

NEW METHODS FOR ASSESSING SUBSTRATE UTILIZATION IN HORSES DURING EXERCISE

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There are two major goals in designing diets and feeding regimens for athletic horses: 1) to meet the caloric requirements for maintenance and the work performed; and 2) to optimize substrate availability and utilization during exercise. Although there is a substantial body of knowledge pertaining to the digestible energy requirements for working horses, the effects of different diets and pre-exercise feeding strategies on substrate utilization during exercise are less well understood. Several important questions arise when considering the effects of a dietary manipulation on substrate metabolism during exercise. First, do such manipulations alter substrate availability during exercise? (e.g. greater availability of muscle glycogen or circulating free fatty acids). Second, does the manipulation alter the mix of substrates utilized during exercise? (e.g. greater use of fat when fed a fat-supplemented diet). Finally, is there a change in the relative contributions by muscle (glycogen, triacylglycerols) vs. non-muscle (blood glucose and fatty acids) fuel sources to energy production? Particularly during prolonged athletic activities, such as endurance rides and the speed and endurance test of a three-day event, both the availability of fuel substrates and the efficiency with which these substrates are utilized can affect exercise performance. Therefore, recommendations for feeding these animals must be based on a sound knowledge of the effects of diet and feeding on substrate availability and utilization during exercise.

A number of equine studies have examined the effects of both acute (pre-exercise feeding) and chronic (diet adaptation) dietary manipulations on the metabolic response to exercise in horses. Unquestionably, the results of these studies have provided important information on the horse's response to different feeding practices. However, the majority of these investigations have used measurement of plasma and muscle substrate concentrations as the primary tool for assessment of substrate metabolism. While these measures provide a qualitative assessment of the effects of diet manipulations on substrate metabolism, they do not allow for quantitative assessment of whole body substrate utilization. Rather, techniques such as indirect calorimetry and isotopic tracer methods are necessary for quantification of rates of substrate use.

The objective of this paper is to discuss the various techniques available for assessment of substrate metabolism in the horse during exercise, with a particular emphasis on indirect calorimetry and the stable isotope tracer method.

Fuels for Energy Production During Exercise

Carbohydrate and lipid are the major fuels used by working muscles during exercise. Although there are alterations in protein metabolism during exercise, data from several species indicate that protein is used minimally for energy production. The main *endogenous* fuel reserves are present in skeletal muscle, liver, and adipose tissue. Glycogen present in liver and skeletal muscle represents the storage form of carbohydrate; during exercise, additional glucose is provided by hepatic gluconeogenesis. Two major sources of fat are oxidized during exercise: non-esterified fatty acids (NEFA) released from triacylglycerols stored in adipose tissue and transported by the bloodstream to skeletal muscle, and NEFA derived from triacylglycerol deposits located within skeletal muscle fibers. Several authors have provided estimates of the quantity of carbohydrate and fat stored in the horse. Although these estimates will vary with the breed, age, size, and training status of the horse, a 450-kg horse has approximately 3000-4000 g muscle glycogen, 100-200 g liver glycogen, 1400-2800 g muscle triacylglycerol, and 35,000-40,000 g as adipose tissue triacylglycerol (Harris, 1997). Whereas the endogenous supply of fat is virtually inexhaustible, carbohydrate stores are more limited. In human athletes, decreases in muscle glycogen content and plasma glucose (liver glucose supply) contribute to the onset of fatigue during exercise. Similarly, recent studies in horses have demonstrated that glucose availability is a limiting factor for both moderate- and high-intensity exercise performance (Farris et al., 1995; Lacombe et al., 1999). These observations highlight the need for a greater understanding of factors that govern the supply and utilization of fuel substrates in the horse during exercise.

The relative contribution of different substrates to fuel metabolism during exercise is determined by a number of factors, including the intensity and duration of exercise, the fitness of the horse, and the availability of substrates in plasma and muscle. In other species, including humans, there is unequivocal evidence that the mix of substrates oxidized during exercise is also influenced by the diet consumed. Furthermore, these diet-induced changes in substrate oxidation can have profound effects on exercise performance. A prime example is the combination of exercise training and a low carbohydrate diet that results in decreased muscle glycogen concentrations. Such depletion of the carbohydrate stores severely limits exercise capacity.

Methods for Study of Energy Metabolism in Exercise

Several methods have been used to study substrate metabolism in horses during exercise. These include the analysis of changes in plasma and tissue concentrations of substrates and respiratory gas exchange (indirect calorimetry). More recently, our laboratory has adapted stable isotopic tracer techniques for the study of glucose metabolism in horses during exercise. In this section, I will review each of these methods, with an emphasis on the advantages and limitations of the technique and application of the method for studies that examine the effects of diet and feeding practices on substrate metabolism in horses during exercise.

1. Plasma substrate concentrations

Measurement of the plasma concentrations of glucose, NEFA, glycerol, and various amino acids have been widely employed in equine studies. There are several advantages of this method. In laboratory studies involving treadmill exercise, blood samples can be readily obtained at frequent intervals. Furthermore, laboratory analysis of these samples is straightforward and relatively inexpensive. However, it is important to recognize that plasma concentrations of a substrate result from the difference between the rate of release of a substrate into plasma and its rate of removal from the plasma compartment (tissue uptake). Using glucose as an example, the plasma concentration is the net result of two simultaneous processes: (1) hepatic glucose production, with release of glucose into circulation; and (2) irreversible uptake of glucose by the tissues. Importantly, plasma concentrations provide no quantitative information on this relationship and, as a result, they provide limited insight into the dynamics of glucose production and utilization. Similarly, interpretation of changes in plasma NEFA concentrations during exercise is difficult without measuring rates of NEFA release from adipose tissue and rates of tissue uptake and oxidation.

It should be noted that, in some circumstances, examination of the changes in plasma substrate concentrations provides a *qualitative* assessment of alterations in substrate availability and use. Studies by Lawrence et al. (1995), Stull and Rodiek (1995), and Pagan and Harris (1999) have clearly demonstrated that the nature and timing of a pre-exercise meal markedly alter plasma glucose concentrations during exercise. For example, in the study by Lawrence et al. (1995), consumption of corn grain 2.5 to 3 hours before exercise resulted in a large glycemic response (Figure 1). At the start of a standardized exercise test, plasma glucose concentrations in the fed horses were 1-2 mmol/l higher when compared to trials in which no grain was fed. Conversely, during

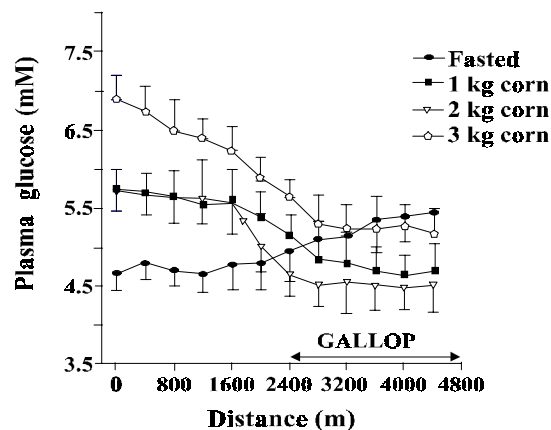


Figure 1. Plasma glucose concentration in exercising horses receiving 0, 1, 2 or 3 kg of corn grain 2.5 to 3 hours before exercise. The first 2400 m of exercise consisted of trotting and walking, while the final 2400 m was undertaken at high intensity (11 m/s). Note 1) the higher glucose concentration at the start of exercise in the horses fed corn grain and 2) that exercise resulted in a decrease in plasma glucose when horses consumed corn but not when they received the controlled treatment (Lawrence et al., 1993)

exercise there were marked decreases in plasma glucose concentrations in trials preceded by corn feeding, whereas plasma glucose was unchanged in the control (no pre-exercise meal) trial. These data provide some evidence for an increase in utilization of plasma glucose under circumstances of increased substrate availability (pre-exercise feeding of carbohydrate). However, estimation of the magnitude of this effect is not possible without measurement of glucose turnover (see below).

2. *Tissue samples*

Samples of skeletal muscle and, less commonly, liver have been obtained from horses before and after exercise in an attempt to quantify various aspects of metabolism in these tissues. In horses, use of the percutaneous muscle biopsy technique (most commonly, sampling of the middle gluteal muscle) has allowed delineation of some aspects of glycogen metabolism during exercise, in particular the effects of the intensity and duration of exercise on rates of muscle glycogen utilization. Given the importance of glycogen for energy production, measurement of glycogen content in skeletal muscle is an important tool in the assessment of substrate stores. Accordingly, several investigators have used this technique to determine the effects of different feeding strategies on storage and mobilization of muscle glycogen in horses during exercise (see Potter, 1998). For example, Essén-Gustavsson et al. (1991) demonstrated a 12% increase in the resting muscle glycogen concentration of horses fed a carbohydrate-rich diet when compared to control or fat-supplemented diets.

The principal advantage of the sampling technique is the ability to quantify changes in tissue substrate storage and use resulting from dietary and exercise interventions. However, interpretation of biopsy data in relation to whole body metabolism is limited by the static and local nature of the sample. As samples can only be obtained at fixed time points (e.g. before and after a period of exercise), it is not possible to determine the dynamics of muscle glycogen use. Rather, the *net rate* of glycogen utilization can be estimated in these circumstances. Furthermore, the change in glycogen content measured in one muscle (e.g. middle gluteal muscle) may not be representative of glycogen utilization in other working skeletal muscle. Even within a single muscle, differences in the fiber-type composition (percentage of type I vs. type II fibers) of samples collected before and after exercise may confound interpretation of the data. Given that the rate of glycogen utilization differs between fiber types, variance in the percentage of type I vs. type II fibers will lead to erroneous estimates of glycogen utilization rates unless single fiber analysis is performed. Finally, measurement of substrate concentrations in muscle samples does not allow for differentiation of the contributions to energy production by intra- vs. extra-muscularly derived substrates. Clearly, this is a major limitation for studies of substrate metabolism during sustained exercise when a substantial proportion of fuel substrate will be derived from non-muscle sources (i.e. liver, adipose tissue, gut).

Compared to use of the muscle biopsy technique, relatively few equine exercise studies have measured changes in liver substrate content. Collection of liver biopsy

samples is an invasive procedure and it can be difficult to obtain an adequate sample, particularly after exercise when the horse's respiratory rate is high. As the liver is the primary source of blood glucose, changes in glycogen content will provide an index of the contribution by liver glycogen to blood glucose supply during exercise. However, during prolonged exercise gluconeogenesis (primarily from lactate, alanine, and glycerol) becomes the predominant mechanism for maintenance of hepatic glucose production. Of course, analysis of liver samples provides no information on the extent to which gluconeogenesis contributes to blood glucose supply during exercise. This disadvantage, together with the invasive nature of the biopsy procedure, limits the usefulness of the liver biopsy technique for equine metabolic studies.

3. Indirect calorimetry

Indirect calorimetry can be used to calculate whole body oxidation rates of carbohydrate and fat. Although the indirect calorimetry technique has been in use since the beginning of the century, there have been surprisingly few equine studies in which this method has been used for estimation of substrate utilization.

Indirect calorimeters are either closed or open. For closed systems, the horse is required to wear a tight-fitting face mask, such that all of the expired air can be collected. Although this method is the most appropriate for resting respiratory gas exchange measurements, closed mask systems severely restrict breathing patterns during exercise and are unsuitable for metabolic studies in this circumstance. On the other hand, open-circuit calorimeters are a valuable tool for equine exercise studies. With open-circuit calorimeters, the horse wears a loose-fitting face mask that does not interfere with normal breathing patterns and, unlike closed systems, does not result in the rebreathing of expired carbon dioxide. This problem is avoided by use of a large fan that draws air through the system at a flow rate several fold higher than the horse's expiratory flow. A sample of the expired air is drawn from the main stream of air flowing away from the face mask and this sample is analyzed for oxygen and carbon dioxide contents. These measurements, together with knowledge of the air flow rate through the system, allow calculation of the horse's oxygen consumption (VO_2), carbon dioxide production (VCO_2), and respiratory exchange ratio (RER). During exercise, these measurements are typically made at frequent intervals (e.g. every 30 s).

From the VO_2 , VCO_2 , and RER values, calculations of rates of carbohydrate and fat utilization can be made. Several simple equations have been developed for these computations. These calculations are based on the recognition that the three major classes of substrates (i.e., carbohydrates, lipid, and protein), when oxidized, consume O_2 and release CO_2 in a ratio specific for each substrate. This ratio of metabolic gas exchange in the combustion of food is termed the respiratory quotient, or RQ. The RQ is 1.0 for carbohydrates, 0.71 for tripalmitatoyl-glycerol, and 0.80 for protein (Livesey and Elia, 1988). The application of the RQ is based on the assumption that the exchange of oxygen and carbon dioxide measured at the lungs reflects the actual gas exchange from nutrient catabolism in the cell. Therefore, the respiratory exchange ratio (VCO_2 divided by VO_2)

provides a measure equivalent to the RQ. As further discussed below, this assumption is valid under steady-state exercise conditions (constant load, low to moderate intensity exercise), but less valid during higher intensity work. When the RER is at or near 1.0, carbohydrate is the sole source of substrate for energy production. Conversely, RER values approaching 0.71 imply that almost all energy is being derived from lipid sources. Quantitatively, rates (grams per minute) of carbohydrate and fat oxidation can be calculated according to the following formulas:

$$\text{Carbohydrate oxidation} = 4.58 \text{ VCO}_2 - 3.23 \text{ VO}_2 \quad (\text{equation 1})$$

$$\text{Fat oxidation} = 1.70 \text{ VO}_2 - 1.69 \text{ VCO}_2 \quad (2)$$

The percentage of energy derived from total carbohydrate and fat oxidation and total energy expenditure (TEE), as kJ/min, can be calculated from:

$$\% \text{CHO} = [(\text{RER} - 0.71)/0.29] \times 100 \quad (3)$$

$$\% \text{Fat} = 100 - \% \text{CHO} \quad (4)$$

$$\text{TEE} = [(\% \text{CHO}/100) \times \text{VO}_2 \times 21.1 \text{ kJ/l}] + [(\% \text{Fat}/100) \times \text{VO}_2 \times 19.7 \text{ kJ/l}] \quad (5)$$

(the values 21.1 and 19.7 represent the number of kilojoules burned per liter of oxygen consumed for 1 gram of carbohydrates and fat, respectively).

The following example is provided to demonstrate the utility of indirect calorimetry for calculation of rates of substrate utilization. In this example, we will assume that a 500 kg horse completes 60 min of treadmill exercise at a workload equivalent to 50% of its maximum rate of oxygen consumption, or $\text{VO}_{2\text{max}}$ (a fast trot that is maintained throughout the 60 min of work). Values for O_2 , VCO_2 (liters of oxygen consumed or carbon dioxide produced per min of exercise), and RER are depicted in Figure 2. During this type of exercise, there will be a steady decline in VCO_2 and RER throughout the exercise bout, indicating greater use of

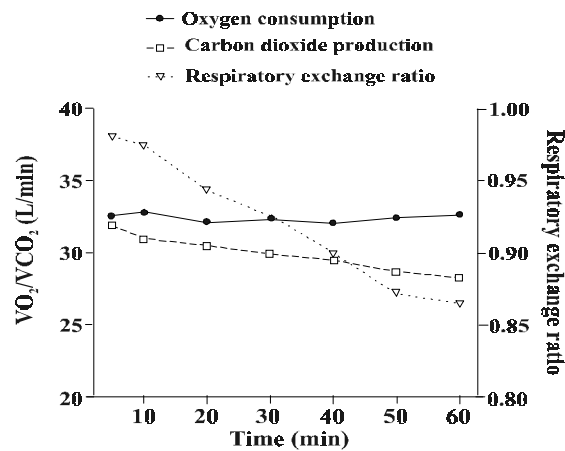


Figure 2. Oxygen consumption (VO_2), carbon dioxide production (VCO_2), and respiratory exchange ratio (RER) in a horse during 60 min of exercise at a workload equivalent to 50% of $\text{VO}_{2\text{max}}$. Note the steady decreases in VCO_2 and RER during exercise, indicating a progressive increase in energy production from lipid sources.

lipids for energy production as exercise progresses. I have used the respiratory gas exchange values at 5, 15, 30, 45, and 60 min of exercise to quantitate this change in the mix of substrates oxidized with increasing duration of exercise. The calculations below are for the 5 and 60 min time points. Data for all time points are presented in Table 1.

Table 1. Respiratory gas exchange and substrate utilization data in a horse during 60 minutes of exercise at a workload equivalent to 60% of maximum oxygen consumption (VO_{2max}).

<i>Time (min)</i>	5	15	30	45	60
VO ₂ (l/min)	32.6	32.2	32.4	32.6	32.7
VCO ₂ (l/min)	31.9	30.8	30	29.2	28.3
RER	0.981	0.956	0.926	0.895	0.865
TEE (KJ/min)	684	668	660	641	628
CHOox (g/min)	40.3	37	32.7	28.4	24
FATox (g/min)	1.7	2.75	4.4	6.1	7.8
%CHO	93.5	84.8	74.5	63.8	53.4
%FAT	6.5	15.2	25.5	36.2	46.6

VO₂ = oxygen consumption; VCO₂ = carbon dioxide production; RER = respiratory exchange ratio; TEE = total energy expenditure; CHOox = rate of carbohydrate oxidation; FATox = rate of lipid oxidation; %CHO = percentage of TEE derived from carbohydrate sources; %FAT = percentage of TEE derived from lipid sources.

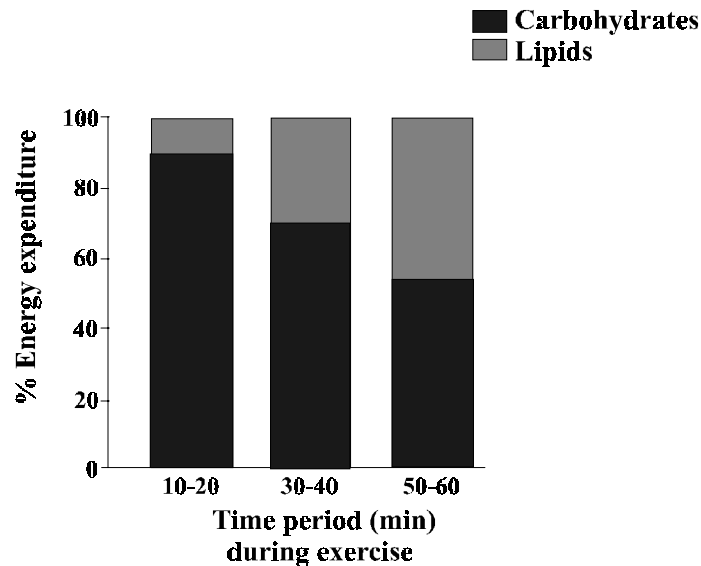
e.g. **5 min:** CHO oxidation = $(4.58)(31.8) - (3.23)(32.6)$
 $= 40.3$ g/min
 Fat oxidation = $(1.70)(32.6) - (1.69)(31.8)$
 $= 1.7$ g/min
 %energy from CHO = $[(0.981-0.71)/0.29] \times 100 = 93.4\%$
 %energy from FAT = $100 - 93.4 = 6.6\%$
 TEE = $[(0.934)(32.6)(21.1)] + [(0.066)(31.8)(19.7)] = 684$ kJ/min

60 min: CHO oxidation = $(4.58)(28.3) - (3.23)(32.7)$
 $= 24.0$ g/min
 Fat oxidation = $(1.70)(32.7) - (1.69)(28.3)$
 $= 7.8$ g/min
 %energy from CHO = $[(0.865-0.71)/0.29] \times 100 = 53.4\%$
 %energy from FAT = $100 - 53.4 = 46.6\%$
 TEE = $[(0.5345)(32.7)(21.1)] + [(0.466)(28.3)(19.7)] = 629$ kJ/min

These data highlight two important concepts concerning energy metabolism during low-to-moderate intensity exercise. First, in the early part of exercise carbohydrates are the predominant substrate for energy production, with minimal contribution from lipid sources. Conversely, after one hour of work the contributions by carbohydrates and fats to energy production are almost equal (Figure 3). During longer but lower intensity work, such as that expected of

an endurance horse, the proportion of energy derived from fats would be even higher. Second, the greater efficiency of energy production from fats is illustrated by the progressive decrease in the rate of energy expenditure over the course of the exercise bout (Table 1). That is, with increasing exercise duration, less energy (and, therefore, lower heat production) is required to sustain the same amount of work.

Figure 3.



As mentioned, few equine studies have employed the indirect calorimetry method for estimation of substrate utilization during exercise. However, the power of this method for detection of the effects of different diets on substrate metabolism was well demonstrated in a study by Pagan and co-workers (1987). In that study, a 3 x 3 Latin square design was used to determine the effects of feeding diets containing different levels of carbohydrate, fat, and protein. The diets were a 12% crude protein (CP)(as fed basis) commercial horse feed (control diet), a 20% CP feed (high protein diet), or an 11% CP feed containing 15% added soybean oil (high fat diet). Each diet was fed for 4 weeks and exercise tests were performed in the 3rd and 4th weeks of each period. During a long, slow exercise test (105 min at a workload of approximately 35% VO_{2max}), values for RER were significantly lower in the high protein and fat diet trials than in the control diet between 30 min and 90 min (the last measurement) of exercise (Figure 4). These differences in values for RER indicate a remarkable increase in the use of fat for the high protein and fat diets when compared to the control diet. For example, at the 90 min time point, there was about a 50:50 contribution by carbohydrates and fats to energy production when horses were fed the control diet. In contrast, for both the high protein and high fat diets, approximately 85% of energy was derived from lipid sources during the corresponding period.

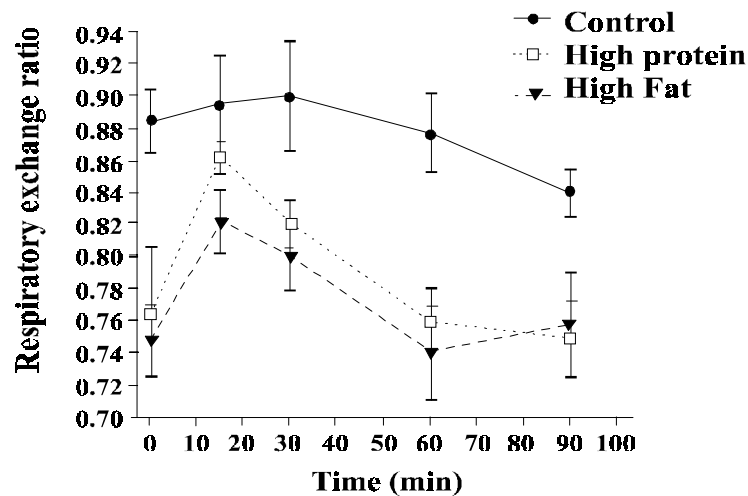


Figure 4. Respiratory exchange ratio (mean \pm SE) in 3 horses at rest and at 3 time points during a submaximal exercise test (trot at 5 m/s) after 4 weeks on each of three diets (control, high protein, and high fat). The lower RER values for the high protein and fat diets indicated a marked increase in use of lipid for energy production when compared to the control diet. (Pagan et al., 1987)

It should be noted that the above formulas and calculations do not include the contributions made by protein to total energy expenditure. Estimates of protein oxidation require measurement of urinary nitrogen excretion. Because the nitrogen content of mixed proteins is approximately 16%, it is assumed that each gram of urinary nitrogen represents the oxidation of 6.25 grams of mixed proteins. For equine exercise studies (and most human studies), the contribution by protein to energy expenditure is considered to be very small and is ignored for the purposes of calculating substrate oxidation rates. Therefore, these estimates are based on the *nonprotein* RER. The validity of these assumptions has not been tested in horses. However, given the technical difficulties associated with complete urine collection during exercise and the likelihood that protein oxidation makes minimal contribution to energy expenditure, use of the nonprotein RER is the most practical approach.

One of the key assumptions with the indirect calorimetry method is that pulmonary gas exchange truly reflects oxygen consumption and carbon dioxide production at the tissue level. Although this assumption is likely true for oxygen (there are negligible oxygen stores in the body), VCO_2 as measured by analysis of expired air is a reliable estimate of tissue CO_2 production only when there is minimal lactate production, i.e. low-to-moderate intensity, predominantly aerobic exercise. During high intensity exercise, when there is significant lactate production in working muscle, a portion of the CO_2 production measured in expired air will be derived from the body's bicarbonate pool. CO_2 production from this source occurs because bicarbonate is used to buffer lactic acid, with the subsequent formation of carbonic acid and release of CO_2 . In this circumstance, estimates of carbohydrate oxidation based on indirect calorimetry

measurements will be erroneously high. Therefore, the indirect calorimetry method is *unsuitable* for calculation of substrate oxidation during brief, high-intensity exercise.

4. Isotopic tracer methods

The isotopic tracer technique has been widely employed in metabolic studies involving a variety of species. Until the last decade, the majority of these studies involved use of radioactive tracer substances. However, given the obvious health and safety concerns associated with use of radiochemicals, there has been a sharp decline in the use of radioactive tracers for studies of energy metabolism. Rather, use of tracers labeled with *stable* isotopes (i.e. nonradioactive) has emerged as an important technique for studying substrate metabolism (Wolfe, 1992). A wide variety of metabolic processes involving carbohydrate, lipid, or protein metabolism can be studied using stable isotopes of carbon (^{13}C), hydrogen (^2H , deuterium), nitrogen (^{15}N), or oxygen (^{18}O). By definition, the term *isotope* refers to all forms of a given element containing different numbers of neutrons (e.g. ^{12}C and ^{13}C are both stable isotopes of carbon). For the purposes of tracer studies, the term *stable isotope* is defined as the nonradioactive isotope that is less abundant than the most abundant naturally occurring isotope. The stable isotopes most commonly used in the synthesis of metabolic tracers are ^2H , ^{13}C , ^{15}N , and ^{18}O (Table 2) (Patterson, 1997). For tracer studies, one or more atoms in the structure of interest is replaced with a stable isotope such as deuterium, e.g. [6,6- ^2H] glucose is frequently used for the study of glucose kinetics. Here, two ^1H atoms at the 6-carbon position on the glucose ring have been replaced by two deuterium (^2H) atoms.

Table 2. The relative abundances of the stable isotopes of elements commonly used in metabolic studies. The isotopes of low natural abundance are used in the synthesis of tracer substances.

Element	Stable Isotope	Atom% Natural Abundance
Hydrogen	1	99.985
	2	0.015
Carbon	12	98.89
	13	1.11
Nitrogen	14	99.63
	15	0.37
Oxygen	16	99.76
	18	0.24

Before further explaining this technique, a few terms must be defined. First, when studying the kinetics of a metabolic substrate (e.g. glucose), we refer to the endogenous unlabeled substrate as the *tracee*. The *tracer* is the form of the tracee substance containing one or more stable isotopes (e.g. [6,6-²H]glucose), and the *isotopic enrichment* is the ratio of tracer to tracee in blood samples. Using glucose as the example, the isotopic enrichment is the ratio of labeled to unlabeled glucose in the sample. One of the underlying principles of this method is that the tracer should not significantly change the tracee pool size (the total amount of tracee substance in the body). Therefore, a typical infusion protocol will result in about a 2-3% enrichment of the blood (i.e. in the case of glucose studies, the tracer is 2-3% of the total glucose pool). Nonetheless, highly sensitive analytical methods (gas chromatography-mass spectrometry) allow detection of very small changes in isotopic enrichment, thus permitting study of the effects of various interventions on the kinetics or turnover of the substrate of interest.

In practical terms, the stable isotopically-labeled tracer substance is administered to “trace” the kinetics of production and utilization of that substance. Two main kinetic parameters are calculated. The *rate of appearance*, or R_a , is the total rate of appearance of the substrate into the sampling pool. Physiologically, the R_a is the production rate of a given substrate. For example, for the glucose system, the R_a reflects hepatic glucose production; for studies of fatty acids, the R_a represents release of NEFA from adipose tissue and lipoproteins. Depending on the feeding state of the animal, a portion of the total R_a may also reflect uptake of the nutrient from the gut. The *rate of disappearance*, or R_d , is the rate of loss of substrate from the sampling pool. Physiologically, the R_d is the rate of irreversible tissue uptake, with perhaps a small component from excretion in urine. During exercise, it is well recognized that more than 90% of the R_d for substrates such as glucose and NEFA reflects uptake and utilization by working muscle (Wolfe, 1992). Taken together, the R_a and R_d provide estimates of the rates of production and utilization of a substrate. Thus, when compared to a static measure such as the blood glucose concentration, a significant advantage of the isotopic tracer method is the ability to monitor the dynamics of substrate metabolism during exercise. Furthermore, it becomes possible to obtain estimates of the sources of carbohydrate and lipid when measurements of plasma kinetics are combined with rates of substrate oxidation (from indirect calorimetry).

The utility of the stable isotope method for measurements of glucose turnover in the horse during exercise is illustrated in Figures 5 and 6. In this example, a primed constant-rate infusion of [6,6-²H] glucose was given over a 90 min period of rest, followed by 90 min of exercise at 40% of VO_{2max} . At rest, the rate of tracer infusion was 0.22 $\mu\text{mol/kg}$ body weight per min. During the transition from rest to exercise, the tracer infusion rate was doubled so as to maintain a relatively steady state in isotopic enrichment. At rest, isotopic enrichment was steady at approximately 3% (Figure 5).

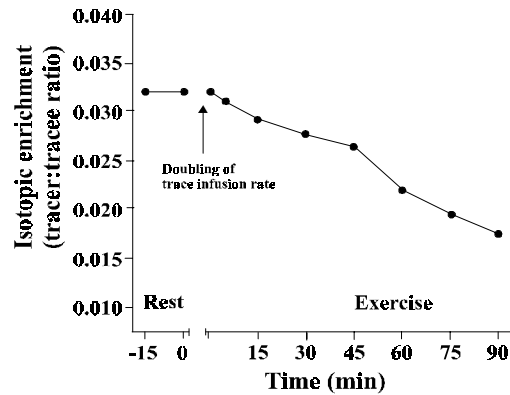


Figure 5. Plasma isotopic enrichment of [6,6-²H] glucose in a horse at rest and during 90 min of exercise at a workload equivalent to 40% of VO_{2max} . Enrichment is expressed as a fraction; therefore, a value of 0.03 is a 3% enrichment. Note the steady decline in enrichment despite the doubling of the tracer infusion rate at the onset of exercise, indicating a large increase in glucose turnover.

Despite the increase in tracer infusion rate, there was a progressive decline in isotopic enrichment during exercise, providing indication of a marked increase in glucose turnover. The magnitude of this change in glucose metabolism is depicted in Figure 6; there were progressive increases in the R_a and R_d of glucose, with values at the end of exercise 6-fold higher than at rest. Importantly, note that plasma glucose concentration was unchanged throughout the trial. Therefore, the tracer method permits detection of large increases in hepatic glucose production (R_a) and tissue glucose utilization (R_d), changes not evident from examination of plasma glucose concentrations. This method offers great potential for future equine studies that examine the effects of different diets on carbohydrate, fat, and protein metabolism.

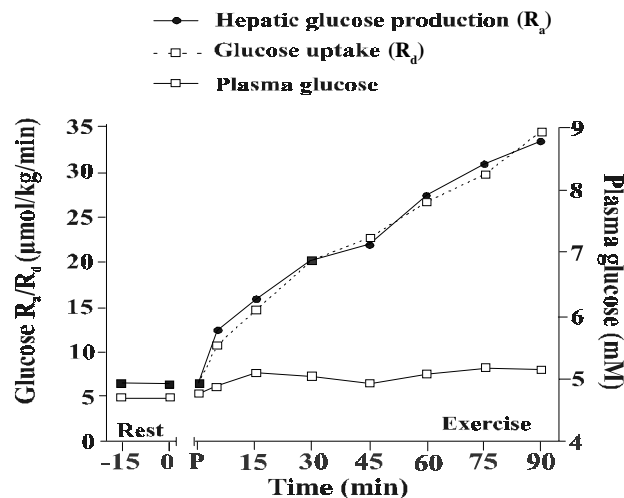


Figure 6. Hepatic glucose production (R_a), glucose uptake (R_d), and plasma glucose concentration in a horse at rest and during 90 min of exercise at a workload equivalent to 40% of VO_{2max} . Note the more than 5-fold increase in glucose production and utilization during exercise, a finding not evident on the basis of plasma glucose concentrations. [P=pre-exercise sample]

Similar to the indirect calorimetry method, the tracer technique is not suitable for assessment of substrate metabolism during high-intensity exercise when the brevity of the exercise task will limit the number of samples that can be obtained and make kinetic analysis difficult. Therefore, the isotopic tracer method is also best suited for studies involving prolonged, low-to-moderate intensity exercise. A further disadvantage of this method is the expense of the instrumentation (gas chromatograph/mass spectrometer) required for measurement of isotopic enrichment. Finally, the cost of the isotope itself can limit applications in the horse. Whereas deuterated tracers are reasonably priced, ^{13}C -labeled tracers (which permit direct measurements of rates of oxidation of that substrate) are currently cost prohibitive for use in horses.

Summary

Several different techniques can be used to study substrate metabolism in the horse during exercise. Although each of these methods has limitations, a combination of indirect calorimetry and stable isotopic tracer methods offers the greatest potential for quantitative analysis of the effects of diet and feeding regimen on substrate metabolism in horses.

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