Effects of diet-induced weight gain and turnout to pasture on insulin sensitivity in moderately insulin-resistant horses

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OBJECTIVE
To quantify insulin sensitivity and monitor glucose, insulin, and lipid concentrations in a group of moderately insulin-resistant horses during induction of obesity by use of a forage diet supplemented with fat and during subsequent turnout to pasture.

ANIMALS
9 adult Standardbred mares (11 to 20 years old).

PROCEDURES
Weight gain of horses was induced during 22 weeks by use of a forage diet supplemented with fat fed in gradually increasing amounts, followed by feeding of that fat-supplemented diet at 2.5 times the daily maintenance requirements. Horses were then turned out to pasture. Insulin sensitivity was measured with the euglycemic hyperinsulinemic clamp method before and after weight gain and after 4 weeks at pasture. Body weight, body condition score, and cresty neck score as well as fasting and postprandial concentrations of plasma insulin, plasma glucose, serum triglyceride, and serum nonesterified fatty acids were measured during the study.

RESULTS
Body weight typically increased by 10%, and body condition score (scale, 1 to 9) increased by > 1.5 from the start to the end of the weight-gain period. There was no difference in insulin sensitivity or metabolic clearance rate of insulin during the weight-gain period. Four weeks at pasture generally improved insulin sensitivity and metabolic clearance rate of insulin by 54% and 32%, respectively, but there was no change in body weight or body condition score.

CONCLUSIONS AND CLINICAL RELEVANCE
Findings indicated that dietary composition played a more important role than did short-term weight gain on alterations in insulin sensitivity of horses. (*Am J Vet Res* 2016;77:300–309)

ABBREVIATIONS

BCS	Body condition score
BW	Body weight
CNS	Cresty neck score
DM	Dry matter
EHC	Euglycemic hyperinsulinemic clamp
I	Mean insulin concentration
I_{60}	Mean insulin concentration during the last 60 minutes of the euglycemic hyperinsulinemic clamp procedure
IR	Insulin resistance
IS	Insulin sensitivity
M	Mean glucose infusion rate
M_{60}	Mean glucose infusion rate during the last 60 minutes of the euglycemic hyperinsulinemic clamp procedure
MCR_{60}	Metabolic clearance rate for insulin during the last 60 minutes of the euglycemic hyperinsulinemic clamp procedure
ME	Metabolizable energy
M:I	Mean rate of glucose disposal per unit of insulin during the euglycemic hyperinsulinemic clamp procedure
$M:I_{60}$	Mean rate of glucose disposal per unit of insulin during the last 60 minutes of the euglycemic hyperinsulinemic clamp procedure
NEFA	Nonesterified fatty acids
NSC	Nonstructural carbohydrates
TG	Triglycerides

Obesity is of increasing concern in equids throughout the world and has been described as one of the major components of equine metabolic syndrome. In addition to obesity, equine metabolic syndrome comprises IR, hyperinsulinemia, dyslipidemia, and a predisposition to develop laminitis.[1] The relationship between the components of equine metabolic syndrome has not been completely defined, and the role of obesity as a cause or a consequence of the metabolic comorbidities of this syndrome has not been established. Investigators have found that obesity is associated with the development of IR,[2–4] whereas weight loss has improved IS in horses,[5–7] which suggests that obesity is the primary cause of IR in horses. Equally important, adaptation to high glycemic diets can induce IR in horses.[2,8–10] A diet high in starch and sugar was used in a study[11] in which the objective was to induce weight gain and evaluate its effect on IS in horses. A study conducted to evaluate weight gain in horses fed a diet low in NSC would make it pos-

sible to evaluate the effects of obesity on IS without the confounding effects of increased amounts of dietary starch and sugars.

Compensatory hyperinsulinemia develops in horses in response to IR.[2,11,12] It is suggested that increased and prolonged postprandial hyperinsulinemia occurs in horses with IR while at pasture because feed intake is not restricted and feed is consumed on an almost continuous basis.[a] Horses and ponies with IR are susceptible to developing hyperinsulinemia and laminitis when grazing on pastures that contain forages high in water-soluble carbohydrates.[13–15] Moreover, sustained elevated concentrations of insulin in human lymphocytes[16] and in mice[17] can induce IR at the level of the insulin receptor or at several sites in the insulin signaling cascade. If this is also true for horses, it is possible that sustained hyperinsulinemia in horses with IR kept on pasture high in water-soluble carbohydrate content will generate a cycle promoting further decreases in IS. The study reported here was designed to test the hypothesis that diet-induced weight gain (by use of a diet low in water-soluble carbohydrates) in horses with reduced IS would have no further impact on tissue sensitivity to insulin, whereas subsequent unlimited access to pasture high in water-soluble carbohydrate content would cause increased hyperinsulinemia as well as a further decrease in IS. The primary objective of the study was to quantify IS by use of the EHC method before and after diet-induced weight gain (achieved by use of a forage diet supplemented with fat) as well as after subsequent turnout to pasture in a group of horses with reduced IS. A second objective was to monitor insulin, glucose, TG, and NEFA concentrations in horses during weight gain (fasting samples) and while at pasture (fasting and postprandial samples).

Materials and Methods

Horses

Nine sedentary Standardbred mares with reduced IS were selected from a research herd owned by the Department of Clinical Sciences of the Swedish University of Agricultural Sciences. Mean ± SD age of the horses was 16 ± 3 years (range, 11 to 20 years). To be eligible to participate, horses had to be healthy with no history of laminitis. Inclusion criteria were a BCS ≤ 6.5 (scale, 1 to 9)[18] and moderate degree of IR defined as M_{60} < 3.5 mg/kg/min. Pituitary pars intermedia dysfunction was excluded by determination of plasma ACTH concentrations. The study was approved by the Ethical Committee for Animal Experiments, Uppsala, Sweden.

Experimental design

A longitudinal study was performed. The study lasted for 30 weeks (end of December 2012 to end of June 2013) and was divided into 5 periods (P1 to P5). Horses were allowed a maintenance period of 3 weeks (P1). Weight gain then was induced by a continuous increase of a forage diet supplemented with fat (low glycemic diet; P2), which was followed by a steady-state period (P3) in which horses were fed the same fat-supplemented diet at 2.5 times the daily maintenance requirement of ME.[19] This period was followed by adaptation to a pasture with limited nutrient availability for 1 week (P4) and subsequently a 4-week period during the most intensive growing season of the pasture grasses (P5). Insulin sensitivity was measured by use of the EHC method before weight gain (end of P1), after weight gain (end of P3), and after pasture turnout (end of P5). Fasting (P1 to P5) and postprandial (P5) blood samples were collected on a regularly scheduled basis to monitor changes in concentrations of plasma insulin, plasma glucose, serum TG, and serum NEFA. Body weight of each horse was measured weekly throughout the study period by use of a portable electronic scale.[b] Body condition score (scale of 1 to 9)[18] and CNS (scale of 0 to 5)[20] were determined by one of the authors (JTB) every third week during weight gain (P2 and P3) and every other week while at pasture (P4 and P5). All horses were dewormed prior to pasture turnout (end of P3).

Housing and feeding of horses

During P1 to P3, horses were individually housed in box stalls, with daily turnout into a sand paddock for 7 h/d. Horses were fed haylage 3 times/d (7 AM, 11 AM, and 3 PM). At 7 AM and 3 PM, each horse was fed in its box stall; at 11 AM, horses (separately or in groups of 2 or 3) were fed haylage in the sand paddock. A fixed amount of haylage (2.5 kg) was fed at 7 AM and 11 AM, and the remaining daily haylage ration was fed at 3 PM. The amount of haylage fed at 3 PM was adjusted for each horse's voluntary haylage consumption, which continuously increased during P2 (initial mean ± SD, 4.0 ± 1.6 kg) to a steady-state maximal consumption during P3 (mean, 11.6 ± 1.3 kg). To increase ME intake, pelleted lucerne was added to the feed ration at week 3 and rapeseed oil was added to the feed ration at week 7. Rapeseed oil and pelleted lucerne were fed 2 times/d (7 AM and 3 PM) in total amounts corresponding to 20 and 25 MJ of ME, respectively. Unconsumed feed was weighed after each meal. During P4, horses were stabled and fed as during P3, except for the 11 AM feeding of 2.5 kg of haylage, which was excluded because the horses were kept in a grass paddock (containing a mixture of perennial grass species) for 7 h/d. During P5, horses were kept at a 10-hectare seminatural grass pasture 24 h/d, with no other feed supplements provided. Water and NaCl (in the form of salt blocks) were provided ad libitum throughout the study.

Collection of feed samples

Samples of haylage and pelleted lucerne were obtained 3 times during P1 through P3. During P4 and P5, samples of pasture grasses were collected every fourth day between 2 PM and 4 PM. Samples were harvested from the part of the pasture where the horses

were grazing at the time of sample collection. All feed samples collected during the study were frozen and stored at –80°C until chemical analysis was performed.

Collection of blood samples

Fasting blood samples were collected at the start of the study (P1), every third week during P2 through P4, and at the end of the study (P5). Postprandial blood samples were collected every fourth day while horses were at pasture (P5). Blood samples were collected via jugular venipuncture into 10-mL evacuated tubes[c]; samples were collected between 6 PM and 7 PM. Lithium heparin tubes were used for analysis of plasma glucose and insulin concentrations, and tubes without additive were used for analysis of serum TG and NEFA concentrations. All tubes were centrifuged for 10 minutes at 2,700 × *g*. Serum and plasma were harvested and stored at –80°C until analysis.

EHC

Each EHC sampling period (end of P1, P3, and P5) consisted of 5 days during which 2 horses were evaluated daily. Prior to P1, horses were assigned by use of a simple randomization procedure (drawing horse names from a hat) to day and time for EHC examination; horses then were maintained on the same schedule for P3 and P5. Food, but not water, was withheld for 12 hours prior to the start of the EHC procedure. The EHC procedure began at 6 AM for the first horse and at 10 AM for the second horse each day. The day before the EHC procedure for P5, horses were moved from pasture at 4 PM, placed in their box stalls overnight, and provided cut pasture grass ad libitum until 12 hours prior to testing.

The day before each EHC test, the area over both jugular veins of each horse was desensitized with local anesthetic gel[d] and a catheter[e] was then inserted aseptically into each jugular vein. One of the catheters was used for collection of blood samples and the other catheter was used for infusion of glucose and insulin. Initiation of insulin and glucose infusion was designated as time 0. Blood samples were obtained before (–10, –5, and –1 minutes) the EHC procedure from the catheter reserved for blood sampling, and baseline blood glucose concentrations were determined by use of a handheld glucometer.[f] Recombinant human insulin[g] was diluted in 500 mL of sterile saline (0.9% NaCl) solution mixed with 5 mL of homologous blood. Insulin and glucose were infused for 3 hours through the catheter in the other jugular vein by use of separate infusion lines with a multichannel volumetric infusion pump.[h] Infusion rate for insulin was 3 mU/kg/min,[21,22] and a variable rate of infusion for glucose[i] was used to maintain a euglycemic blood glucose concentration (defined as 5 mmol/L). During the insulin and glucose infusion, blood samples (< 3 mL) were obtained every 5 minutes for analysis of blood glucose concentration with the handheld glucometer. The glucose infusion rate was adjusted if the concentration deviated by > 0.2 mmol/L from euglycemia. Larger blood samples (20 mL) were collected at –10 and –1 minutes as well as every 10 minutes throughout the EHC procedure for subsequent determination of plasma concentrations of glucose (10-minute intervals) and insulin (20-minute intervals). After collection of each blood sample, the catheter was immediately flushed with 10 mL of sterile saline solution.

Analysis of blood samples

An automated clinical chemistry analyzer[j] and commercially available enzymatic colorimetric method reagents were used for measurement of serum NEFA[k] and serum TG[l] concentrations. Plasma glucose concentrations were measured enzymatically with an automated clinical chemistry analyzer.[m] Plasma insulin concentrations for the continuous rate infusion of recombinant human insulin during the EHC procedures were determined by use of a commercial human ELISA,[n] and a commercial kit[o] was used as a control measure. Fasting and postprandial plasma insulin concentrations were determined by use of a commercial equine-optimized ELISA[p] validated for use in horses,[23] and a commercial kit[q] was used as a control measure. All analyses of plasma glucose and insulin concentrations were performed in duplicate. Intra-assay coefficient of variation was 0.8% for TG and 2.2% for NEFA, as determined on the basis of results for 10 replicates of the same sample for each method. Mean intra-assay coefficient of variation for glucose, insulin (equine-optimized ELISA[p]), and insulin (human ELISA[n]) was 0.5%, 2.7%, and 3.3%, respectively.

Analysis of feedstuffs

Analysis of the contents of free glucose, free fructose, sucrose, fructan, and starch in feed samples was performed by use of an enzymatic-spectrophotometric method described elsewhere.[24] Total NSC content was calculated as the sum of the free glucose, free fructose, sucrose, fructan, and starch concentrations. Total water-soluble carbohydrate content was calculated as the sum of the free glucose, free fructose, sucrose, and fructan concentrations. Product information was used for energy content of rapeseed oil. The DM, digestible crude protein, and ME content of forages for horses were analyzed or estimated as described in another study.[25]

Data analysis

The first 120 minutes of the EHC procedure was considered an equilibration period. The M was calculated for each 10-minute interval by use of the following equation:

$$M = GIR - SC$$

where GIR is the glucose infusion rate, and SC is the space correction. The space correction represented an adjustment for glucose added to or removed from the glucose space by factors other than metabolism. The SC was calculated by use of the following equation:

$$SC = (G2 - G1) \times 0.19/T$$

where G1 and G2 are plasma glucose concentrations

at the beginning and end of the time interval, and T is the time interval (ie, 10 minutes).

Data for the final 60 minutes of an EHC procedure (which was considered a steady-state period) were used for calculations of M_{60}, $M:I_{60}$, MCR_{60}, and I_{60}.[26] The value for M_{60} was the mean of the M values obtained at 10-minute intervals during the final 60 minutes of an EHC procedure. The M:I was calculated for each 20-minute interval during the final 60 minutes of an EHC procedure. The $M:I_{60}$ was the mean of the M:I values obtained at 20-minute intervals during the final 60 minutes of an EHC procedure. The MCR_{60} for insulin was calculated by use of the equation:

$$MCR_{60} = CRI_{INS}/\Delta I$$

where CRI_{INS} is the constant rate infusion for insulin, and ΔI is the increase in plasma insulin concentration above the basal concentration during the final 60 minutes of an EHC procedure.

Data were analyzed by use of a mixed model procedure.[r] Because time points were not equidistant, a spatial power covariance structure was used to model the within-horse covariance over time. A 1-way ANOVA for repeated measures was used to test the effect of time on the variables BCS, CNS, BW, and fasting and postprandial blood concentrations (insulin, glucose, TG, and NEFA) and on EHC variables (M_{60}, $M:I_{60}$, MCR_{60}, and I_{60}). Comparisons between time points were performed by use of the Tukey-Kramer post hoc test. Values of $P < 0.05$ were considered significant. Logarithmic transformations were used to obtain normally distributed residuals for fasting and postprandial plasma insulin and serum NEFA concentrations. Logarithmic means were then back transformed and reported as the geometric mean ± 95% confidence interval. All other data were reported as mean ± SD.

Results

Horses

All horses fulfilled the inclusion criteria for the study. None of the horses had clinical signs of disease during the study period. All horses gained weight during the study **(Figure 1)**.

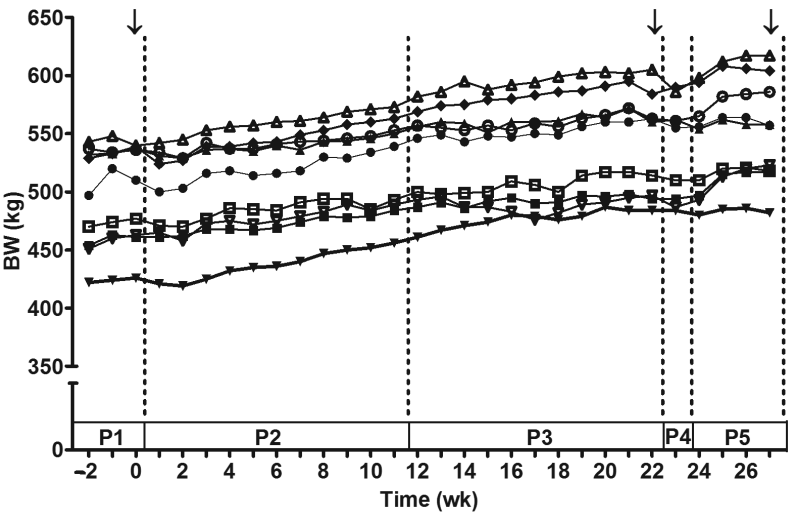

Figure 1—Body weight for each of 9 horses during a study that lasted 30 weeks. There was an initial maintenance period (P1). Weight gain then was induced by a continuous increase of a forage diet supplemented with fat (P2), which was followed by a steady-state period in which horses were fed the same fat-supplemented diet at 2.5 times the daily maintenance requirement of ME (P3). This was followed by adaptation to a pasture with limited nutrient availability for 1 week (P4) and subsequently a 4-week period during the most intensive growing season of the pasture grass (P5). Testing with EHC procedures was performed 3 times during the study (arrows).

Table 1—Chemical composition of samples of pelleted lucerne (n = 3) and haylage (3) collected during weight gain of horses as well as samples of pasture grass collected every fourth day during pasture turnout.

Dietary component	WSC	Free glucose	Free fructose	Sucrose	Fructan	Starch	dCP	ME_h (MJ)	DM (g/kg)
Rapeseed oil	NA	NA	NA	NA	NA	NA	NA	33.0	100.0
Pelleted lucerne*	51 ± 7.2	8 ± 1.9	12 ± 1.0	16 ± 0.2	15 ± 4.6	74 ± 30.3	93 ± 5.3	9.3 ± 0.5	901 ± 12.4
Haylage*	73 ± 13.5	14 ± 5.4	44 ± 7.2	7 ± 1.0	10 ± 3.9	NA	56 ± 6.9	9.1 ± 0.3	518 ± 56.3
Pasture sample									
1	98	22	19	34	23	NA	114	11.3	250
2	111	27	26	29	29	NA	112	11.1	250
3	159	33	32	31	63	NA	86	11.5	290
4	133	30	29	23	51	NA	97	11.1	250
5	141	33	32	25	51	NA	88	11.0	250
6	103	24	24	18	38	NA	109	10.9	240
7	108	28	26	18	36	NA	88	10.2	270

Data reported are g/kg of DM unless indicated otherwise. Energy (ME) and DM content of rapeseed oil is as reported by the manufacturer.
*Value reported is mean ± SD.
dCP = Digestible crude protein. ME_h = The ME for horses estimated from in vitro digestible organic matter. NA = Not applicable. WSC = Water-soluble carbohydrates; calculated as the sum of the free glucose, free fructose, sucrose, and fructan concentrations.

Feeding of horses and nutrient composition

Chemical composition of haylage, pelleted lucerne, and pasture was determined **(Table 1)**. From P1 to the end of P2, daily intake of ME as a percentage of maintenance requirements increased from 97 ± 19% to 242 ± 11% **(Figure 2)**. Mean consumption of NSC during the same period increased from 55 ± 17 g of diet/100 kg of BW to 183 ± 19 g of diet/100 kg of BW. During P3, the mean intake of ME as a percentage of maintenance requirements ranged from 233 ± 10% to 260 ± 11%. Mean consumption of NSC during the same period ranged from 167 ± 13 g of diet/100 kg of BW to 182 ± 18 g of diet/100 kg of BW.

BW, BCS, and CNS

The BW for each horse increased significantly over time (Figure 1). Typically, there was a significant increase in BW (10%; $P < 0.001$), BCS (> 1.5; $P < 0.001$), and CNS (approx 0.5; $P = 0.002$) from the start to the end of weight gain (beginning of P2 to end of P3; **Table 2**). During P5, all horses were obese (BCS ≥ 7), as defined in another study.[2]

Blood variables

Fasting insulin concentrations were significantly ($P = 0.02$) higher at the end of P2 and during P3, compared with concentrations at P1 **(Figure 3)**. Fasting insulin concentrations decreased during P4 and P5 and were not significantly ($P = 0.69$) different from those at P1. Postprandial insulin concentrations did not differ significantly ($P = 0.51$) over time **(Figure 4)**. There was no significant effect of time on fasting ($P = 0.10$) or postprandial ($P = 0.08$) glucose concentrations. All samples were defined as normoglycemic (reference range, 4.2 to 6.4 mmol/L).[27] Fasting NEFA concentrations were significantly ($P < 0.001$) lower during P2 through P4, compared with concentrations at P1, but increased during P5 and then were not significantly ($P = 0.69$) different from the concentration at P1. Postprandial NEFA concentrations differed significantly ($P < 0.001$) over time, with the concentration at 1 time point being significantly ($P = 0.03$) higher than the first postprandial sample during P5.

There was no significant ($P = 0.34$) difference in fasting TG concentrations during P2 through P5, compared with the concentration at P1. Postprandial TG concentrations differed significantly ($P < 0.001$) over time, with 2 time points at the beginning of P5 having significantly ($P = 0.003$) higher concentrations, compared with the first postprandial concentration of P5.

EHC

As determined on the basis of results of EHC testing, there was no significant change in $M:I_{60}$ ($P = 0.78$) or MCR_{60} ($P = 0.80$) over the weight gain period (P2 and P3). However, after 4 weeks at pasture, there was significant ($P < 0.001$) improvement in the $M:I_{60}$ (54%) and MCR_{60} (32%; **Table 3**).

Discussion

Previous studies of horses have been conducted to evaluate the effect of weight gain on IS, but to our knowledge, the study reported here was the first in which investigators assessed the effect of weight gain

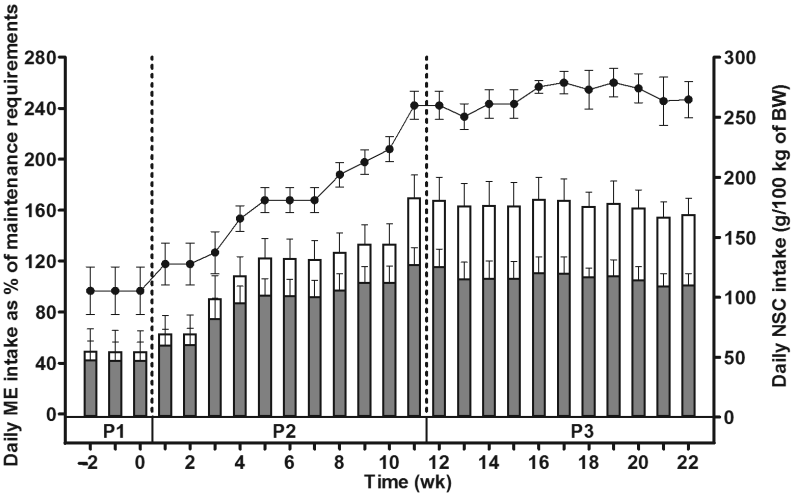

Figure 2—Daily intake of ME as a percentage of maintenance requirements (black circles) and NSC in the diet (bars) during periods of maintenance and weight gain for horses during the study. The NSC intake comprises the sum of the free glucose, free fructose, and sucrose concentrations (gray bars) and the sum of the fructan and starch concentrations (white bars). Results represent the mean ± SD intake on the first day of each week. ***See*** Figure 1 for remainder of key.

Table 2—Mean ± SD (range) of BW, BCS, and CNS determined for 9 horses during a maintenance period (P1), after weight gain (P3), and after 4 weeks at pasture (P5).

Variable	P1	P3	P5
BW (kg)	497 ± 44[a] (422–548)	542 ± 47[b] (479–603)	545 ± 44[b] (481–604)
BCS	5.5 ± 0.6[a] (4.5–6.5)	7.1 ± 0.4[b] (6.5–7.5)	7.3 ± 0.4[b] (7.0–8.0)
CNS	2.4 ± 0.4[a] (1.5–3.0)	2.8 ± 0.3[b] (2.5–3.0)	2.9 ± 0.3[b] (2.5–3.5)

The BCS was scored on a scale of 1 to 9, and CNS was scored on a scale of 0 to 5.
[a,b]Within a row, means with different superscript letters differ significantly ($P < 0.05$).

in horses with moderate IR by use of a diet low in starch and sugar. Furthermore, the effect of pasture turnout on IS was evaluated after weight gain. The primary findings were that weight gain induced by a low glycemic diet did not further decrease IS in the horses, whereas subsequent pasture turnout after weight gain did not decrease IS but instead increased it. Moreover, diet caused an increase in fasting concentrations of insulin and a decrease in fasting concentrations of NEFA during weight gain (P1 through P3) but did not affect fasting TG concentrations during the study (P1 through P5).

Obesity has been implicated as a contributing factor to the development of IR and hyperinsulinemia in horses[2–4] and several other species.[28–31] Furthermore, weight reduction in ponies with IR has been associated with improved IS.[5–7] Nevertheless, in an aforementioned study[5] of obese ponies, only approximately 50% of the ponies were IR before weight reduction,[5] which suggests that obesity is not always linked to IR in horses. This is further supported by the fact that hyperinsulinemia can be detected in lean ponies.[32] Thus, the relationship between obesity and alterations in IS is not fully understood. Short-term weight gain induced by a diet rich in starch and sugar reportedly decreases IS.[11] In contrast, short-term weight gain induced by a diet low in starch and sugar in the present study did not further decrease IS. Results of the present study are in agreement with those of another study[10] in which investigators were unable to identify an effect on IS when a diet high in fat and fiber was used to encourage weight gain in horses. In addition, adaptation to diets rich in starch and sugar contributes to development of IR in horses independent of the effects of obesity.[2,8–10] Studies of horses comparing diets high in fat and fiber content or high in starch and sugar content have revealed a 30%[9] and 37%[8] decrease in IS when the diets high in starch and sugar were fed. The decrease in IS in horses fed a diet supplemented with sugar and starch can be observed after a relatively short period (4 to 6 weeks).[9,10] Thus, it is conceivable that, at least in the short term, other factors such as diet, rather than obesity, are more important for causing IR.

The degree of weight gain is another factor that might have an impact on when IS starts to decrease in a horse. The 10% weight gain in the Standardbred horses of the present study had no impact on IS, whereas a 20% weight gain in Arabian horses in another study[11] decreased IS (mean, 71%). On the other hand, a weight gain of 17% in Thoroughbreds failed to alter IS,[10] which suggested that the degree of weight gain may be of subordinate importance for the development of IR. These weight gain studies involved the use of various breeds, and discrepancies in the results between studies highlight the possibility that breed

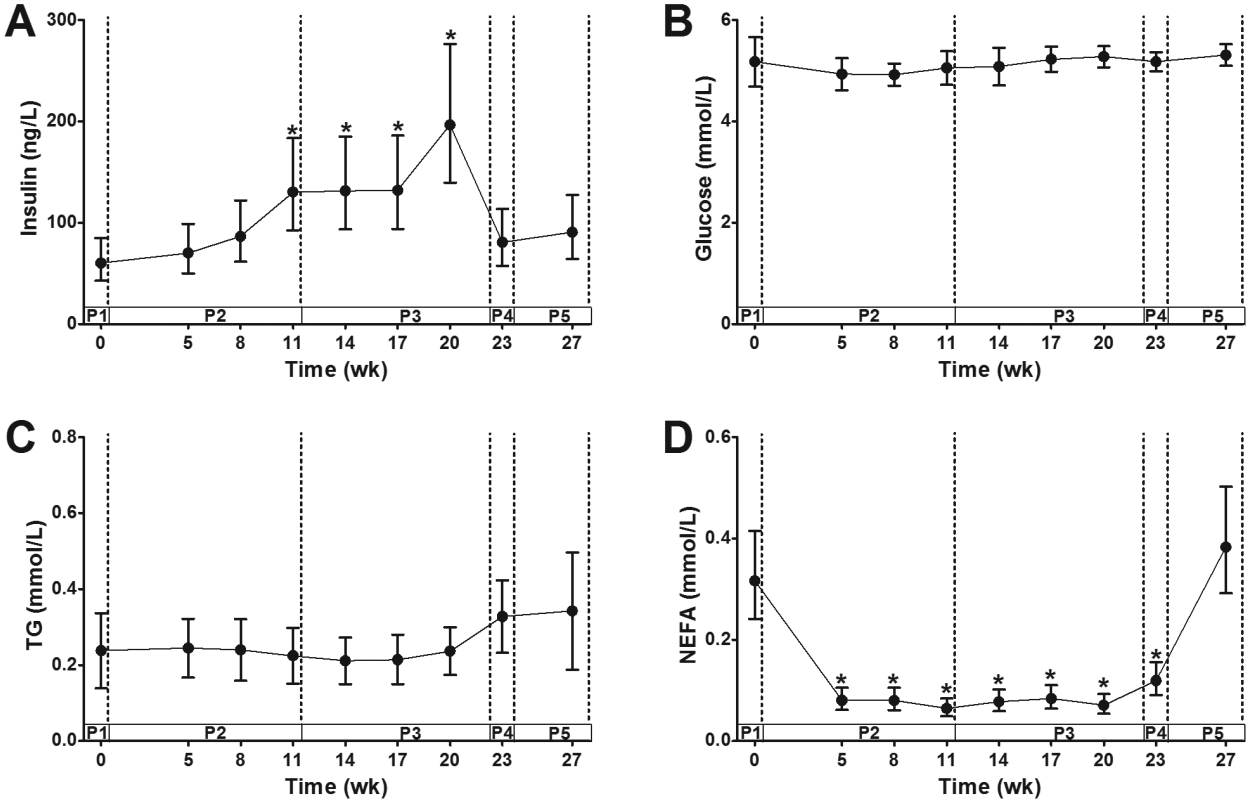

Figure 3—Fasting plasma insulin (A), plasma glucose (B), serum TG (C), and serum NEFA (D) concentrations for 9 horses during the study. Values reported are the geometric mean ± 95% confidence interval for plasma insulin and serum NEFA concentrations and mean ± SD for plasma glucose and serum TG concentrations. *Within a panel, mean value of a week differs significantly ($P < 0.05$) from the mean of week 0. ***See*** Figure 1 for remainder of key.

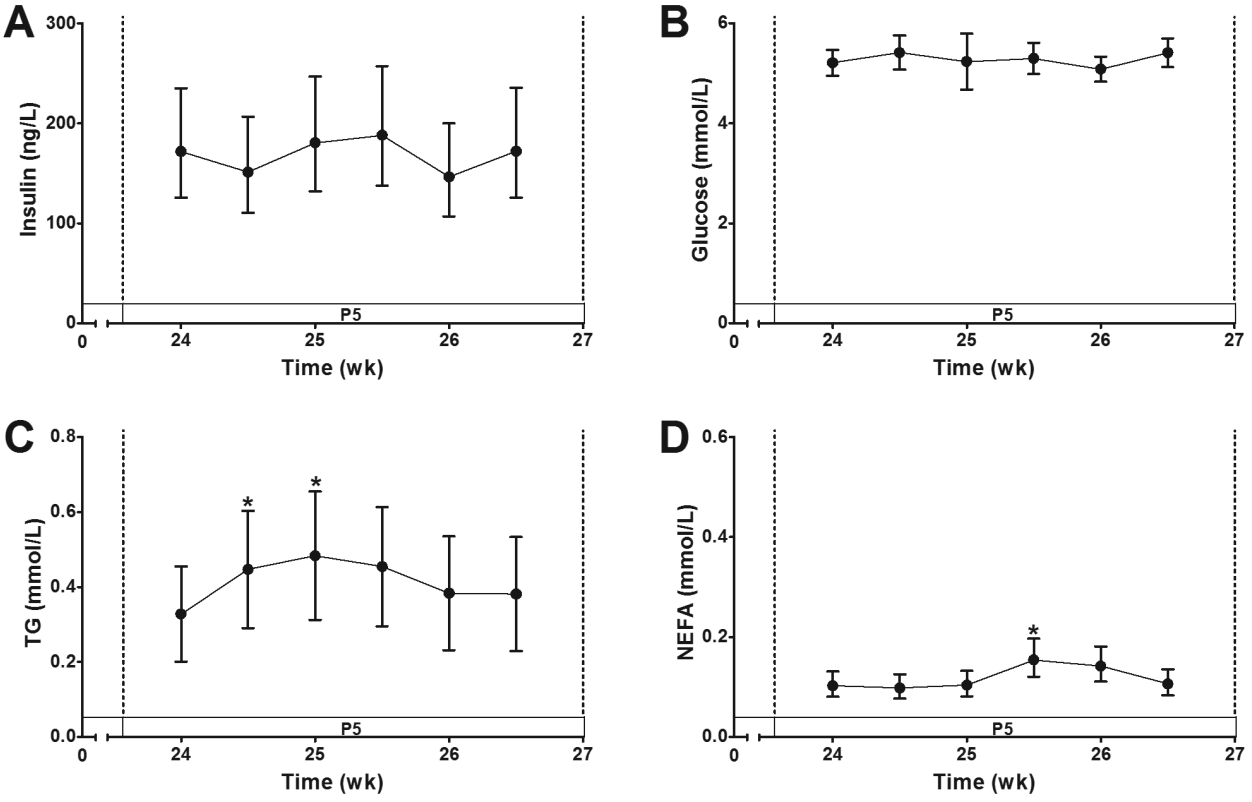

Figure 4—Postprandial plasma insulin (A), plasma glucose (B), serum TG (C), and serum NEFA (D) concentrations for 9 horses during P5. Values reported are the geometric mean ± 95% confidence interval for plasma insulin and serum NEFA concentrations and mean ± SD for plasma glucose and serum TG concentrations. *Within a panel, mean value of a week differs significantly ($P < 0.05$) from the mean of week 24. ***See*** Figure 1 for remainder of key.

Table 3—Mean ± SD values for EHC testing performed on 9 horses during a maintenance period (P1), after weight gain (P3), and after 4 weeks at pasture (P5).

Variable	P1	P3	P5
M_{60} (mg/kg/min)	2.56 ± 0.56[a]	2.98 ± 0.60[a,b]	3.35 ± 0.74[b]
$M:I_{60}$ ([mg/kg/min X 10^3]/[mU/L])	4.27 ± 1.17[a]	4.75 ± 1.13[a]	7.32 ± 2.14[b]
MCR_{60} (mL/kg/min)	5.07 ± 0.56[a]	4.90 ± 0.63[a]	6.68 ± 0.78[b]
I_{60} (mU/L)	605 ± 66[a]	634 ± 93[a]	466 ± 63[b]

The value for M_{60} is the mean of the M values obtained at 10-minute intervals during the final 60 minutes of an EHC procedure. The $M:I_{60}$ is the mean of the M:I values obtained at 20-minute intervals during the final 60 minutes of an EHC procedure. The MCR_{60} for insulin is calculated by use of the equation: $MCR_{60} = CRI_{INS}/\Delta I$, where CRI_{INS} is the constant rate infusion for insulin, and ΔI is the increase in plasma insulin concentration above the basal concentration during the final 60 minutes of an EHC procedure.

[a,b]Within a row, means with different superscript letters differ significantly ($P < 0.05$).

variations exist concerning the effect of obesity on metabolic functions.

Horses with IR are prone to develop hyperinsulinemia and laminitis when exposed to diets with high concentrations of NSC.[11,13–15] Prolonged and increased postprandial hyperinsulinemia in horses with IR is a concern when horses are grazing because there is an almost continous intake of nutrients.[a] Hyperinsulinemia develops as a compensatory response to IR through 2 mechanisms: increased insulin secretion from beta cells in the pancreas and reduced insulin clearance by the liver.[2,11,12,29,33,34] Moreover, a study[35] of rodents found that hyperinsulinemia can contribute to development of IR. In accordance with our first hypothesis, weight gain induced by a forage diet supplemented with fat resulted in obesity in the horses of the present study without any further impact on IS. Thus, prior to pasture turnout, the horses were obese and remained in a state of reduced IS with a decreased metabolic clearance rate for insulin. Our second hypothesis was that the horses would develop postprandial hyperinsulinemia as well as a subsequent decrease in IS when exposed to increased amounts of water-soluble carbohydrates while foraging on pasture. Unexpectedly, after 4 weeks at pasture, IS was improved, which was accompanied by a proportional increase in the MCR_{60}. The reason that

IS was not further reduced during pasture turnout could have several explanations. First, the horses did not continue to increase in BW or BCS during pasture turnout, which suggested a decrease in nutrient intake from that of the weight gain period, despite unlimited access to pasture forages. A lower intake of nutrients and sugar during pasture turnout, compared with that during the weight gain period, was supported by the fact that postprandial insulin concentrations during pasture turnout (P5) were the same as the fasting insulin concentrations at the end of weight gain (P3). Interestingly, a study[a] of grazing Thoroughbred mares revealed a variation in hyperinsulinemia as well as in proxies for estimates of IS during the pasture period. Hyperinsulinemia and the decrease in basal proxies for IS (reciprocal of the square root of insulin) were apparent when the mean water-soluble carbohydrate content of pasture grass was 190 g/kg of DM. When the mean water-soluble carbohydrate content was ≤ 102 g/kg of DM, basal proxies for IS and postprandial insulin concentrations were not different between grazing horses and control horses fed a hay diet.[a] In the present study, the mean water-soluble carbohydrate content of pasture grass was 122 g/kg of DM, which suggested that a higher water-soluble carbohydrate content would have been required to obtain a further decrease in IS and development of hyperinsulinemia during pasture turnout. However, even if this could explain the reason that the horses did not have a decrease in IS during pasture turnout, it is harder to clarify why it improved. Increased physical activity during pasture turnout could have been a contributing factor. Physical activity, with variations in intensity and duration, increases IS in humans[36,37] and horses.[9,38,39] Although horses in the study reported here were not exposed to forced exercise during pasture turnout, it is reasonable to believe that the increase in physical activity, compared with that for a previous stage of inactivity during the weight gain period when horses were stabled in box stalls and only allowed access to small sand paddocks, was enough to improve IS in horses with IR.

One of the inclusion criteria for the present study was a moderate degree of IR (defined as M_{60} < 3.5 mg/kg/min). The M:I is considered to be a more reliable and accurate measurement of IS than is the M value, but it requires measurement of plasma insulin concentrations during EHC procedures. Because different methods have been used for analysis of insulin concentrations among studies, results are not comparable. Thus, use of M:I for comparison of IS among studies is unreliable. Induction of IR by dexamethasone treatment of Standardbred horses reportedly yielded mean M values of 2 mg/kg/min.[40] Trained healthy Standardbred horses reportedly have mean M values of 9 mg/kg/min,[41] whereas lower mean M values of 5 mg/kg/min have been detected in healthy Belgian horses.[22] Therefore, it was considered reasonable to use an M value halfway between 2 and 5 mg/kg/min when defining the cutoff value for moderate IR in the present study. The low IS of Standardbred horses in the present study likely was related to their relatively high age and sedentary lifestyle and not to a metabolic abnormality linked to equine metabolic syndrome. It is therefore possible that a more pronounced IR or an underlying metabolic abnormality is required for hyperinsulinemia and progression in IR to occur during pasture turnout or for other conditions in which there is ad libitum access to feedstuffs high in NSC. This was further supported by the fact that an increase in adipose tissue mass in the Standardbred horses of the present study was not associated with an increase in fasting plasma TG concentrations. Hypertriglyceridemia has been associated with equine metabolic syndrome in ponies in several studies,[15,42,43] and there are indications that elevated fasting insulin and TG concentrations are important features of the metabolic phenotype of horses or ponies with equine metabolic syndrome.[5]

One limitation of the present study was the lack of a control group. Because one of the inclusion criteria was a moderate degree of IR, it was impossible to recruit a sufficient number of horses for a control population. Insulin sensitivity was measured with the EHC procedure, which is a method considered to be suitable for longitudinal studies because of high repeatability of the test.[21] In longitudinal studies that last for several months, it is possible that factors such as seasonal variation might influence the results. However, seasonal effects on IS were not identified when horses were housed and fed in constant conditions for almost a year.[44] In the present study, IS was unchanged after a 5-month period of weight gain (P1 through P3) from January to the end of May, but it increased after only 4 weeks at pasture in June, which further supported the lack of seasonal variation on IS.

Because only mares were used in the present study, it is possible that other factors such as the stage of the estrous cycle could have influenced the results. We attempted to minimize this effect by regularly testing the mares with a teaser stallion during P1 through P4. The day when each EHC period started could thereby be planned in advance and adjusted if necessary to avoid determination of IS during estrus.

Although previous studies in horses have found an association between IR and obesity, results of the present study indicated that obesity per se did not appear to impair IS. Obesity induced by a diet low in NSC was not associated with a decrease in IS. Contrary to the expected result, continuous access to pasture grass did not cause hyperinsulinemia or a further decrease in IS, which may have been related to the use of a breed that lacks the underlying metabolic abnormality required for development of equine metabolic syndrome. An interpretation of these findings is that obesity is not the primary requisite for the equine metabolic syndrome phenotype but, rather, a marker of an underlying metabolic dysfunction. If this is true,

obesity cannot be used as a diagnostic criterion for identification of equine metabolic syndrome.

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Footnotes

a. McIntosh B. *Circadian and seasonal variation in pasture nonstructural carbohydrates and the physiological response of grazing horses*. PhD thesis, Department of Animal and Poultry Sciences, College of Agricultural and Life Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Va, 2007.
b. Botek scale DT2250, Botek, Stockholm, Sweden.
c. Vacuette 9 mL, Greiner Bio-One GmbH, Kremsmünster, Austria.
d. EMLA, AstraZenica AB, Södertälje, Sweden.
e. Intranule, 2.0 X 105 mm, Vygon, Ecouen, France.
f. Accu-Check Aviva, Roche Diagnostics Scandinavia AB, Bromma, Sweden.
g. Humulin Regular, Eli Lilly Sweden AB, Solna, Sweden.
h. Colleague, volumetric infusion pump, Baxter Healthcare SA, Zurich, Switzerland.
i. Glucose Fresenius Kabi 500 mg/mL, Fresenius Kabi AB, Uppsala, Sweden.
j. Architect c4000, Abbott Scandinavia AB Diagnostics, Solna, Sweden.
k. NEFA-HR(2), ACS-ACOD method, Wako Chemicals GmbH, Neuss, Germany.
l. Triglyceride, Abbott Laboratories, Abbott Park, Ill.
m. YSI 2300 Stat Plus analyzer, YSI Inc, Yellow Springs, Ohio.
n. Mercodia insulin ELISA, Mercodia AB, Uppsala, Sweden.
o. Mercodia diabetes antigen control (low, high)/human, Mercodia AB, Uppsala, Sweden.
p. Mercodia equine insulin ELISA, Mercodia AB, Uppsala, Sweden.
q. Mercodia animal insulin control (low, medium, high), Mercodia AB, Uppsala, Sweden.
r. JMP Pro 11.0.0, SAS Institute Inc, Cary, NC.
s. McCue M, Geor R, Schultz N. Re-defining equine metabolic syndrome (abstr), in *Proceedings*. 32nd Am Coll Vet Intern Med Forum 2014;254–257.

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