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Original Article

Effect of intravenous glucose and combined glucose-insulin challenges on energy-regulating hormones concentrations in donkeys

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ABSTRACT

Metabolic disorders are highly prevalent in donkeys. Differences in energy regulatory hormones and glucose dynamic testing, including the intravenous glucose tolerance test (IVGTT) and combined glucose-insulin test (CGIT), have been documented between donkeys and horses. The aims of this study were to characterise the insulin:glucagon (IGR) and glucagon:insulin (GIR) molar ratios, at baseline and in response to the IVGTT and CGIT in healthy donkeys, and to determine their correlation with endocrine (leptin, ghrelin and adiponectin) and morphometric variables. Median values and interquartile ranges (IQRs) for IGR and GIR in 49 healthy adult donkeys were 1.5 (IQR, 1.0–1.8) and 0.7 (IQR 0.5–0.9), respectively. IVGTT and CGIT were each performed on eight donkeys, while dynamic testing was performed on six donkeys due to loss of two donkeys from the study. IVGTT induced an increase in IGR (and a decrease in GIR) from 15 to 180 min after the onset of the test, but had no effect on leptin, adiponectin or ghrelin concentrations. CGIT resulted in a significant elevation in IGR (and a decrease in GIR) from 15 to 120 min after the onset of the test. Plasma leptin concentrations increased significantly at 240 min. No correlations were found between ratios, hormones and morphometric measurements. The findings support differences between donkeys and horses, which are likely to be related to proportionally higher glucagon compared to insulin concentrations in donkeys, and may be relevant to disorders related to energy dysregulation in donkeys, including metabolic syndrome and dyslipidaemias.

Introduction

Donkeys are 'thrifty' animals, with lower energy requirements and a higher ability to digest poor quality forages than horses and ponies (Smith and Pearson, 2005). Metabolic related pathologies, such as dyslipidaemia and metabolic syndrome, are highly prevalent in this species (Mendoza et al., 2018). Differences in energy regulating hormones, and their association with morphometric variables, age and sex, have been documented between donkeys and horses (Mendoza et al., 2015b). Glucose and insulin responses after glucose challenges, including the intravenous glucose tolerance test (IVGTT) and combined glucose-insulin test (CGIT), also differ between equid species, which should be taken into consideration when making diagnostic decisions (Mendoza et al., 2015a).

Energy regulating hormones in equids include insulin, glucagon, leptin, adiponectin and ghrelin. Insulin and glucagon have opposing actions; insulin induces an anabolic state, while glucagon has catabolic effects (Kalra and Gupta, 2016). Leptin and adiponectin are adipocyte-derived hormones controlling satiety and free fatty acid breakdown; in addition, both hormones have reproductive functions. Ghrelin is mainly synthesised by the stomach and it has an opposite effect to leptin, stimulating hunger (Meier and Gressner, 2004).

It has been demonstrated that secretion of these energy regulating hormones is influenced by glucose and insulin in horses, human and rodents (Meier and Gressner, 2004; Gordon and McKeever, 2006), but whether this is also true in donkeys is unknown. There are several tests for glucose tolerance and insulin sensitivity in equids (Frank, 2011), of which the IVGTT and CGIT have been used frequently to reduce variability introduced by differences in gastroenteric function and feeding status in oral tests.

Insulin:glucagon and glucagon:insulin bipolar axes regulate carbohydrate and lipid metabolism; therefore, determining the insulin-to-glucagon molar ratio (IGR) and glucagon-to-insulin molar ratio (GIR) could yield information about catabolic and anabolic states in donkeys, since these ratios are influenced by endogenous glucose, ketone bodies, fatty acids and glycerol (Unger, 1971; Parrilla et al., 1974; Kalra and Gupta, 2016). A high IGR is

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related to free fatty acid storage, glycogenogenesis and protein biosynthesis, whilst a low IGR has been linked to lipolysis, protein breakdown and gluconeogenesis (Kalra and Gupta, 2016).

Despite the importance of endocrine and metabolic pathologies in donkeys, information on how the IVGTT and CGIT affect energy-regulating hormones, IGR and GIR is lacking in this species; to our knowledge, only one study has demonstrated the influence of carbohydrate dynamic tests (oral and intravenous) on hormones such as ghrelin, leptin and adiponectin in horses (Gordon and McKeever, 2006). The aims of this study were: (1) to establish baseline IGR and GIR reference ranges, and determine their association with glucose and triglyceride concentrations, as well as morphometric variables, in healthy adult donkeys; (2) to evaluate changes in IGR and GIR in response to the IVGTT and CGIT; and (3) to investigate the glucagon, leptin, ghrelin and adiponectin responses to the IVGTT and CGIT.

Materials and methods

Animals

Forty-nine healthy, non-pregnant, adult, Andalusian and crossbred female donkeys (jennies), 8.1 ± 0.6 years of age (mean ± standard error of the mean), with a mean weight of 301.2 ± 8.3 kg, were used to generate baseline IGR and GIR values. Eight healthy, non-pregnant, adult, Andalusian jennies (7.7 ± 1.6 years of age), weighing 281.9 ± 8.1 kg, were used to investigate the IGR, GIR, glucagon, ghrelin, leptin and adiponectin responses to IVGTT and CGIT. Dynamic testing was performed on six of these donkeys, since two were sold during the study.

All donkeys were kept in a semi-intensive system, with free access to water and forage, and supplemented with oat hay and beet pulp twice a day. Donkeys were considered to be healthy on the basis of clinical history, physical examination, haematology and blood chemistry profile. Donkeys were under a regular parasite control programme. Donkeys included in the study had fasting glucose concentrations < 110 mg/dL and insulin concentrations < 20 μIU/mL (Carter et al., 2009), neck scores of 2–3 (Mendoza et al., 2015b; Pearson and Ouassat, 2000) and no evidence of abnormal hoof wall growth patterns. All animals received care in compliance with the Spanish Guide for the Care and Use of Laboratory Animals. The study was approved by local (approval number 2015PI/04; date of approval 23 February 2015) and regional (approval number 19-03-15-214; date of approval 19 March 2015) animal welfare committees.

Body morphometric measurements

Morphometric variables used in this study were: (1) calculated body weight (BW); (2) body mass index (BMI); (3) body condition score (BCS); and (4) neck score (NS) (Mendoza et al., 2015b; Pearson and Ouassat, 2000). BW was calculated using the formula: BW (kg) = [girth (cm)$^{2.12}$ × length (cm)$^{0.688}$]/3801. BMI was expressed as weight (kg)/height (m)^2. BCS (range 1–9) and NS (range 0–4) were evaluated by three independent evaluators according to scoring systems validated previously (Mendoza et al., 2015b).

Intravenous glucose tolerance test and combined glucose-insulin test

Donkeys were housed overnight (10:00 pm to 8:00 am) with one 'flake' of oat hay and free access to water (Frank, 2011). Only water was available during the tests. Both IVGTT and CGIT were performed in six donkeys following protocols previously validated for donkeys (Mendoza et al., 2015a), with a 1 month washout period between tests. In the IVGTT, glucose (300 mg/kg; 50% glucose solution) was administered intravenously as a bolus. In the CGIT, glucose (150 mg/kg) was administered intravenously, followed by intravenous recombinant human insulin (0.1 IU/kg diluted in 1 mL of saline solution). In both tests, blood samples were collected at 0 (baseline), 15, 30, 60, 120, 180 and 240 min.

Insulin:glucagon ratio, glucagon:insulin ratio and proxy calculation

The IGR and GIR were calculated in overnight fasted donkeys using formulas described previously (Jin et al., 2014; Kalra and Gupta, 2016). Although these ratios are reciprocal, they have been described independently and studied in previous reports; therefore, both are listed in this manuscript in order to provide proper comparative information.

Glucose proxies were calculated using formulas described for donkeys and horses (Treiber et al., 2005; Durham et al., 2008): (1) modified insulin:glucose ratio (MIRG): (800 − 0.3 × [fasting insulin − 50]^2)/(fasting glucose − 30); (2) reciprocal of the square root of insulin (RISQI): 1/(fasting insulin$^{-0.5}$); (3) quantitative insulin sensitivity check index (QUICKI): 1/(log fasting insulin/log fasting glucose); (4) homeostasis model assessment for IR (HOMA-IR): (fasting insulin × fasting glucose)/22.5; and (5) homeostasis model assessment of percentage β cell function (HOMA-B%): (20 × fasting insulin)/(fasting glucose − 3.5).

Biochemical determinations

Blood samples were collected into tubes with sodium fluoride for glucose determination and into lithium heparin or clot accelerator for hormone and triglyceride determinations. Blood samples were chilled on ice, centrifuged at 1500 × *g* for 10 min and stored at −20 °C. Plasma glucose and triglyceride concentrations were measured by spectrophotometry (Biosystems). Hormone concentrations were determined using commercially available radio immunoassays validated for donkeys and horses (Gordon and McKeever, 2005; Mendoza et al., 2015b): (1) insulin (sensitivity limit 1.7 μIU/mL, intra-assay coefficient of variation, CV, < 2.1%; DIASource Immunoassays S.A.); (2) glucagon (sensitivity limit 6.8 pg/mL, linearity limit 400 pg/mL, intra-assay CV < 6.8%; Millipore); (3) leptin (sensitivity limit 0.8 ng/mL, linearity limit 50 ng/mL, intra-assay CV < 3.6%; Millipore); (4) total adiponectin (sensitivity limit 1 ng/mL, linearity limit 200 ng/mL, intra-assay CV < 6.2%; Millipore); and (5) active ghrelin (sensitivity limit 7.8 pg/mL, linearity limit 2000 pg/mL, intra-assay CV < 9.5%; Millipore). Results for active ghrelin and total adiponectin are expressed as human equivalents (HE) units.

Statistical analysis

Normality was assessed using the Shapiro-Wilk test. Since all data were not normally distributed, results are expressed as medians with interquartile ranges (IQRs); percentiles (75th–25th percentiles) were calculated using the Tukey's-Hinges test. Friedman's test was used to determine differences over time, followed by Wilcoxon's test to further assess differences between two time-points. Correlations were assessed using Spearman's test. Areas under the curve (AUCs) for insulin (AUCins), glucagon (AUCgon), leptin (AUClep), adiponectin (AUCadi), ghrelin (AUCghr), IGR (AUCigr) and GIR (AUCgir) were calculated using the trapezoidal method. $P < 0.05$ was considered to be statistically significant. Statistical analysis was performed using a commercial statistical software (SPSS 17, IBM).

Results

Insulin:glucagon ratio, glucagon:insulin ratio and proxy calculation

Medians for IGR and GIR in 49 healthy adult donkeys were 1.45 (IQR 1.0–1.8) and 0.7 (IQR 0.5–0.9), respectively. There were no significant correlations between IGR or GIR and morphometric variables, glucose surrogates, and glucose, triglyceride, leptin, adiponectin and ghrelin concentrations. Glucose proxies were within reference ranges for donkeys (Du Toit and Trawford, 2010; Mendoza et al., 2015a) (Table 1).

Effect of intravenous glucose tolerance and combined glucose-insulin tests on insulin:glucagon and glucagon:insulin ratios

Morphometric measurements and biochemical parameters in the donkeys in this study were similar to values reported previously for healthy donkeys (Table 2) (Mendoza et al., 2015b; Burden et al., 2016). In response to the IVGTT, IGR increased from 15–120 min ($P = 0.043$), returning to baseline at 180 min (Fig. 1A; $n = 6$). Similarly, GIR was decreased significantly in relation to baseline values from 15 to 120 min ($P = 0.043$), returning to baseline at 180 min (Fig. 1A; $n = 6$). The AUCigr and AUCgir were 1788.1 (IQR 1136.7–1810.5) and 78.8 (IQR 70.9–79.6), respectively. The CGIT induced a significant rapid IGR increase at 15 min ($P = 0.028$); this lasted until 120 min (Fig. 1A). The GIR curve mirrored that reported for IGR (Fig. 1A). The AUC was 3151.5 (IQR 3063.1–3596.5) and 128.5 (IQR 110.6–128.9) for IGR and GIR, respectively.

Effect of intravenous glucose tolerance test on energy hormones

Intravenous glucose administration induced a significant decreased in glucagon concentration from 15 min ($P < 0.05$) to 120 min ($P < 0.024$), returning to baseline by 180 min (Fig. 2A; $n = 6$). This was associated with a significant ($P < 0.05$) increase in insulin concentrations; these returned to baseline by 120–180 min