The Veterinary Journal 206 (2015) 371–376

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Characterization of the intravenous glucose tolerance test and the combined glucose–insulin test in donkeys

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ARTICLE INFO

Article history:
Accepted 10 August 2015

Keywords:
Donkeys
Dynamic tests
Energy metabolism
Glucose
Insulin

ABSTRACT

Glucose–insulin dynamic challenges such as the intravenous glucose tolerance test (IVGTT) and combined glucose–insulin test (CGIT) have not been described in donkeys. The objectives of this study were (1) to characterize the IVGTT and CGIT in healthy adult donkeys, and (2) to establish normal glucose–insulin proxies. Sixteen donkeys were used and body morphometric variables obtained each. For the IVGTT, glucose (300 mg/kg) was given IV. For the CGIT, glucose (150 mg/kg) followed by recombinant insulin (0.1 IU/kg) were administered IV. Blood samples for glucose and insulin determinations were collected over 300 min.

In the IVGTT the positive phase lasted 160.9 ± 13.3 min, glucose concentration peaked at 323.1 ± 9.2 mg/dL and declined at a rate of 1.28 ± 0.15 mg/dL/min. The glucose area under the curve (AUC) was 21.4 ± 1.9 × 10^3 mg/dL/min and the insulin AUC was 7.2 ± 0.9 × 10^3 μIU/mL/min. The positive phase of the CGIT curve lasted 44 ± 3 min, with a glucose clearance rate of 2.01 ± 0.18 mg/dL/min. The negative phase lasted 255.9 ± 3 min, decreasing glucose concentration at rate of –0.63 ± 0.06 mg/dL/min, and reaching a nadir (33.1 ± 3.6 mg/dL) at 118.3 ± 6.3 min. The glucose and insulin AUC values were 15.2 ± 0.9 × 10^3 mg/dL/min and 13.2 ± 0.9 × 10^3 μIU/mL/min. This is the first study characterizing CGIT and IVGTT, and glucose–insulin proxies in healthy adult donkeys. Distinct glucose dynamics, when compared with horses, support the use of species-specific protocols to assess endocrine function.

Introduction

The donkey evolved and adapted to hostile environmental conditions characterized by low-quality diets, extreme temperatures, diseases, and high workloads (farming, transportation). Part of this evolutionary success was due to the development of morphometric and metabolic traits typical of thrifty species (Starkey and Starkey, 2004). Of interest, these characteristics directly and indirectly influenced human social, geographical, territorial, and economic progress. To date, donkeys still play important roles in the economy of developing countries, including transportation of goods, agriculture and tourism (Burden and Thiemann, 2015). Additionally, interest in this species as companion animals as well as a source of valuable nutrients for humans is increasing in developed countries (Martemucci and D'Alessandro, 2012).

Metabolic and endocrine differences between donkeys and horses have been recently demonstrated (Mendoza et al., 2011, 2013, 2015). Therefore, considering both species as similar or extrapolating laboratory information from horses to donkeys could lead to erroneous diagnosis, improper treatment, and/or inaccurate prognosis. There is therefore a need to further generate donkey-specific biochemical, metabolic and endocrine information to enhance our understanding of the physiology of this species and to improve data interpretation, diagnosis and treatment.

Metabolic disturbances such as insulin dysregulation, obesity, metabolic syndrome and pituitary pars intermedia dysfunction (PPID) are observed in donkeys (Frank, 2009). Although there is some information on these disorders in horses, data from donkeys are scarce, and reference values for common energy-regulating hormones were not established until recently (Mendoza et al., 2015).

In horses, insulin resistance (IR) is diagnosed by either measuring fasting blood glucose and insulin concentrations or by calculating proxies and performing dynamic challenges such as oral and intravenous (IV) glucose tolerance tests (OGTT and IVGTT, respectively), the combined glucose–insulin test (CGIT), the euglycemic–hyperinsulinemic clamp test or the frequently sampled IV glucose tolerance test (FSIGTT) (Kronfeld et al., 2005; Firshman and Valberg, 2007). Due to a combination of ease, low cost, low blood sample numbers and volumes, and reliability, the IVGTT and CGIT are the

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http://dx.doi.org/10.1016/j.tvjl.2015.08.015
1090-0233/

most common glucose dynamic challenges used clinically in order to test IR in horses (Frank, 2011; Johnson et al., 2011); neither test has been characterized in donkeys although both the IVITT and the FSIGTT have been previously evaluated in the species (Forhead and Dobson, 1997; McLean et al., 2009). The FSIGTT is considered the gold standard method for assessing insulin sensitivity in horses, and is also used to quantify in vivo insulin activity; however, due to their tedious technical nature, the tests are rarely used in the clinical setting (Kronfeld et al., 2005; Pratt et al., 2009).

In order to enhance our understanding of glucose homeostasis in donkeys and to use this information as a diagnostic tool, the objectives of this study were (1) to characterize the IVGTT and the CGIT in healthy adult donkeys, and (2) to establish normal glucose–insulin proxies that assess glucose mobilization, pancreatic β-cell function (insulin release) and insulin sensitivity.

Material and methods

Animals

Sixteen adult female healthy non-pregnant Andalusian donkeys were used in the study. For the CGIT, 10 donkeys with an age of 7.3 ± 1.5 years (range 2–14 years) were used. The IVGTT was performed in 10 of the donkeys (four of which were also used in the CGIT) and had a mean age of 8.1 ± 1.3 years (range 2–14 years). All animals were housed semi-extensively on the same farm, with free access to drinking water and forage supplemented with hay and beet pulp twice a day. The donkeys were considered healthy based on clinical history, physical examination (heart and respiratory rates, rectal temperature, mucous membrane colour, capillary refill time, intestinal borborygmi and digital pulses) and blood work profile. They had been dewormed every 6 months. The donkeys had normal hair coat for the season (spring) with no evidence of PPID; however, they were not tested for this condition (Frank and Tadros, 2014). Hoof walls were evaluated for abnormal growth patterns and animals with clinical evidence of laminitis were not included in the study. Another inclusion criteria was fasting concentrations of glucose <110 mg/dL and insulin <20 μIU/mL (Carter et al., 2009).

All animals received care in compliance with the Spanish Guide for the Care and Use of Laboratory Animals. This study was approved by the Animal Care and Ethics Committee of the University of Cordoba (2015PI/04, 20 February 2015).

Body morphometric measurements

The following body morphometric measurements were obtained: height at withers (distance from the ground to the highest point at the withers); length (distance from the tuber ischii to the elbow in a straight line); girth (measurement around the chest just behind the elbows), and neck circumference (NC, measurement around the neck at the middle point between the poll and the withers, with the neck flexed at a 45° angle and completely relaxed). Bodyweight (BW) was calculated using the previously established formula for donkeys (Pearson and Ouassat, 2000):

$$\text{BW (kg)} = \left(\text{girth [cm]}^{2.12} \times \text{length (cm)}^{0.688}\right)/3801$$

Body mass index (BMI) and NC to height ratio (NCHR) were estimated as weight (kg)/height (m)^2 and NC (cm)/withers height (m), respectively (Pleasant et al., 2013). Body condition score (BCS, range 1–9) and neck score (NS, range 0–4) were evaluated by three independent evaluators according to scoring systems previously validated for donkeys (Pearson and Ouassat, 2000; Mendoza et al., 2015). Donkeys used in this study were considered to be in optimal body condition based on NS and BCS.

Testing protocols

Protocols for CGIT and IVGTT were adapted from those reported for horses (Eiler et al., 2005; Frank et al., 2010; Johnson et al., 2011). Catheters (14G) were placed in the left jugular vein under sterile conditions for glucose and insulin administration, and for blood collection. Patency was maintained with sterile heparinized saline solution. The donkeys were housed overnight (22:00–08:00 h) with a flake of hay and free access to water (Frank, 2011). Only water was available during the tests, since food was removed before of starting the test. All tests were performed in the spring time.

Intravenous glucose tolerance test

Glucose (300 mg/kg, 50% glucose solution) was administered IV as a bolus and the infusion line flushed with heparinized solution. Glucose concentrations were determined at the following time points: 0 (baseline), 5, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240 and 300 min. Insulin concentrations were measured at time 0 (baseline), 5, 15, 30, 45, 60, 75, 90, 120, 180, 240 and 300 min.

Combined glucose–insulin test

Glucose (150 mg/kg) was administered IV as a bolus, followed immediately (<10 s), by recombinant human insulin (0.1 IU/kg diluted in 1 mL of saline solution) IV. Glucose and insulin concentrations were determined at the same time points as for the IVGTT. One donkey was excluded from the study due to minor clinical signs (pawing, slight sweating and tremors) during the lowest point of the curve (lowest glucose concentration was 18 mg/dL at 75 min).

Parameters analyzed in both tests included: positive phase duration (time from the start to the time glucose returned to baseline); positive glucose clearance rate (ratio between the difference the highest measured and baseline glucose by the difference in time from the highest measured glucose to the end of the positive phase); negative phase duration (time from to the end of positive phase to glucose returned to baseline); start to nadir interval (time from the start to lowest measured glucose); nadir concentration; valley duration (when applicable); negative glucose clearance rate (ratio between the difference the baseline glucose and the glucose nadir by the difference in time from the end of the positive phase and the lowest glucose), and valley to baseline interval (time from minimal glucose until glucose returned to baseline) (Eiler et al., 2005; Funk et al., 2012). The areas under the curve for glucose (AUCg) and insulin (AUCi) were calculated using the trapezoidal method (Funk et al., 2012).

Biochemical determinations

Blood samples were collected into tubes with sodium fluoride for glucose determination and lithium heparin for plasma triglyceride and insulin measurements. Blood samples were chilled on ice, centrifuged at 1500 *g* for 10 min, aliquoted and stored at –20 °C until measurements. Glucose and triglyceride concentrations were determined by spectrophotometry, and insulin concentration using commercial radioimmunoassays previously validated for donkeys (Mendoza et al., 2015).

Calculation of proxies for IR, insulin sensitivity and β-cell function

Glucose and insulin concentrations were used to calculate the following proxies for either IR, insulin sensitivity or pancreatic β-cell function: glucose/insulin ratio; insulin/glucose ratio; modified insulin to glucose ratio (MIRG): (800 – 0.3 × [fasting insulin – 50]^2)/(fasting glucose – 30); reciprocal of the square root of insulin (RISQI): 1/(fasting insulin$^{-0.5}$); quantitative insulin-sensitivity check index (QUICKI): 1/(log fasting insulin/log fasting glucose); homeostasis model assessment for IR (HOMA-IR): (fasting insulin × fasting glucose)/22.5), and homeostasis model assessment of percentage β-cell function (HOMA-B%): (20 × fasting insulin)/(fasting glucose – 3.5) (Treiber et al., 2005; Durham et al., 2008).

Data analysis

Results are expressed as the mean ± standard error of the mean (SEM), median and 25th to 75th percentiles. Normality was assessed with the Shapiro–Wilk test. The 95% confidence intervals and 25th and 75th percentiles were provided by Tukey's Hinges test. Mann–Whitney test was used in order to determine differences between each time point. $P < 0.05$ was considered statistically significant. Statistical analysis was performed using commercial statistical software.

Results

Body morphometric measurements

Morphometric variables are shown in the Table 1. No differences were observed between donkeys used for the IVGTT and the CGIT.

Biochemical determinations and proxies calculations

Plasma glucose, triglyceride and insulin concentrations as well as proxy results are shown in Table 2. No differences in biochemical parameters and proxies were found between donkeys used in both protocols.

Intravenous glucose tolerance test analysis

Results of the IVGTT are shown in Table 3. The positive phase lasted 160.9 ± 13.3 min, glucose concentration peaked at 323.1 ± 9.2 mg/dL (384%), declining at a rate of 1.28 ± 0.15 mg/dL/min to reach baseline between 150 and 180 min (Fig. 1A). The AUCg was 21.4 ± 1.9 × 10^3 mg/dL/min and the AUCi was 7.2 ± 0.9 × 10^3 μIU/mL/min. From 5 min to 120 min post injection, insulin concentration