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Energy-related parameters and their association with age, gender, and morphometric measurements in healthy donkeys

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ABSTRACT

Donkeys are commonly afflicted by endocrine and metabolic disturbances but few studies have investigated endocrine variables involved in energy regulation and their association with morphometric indices, age or gender in this species. Hemostatic and clinical differences have been demonstrated between horses and donkeys, so to consider both species as metabolically and endocrinologically similar could lead to misdiagnosis. In this study, plasma concentrations of glucose, triglycerides and endocrine factors involved in energy homeostasis (insulin, glucagon, leptin, adiponectin, ghrelin and insulin-like growth factor [IGF]-1) were measured and their association with morphometric variables (body condition score, neck scoring and body mass index), gender and age was determined in 62 healthy donkeys. In addition, a neck scoring system specific for donkeys was developed.

Insulin, glucagon, leptin and IGF-1 concentrations were found to be similar between donkeys and other species, but adiponectin and active ghrelin were lower in donkeys than horses. Donkeys with larger neck scores and body mass indices had higher triglyceride, leptin and IGF-1 concentrations. A sexual dimorphism was observed on all morphometric measurements and plasma glucose concentrations independent of adiposity. Younger animals had lower morphometric measurements and triglyceride and leptin concentrations.

Introduction

Donkeys play an important role in the livelihood of many people in the developing world; however, research on their physiology has been minimal. Extrapolating reference values from horses could lead to inaccurate diagnosis and treatment. For example, significant differences between the two species have been demonstrated for thyroid hormones and the hemostatic system (Mendoza et al., 2011, 2013). Thus, it is essential to characterize donkey-specific biochemical and endocrine parameters.

Endocrine and metabolic disorders that are well documented in both horses and donkeys include obesity, insulin resistance, pituitary pars intermedia dysfunction (PPID), and metabolic syndrome (Frank, 2009). However, in contrast to the horse, for which there are extensive data on energy regulation and dysregulation, information is limited for the donkey. Similarly, morphometric parameters have been associated with energy variables (glucose, triglycerides, hormones) in horses (Carter et al., 2009), but similar data are not available for donkeys.

Regulators of energy metabolism in equids include insulin, glucagon, leptin, adiponectin, ghrelin, growth hormone (GH), insulin-like growth factor (IGF)-1 and thyroid hormones. Briefly, insulin decreases gluconeogenesis, the activity of lipoprotein lipase and hormone-sensitive lipase, and inhibits glucogenolysis and lipolysis (Barsnick and Toribio, 2011). Glucagon has opposing glycemic effects to insulin, stimulating glucogenolysis and lipolysis, and inhibiting glucogenogenesis (Quesada et al., 2008). Leptin is an adipocyte-derived hormone that controls satiety, lipolysis, and fatty acid oxidation, as well as having reproductive and immune functions (Dardeno et al., 2010). Adiponectin is another adipocyte-derived hormone involved in energy metabolism and in reproductive functions (Kadowaki and Yamauchi, 2005); it also plays a role in the pathogenesis of insulin resistance and metabolic syndrome in human beings (Kadowaki and Yamauchi, 2005). Ghrelin promotes hunger and growth hormone release (Barsnick and Toribio, 2011). In equids, serum ghrelin concentrations increase in response to fasting, hypoglycemia and anorexia (Barsnick and Toribio, 2011). IGF-1 is mainly secreted by the liver in response to growth hormone and contributes to somatic growth, tissue healing and energy metabolism (Laron, 2001).

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The objectives of this study were to determine the plasma concentrations of glucose, triglycerides and endocrine factors involved in energy regulation, and to determine their association with morphometric variables, gender and age in healthy donkeys.

Materials and methods

All animals received care in compliance with the Spanish Guide for the Care and Use of Laboratory Animals. The Animal Care and Ethics Committee of the University of Cordoba (Spain) concluded that the study did not require ethical approval under Spanish Law (RD 53/2013).

Blood samples were collected from 62 healthy donkeys (9 geldings and 53 non-pregnant jennets), with a mean age of 6.8 ± 0.6 years (range 1–19 years) and a mean estimated weight of 267.4 ± 10.6 kg. Most donkeys were of the Andalusian breed ($n = 45$), although other breeds (one Leones-Zamorano) and crossbreds ($n = 16$) were also included. Donkeys were housed semi-extensively on the same farm, with free access to drinking water and forage. All donkeys were supplemented with straw, beet pulp pellets and carrots twice a day. Animals were treated with anthelmintics every 6 months.

The selected donkeys were considered healthy based on clinical history, physical examination (heart and respiratory rates, temperature, mucous membrane color, capillary refill time, intestinal motility and digital pulses) as well as hematology and routine biochemistry tests. Hoof walls were evaluated for evidence of abnormal growth patterns and donkeys with evidence of laminitis were excluded.

Animals were not fed the night before blood collection (approximately 12 h). Blood samples were collected by jugular venepuncture using an 18 G needle and a 20 mL syringe (Terumo) and transferred to one of four tubes (Aquisel): (1) 8 mL tube with lithium heparin for plasma collection; (2) 8 mL tube with clot accelerator for serum collection; (3) 2 mL tube with sodium fluoride for glucose determination, and (4) 2 mL tube with K_3-EDTA for hematology. Blood samples were chilled on ice, centrifuged at 1500 *g* for 10 min, aliquoted and stored at –20 °C until used for analysis.

Body morphometric measurements

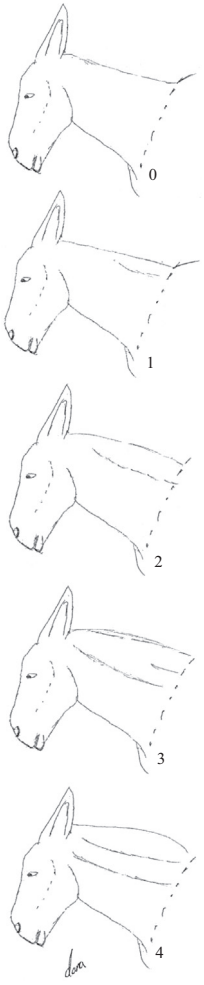
The following body measurements were taken on every animal: (1) height at the withers (distance from the ground to the highest point of the withers); (2) length (distance from the tuber ischii to the elbow in a straight line); (3) girth (measurement around the chest just behind the elbows), and (4) neck circumference (NC, measurement around the neck at the middle point between the poll and the withers, with the neck flexed to a 45° angle and completely relaxed). Bodyweight was calculated using the previously established formula for donkeys (Pearson and Ouassat, 2000): BW (kg) = [girth (cm)$^{2.12}$ × length (cm)$^{0.688}$]/3801. Body mass index (BMI) and neck circumference to height ratio (NCHR) were estimated as weight (kg)/height^2 (m^2) and NC (cm)/wither height (m), respectively (Pleasant et al., 2013).

Five independent evaluators graded the body condition score (BCS) and the neck score (NS). The BCS ranged between 1 (very thin) to 9 (obese) and was based on a scoring system previously established for donkeys (Pearson and Ouassat, 2000). Since no neck scoring system had been validated for donkeys, the authors developed a new system (range 0–4; Fig. 1).

Biochemical determinations

Glucose and triglyceride concentrations were measured by spectrophotometry (Biosystems). Leptin, total adiponectin and active ghrelin concentrations were determined using commercially available radioimmunoassays validated for horses or donkeys (Gordon et al., 2007; Salimei et al., 2007; Barsnick et al., 2014). Technical parameters for these assays were: leptin (sensitivity limit 0.8 ng/mL, Millipore); total adiponectin (sensitivity limit 1 ng/mL, Millipore), and active ghrelin (sensitivity limit 7.8 pg/mL, Millipore). Purified equine active ghrelin and adiponectin were not available for comparison so our results are expressed as human equivalents (HE) of immunoreactive ghrelin (ir-ghrelin HE) and adiponectin (ir-adiponectin HE) units.

Human insulin (DIASource ImmunoAssays S.A.), glucagon (Millipore) and IGF-1 radioimmunoassays (Mediagnost) were validated. The IGF-1 assay did not require extraction steps or blocking of IGF-binding proteins prior to determination. A basic assay validation was performed by assessment of specificity, sensitivity and intra-assay precision (Midgley et al., 1969). Briefly, to determine specificity, plasma from a healthy donkey with experimentally-induced hyperglycemia (300 mg/kg of glucose 50%, intravenously) was used for insulin and glucagon measurement. For IGF-1 validation, plasma from a healthy donkey was serially diluted (dilutional parallelism) with the zero standard. Measurements were carried out in duplicate. Concentrations were plotted against their respective dilution factors. In addition, measured concentrations corrected by the dilution factor (apparent recovery) were compared with undiluted values. Sensitivity was calculated from the dilution curves, as the point at which the lower 95% confidence limit of the dilution intercepted the X-axis. For intra-assay precision, one sample was measured five consecutive times and the coefficient of variation calculated as the standard deviation/mean × 100.



Score	Description
0	Neck thin with absence of a visible and palpable crest.
1	Neck still slightly thin. Crest no visible but palpable. Normal appearance.
2	Neck moderately fatty. Noticeable crest that can be palpated from withers to poll. Patchy fat deposits can be palpated. Bony prominences cannot be felt.
3	Neck thick and rounded. Crest is enlarged, thickened and hard. It is palpable from withers to poll. Crest begins to make longitudinal fat deposit to both sides of the neck. Fat deposited from middle of the neck to withers. Crest width is increased.
4	Neck thick and rounded. Crest grossly enlarged and thickened. Large fat deposits from poll to withers, forming longitudinal hard bands of fat at both neck sides that can be grasped with the hand. Crest width is grossly expanded and it cannot be grasped with one hand. Occasionally in large breeds crest may drop to one side.

Fig. 1. Neck scoring system descriptions and illustrations. Grading score from 0 to 4.

Data analysis

Results are expressed as the mean ± standard error of the mean (SE), median and 25th–75th percentiles. Normality was assessed using the Shapiro–Wilk test. The 95% confidence intervals and 25th and 75th percentiles were determined by using Tukey's Hinges test. Differences between two groups were determined by the Mann–Whitney test. Comparisons for more than two groups were carried out using the Kruskal–Wallis test with the Bonferroni correction being used for post-hoc analysis. Correlations were determined using Spearman's test. Intra-assay precisions and inter-observer coefficients of variation for BCS and NS were calculated as the standard deviation/mean × 100. Statistical analyses were performed using SPSS 17.0 (IBM).

In order to determine whether age was associated with body measurements and metabolism, donkeys were grouped into the following four groups: (1) <1 year old ($n = 14$); (2) 1–5 years old ($n = 14$); (3) >5–10 years old ($n = 19$), and (4) >10 years old ($n = 15$).

The BCS and NS scores from the observers were averaged and rounded up to the closest integer score. BCS was categorized as follows: (1) under condition (score <4 points); (2) optimal condition (4–7 points); (3) overweight/obese (>7 points). NS was grouped as: (1) suboptimal neck (score <2); (2) optimal neck (score 2 or 3), and (3) cresty neck (score >3).

Results

Morphometric measurements

Measurements are reported in Table 1. Inter-observer coefficient of variation (CV) for BCS and NS were <20%, indicating a good precision for these subjective measurements (11.3% and 18.5%,