold difference in mass-specific $\dot{V}O_2$ and energy expenditure. However, at approximately 60% $\dot{V}O_{2max}$ the contribution to energy expenditure from carbohydrate (57–60%) versus fat (40–43%) oxidation is similar between dogs and goats, unlike the comparison between horses (75% from carbohydrate, 25% from fat) and humans (40% from carbohydrate, 60% from fat)[11,22,23] (Table 2).

Horses and dogs, when compared with humans and goats exercising at a similar % of $\dot{V}O_{2max}$, have a two-fold higher energy flux. Horses, when compared with humans, dogs and goats exercising at the same intensity, have a greater proportion of energy derived from carbohydrate. Therefore, observations made in humans or other animals about carbohydrate and fat metabolism and energy requirements during exercise at an apparently similar intensity (same % $\dot{V}O_{2max}$) may not apply to horses.

Estimation of substrate utilization during moderate- to high-intensity exercise in horses has limitations not encountered in humans exercising at the same % $\dot{V}O_{2max}$. Estimates of energy expenditure and contributions from fat and carbohydrate oxidation are obtained using indirect calorimetry and gaseous exchange measurement. Simultaneous infusion of stable isotopes of glucose or fatty acids allows a complete assessment of intramuscular versus extramuscular substrate oxidation. Estimates of whole-body carbohydrate and fat oxidation during exercise by indirect calorimetry are based on some assumptions; one of the assumptions is that the rates of oxygen consumption ($\dot{V}O_2$) and of carbon dioxide production ($\dot{V}CO_2$), as measured in exhaled gas, reflect the consumption of O_2 and production of CO_2 at a cellular level. However, at high exercise intensities exhaled CO_2

Table 2 Mass-specific maximal rates of oxygen consumption ($\dot{V}O_{2max}$), as well as rates of oxygen consumption ($\dot{V}O_2$), total energy expenditure (TEE) and contributions from carbohydrate (CHO) and fat oxidation during moderate-intensity exercise in athletic (horses and dogs) and non-athletic mammals (humans and goats)[11,22,23]

	Horses 60% $\dot{V}O_{2max}$	Humans 65% $\dot{V}O_{2max}$	Dogs 60% $\dot{V}O_{2max}$	Goats 60% $\dot{V}O_{2max}$
$\dot{V}O_{2max}$ (ml $kg^{-1}\,min^{-1}$)	137	67	146	68
$\dot{V}O_2$ (ml $kg^{-1}\,min^{-1}$)	77	44	84	39
TEE (cal $kg^{-1}\,min^{-1}$)	400	200	410	190
Contribution from CHO ox. (%)	75	40	60	57
Contribution from fat ox. (%)	25	60	40	43

may not accurately reflect gas exchange at a cellular level due to substantial loading of carbon dioxide into the arterial and venous blood and other body fluid compartments, as well as additional carbon dioxide produced from buffering of hydrogen ions by bicarbonate as concentrations of protons increase in muscle and plasma. Therefore, $\dot{V}CO_2$ as measured by indirect calorimetry at high exercise intensity is an overestimation of the actual production of CO_2 at a mitochondrial level, and consequently the estimates of whole-body carbohydrate and fat oxidation are erroneous. Horses running at exercise intensities at or below 60% $\dot{V}O_{2max}$ have a respiratory exchange ratio (RER = $\dot{V}CO_2/\dot{V}O_2$) of 0.90–0.96 and contributions from carbohydrate and fat oxidation can be estimated by indirect calorimetry[23,24]. However, in horses performing an incremental exercise test the RER is above 1 at exercise intensities higher than 75% $\dot{V}O_{2max}$[25]. Therefore, indirect calorimetry estimation of contributions from carbohydrate and fat oxidation to energy expenditure cannot be reliably estimated at exercise intensities above 60% $\dot{V}O_{2max}$ in horses. Validation of estimates of carbohydrate and fat oxidation by indirect calorimetry during high-intensity exercise requires measurement of the absolute ratios of $^{13}C/^{12}C$ in expired air, in endogenous glucose, fat and protein in addition to $\dot{V}O_2$ to obtain carbohydrate and fat oxidation rates independently of CO_2 production[26]. Unlike in horses, measurements of rates of substrate oxidation by indirect calorimetry have been validated in humans exercising up to 85% $\dot{V}O_{2max}$, an exercise intensity that results in a RER of ≈ 0.9[26]. Consequently, the accuracy of estimates of fat and carbohydrate oxidation provided by indirect calorimetry is likely to decline as the RER approaches 1.0. Values are probably reliable at lower work intensities, but the intensity, or RER, at which the inaccuracy becomes important has not been determined in horses.

The regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity has not been formally investigated in horses. However, a similar trend is observed of increasing contribution from carbohydrate oxidation and decreasing contribution from fat oxidation, as a % of the energy used to fuel exercise, with increasing exercise intensity if we compare two studies: (1) horses exercised at 35% $\dot{V}O_{2max}$ for 90 min have an energy expenditure of $\approx 210\,cal\,kg^{-1}\,min^{-1}$ with relative contributions to energy of $\approx 42\%$ from fat oxidation and $\approx 58\%$ from carbohydrate[27]; and (2) horses exercised at 50% $\dot{V}O_{2max}$ for 60 min have an energy expenditure of $\approx 325\,cal\,kg^{-1}\,min^{-1}$ with relative contributions to energy of $\approx 30\%$ from fat oxidation and $\approx 70\%$ from carbohydrate[28]. In addition, at the same exercise intensity the pattern of substrate oxidation is dependent on duration of exercise. In horses exercised at 35% $\dot{V}O_{2max}$ for 90 min, during the 0–30, 30–60 and 60–90 minute intervals, fat oxidation accounts for $\approx 43\%$, $\approx 55\%$ and $\approx 68\%$, and carbohydrate oxidation accounts for $\approx 57\%$, $\approx 45\%$ and $\approx 32\%$[27]. Therefore, there is a shift in the contribution to energy expenditure from carbohydrate to fat oxidation as duration of an exercise bout increases.

Carbohydrate metabolism: muscle glycogen, liver glycogen and plasma glucose

The fuel reserves of a horse are in the form of fat, carbohydrates and protein. Protein is a quantitatively important source of energy production only in starvation. Since fats are more energy-dense and the amount of fat reserves is greater, horses have as much as 20–25 times more energy stored in the form of fat than carbohydrate[29] (Table 1). Therefore, similar to other species, carbohydrate stores of horses are relatively limited when compared to fat. In addition, exercise performed by horses in athletic events requires concurrent oxidation of carbohydrate and fat. For these reasons carbohydrate availability and oxidation by working skeletal muscle may become a limiting factor for exercise performance. The impact of carbohydrate availability and oxidation on exercise performance has been demonstrated in horses in two situations: (1) as an increase in the time to fatigue in horses administered supplemental glucose by intravenous infusion during moderate-intensity exercise[30], and (2) by depletion of muscle glycogen prior to exercise and demonstration of subsequent lower exercise performance[31,32]. These studies are discussed below in greater detail.

Carbohydrates are either absorbed from the gastrointestinal tract or synthesized *de novo* by liver gluconeogenesis. Carbohydrates are stored in the body in the form of glycogen. Other forms of carbohydrate that contribute to energy supply during exercise are plasma glucose and lactate. The rest of the carbohydrates in the body are mostly in the form of glycosylated proteins or lipids and are not quantitatively relevant to exercise metabolism.

Liver glycogen is used to maintain normal glucose concentrations in periods when glucose availability from intestinal absorption is decreased. Those tissues dependent solely on glucose for their metabolism, such as neurons and red blood cells, require a constant supply of glucose from the liver via breakdown of glucose from glycogen or synthesis of glucose from gluconeogenic precursors, such as lactate, glycerol and most amino acids. Propionate, which is one of the VFAs absorbed in the hindgut from microbial fermentation of plant fibre, is considered a very important gluconeogenic precursor in resting horses,

and it may account for as much as 50–60% of hepatic glucose production in non-exercising horses[33].

Unlike liver glycogen, muscle glycogen does not contribute to maintenance of normoglycaemia. The majority of muscle glycogen is stored in fast-twitch high oxidative (Type-IIA) and fast-twitch low oxidative (Type-IIB) muscle fibres. These muscle fibre types are those most dependent on glycogen for synthesis of ATP via oxidative phosphorylation and/or glycolysis resulting in lactic acid formation. Glycogen granules are particles of carbohydrate with complexed proteins (glycogenin, glycogen synthase, glycogen phosphorylase and phosphorylase kinase) found in subsarcolemmal and myofibrillar locations. In the *vastus lateralis* of humans, most of the glycogen granules are found between myofibrils, but the subsarcolemmal space is also densely packed with glycogen granules[34].

Glucose metabolism during exercise in horses

Plasma glucose concentrations and glucose kinetics during exercise

In horses, unlike humans and dogs, plasma glucose concentrations increase (2–4 mM, 36–72 mg dl^{-1}) even during moderate-intensity exercise (50% $\dot{V}O_{2max}$)[30]. This indicates a mismatch between the rate of appearance of glucose in blood (glucose R_a) and the rate of disappearance of glucose from blood (glucose R_d). Using stable isotope techniques it has been determined in horses that both glucose R_a (hepatic glucose production) and R_d (net glucose disposal by peripheral tissues) increase four-fold during exercise at 35% $\dot{V}O_{2max}$ compared with resting values[27]. However at 50% $\dot{V}O_{2max}$ glucose R_a increases seven-fold but glucose R_d increases by only four-fold compared with resting values[35]. This mismatch in the glucose turnover rates may be partly or completely due to sympathoadrenergic mechanisms operating directly via hepatic sympathetic innervation or indirectly via circulating adrenaline, as discussed below.

Insulin, glucagon and insulin : glucagon ratio during exercise

Insulin and glucagon act to maintain glucose homeostasis and prevent hypoglycaemia despite large increases in glucose uptake by working skeletal muscle. Similar to other species, during low- and moderate-intensity exercise plasma insulin concentration of horses decreases, plasma glucagon concentration increases and the insulin : glucagon ratio decreases[23,27]. However, these responses will be altered if plasma glucose concentrations are elevated by oral or intravenous glucose administration. Plasma insulin concentration during exercise is higher, and plasma glucagon is lower, when plasma glucose concentration is high during exercise because of administration of glucose before or during exercise in horses[27,35].

Catecholamines and glucose turnover rates

Sympathoadrenergic mechanisms play an important role in the control of plasma glucose concentrations during exercise. The mismatch between glucose production and glucose oxidation during moderate-intensity exercise leads to hyperglycaemia in horses. An excessive rate of hepatic glucose production and lower rate of peripheral glucose disposal partly account for this phenomenon, as evidenced by β-adrenergic blockade by the non-selective β-blocker propranolol augmenting, and adrenaline infusion inhibiting, the rate of glucose disposal by skeletal muscle[23,35].

Effect of training status on glucose kinetics in horses

In humans, training results in a lower rate of glucose oxidation during exercise at the same absolute intensity when compared with pretraining values[36]. Similarly, training decreases reliance on glycogenolysis and blood-borne glucose oxidation during exercise in horses when compared with the response before training at the same absolute, but not relative, exercise intensity[37].

Glucose availability and exercise performance in horses

Blood glucose is an important fuel for contracting muscle. Studies in human subjects have demonstrated that over 90% of the whole-body glucose uptake during moderate-intensity exercise is oxidized by skeletal muscle[36]. Increased glucose availability during prolonged moderate-intensity exercise by ingestion of glucose, glucose polymers, sugar-rich snacks or carbohydrate meals prior to and/or during exercise in humans enhances performance, measured as an increased time to fatigue or an improvement in time to complete a set distance[38]. Similarly, the time to fatigue is prolonged by 14–20% in horses exercised to exhaustion at 50–60% $\dot{V}O_{2max}$, when plasma glucose availability is enhanced by intravenous administration of glucose[30,39]. However, the relative contribution to energy expenditure from blood-borne glucose oxidation during exercise in horses is modest at best when compared with oxidation of muscle glycogen and intra/extramuscular sources of fat. At exercise intensities varying from 30–60% of $\dot{V}O_{2max}$ muscle glycogen oxidation and fat oxidation account for 32–68% and 25–56% respectively of the energy expenditure, whereas oxidation of blood-borne glucose accounts for only 6–12% of the energy expenditure during exercise[23,27,28].

Increased glucose availability by intragastric administration of a glucose solution (2 g kg^{-1}) prior to exercise increases the rate of blood-borne glucose oxidation and the rate of whole body carbohydrate oxidation but muscle glycogenolysis is unchanged[35]. However, the effect of increased glucose availability by oral/intragastric administration on exercise performance in horses has not been determined.

Intravenous or oral glucose administration is not a practical intervention in athletic field events. A more practical strategy to increase glucose availability prior to exercise is to provide a starch-rich meal, such as corn or oats, prior to exercise in order to increase plasma glucose availability by intestinal digestion and absorption of glucose. Increasing glucose availability prior to exercise in horses by providing a grain meal does alter carbohydrate and fat metabolism but the effect of such a meal prior to exercise on athletic performance has not been determined in horses[28].

Muscle glycogen and exercise performance in horses

Muscle glycogen depletion and rates of muscle glycogenolysis in exercising horses

The normal glycogen concentration in the horse's muscle is 130–140 mmol kg^{-1}ww (560–600 mmol kg^{-1}dw), which is greater than the values in human athletes of 80–100 mmol kg^{-1}ww (340–425 mmol kg^{-1}dw)[40–43]. This greater concentration of muscle glycogen may contribute to the greater athletic capacity of horses. However, despite this greater concentration muscle glycogen concentrations are substantially depleted by exercise.

During a competitive 50 to 100 km endurance ride (≈4–9 h events) muscle glycogen depletion was found to be 57–65% and the rate of muscle glycogenolysis during these events was 0.14–0.30 mmol kg^{-1} ww min^{-1} (refs 4 and 5). In a simulated 80 km endurance ride that lasted 4 h muscle glycogen depletion was found to be greater than 90% and the average rate of muscle glycogenolysis was 0.5 mmol kg^{-1} ww min^{-1} (ref. 43). Therefore, substantial muscle glycogen depletion is observed in horses performing long distance, low- to moderate-intensity exercise. During prolonged exercise glycogen depletion in different muscle fibre types occurs progressively, with initial depletion occurring in Type-I fibres and depletion of glycogen in Type-IIB fibres occurring later. This pattern of depletion is related to progressive recruitment of muscle fibre types[5,44].

High-intensity exercise results in exponential increases in the rate of muscle glycogenolysis in humans. Similarly, in horses running an 800 m sprint (14.3 m s^{-1}, 32 mph for 50–60 s) or a 2000 m sprint (13.4 m s^{-1}, 30 mph for 2.5 min) the rates of muscle glycogenolysis were found to be 37 mmol kg^{-1} ww min^{-1} and 16 mmol kg^{-1} ww min^{-1}, respectively[42]. Muscle glycogen depletion at these exercise intensities varies from 20% to 40% of values before exercise[42,45–47]. The pattern of glycogen depletion during high-intensity exercise in Thoroughbred and Standardbred races shows that significant glycogen depletion occurs in Type-IIA and -IIB muscle fibres[3,44]. These estimates of muscle glycogenolysis should approximate to the rates of glycogen utilization in competitive Thoroughbred or Standardbred races.

The differences observed between humans and horses in resting muscle glycogen concentration, rate of muscle glycogenolysis during exercise and rate of muscle glycogen synthesis after exercise may be partly explained by differences in muscle fibre composition. The *vastus lateralis* muscle of human marathon runners has up to 80% of Type-I fibres, while that of elite sprinters contains up to 60% of the Type-II fast-twitch fibres[48,49]. In contrast, muscle fibre type composition in the *gluteus medius* muscle of horses is not nearly as variable as in humans. Horses competing in endurance events have 18–32% Type-I, 36–46% Type-IIA and 20–38% Type-IIB muscle fibres in their *gluteus medius*[2,4,5], whereas Thoroughbred racehorses have 7–11% Type-I, 57–61% Type-IIA and 28–32% Type-IIB in their *gluteus medius* muscle[50]. Therefore, the high muscle glycogen concentration, fast rate of glycogenolysis during exercise and slow rates of muscle glycogen synthesis after exercise observed in horses when compared with humans may be partly related to a lower percentage of slow-twitch Type-I muscle fibres in major locomotory muscles.

Exercise performance and muscle glycogen

In human athletes low muscle glycogen concentrations before exercise are associated with decreased exercise performance and conversely, high muscle glycogen concentrations enhance performance[40]. Increased muscle glycogen availability prior to exercise has been shown to enhance endurance exercise performance in humans[38,51], and it is common practice in cyclists and marathon runners to reduce training and increase carbohydrate intake during the days prior to a competitive event. However, each gram of glycogen is stored with ≈2.7 g of water, and glycogen loading may result in a weight gain, which may be detrimental for high-intensity exercise.

The relationship between muscle glycogen and exercise performance has not been investigated as extensively in horses. However, low muscle glycogen concentrations prior to exercise in horses appear to decrease exercise performance at moderate- and high-intensity exercise. Time to exhaustion in horses trotting at 6.5 mph (3 m s^{-1}) decreases by 35% when

muscle glycogen prior to exercise is 70% lower than normal[52]. Anaerobic work performance, as measured by pulling increasing weight loads on a sled, decreases by 31% in horses when muscle glycogen is 42% lower than normal[53]. Anaerobic work performance tests are designed to estimate the capacity to perform brief maximal-intensity exercise that relies mostly on utilization of the intramuscular ATP–phosphocreatine pool and anaerobic glycolysis to fuel work during high-intensity exercise. In a different study under controlled laboratory conditions, horses undergoing three consecutive days of aerobic intense exercise followed by 1 min sprints had a depletion of muscle glycogen of 55–75%[31,32]. When muscle glycogen remained decreased by 60%, maximum accumulated oxygen deficit, which is another estimate of anaerobic capacity, and run time to fatigue during an 'all out' sprint of 2 min at 25 mph, decreased by 26% and 28% respectively[32].

In summary, exercise- and dietary-induced muscle glycogen depletion is associated with decreased exercise performance both at low- and high-intensity exercise in horses. However, attempts to increase muscle glycogen concentration above that usually found have proved unfruitful and the effect of high muscle glycogen concentrations on exercise performance in horses is unknown.

Muscle glycogen synthesis

Muscle glycogen synthesis after exercise will depend on substrate availability and interval from completion of the exercise bout to delivery of substrate. Muscle glycogen replenishment is enhanced in horses when supplemental glucose is administered as an intravenous infusion ($6\,g\,kg^{-1}$)[54]. However, intragastric administration of an oral glucose polymer at $3\,g\,kg^{-1}$ does not enhance muscle glycogen synthesis in horses[55], unlike ingestion of the same amount of glucose in humans. In these studies, by 24 h after exercise muscle glycogen did not return to concentrations found prior to exercise, even for those horses administered supplemental oral or intravenous glucose[54,55]. In contrast, humans with similar degrees of glycogen depletion have replenished or even supercompensated muscle glycogen stores by 24 h when glucose is ingested as a solution or as meals with a high glycaemic index.

In Thoroughbreds and trotting Standardbreds, the rate of muscle glycogen synthesis after high-intensity sprinting exercise, which lowers muscle glycogen concentration by 30–40%, varies between 0.6 and $1.5\,mmol\,kg^{-1}\,ww\,h^{-1}$ (refs 46, 47 and 56). Unlike other animals, horses fed increasing amounts of digestible carbohydrate have only a minimal increase in the rate of muscle glycogen synthesis[57], and the rate of glycogen synthesis after high-intensity sprint exercise is $\approx 1.5\,mmol\,kg^{-1}\,ww\,h^{-1}$ (ref. 46) which is four times lower than values observed in human athletes[58,59]. In a controlled laboratory study, horses which had undergone three consecutive days of aerobic intense exercise followed by 1 min sprints had a depletion of muscle glycogen of 55–75%[31] and were subsequently fed one of three isocaloric diets containing increasing amounts of soluble carbohydrates for 3 days[57]. For those horses fed a diet high in grain and low in roughage, the rate of muscle glycogen synthesis was higher and replenishment was complete by 3 days, whereas those horses fed a mixed hay and grain diet, or mostly hay, did not attain complete replenishment of muscle glycogen. The rate of muscle glycogen synthesis was $\approx 1.5\,mmol\,kg^{-1}\,ww\,h^{-1}$ (ref. 57). Therefore, muscle glycogen synthesis in horses is complete in 3 days when the diet fed after exercise contains sufficient starch. However, long-term feeding of such a high proportion of starch may not be well tolerated by horses.

The reasons for the relatively slower rate of muscle glycogen replenishment after exercise in horses, when compared with other species, have not been elucidated. One possibility, as discussed below, is that the gastrointestinal function of the horse is not well suited to digest starch and other soluble carbohydrates that will be a source of glucose for glycogen replenishment. If this is the case, the limiting factor is glucose availability from the gastrointestinal tract. This possibility is supported by the fact that intravenous glucose supplementation enhances muscle glycogen synthesis in horses after exercise, whereas oral glucose polymer administration does not[54,55]. A second possibility is that those molecular mechanisms involved in insulin-stimulated glycogen synthesis are not as functional as in other species. Two of the mechanisms involved in glycogen synthesis are: (1) the insulin-stimulated translocation of glucose transporters (glucose transporter type 4) from intracellular vesicle pools to the sarcolemma, and (2) the activity of glycogen synthase. The molecular mechanisms underlying glycogen synthesis in horses are an active area of research but there are no published studies at this point in time.

Nutritional interventions to alter/optimize carbohydrate metabolism during exercise in horses

Effect of meal type prior to exercise on carbohydrate metabolism during exercise

Studies performed in horses have described the effects of different meal types prior to exercise on a number of plasma substrates and hormones during and after moderate-intensity exercise[60–66]. In summary, ingestion of a high-glycaemic meal, such as corn, 2–4 h prior to a moderate-intensity exercise bout results in transient decrease in plasma glucose concentration

during exercise, attenuation of exercise-induced increase of NEFA concentration and increased serum insulin concentration during exercise when compared with horses not fed or fed a hay meal. These alterations in plasma and serum concentrations of substrates and hormones have been hypothesized to be deleterious for performance in horses, presumably because they lead to impaired substrate use during exercise. However, a complete quantitative analysis by indirect calorimetry and stable isotopic tracer methods of the effects of meal type prior to exercise on substrate metabolism has not been conducted until recently[28]. Horses fed a corn meal an hour prior to exercise at 50% $\dot{V}O_{2max}$ for 60 min have greater rates of blood-borne glucose oxidation and whole-body carbohydrate oxidation when compared with those eating an isocaloric meal of hay or those from which food has been withheld; however, ingestion of a meal of corn had no sparing effect on muscle glycogen utilization[28]. In addition, neither the latter nor previous studies have determined the effect of meal type, or of withholding feed prior to exercise, on athletic performance.

Dietary manipulations intended to minimize muscle glycogenolysis during exercise and/or optimize muscle glycogen synthesis after exercise

Horses fed a fat-supplemented diet (10% of fat as weight) for 3 weeks have greater muscle glycogen concentration prior to exercise and greater muscle glycogenolysis during sprinting exercise when compared with those fed a control diet of hay and grain[67]. However, the diets fed in this study appear not to be isocaloric. In a study in which horses were fed a diet of 15% added-fat, when compared to no-fat feeding (diets were not isocaloric), muscle glycogen concentration was lower before and after exercise and net muscle glycogenolysis was not different[68]. In another study in which horses were fed isocaloric diets after exercise, one of the two diets containing 5% of fat, muscle glycogen synthesis was not different[69]. In summary, some authors claim that feeding horses a high-fat diet has a muscle glycogen-sparing effect or increases muscle glycogen concentration; however the evidence is conflicting, partly due to differences in study design, and further study is required.

One of the causes of the horse's limited ability to digest starch in grains may be its low rate of amylase secretion. Amylase is the enzyme, released by the pancreas, responsible for degradation of starch. The output of amylase per unit weight of pancreatic tissue in horses is only 5–6% that of pigs, therefore the amount of starch horses can tolerate is relatively small compared with other monogastric animals[70,71]. Similarly, horses, when compared with cattle, have a pancreatic flow that is three times higher but amylase concentration in pancreatic secretion is 1/10 of that in cattle[72,73]. Therefore, the horse's ability to secrete amylase into the small intestine is lower than in other herbivores or other monogastric animals. The maximal starch ingestion tolerated by horses is 0.3–0.4% of body weight; in other words a 450-kg horse (≈1000 lb) has a maximal starch digestibility of 1.4–1.8 kg (3–4 lb) of starch, which is equal to 2.3–3 kg (5–6.7 lb) of corn[74].

As described previously under 'Muscle glycogen synthesis', horses that undergo three consecutive days of muscle glycogen-depleting exercise will have an enhanced rate of muscle glycogen synthesis when fed a diet high in starch. In order to avoid the gastrointestinal complications of feeding excessive amounts of grain to horses, one possibility is to increase the number of feedings per day and decrease the amount of each meal. In doing so, one would minimize the risks of diets high in grain intake without compromising even further muscle glycogen synthesis.

In summary: (1) energy metabolism of horses during exercise is different to that of humans and dogs, therefore data obtained in other athletic species should not be directly extrapolated; (2) carbohydrate availability may limit performance in horses as demonstrated by decreased exercise performance when muscle glycogen concentrations are low, as well as by increased exercise performance when glucose is supplemented intravenously; and (3) horses have a limited capacity for rapid muscle glycogen synthesis when compared with other species, which may be related to differences in diets and in adaptations of the gastrointestinal tract.

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