

Dietary Energy Source Affects Glucose Kinetics in Trained Arabian Geldings at Rest and during Endurance Exercise^{1,2}

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Abstract

Advances in modeling and tracer techniques provide new perspective into glucose utilization and potential consequences to health or exercise performance. This study used stable isotope and compartmental modeling to evaluate how adaptation to a feed high in sugar and starch (SS) compared with a feed high in fat and fiber (FF) affects glucose kinetics at rest and during exercise in horses. Six trained Arabians adapted to each feed underwent similar tests at rest and while running ~4 m/s on a treadmill. For both tests, horses received 100 $\mu\text{mol/kg}$ body weight [6,6-²H]glucose through a venous catheter. Circulating tracer glucose was described for 150 min by exponential decay curves and compartmental analysis. All parameters of glucose transfer increased with exercise ($P \leq 0.004$). Compared with FF horses, SS horses had higher circulating glucose ($P = 0.022$) and fractional glucose transfer rates (min^{-1}) at rest ($P = 0.055$). Exercise increased glucose irreversible loss (mmol/min) more in SS horses ($P = 0.037$). Total glucose transfer during exercise tended to be greater in SS horses (0.027 ± 0.002 mmol/min) compared with FF horses (0.023 ± 0.002 mmol/min) ($P = 0.109$). This study characterized the effect of diet on glucose kinetics in resting and exercising horses using new modeling methods. Horses adapted to a fat-supplemented feed utilized less glucose during low-intensity exercise. Fat supplementation in horses may therefore promote greater flexibility in the selection of substrate to meet energy demands for optimal health and performance. *J. Nutr.* 138: 964–970, 2008.

Introduction

Dietary energy source determines the fuel available to maintain normal biological function such as muscle contraction. Improper regulation of energy substrate is a unifying factor in metabolic diseases, including obesity, insulin resistance, type 2 diabetes, and possibly equine pasture-associated laminitis (1). Exercise is also recommended to normalize metabolic function, as pathways of energy utilization are upregulated to meet increased energy demands during exercise. In horses, exercise may be equally important to health in addition to representing their primary production value.

Considerable work in humans and horses has focused on an optimal diet regimen for athletes. In horses, fat supplementation has been recommended to spare limited muscle glycogen stores and improve exercise endurance (2). Fat supplementation facilitates reduced dietary soluble carbohydrates, thus mitigating postprandial hyperglycemia and hyperinsulinemia, which potentially disrupt energy regulation and contribute to insulin resis-

tance (3,4). In humans, insulin resistance and type 2 diabetes is further associated with altered energy utilization during exercise (5). Evaluating the effect of fat supplementation on energy substrate utilization during rest and exercise may therefore provide important clues into the management of horses both to maintain health and optimize performance.

The diets selected for this study were a feed similar to commercial sweet feeds rich in sugar and starch (SS)⁶ and a feed utilizing fat and fiber (FF) to replace soluble carbohydrate energy sources (3). Exaggerated postprandial hyperglycemia and hyperinsulinemia have been observed following an SS meal, whereas the response to an isocaloric meal of the FF feed resembled that of a horse grazing pasture (6).

Adaptation to the SS feed has also been shown to reduce insulin sensitivity in untrained resting horses compared with the FF feed (3,4). The exercise-trained horses used in the current study previously demonstrated similar insulin sensitivity at rest; however, insulin sensitivity was lower in the SS-adapted horses

¹ Supported by the John Lee Pratt Fellowship, the WALTHAM Centre for Pet Nutrition, and Equine Guelph.

² Author disclosures: K. H. Treiber, R. J. Geor, R. C. Boston, T. M. Hess, P. A. Harris, and D. S. Kronfeld, no conflicts of interest.

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⁶ Abbreviations used: ADF, acid detergent fiber; BC, body condition score; BW, body weight; EGP, endogenous glucose production; FF, fat and fiber feed; MRT, mean residence time; NDF, neutral detergent fiber, NFC, nonfiber carbohydrates; SS, sugar and starch feed.

during an exercise protocol identical to that performed in the current study (7). The study reported here evaluated the concomitant changes in glucose transfer independent of insulin action resulting from adaptation to the high-carbohydrate SS feed compared with the fat-supplemented FF feed.

Kinetic models describe the appearance (mobilization) of fat or glucose in the circulation and clearance of these energy substrates (presumably into cells where they are either stored or, during exercise, oxidized for ATP synthesis). Such models have been used extensively in human research to evaluate diet and exercise recommendations for exercise performance and reducing health risks associated with metabolic dysfunction (8). Recently primed, constant rate infusions have been applied to the horse to evaluate the effects of exercise and energy substrate on glucose kinetics (9–11). For this study, single-injection tracer technique and compartmental modeling were introduced to explore new characteristics and previous assumptions of the glucose system, including compartment volumes and masses. This technique was applied to resting horses over 30 y ago to evaluate the effect of diet on glucose transfer (12–14). Since then, technological advancement in stable-isotope tracers and mathematical modeling has increased the accessibility of single-injection kinetic studies and the information that can be gleaned from analysis.

The single injection method and compartmental modeling complement the increasing use of physiological models in horses, namely the minimal model of glucose and insulin dynamics (15,16). Although the minimal model has been used to evaluate the impact of dietary adaptation on insulin action and glucose regulation in horses (7), underlying changes in glucose transfer independent of insulin (the primary component of glucose delivery during exercise) remain to be explored by compartmental modeling.

This study applied single-injection stable isotope tracer methodology and compartmental modeling to glucose tracer disposal curves from 12 trained Arabian geldings adapted to SS ($n = 6$) or FF feeds ($n = 6$) during rest or constant, low-intensity exercise, to evaluate the effect of dietary energy source on glucose kinetics.

Materials and Methods

Twelve Arabian geldings were maintained on mixed grass/legume pasture and adapted to a FF or SS feed. Horses were matched by body condition score (BC) and age with 1 horse from each pair assigned to each feed group. Diet groups were kept separate with pastures rotated monthly to ensure all subjects were exposed to similar pasture conditions. Horses received 1 kg feed at 0700 and 1400 daily and all feed was eaten. Diet groups were fed collectively, but each horse was offered a single portion in an individual pan. The feed provided ~30% of the recommended daily dietary energy for light work (17). We weighed and scored the horses for BC before beginning feed adaptation and just prior to testing (18). The Virginia Tech Institutional Animal Care and Use Committee approved the study protocols.

The feeds were formulated to be isocaloric and isonitrogenous with vitamin and mineral contents designed to complement the pasture and fulfill present recommendations (17,19). Feed compositions were reported previously (20). Pasture and feeds were sampled monthly and submitted to a commercial laboratory (Dairy One) for proximate analysis (Table 1). Previous studies demonstrated a higher glycemic and insulinemic response to the SS feed (2-fold and 6-fold that of the FF feed, respectively) (6); accordingly, the SS feed has been assigned a high glycemic index and the FF feed a moderate glycemic index (21).

All horses were conditioned for 10 mo to running on a treadmill (EquiGym Products) before undertaking the present tests. Each horse

TABLE 1 Partial proximate analysis of SS and FF feeds¹

	SS feed, 2 kg/d	FF feed, 2 kg/d	Pasture ²
<i>n</i>	6	6	12
Dry matter, %	87.4 ± 0.2*	91.1 ± 0.5	240 ± 22
Feed analysis, g/kg dry matter			
Crude protein	154 ± 8	140 ± 5	183 ± 9
ADF	147 ± 8*	326 ± 6	350 ± 10
NDF	242 ± 12*	451 ± 11	624 ± 12
NFC	522 ± 21*	264 ± 10	116 ± 11
NSC ³	447 ± 21*	131 ± 8	102 ± 9
Crude fat	33 ± 1*	106 ± 8	33 ± 3
Ash	72 ± 6	84 ± 1	105 ± 7
DE, ⁴ MJ/d	22.2 ± 0.4	21.7 ± 0.4	N/A

¹ Values are means ± SEM. *Different from FF, $P < 0.001$.

² Pastures did not differ for diet groups for any variable, $P > 0.26$, and results for all pasture samples were combined.

³ Nonstructured carbohydrates (NSC) determined as water soluble carbohydrate + starch (19).

⁴ MJ/kg feed calculated according to Harris and Kronfeld (60). DE, dietary energy.

exercised for ~1 h, 3 times per week. Exercise consisted primarily of treadmill work (at least once per week) and work in an automatic walker (walk, trot). Horses were also exercised an equivalent amount by riding or lunging when the treadmill and walker were not available.

Each horse underwent 2 similar tests, 1 at rest and 1 during exercise. Horses were randomly assigned to test days and 3 horses from each feed group shared the same test sequence. For all tests, horses were maintained on pasture until the morning of the test and given no feed for at least 18 h prior to the test. A catheter was inserted into the jugular vein the morning of each test. Horses were allowed at least 30 min to rest following the procedure. Basal samples were then taken, followed by initiation of the test. Horses had at least 8 d to recuperate between tests and missed 1 training session before and 1 training session after their exercise test. We initiated all tests between 0900 and 1000.

Rest. Horses consumed grass hay and water ad libitum throughout this test. Although horses consumed and drank only a small amount during the test, the availability of feed and water ensured the animals remained calm during the test. Consumption of hay during similar tests has not been shown to significantly impact circulating glucose concentrations. Glucose arriving in circulation would be predominantly from gluconeogenesis following hindgut fermentation of forage, which would have been consumed in all horses prior to the test. Feed deprivation, which is typical for glucose studies in other species, was deemed inappropriate, because horses are continuous feeders. Feed deprivation potentially alters glucose kinetics and dynamics as the subject switches to stored energy sources and energy conservation, a condition which should be carefully considered when interpreting the results of metabolic studies, particularly in animals accustomed to continuous intake.

Following baseline (0 min) samples, an i.v. glucose tracer dose (100 $\mu\text{mol/kg}$ body weight [BW] of [6,6-²H] glucose, 98% enriched; Sigma-Aldrich) in 0.9% saline solution was administered rapidly through the catheter. Blood samples were collected at 1- to 30-min intervals for 150 min following the glucose injection.

Exercise. Following baseline samples at rest, each horse was warmed up by walking for 10 min at 1.8 m/s at a 0% slope then trotting for 15 min at a 2% slope and 60% of its lactate threshold (~4 m/s) as determined by a lactate breakpoint test performed previously (7). Samples were collected at 10 and 20 min of warm-up followed by a sample at 25 min of warm-up, which constituted the 0-min sample for the test. The horse continued at the same speed (60% of its lactate breakpoint) for the remainder of the test. Dosing and sampling during the exercise test were identical to the test at rest, with the i.v. glucose tracer dose administered followed by blood collection for 150 min. We measured heart rate throughout the exercise test using a commercial digital heart rate monitor (Polar Pacer, Polar CIC).

Blood sample handling. Blood was withdrawn into sample tubes containing EDTA anticoagulant (Vacutainer evacuated blood collection tubes, Fisher Health Care) and placed in ice water until centrifuged at $3000 \times g$; 10 min. Plasma was removed within 30 min of collection and frozen at -20°C until analysis. Plasma glucose was analyzed by enzymatic assay (Beckman Instruments, glucose procedure no. 16-UV, Sigma Diagnostics). The intra-assay CV of duplicate samples was $<1\%$.

Plasma $[6,6\text{-}^2\text{H}]$ glucose enrichments were analyzed by GC-MS analysis with electron impact for penta-acetate derivatives of glucose. Protein was precipitated from 0.2 mL of sample plasma by adding 2 mL acetone. The mixture was centrifuged at $3000 \times g$; 10 min at 4°C and the removed supernatant was vacuum dried. The dried remnant was dissolved in 0.5 mL of a 2:1 v:v acetate anhydride:pyridine and heated on a heating block at 60°C for 10 min. After cooling to room temperature (5–10 min), 1.0 μL of the solution was injected on the GC-MS system (Hewlett-Packard 6890 GC with 5973N Mass Selective Detector, Hewlett-Packard). Percent isotope molar enrichment was determined using the selective ion-monitoring mode with the ratio of peak areas for ions m/z 100 (labeled glucose) and m/z 98 (unlabeled glucose) applied to a calibration curve (22). The intra-assay CV of duplicate samples was $<1\%$.

Modeling. Tracer as fraction of dose per liter was calculated as: $\text{Tr}(t) = [E \cdot G(t)]/D$, where t is the time in min, E is the plasma isotopic enrichment (%), $G(t)$ is the plasma glucose concentration (mmol/L) at t min, and D is the tracer dose (mmol). Curves of $\text{Tr}(t)$ were modeled using WINSAAAM software for a 2-compartment model with loss from the secondary compartment only. Tracer curves from rest and exercise were modeled simultaneously with resting curves providing a baseline for rate constants (k_{ij} , min^{-1}) describing movement of glucose to compartment i from compartment j . An exercise effect parameter (p_{ij}) was added to each rate constant for fitting of the exercise curve such that: $k_{ij\text{exercise}} = k_{ij\text{rest}} + p_{ij}$. From the determined curves and rate constants, the remaining model parameters (compartment volumes, masses, rate constants, and mass transfers) could be determined (23–25). We also determined noncompartmental parameters (clearance rate, mean residence times (MRT), turnover rate constants, turnover times, and turnover rates). Equations for parameter determinations are described in the Appendix and elsewhere (26).

Statistics. Statistics were performed using Intercooled Stata version 9 (StataCorp). Differences between exercise and rest for BW, plasma glucose, and model parameters were determined using a 2-way ANOVA with horses nested in diet and, where appropriate, time was entered as a repeated measure. Interaction effects were evaluated by regression with interaction expansion and clustering by horse. Diet effects within the resting test, exercise test, or the exercise effect parameters were determined using the 2-sample Kruskal Wallis statistic. Because the nonparametric tests resulted in finite combinations for rank comparisons between diet groups where $n = 6$, P -values approaching significance were limited to $P = 0.109$, $P = 0.078$, $P = 0.055$, and $P \leq 0.037$. To avoid overlooking results of potential physiological relevance, we chose to report significance as $P \leq 0.055$ and trends as $P = 0.109$ or 0.078 . P -values are clearly indicated for the sake of interpretation. Results are reported as means \pm SEM unless otherwise indicated.

Results

Feeds differed as expected in energy sources and provided similar daily energy (Table 1). The estimated total dietary energy was 78.2 MJ/d with the feeds offering 28% of this requirement. Prior to diet adaptation, diet groups did not differ by BW, BC, or age. Horses weighed 456 ± 13 kg, had BC scores of 5.3 ± 0.3 (range 4–7), and were 11 ± 1 y old (range 5–16 y). There was no effect of diet adaptation on BW nor was there a difference in BW between the exercise and rest test. Ambient temperature on the days of the test did not differ between diet groups or trials. Temperature was $7 \pm 1^{\circ}\text{C}$.

Basal heart rate did not differ between diet groups or trials. Mean heart rate during exercise ranged from 90 to 130 beats per minute (bpm) for individual horses. Heart rate during exercise did not differ across feeds and declined from 113 ± 4 bpm at 40 min to 108 ± 3 bpm at 150 min ($P = 0.086$). Because predetermined lactate breakpoints did not differ, speed did not differ during the exercise test for FF horses (4.0 ± 0.1 m/s) compared with SS horses (3.7 ± 0.1 m/s).

Plasma glucose. Plasma glucose increased $\sim 15\%$ immediately following the glucose tracer dose in both tests. Glucose was ~ 0.44 mmol/L higher in SS-adapted horses across both tests (overall diet effect $P = 0.022$), but diet did not affect changes in glucose during tests (Fig. 1). The small fluctuations in plasma glucose concentrations were not expected to invalidate steady-state assumptions of the modeling (26,27) and were similar across diet groups.

Model analysis. Effects of exercise independent of diet are reported elsewhere (26). Briefly, exercise increased all rate constants, mass transfer rates, clearance rate, MRT, and turnover rates. Exercise also decreased the volume and mass of the primary glucose compartment and MRT ($P \leq 0.004$).

Tracer curves demonstrated an interaction ($P = 0.034$) between test and feed adaptation, a noncompartmental indication of a greater rate of glucose disposal for SS horses during exercise (Fig. 2). This was supported by a trend ($P = 0.077$) for

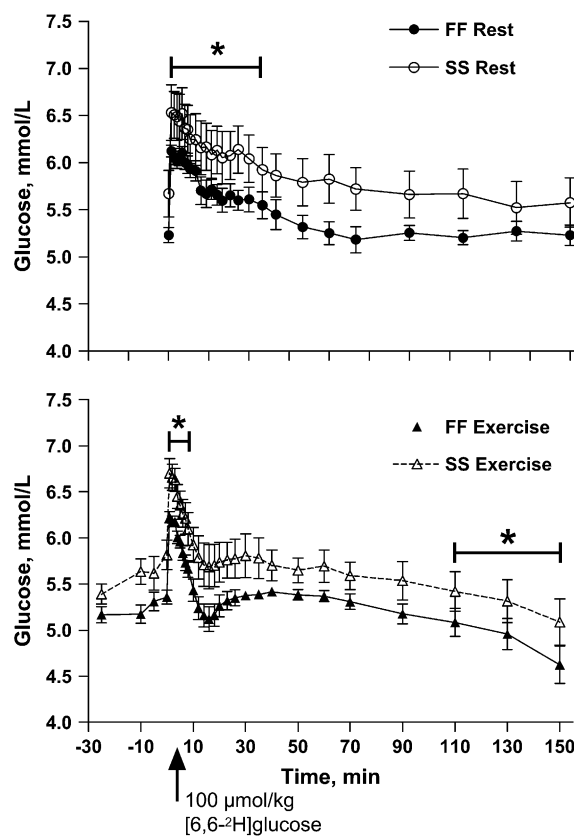


FIGURE 1 Plasma glucose concentrations at rest (*top*) and during exercise (*bottom*) in trained Arabians adapted to SS feeds, $n = 6$, or FF feeds, $n = 6$. At 0 min, an i.v. bolus of $100 \mu\text{mol/kg}$ BW of $[6,6\text{-}^2\text{H}]$ glucose was administered. For the exercise test, a 25-min warm-up occurred before the 0 min dose. Values are means \pm SEM. *Values bracketed differ from 0 min across both diet groups, $P < 0.05$.

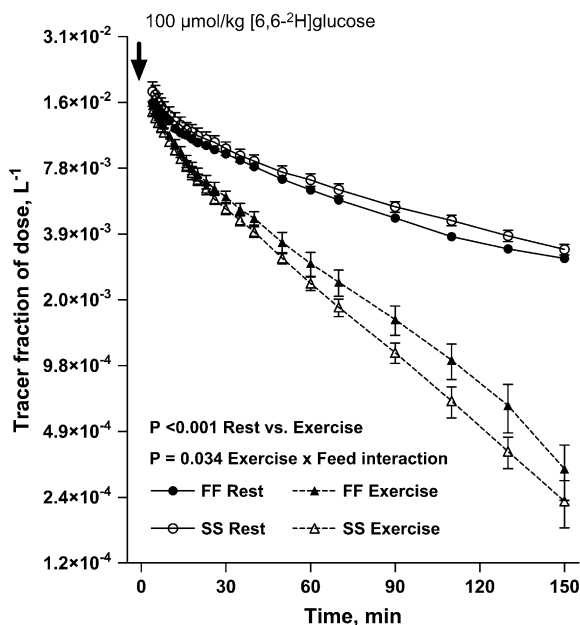


FIGURE 2 Tracer curves at rest and during exercise in trained Arabians adapted to SS feeds, $n = 6$, or FF feeds, $n = 6$. Values are means \pm SEM.

lower tracer fraction during exercise in SS horses compared with FF horses (Fig. 1).

At rest, SS-adapted horses demonstrated greater fractional glucose transfer rates (k_{12} , $k_{21} = -k_{11}$ and $-k_{22}$; min^{-1}) ($P = 0.055$) (Fig. 3). The corresponding exercise effect parameters or the resulting exercise values did not differ ($P \leq 0.20$). However, exercise did not affect k_{12} in 3 SS horses, whereas all FF horses demonstrated increased k_{12} with exercise.

Total glucose irreversible loss [which in steady state is equivalent to endogenous glucose production (EGP) $\text{mmol}\cdot\text{min}^{-1}\cdot\text{kg BW}^{-1}$] revealed a greater exercise effect in SS horses ($P = 0.037$), mirrored by an interaction ($P = 0.044$) between feed adaptation and test. As a result, although feed groups had similar EGP at rest, EGP tended to differ between FF and SS horses during exercise ($P = 0.109$) (Fig. 3).

The volume of the primary compartment (V_1 ; $\text{mL}/\text{kg BW}$) decreased more from rest to exercise in FF horses than in SS horses (feed by test interaction, $P = 0.023$). As FF horses tended ($P = 0.109$) to have a greater V_1 at rest, this difference resulted in similar V_1 during exercise for the feed groups. The same pattern was observed for the mass of the primary compartment (Q_1 ; $\text{mmol}/\text{kg BW}$) (Fig. 3).

Feed adaptation did not affect plasma glucose clearance rates or MRT (Fig. 4). At rest, pool turnover times (min) were longer ($P = 0.055$) for FF horses compared with SS horses, reflecting the differences in k_{11} , k_{22} , or Q_1 , although feed adaptation did not affect turnover rates (mmol/min) (Fig. 4).

Discussion

This study demonstrated that glucose kinetics are upregulated during constant low-intensity exercise but that this upregulation is affected by dietary adaptation. Advancing techniques such as stable-isotope method and compartmental modeling elucidate these diet effects, facilitating improved nutrition management of horses to avoid metabolic health risks and optimize exercise performance. The methods used in this study have not been applied to horses in over 30 y and, to our knowledge, never during exercise. Nevertheless, the results agree with those from previous studies of glucose kinetics in horses.

Structurally, the 2-compartment model applied in our study is consistent with most whole-body glucose kinetics models in animals (12,28,29). Theoretically, this model represents a primary compartment of plasma and rapidly exchanging interstitial fluid and a secondary compartment of slowly exchanging interstitial fluid. The volumes estimated in this study are consistent with this interpretation, with the primary compartment representing 12–14% of BW at rest and the total glucose distribution space 21–26% of BW at rest (30–33).

The volume of glucose distribution during exercise has not previously been evaluated in horses to our knowledge, although related assumptions are necessary for some infusion studies (9). This study revealed decreased V_1 during exercise, consistent with reported values for exercise in horses and other species independent of sweat loss (34–36) and attributable to fluid shifts

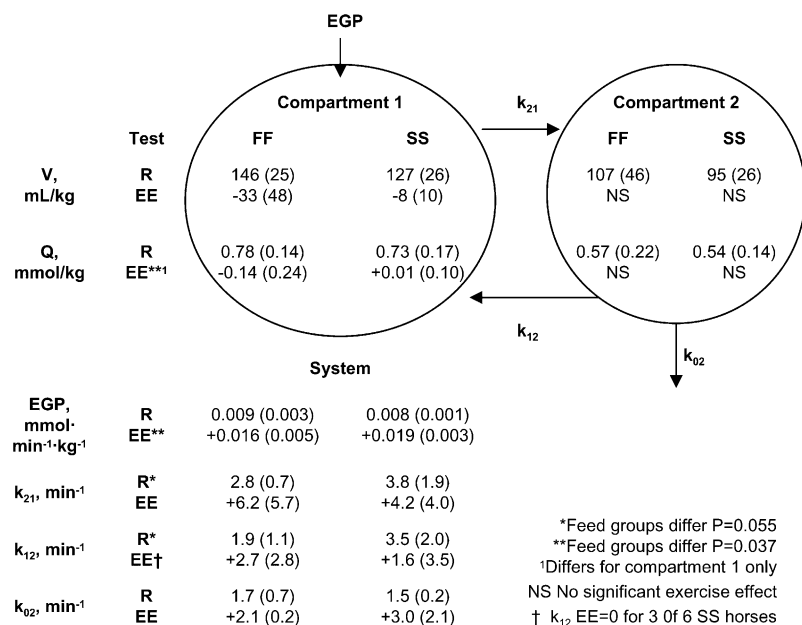


FIGURE 3 Two-compartment model of the glucose space in trained Arabians adapted to SS feeds, $n = 6$, or FF feeds, $n = 6$. Values are median resting value (interquartile range) for rest (R) and the effect of constant low intensity exercise (EE). Rate constants (k_{ij}) are expressed as percent (i.e. $\times 100$).

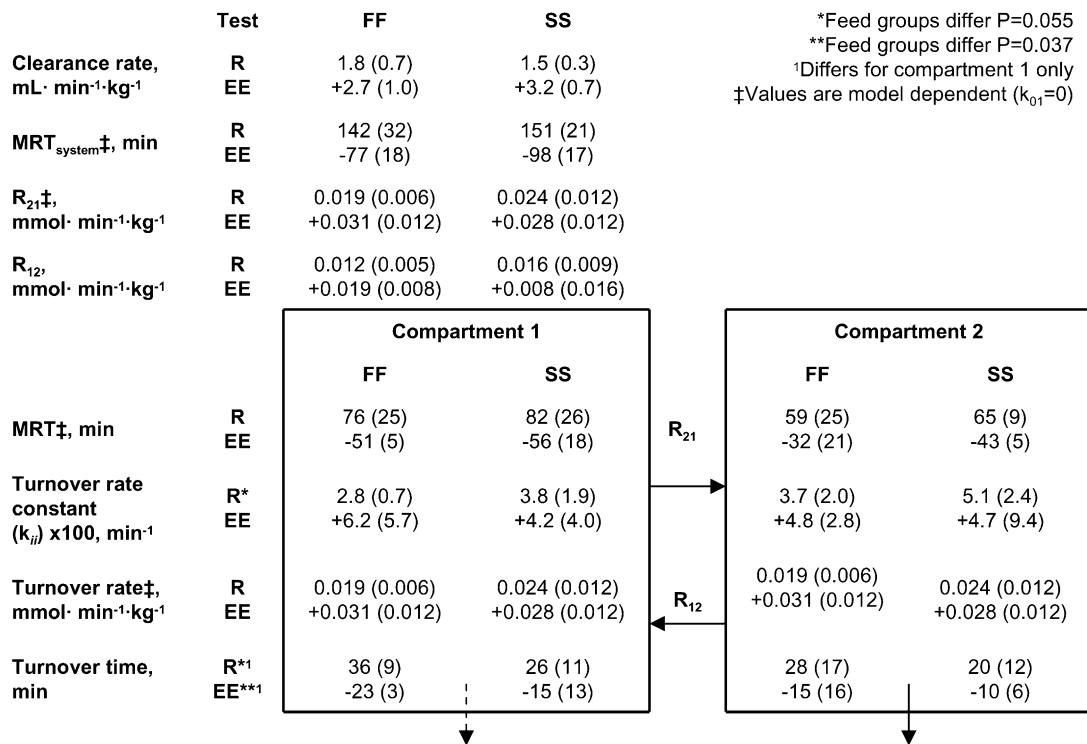


FIGURE 4 Noncompartmental parameters of glucose kinetics for a 2-compartment model in trained Arabians adapted to SS feeds, $n = 6$, or FF feeds, $n = 6$. Values are median resting value (interquartile range) for rest (R) and the effect of constant low intensity exercise (EE).

into active muscle (37). Such changes in fluid distribution emphasize the importance of using descriptive compartmental models when making comparisons across physiological states. The explanations and implications for the diet-induced differences in the volume and Q_1 are unclear. However, it is important to note that although circulating glucose concentrations were higher in SS horses than in FF horses, this did not necessarily reflect the total glucose available in circulation (Q_1 ; mmol/kg BW) (Fig. 3).

The effect of diet on glucose kinetics observed in the resting horses in this study agreed with that observed previously in ponies (12,13,38) and other species, including pigs, cows, sheep, camels, and fish (39–45). In this study, resting horses adapted to a carbohydrate-rich diet (SS) demonstrated higher fractional glucose transfer rates (k_{ij} , min^{-1}) and mass transfer rates (r_{12} , mmol/min) compared with horses adapted to fat and fiber (FF). Ponies adapted to a diet comprising oats (i.e. high in soluble carbohydrate) demonstrated increased glucose entering and leaving the sampled glucose compartment compared with ponies adapted to alfalfa and beet pulp (13). These increases may represent adjustments such as the upregulation of glycolysis to increase the utilization of carbohydrate energy to deal with regular postprandial glucose loads and carbohydrate availability (13).

Although glucose transfer rates (min^{-1}) in SS-adapted horses were higher compared with FF horses at rest, due to the slight differences in glucose distribution at rest, absolute glucose losses did not differ (mmol/min, r_{21} , EGP or turnover rates) between diet groups at rest. The lack of a diet effect may be attributable to the fact the subjects were exercise trained. Exercise training has been shown to alter both glucose and fat energy utilization in various species (46–48) and may have overwhelmed the influence of diet in this study. A previous study in trained horses also found no diet difference in resting glucose transfer in horses adapted to a control feed compared with horses adapted to a fat-

supplemented feed (11). Another study, however, showed no effect of training alone on resting glucose transfer (49). It is possible that exercise training interacts with diet adaptation to reverse diet-induced changes in glucose transfer at rest.

In this study in trained Arabian geldings, resting values for EGP determined by compartmental modeling ($\sim 0.008 \text{ mmol}\cdot\text{min}^{-1}\cdot\text{kg BW}^{-1}$) were similar to those previously determined for trained, overnight feed-deprived Thoroughbred, Arabian, and Standardbred horses using glucose tracer primed-infusion approaches (9,11,50). EGP was also shown to increase with exercise according to both methods, a response partially attributable to catecholamines, as has been clearly demonstrated in exercising horses (50,51). The mean absolute running speed in this study ($3.9 \pm 0.1 \text{ m/s}$) increased EGP by 240% in SS-adapted horses and 180% in FF-adapted horses. Similarly, a glucose tracer-infusion study ran horses at 35% $\text{VO}_{2\text{max}}$ ($4.4 \pm 0.2 \text{ m/s}$), increasing EGP by $\sim 250\%$ in horses adapted to a control diet and $\sim 150\%$ in fat-supplemented horses. Thus, EGP in horses exercising at low intensity demonstrates consistency despite differences in breed, training, modeling techniques, and overnight food-deprived vs. grazing state.

Greater EGP during exercise in SS- compared with FF-adapted horses (and the greater effect of exercise on EGP in SS in the current study) indicates greater utilization of plasma glucose for energy during exercise by SS horses, because glucose leaving circulation is expected to be entering working tissue for metabolism (52). Increased uptake of glucose in SS horses could be attributed to upregulation of the glycolytic pathway, downregulation of fat metabolism, or higher circulating glucose concentrations. The latter is supported by the similar clearance rates observed between diet groups during exercise in this study. Increased circulating glucose appears to improve exercise performance only in subjects at risk for developing hypoglycemia (53). When glucose availability is increased in fat-adapted horses

via glucose infusion, rates of glucose transfer remain lower, suggesting that carbohydrate utilization, not availability, is the limiting factor.

Lower carbohydrate utilization is likely compensated for by increased utilization of FFA as demonstrated in horses during primed-infusion studies (54). Compensation for decreased glucose and glycogen utilization by increased utilization of lipid energy sources has been demonstrated in fat-adapted humans (55,56). In horses, adaptation to the FF and SS diets has not been shown to affect muscle glycogen loss during exercise (57). Such findings agree with previous work indicating that plasma glucose utilization does not compensate as a source of carbohydrate energy during exercise to spare muscle glycogen (9,58).

Increased reliance on carbohydrates as an energy source has been suggested to represent a reduced capacity to switch between carbohydrate and fat energy supplies, also termed reduced metabolic flexibility (59). Interestingly, in this study, fractional glucose transfer rate from the secondary compartment back to the primary compartment (k_{12}) increased with exercise in all FF-adapted horses but only 3 of the SS-adapted horses. Failure to upregulate k_{12} could be attributed to higher resting values in SS horses or the observed differences in glucose distribution between diet groups. However, the lack of upregulation resulted in k_{12} below the FF median during exercise for these 3 SS horses. The lack of exercise-induced increase in this parameter may therefore also suggest a reduced flexibility for SS-adapted horses to adjust metabolism between rest and exercise.

In conclusion, adaptation to a diet that replaces soluble carbohydrates (sugar and starch) with fat as an alternative energy source may avoid reliance on glucose substrate during exercise. The findings of this study could reflect a greater capacity for fat-supplemented horse to select alternate fuel sources. This capacity could spare limited energy sources (i.e. muscle glycogen) during endurance exercise and may reduce the risk of metabolic dysfunction such as insulin resistance. Similarly, exercise-induced upregulation of glucose utilization may promote metabolic efficiency independent of diet, thus avoiding or even reversing metabolic dysfunction.

Acknowledgments

Thanks to Leah Larsen and Louisa Gay for technical assistance.

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Appendix 1: Determination of Kinetic Parameters

Rest and exercise curves of tracer fraction of dose were fit simultaneously to the 2 compartment model using WINSAAAM software to determine k_{ij} and respective exercise effects. The remaining parameters were determined algebraically according to the following equations:

$$V_1 = \frac{1000mL}{L} \cdot \left[\frac{1}{A} \right] \cdot BW^{-1},$$

where A is the y-intercept of the tracer fraction of dose curve.

$$Q_1 = V_1 \cdot C \cdot \frac{1L}{1000mL},$$

where C is the known concentration (mmol/l) of plasma glucose.

$$R_{ij} = Q_j \cdot k_{ij}$$

and, according to steady state assumptions:

$$\sum_{i=0}^n R_{ij} = \sum_{i=0}^n R_{ji},$$

thus:

$$Q_2 = \frac{Q_1 \cdot k_{21}}{-k_{22}}.$$

$$\text{Because: } V_i = \frac{Q_i}{C},$$

then:

$$V_2 = \frac{V_1 \cdot k_{21}}{-k_{22}}$$

$$\text{and: } V_d = V_1 + V_2$$

$$R_{12} = \frac{-Q_1 \cdot k_{12}k_{21}}{k_{22}} \text{ and: } R_{21} = -Q_2 \cdot k_{22}$$

$$R_{01} = EGP = Q_1 \cdot \left(\frac{k_{12}k_{21}}{k_{22}} - k_{11} \right)$$

$$\text{ClearanceRate} = \frac{R_{01}}{C}$$

$$MRT_{total} = \frac{Q_1 + Q_2}{R_{01}}, \text{ } MRT_1 = \frac{-k_{22}}{k_{11} \cdot k_{22} - k_{12}k_{21}}$$

$$\text{and: } MRT_2 = \frac{k_{21}}{k_{11} \cdot k_{22} - k_{12}k_{21}}$$

$$\text{TurnoverTime}_i = \frac{-1}{k_{ii}} \text{ and: } \text{TurnoverRate}_i = -Q_i \cdot k_{ii}.$$