Effects of diet on Glucagon-Like Peptide-1 (GLP-1), Glucose and Insulin response of young horses

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Introduction

Glucagon-like peptide-1 (GLP-1) is secreted by intestinal L-cells in response to nutrients, especially carbohydrates and fat, in the lumen of the intestine and serves to enhance insulin secretion by pancreatic β-cells in other species (*Roberge* and *Brubaker* 1991, *Holst* 1994, *Perfetti* and *Merkel* 2000). Fasting plasma GLP-1 concentrations are similar in horses to those reported in other species and responses to meals of pelleted concentrates were within the range reported in humans consuming mixed meals (*Duarte* et al. 2005).

Simple and complex carbohydrates are the main sources of energy present in forages and grain-based concentrates commonly offered to horse, with the average fat concentrations in "natural" rations being <5% (NRC 1989). Increased starch content of horse rations has long been known to affect responses to standardized dextrose challenges (*Argenzio* and *Hintz* 1972, *Jacobs* and *Bolton* 1982). However, the use of fat as a major source of energy (>7% in total ration) has recently gained popularity for use in a wide variety of horses (*Crandell* et al. 1999, *Ropp* et al. 2003, *Cubitt* et al. 2005, *Kronfeld* et al. 2005), purportedly due to "glucose-sparing" and reduction of insulin responses to meals. The long-term effects of high fat rations on glucose/insulin metabolism and GLP-1 secretion in horses have not yet been elucidated.

The present study was designed to determine the effects of adaptation to a high fat and fiber (FF) versus a high starch and sugar (SS) ration on GLP-1, glucose and insulin responses of weanlings to a low dose oral dextrose tolerance test (LDOD, *Ralston* et al. 2001).

Material and Methods

Twelve Belgian X Quarter Horse weanling fillies, 4 to 5 months of age initially, were used. The horses were individually housed in box stalls overnight and allowed access to a dry-lot paddock from 08.00 to 15.30 hours with free access to water and salt. The rations were formulated to meet or exceed the requirements for moderate growth (0.75 kg/day, NRC, 1989), supplied by alfalfa/timothy mix hay cubes (50% of total calories, Semican, Plessisville, Quebec, Canada) and a 14% protein, 5% fat, 12% fiber custom pellet (50% of total calories, Rutgers Research Mix, F. M. Brown's Son, Inc, Birdsboro, PA). After a two week period of acclimatization to research conditions the horses were randomly assigned to one of two treatment groups based on size and temperament.

The treatments consisted of two custom (Nutrena-Cargill, Mankato, MN) pelleted concentrates formulated to meet or exceed nutrient requirements (NRC, 1989) when fed to provide 50% of the caloric requirements for moderate growth. Treatment FF had high fat (10%) and fiber (15%) and low non-structural carbohydrates (NSC) (30%), treatment SS concentrate was a high NSC (50%), low fat (3%) and fiber (7%) concentrate. Protein and mineral content of the two rations were similar. The horses were fed their assigned experimental rations for 18 weeks. Low dose oral dextrose challenges were administered on weeks 0, 3, 6 and 15 and 18. For each trial jugular catheters were pre-placed the evening before tests and horses were fasted overnight with free access to water and salt. At 07.30-08.00 the following morning 0.25g of dextrose/kg of body weight in 50 cc of unsweetened applesauce was administered orally with a dosing syringe to each horse. Blood samples were collected –10, 0, 5, 15, 30, 45, 60, 90, 120, 150 and 180 minutes relative to challenge administration and 10 ml of whole blood immediately placed in chilled labeled tubes pretreated with 100µL of 15% EDTA solution (10µL/mL of blood), 400µL of trasylol (40µL/mL of blood), and 100µL of anti-dipeptidyl peptidase-4 (anti-DPP-IV) enzyme (10µL/mL of blood). The samples were centrifuged within 60 min of collection and plasma was stored frozen pending analysis for GLP-1 (LINCO Research, St. Charles, MO) and insulin (Mercodia Insulin ELISA, ALPCO Diagnostics, Windham, NH). Glucose plasma concentrations were determined by an enzymatic colorimetric kit (Glucose-SL Assay, Diagnostic Chemicals Limited, Oxford, CT.). All three assays were validated for use in horses in our laboratory (*Duarte* 2003).

Statistical Analysis

Data were evaluated using GLM-ANOVA (Statistix 8, Analytical Software, Tallahassee, FL) factoring the effects of Treatment, horse and test. Significant effects were evaluated using Tukeys' LSD or Student's T-test where appropriate. Correlations between GLP-1, glucose and insulin concentrations were determined using Pearson's Correlation coefficients. Significance was set at $P<0.05$ with trends identified at $P<0.1$.

Results

One filly in the SS group had developed severe diarrhea prior to the baseline trial and was clinically ill for two weeks. She had higher ($P<0.05$) mean GLP-1 at week 0 (30.0±0.08 pMol/L) and week 3 (52.9±3.3 pMol/L) than the other horses (week 0: 9.6±0.3 pMol/L, week 3: 9.6±0.5 pMol/L) despite being clinically normal during the tests and having "normal" glucose/insulin responses. Her data were excluded from the analyses, though her GLP-1 had returned to normal by week 6.

After 6 weeks all of the horses (7 to 8 mo old at the time) had decreased fasting plasma GLP-1 (5.7±0.7 pMol/L, $P<0.05$) when compared to week 0 or 3 (9.2±0.7 pMol/L, 10.3 ±1.2 pMol/L, respectively) and lower ($P<0.05$) GLP-1 AUC responses. These lower concentrations and responses persisted through weeks 15 and 18, with peak GLP-1 responses at 37 to 46 min in the later trials as opposed to 26 minutes at week 3. The GLP-1/insulin ratio was decreased ($P<0.05$) at 6 weeks and remained at the same level throughout the other two tests.

Horses on FF had lower ($P<0.05$) fasting GLP-1 (4.0 to 4.8 pMol/L) than horses on SS (6.2 to 7.4 pMol/L) at weeks 6-18 and lower ($P<0.05$) peak GLP-1 (FF: 5.2 to 7.4 pMol/L, SS: 8.2 to 10.3pMol/L) and AUC at weeks 15 and 18.

Fasting plasma glucose was not affected ($P>0.1$) by time or treatment, however horses on SS had lower ($P<0.05$) glucose AUC and peak glucose compared to those on FF. Fasting insulin was lower ($P<0.05$) in the FF horses than in SS however insulin responses to the LDOD were not different ($P>0.1$) between the two groups. Overall the glucose/insulin ratio was decreased ($P<0.05$) at 3 weeks relative to baseline and was even lower ($P<0.05$) at 6 and 18 weeks. The glucose/insulin ratio was higher ($P>0.05$) in the FF horses than the SS fed animals at week 18 with the trend toward separation between the two groups apparent at 15 weeks.

Fasting GLP-1 concentrations over all horses and trials were not correlated ($P>0.1$) with fasting glucose/insulin, despite the treatment differences in the later trials. There were actually negative correlations between insulin AUC and GLP-1 AUCs ($r^2=-0.30$, $P=0.02$) and between the GLP-1 AUC and peak glucose responses ($r^2=-0.38$, $P=0.005$) and perhaps peak insulin response ($r^2=-0.26$, $P=0.059$) across all times and treatments. The SS fed horses had correlations between GLP-1 and glucose ($r^2=0.20$, $P=0.0003$) and insulin ($r^2=-0.17$, $P=0.001$) that were stronger than in the FF group (glucose: $r^2=-0.06$, $P=0.24$; Insulin: $r^2=-0.06$, $P=0.23$).

Discussion

High starch rations stimulate greater GLP-1 secretion than high fat/fiber rations in young, growing horses. This may contribute to the higher insulin secretion and more rapid clearance of dextrose challenges reported in horses fed high amounts of grain versus only forage (*Argenzio* and *Hintz* 1972, *Jacobs* and *Bolton* 1982). Similarly, the low resting GLP-1 in horses adapted to high fat rations may account in part for the lower insulin secretion and apparent higher insulin sensitivity reported in other studies (*Crandell* et al. 1999, *Hoffman* et al. 2003, *Ropp* et al. 2003, *Cubitt* et al. 2005, *Kronfeld* et al. 2005). The negative correlations between GLP-1 and insulin were unexpected, due to GLP-1 enhancement of pancreatic β-cell function in other species (*Perfetti* and *Merkel* 2000). It may be possible that high fat versus high starch rations' influence on other hormonal systems (*Ropp* et al. 2003) may affect insulin sensitivity at the cellular level, over riding the effects on GLP-1 secretion and action.

The apparent dietary effect on GLP-1 and glucose metabolism was apparently confounded by maturation of the young horses' metabolism and/or digestive systems. Horses <12 months of age have been reported to be relatively insulin resistant (higher insulin secretion relative to glucose load) to standardized challenges (*Ralston* 1996, *Krusic* et al. 1997, *Cubitt* et al. 2005). In this study there were significant changes over time, regardless of treatment, that occurred when the horses were 7-12 months old with the most significant changes seen at 7-8 months of age. The lower GLP-1 in the horses when tested at 7-12 months of age versus 5-7 months would be consistent with previously reported reductions in insulin response with age in young horses (*Ralston* 1996, *Krusic* et al. 1997), however the lower glucose/insulin ratio in the yearlings would suggest a decreased sensitivity to insulin action at the cellular level rather than the increase previously reported in Thoroughbreds and Lippizaners (Ralston 1996, Cubitt et al. 2005). The greater apparent decrease in insulin sensitivity despite more rapid clearance of the glucose challenge in the Belgian/Quarterhorse cross horses fed the SS ration is consistent, however with previous reports in Thoroughbreds (*Pagan* et al. 2001, *Cubitt* et al. 2005). It could be that the differences in challenges employed (IV dextrose and resting values {*Krusic* et al. 1997, *Cubitt* et al. 2005} versus LDOD), breed and composition of rations employed could have contributed to the discrepancies.

We can conclude, however, that long term feeding of high fat/fiber rations versus a high starch feed will alter GLP-1 secretion and enhance insulin sensitivity in young horses. The hormonal/cellular mechanisms of maturation of the 4 to 12 month old horse and effects of different rations need to be further elucidated.

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Investigation on immunoglobulin G concentration by colostrometry, refractometry and an ELISA-technique in colostrum of mares

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Introduction

Insufficient absorption of immunoglobulin G (IgG) by the foal in the first hours after birth leads to an inadequate immunological status and correlates with a high predisposition for neonatal infections (*McGuire* et al. 1977, *Thein* et al. 1983, *Gènin* und *Clèment* 1989). As the transfer of immunoglobulin from the mare to the foal is minimal prepartum and the passage of these molecules at a high range is possible only during the first 12 hours of live, the quality of colostrum is a crucial parameter for the future health of the foal (*Crawford* et al. 1977, *Chavatte-Palmer* et al. 2001). The evaluation of the colostrum quality is one of the most important steps in the prevention of the failure of immunoglobulin transfer. The goals of the present study were to compare different methods of measuring the IgG-concentration in the colostrum of mares, to determine the kinetic of the IgG-level in the first 12 hours post partum and to determine if the number of parturitions has an influence on the amount of IgG provided by the mare.

Material and methods

Colostrum samples were collected five times within the first 12 h post partum (p.p.) (n=360) of each half of the udder from 36 Warmblood mares of one stud farm. The foals were muzzled over the first 6 hours post natum. Additionally blood sample were taken from each mare. The mares were divided into three groups (group I: 1. parturition, three and four year-old mares; group II: 3. parturition, five to seven year-old mares; group III: 4. and > 4. parturition, older than seven year-old mares). The density (colostrometry) and the refraction index (refractometry) as non-specific parameters were measured in the native colostrum immediately after sample collection. The concentration of immunoglobulin G (IgG) measured by ELISA as a specific method were determined from deep frozen samples.

Results

- There was no difference in the colostral volume between the two halves of the udder in the 36 healthy mares.
- A significant correlation was determined between the refraction index and the colostral IgG-concentration (r = 0.93), and between the density and the colostral IgG-con centration (r=0.88).
- Primiparous mares have a greater mean concentration of IgG than multiparous mares do (68 mg/ml and 51 mg/ml, respectively). Within the first three hours however, primiparous mares have a significantly lower amount of IgG (31.51 g and 48.56 g, respectively).
- Multiparous mares have a mean colostral volume of 1020 ml and, in primiparous mares, a mean volume of 527 ml was determined within the first three hours post partum.