ORIGINAL ARTICLE

Equine Veterinary Journal WILEY

Characterisation of the oral glucose and sugar tolerance tests and the enteroinsular axis response in healthy adult donkeys

Francisco J. Mendoza[1] | Antonio Buzon-Cuevas[1] | Ramiro E. Toribio[2] | Alejandro Perez-Ecija[1]

[1]Department of Animal Medicine and Surgery, University of Cordoba, Cordoba, Spain

[2]Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, Ohio, USA

Correspondence
Francisco J. Mendoza, Department of Animal Medicine and Surgery, University of Cordoba, Campus Rabanales, Road Madrid-Cadiz km 396, 14104 Cordoba, Spain.
Email: fjmendoza@uco.es

Summary

Background: Insulin dysregulation (ID) is diagnosed in horses and ponies using oral glucose (OGTT) and oral sugar (OSTT) tolerance tests. The enteroinsular axis plays a major role in postprandial glucose disposal and insulin response in horses, ponies and foals. The insulin and incretin response to oral carbohydrate challenges has not been characterised in donkeys.

Objectives: (a) To characterise OGTT and OSTT, and (b) to assess the plasma incretin response to OGTT and OSTT in healthy donkeys.

Study design: In vivo experiments.

Methods: Six healthy adult female Andalusian donkeys were challenged with OGTT (1 g/kg glucose, 20% solution by nasogastric tube) and OSTT (0.45 mL/kg corn syrup orally by syringe) with a 1-week washout. Blood samples were collected for glucose (spectrophotometry), insulin (radioimmunoassay), glucose-dependent insulinotropic polypeptide (GIP, ELISA) and active glucagon-like peptide-1 (aGLP-1, ELISA) determination over 6 hours. Curves were analysed and proxies calculated.

Results: Glucose and insulin concentrations peaked at 180 minutes in OGTT, but at 300 and 150 minutes in OSTT, respectively. Plasma GIP concentrations increased in the OGTT and OSTT (peaked at 180 and 360 minutes, respectively), but aGLP-1 increased only in OGTT (240 minutes).

Main limitations: Single breed, narrow age and sample, diet, season and not having donkeys with evidence of ID to provide clinical validation.

Conclusions: Donkeys have a functional enteroinsular axis that is activated by enteral carbohydrates. Donkeys have evident endocrine differences with horses, supporting the validation of the OSTT and OGTT to assess insulin sensitivity in this species to avoid extrapolation from horses.

KEYWORDS
donkeys, glucose, horse, incretins, insulin dysregulation, metabolic syndrome

The abstract is available in Portuguese in the Supporting Information section of the online version of this article.

1 | INTRODUCTION

Physiological and clinical differences between horses and donkeys have been reported in recent years,[1-3] supporting the need for species-specific data and studies. Knowledge gaps remain in donkeys and data extrapolation from horses increases the risk of misdiagnosis, unnecessary treatments, potential complications and additional costs.

The prevalence of endocrine and metabolic disturbances, including hyperlipaemia, donkey metabolic syndrome (DMS) and pituitary pars intermedia dysfunction is increasing in donkeys,[4-7] thus there is a need to develop donkey-specific diagnostics. Guidelines for the diagnosis of equine metabolic syndrome (EMS) were recently updated,[8] with resting glucose and insulin concentrations together with overt clinical signs being the first screening for the diagnosis of EMS, although both having low diagnostic sensitivity, and dynamic testing being the hallmark for further diagnostic confirmation of insulin dysregulation (ID).[8] Numerous endocrine and metabolic differences between donkeys and horses have been reported,[5-7,9-11] but guidelines to diagnose ID in donkeys have not been generated.

The revised EMS diagnosis guidelines recommend the oral sugar tolerance test (OSTT) in horses with ID[8]; however, neither the OSTT nor the oral glucose tolerance test (OGTT) have been characterised in donkeys. The frequently sampled intravenous glucose-insulin tolerance test, the combined glucose-insulin test, the intravenous glucose tolerance test and the intravenous insulin tolerance test have been evaluated in healthy donkeys.[7,10,12,13]

The enteroinsular axis comprises enteroendocrine factors (incretins) and insulin and plays a central role in postprandial glucose disposal and insulin response in horses, ponies and foals.[14,15] The best described incretins are glucose-dependent insulinotropic polypeptide (GIP) secreted by K cells in the proximal small intestine and glucagon-like peptide-1 (GLP-1) produced by L cells in the distal small intestine and proximal large intestine.[16] At least in other species, incretins account for most of the insulin released after a meal.[16] Exaggerated incretin secretion has been proposed as a player in the pathogenesis of ID in horses.[17] Increased insulin and incretin response to oral non-structural carbohydrates and pasture were documented in ID ponies, increasing the risk of endocrinopathic laminitis.[17,18]

Considering the unique metabolic features of the donkey and the consequences of endocrine dysregulation in these animals, evaluating dynamic oral challenges and the response of the enteroinsular axis will provide important insight on asinine metabolic physiology, with diagnostic implications. Therefore, the objectives of this study were: (a) to characterise OGTT and OSTT; and (b) to assess the plasma incretin response to OGTT and OSTT in healthy donkeys.

2 | MATERIALS AND METHODS

2.1 | Animals and body morphometric measurements

Six healthy nonpregnant Andalusian jennies, weighing 238 (55) kg and 4.6 (1.2) years old (data expressed as median and -interquartile range-), were housed together in a sand paddock. Jennies were accustomed to the paddock and herd and were healthy based on physical examination (heart and respiratory rates, mucous membrane colour, capillary refill time, digital pulse and rectal temperature), normal blood work (haematology and biochemistry), and had no history or clinical evidence of laminitis. All animals were under a regular deworming program and were fed alfalfa hay twice a day (1.5% of bodyweight per day) for 1 month prior to the study. Donkeys had a baseline plasma glucose concentration <110 mg/dL and plasma insulin concentration <20 μIU/mL. These values were used according to studies previously published by our research group in donkeys, although a lower insulin cut-off value has also been proposed by other authors in horses and ponies in order to increase the sensitivity of baseline insulin for ID diagnosis.[19,20]

The following morphometric variables were measured: body mass index (BMI), body condition score (BCS), neck score (NS), neck circumference (NC) and neck circumference to height ratio using formulas described for donkeys and horses.[21,22] Three independent evaluators graded BCS and NS using previous scales: BCS from 1 (very thin) to 9 (obese) and the NS from 0 to 4.[9,21]

2.2 | Testing protocols and interpretation

The night (8.00 PM) prior to the test, donkeys were offered alfalfa hay (1.5 kg/donkey) and had free access to water until the morning. Carbohydrate challenges were carried out between 7.00 and 8.00 AM inside the paddock, with no access to food or water during sampling.

Both OGTT and OSTT were performed using protocols previously described for horses.[23,24] For the OGTT, glucose (glucose monohydrate, Merck Life Science) (1 g/kg BW, 20% solution in water) was administered by nasogastric tube. For the OSTT, Karo® light corn syrup (ACH Food Companies) (0.45 mL/kg, estimated to contain 450 mg/kg glucose-based digestible carbohydrates)[25] was administered orally using a 100-mL syringe. Blood samples in both tests were collected at the following time-points: baseline, 30, 60, 90, 120, 150, 180, 210, 240, 300 and 360 minutes. Corn syrup was administered to each donkey by the same operator. After 1 week washout period, the other test was performed. Studies were carried out in spring (February and March) in the Northern hemisphere.

2.3 | Sample handling and endocrine measurements

A sterile catheter was placed in the left jugular vein before experiments for blood sample collection. Blood samples were collected into heparin-containing tubes for insulin, EDTA-containing tubes for incretins and oxalate-containing tubes for glucose measurements. After collection, blood samples were placed immediately on ice, centrifuged at 1500 g for 10 minutes at 4°C, aliquoted and stored at −20°C until analysis.

Plasma insulin (sensitivity limit 2.7 μIU/mL, intra-assay CV < 4.4%) concentration was measured with a human-specific immunoradiometric assay (DIASource ImmunoAssays SA) previously used in donkeys.[9,26] Plasma GIP (lowest sensitivity: 4.2 pg/mL; intra-assay CV: <8.8%, detection limit: 1.83 pg/mL) (Human GIP total ELISA

[EZHGIP-54K]; Millipore Corporation), and aGLP-1 (lowest sensitivity: 2 pM; intra-assay CV: <9%, detection limit: 0.14 pM) (Glucagon-like Peptide-1 active ELISA [EGLP-35K]; Millipore Corporation) concentrations were measured using ELISA kits previously validated for horses and ponies[18,27] at the following times: baseline, 30, 60, 120, 180, 240 and 360 minutes. Glucose concentrations were measured by spectrophotometry (A15 Biosystems).

2.4 | Proxies and curves parameters calculation

Fasting baseline glucose and insulin concentrations were used to calculate the following proxies: glucose:insulin ratio, insulin:glucose ratio, modified insulin to glucose ratio, reciprocal of the square root of insulin, quantitative insulin-sensitivity check index, homeostasis model assessment for insulin resistant and homeostasis model assessment of percentage β-cell function.[7,28]

The following parameters were obtained from the curves of each dynamic test: 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 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), valley to baseline interval (time from minimal glucose until glucose returned to baseline), and area under the curve (trapezoidal method).[10,29,30]

2.5 | Data analysis

Normality was assessed with the Shapiro-Wilk test. Data were not normally distributed. Results are expressed as median and interquartile range (IQR: 75th-25th percentile). Percentiles were calculated using the Tukey's-Hinges test. A Friedman analysis followed by a Wilcoxon test was carried out to determine differences between repeated measures. Correlations were calculated using the Spearman test. A P value <.05 was considered significant. Statistical analysis was performed using a commercial statistical software (IBM SPSS Statistics 25).

3 | RESULTS

3.1 | Morphometric variables, basal concentrations and proxies

All donkeys used in this study had an optimal BCS (4-6) (Table S1). Donkeys were normoglycaemic and normoinsulinaemic (Table S1).[31,32] Baseline plasma glucose, insulin, GIP and aGLP-1 concentrations and proxies are listed in Table S1.

Baseline plasma insulin concentrations were positively correlated with baseline aGLP-1 concentrations ($r = .876$, $P = .02$), but not with GIP (Table 1). Baseline aGLP-1 concentration was also correlated with NS ($r = .84$, $P = .04$) and proxies (Table 1). No correlation was observed between GIP and aGLP-1.

3.2 | Oral glucose tolerance test

All donkeys tolerated the OGTT without complications. Administration of glucose intragastrically led to a significant increase of plasma glucose concentration from 30 minutes to the end of the experiment (Figure 1A), peaking at 180 minutes (240% of baseline concentration, $P < .05$). Plasma insulin concentrations followed a pattern similar to glucose, peaking at 180 minutes (Figure 1B), there was no statistical difference at 360 minutes compared with baseline or peak. Parameters obtained from the analysis of glucose and insulin curves are shown in Table S2.

Plasma GIP concentrations peaked at 180 minutes and were significantly higher than baseline from 30 minutes to the end of the experiment (360 minutes) (Figure 2A, $P < .05$). Plasma aGLP-1 concentrations were significantly higher than baseline from 30 to 360 minutes, peaking at 240 minutes (Figure 3A, $P < .05$). Parameters obtained from the incretin curve analysis are shown in Table 2.

3.3 | Oral sugar tolerance test

All donkeys tolerated the OSTT without complications. Oral administration of corn syrup resulted in a significant increase of glucose (148% of baseline concentration) and insulin concentrations (Figure 1B, $P < .05$), peaking at 300 and 150 minutes, respectively. Glucose and insulin curve analyses are shown in Table S3.

Plasma GIP concentration increased progressively and slowly, reaching maximum concentrations at 360 minutes (Figure 2B). Plasma aGLP-1 concentrations peaked at 120 minutes, returning quickly to baseline at 180 minutes (Figure 3B). Parameters obtained from the incretin curve analysis are shown in Table 2.

4 | DISCUSSION

In this study we evaluated the OGTT and OSTT and assessed the effect of enterally-administered soluble carbohydrates on the enteroinsular axis of healthy donkeys and showed that both glucose and corn syrup triggered an increase in glucose and insulin concentrations that followed different patterns. In addition, these tests elicited a GIP and aGLP-1 response that was more evident with the OGTT. This is the first study characterising the OGTT and OSTT, and reporting the GIP and aGLP-1 response to these tests in healthy donkeys. This information enhances our understanding of metabolic regulation in donkeys and could be relevant in the diagnosis of endocrinopathies, including DMS, in this species.

TABLE 1 Spearman correlations for morphometric variables, baseline glucose and hormone concentrations and proxies (n = 6)

Variable	BWT	BCS	BMI	NC	NS	NCHR	Glucose	Insulin	GIP	aGLP-1	G:I	I:G	RISQI	MIRG	QUICKI	HOMA-IR	HOMA-B%
BWT	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
BCS	0.03	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
BMI	0.91*	0.43	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–
NC	0.69	0.71	0.89*	1	–	–	–	–	–	–	–	–	–	–	–	–	–
NS	0.36	0.90*	0.68	–	1	–	–	–	–	–	–	–	–	–	–	–	–
NCHR	0.46	0.88*	0.77	0.95**	0.96**	1	–	–	–	–	–	–	–	–	–	–	–
Glucose	0.47	0.16	0.41	0.53	0.51	0.37	1	–	–	–	–	–	–	–	–	–	–
Insulin	0.53	0.75	0.79	0.89*	0.94**	0.92**	0.52	1	–	–	–	–	–	–	–	–	–
GIP	0.83*	-0.43	0.55	0.29	-0.17	-0.01	0.21	-0.01	1	–	–	–	–	–	–	–	–
aGLP-1	0.21	0.69	0.49	0.59	0.84*	0.70	0.46	0.88*	-0.36	1	–	–	–	–	–	–	–
G:I	-0.59	-0.75	-0.84*	-0.93**	-0.95*	-0.93*	-0.53	-0.99**	-0.06	-0.85*	1	–	–	–	–	–	–
I:G	0.56	0.74	0.82*	0.89*	0.92**	0.93**	0.43	0.99**	0.03	0.84*	-0.99	1	–	–	–	–	–
RISQI	-0.61	-0.71	-0.83*	-0.93**	-0.94*	-0.91*	-0.63	-0.98**	-0.09	-0.83*	0.99**	-0.97**	1	–	–	–	–
MIRG	0.49	0.77	0.79	0.86*	0.89*	0.92**	0.29	0.97**	-0.02	0.83*	-0.96**	0.99**	-0.92**	1	–	–	–
QUICKI	-0.61	-0.67	-0.79	-0.91*	-0.92**	-0.88*	-0.73	-0.95**	-0.11	-0.81	0.96**	-0.92**	0.99**	-0.86	1	–	–
HOMA-IR	0.53	0.74	0.78	0.88*	0.95**	0.91*	0.56	0.99**	-0.02	0.89*	-0.99**	0.98**	-0.99**	0.95**	-0.96**	1	–
HOMA-B%	0.53	0.75	0.81	0.89*	0.93**	0.93**	0.469	0.99**	0.01	0.86*	-0.99**	0.99**	-0.97**	0.98**	-0.93*	0.99**	1

Abbreviations: aGLP-1, active glucagon-like peptide-1; BCS, body condition score; BMI, body mass index; BWT, bodyweight; G:I, glucose to insulin ratio; GIP, glucose-dependent insulinotropic polypeptide; Glu, glucose concentration; HOMA-B%, homeostasis model assessment of percentage β-cell function; HOMA-IR, homeostasis model assessment for IR; I:G, insulin to glucose ratio; MIRG, modified insulin to glucose ratio; NC, neck circumference; NCHR, neck circumference to height ratio; NS, neck score; QUICKI, quantitative insulin-sensitivity check index; RISQI, reciprocal of the square root of insulin.

*P < .05; **P < .01.

FIGURE 1 Plasma glucose (continuous black line) and insulin (dashed grey line) concentrations after oral glucose (A, OGTT) and oral sugar (B, OSTT) tolerance tests in healthy adult Andalusian female donkeys (n = 6). [a]$P < .05$ vs baseline; [b]$P < .05$ vs 180 min; [c]$P < .05$ vs 150 min
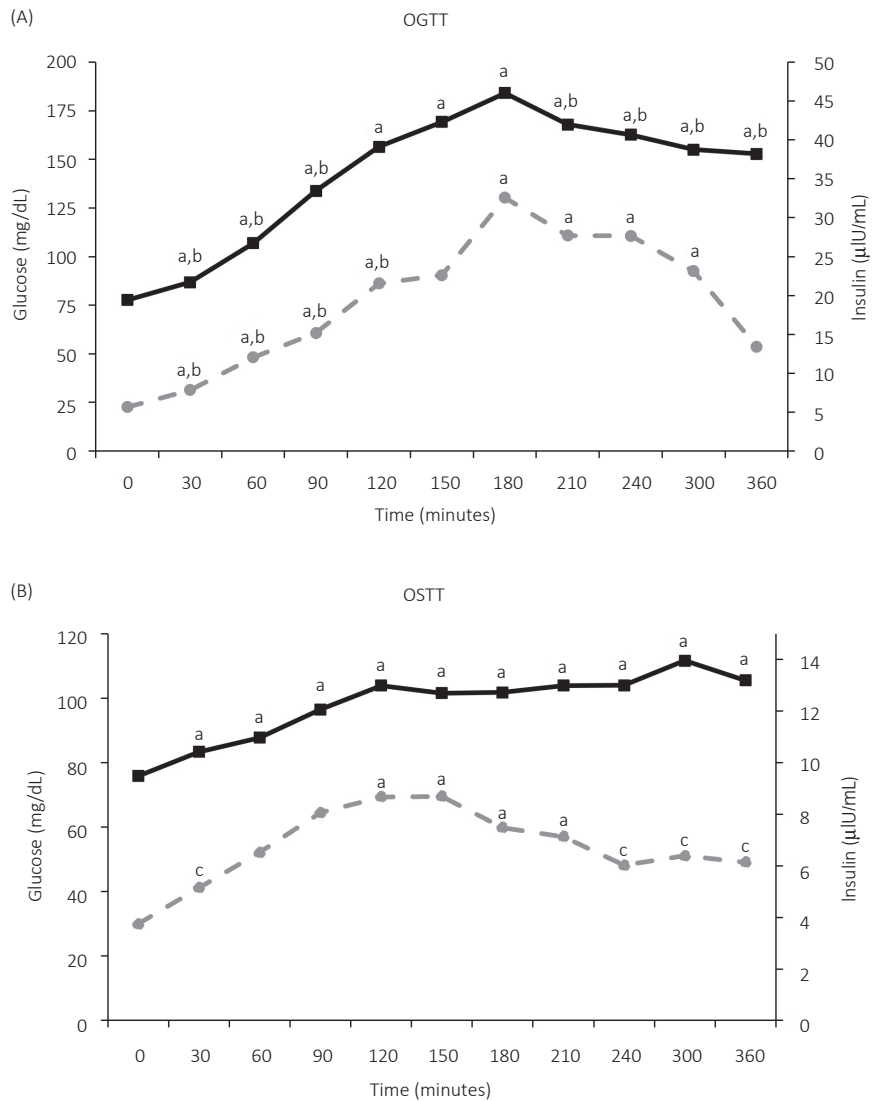
In the OGTT, plasma glucose concentrations in the donkeys of this study peaked later (180 minutes) than previously reported in donkeys (150 minutes),[32] but insulin concentrations were similar. Since fasting and diet influence glucose disposal,[26,33] this difference could be explained by the longer fasting (24 hours) and different diets used (hay and sweet feed).[32] Compared to horses and ponies, glucose and insulin curves in donkeys were right-shifted and returned slower to baseline, although peaks were of similar magnitude,[23,34] and insulin concentration at 180 minutes was lower than cut-off established for ID horses or ponies at 120 minutes (85 μIU/mL).[35] This finding supports the use of both glucose and insulin concentrations to increase diagnostic accuracy and reduce the risk of misdiagnosis of ID in donkeys. Differences between species could be attributable to inter-species effect, idiosyncrasies in gastric emptying, intestinal glucose sensing, intestinal glucose absorption or incretins release; since repeatability for these challenges have not been evaluated in donkeys, it has been reported to be good in other equids.[35]

The OSTT was performed using the high dose described for ponies and horses (0.45 mL/kg),[20,24] however, the response in the donkeys of this study was lower for glucose (100-120 mg/dL vs 130-190 mg/dL)

and insulin (6-8 μIU/mL vs 135 μIU/mL, using both radioimmunoassay technique), but similar to values reported with the low-dose protocol in ponies (0.15 mL/kg). Glucose and insulin curve peaks (120-300 minutes and 120-150 minutes, respectively) were right-shifted in our donkeys compared with values reported for healthy horses and ponies using radioimmunoassay (glucose peak at 60 and 55 minutes and insulin peak at 60 and 75 minutes, horses and ponies, respectively).[24,36,37] Moreover, glucose concentrations remained elevated longer in our donkeys compared with healthy horses and ponies, despite insulin concentrations returning to baseline.[24,36] No donkey was classified as ID using equine insulin cut-off values (>45 μIU/mL at 60-75 minutes).[38] In addition, other factors that could potentially influence results include breed, age, unknown sugar content of the corn syrup, pre-intestinal sugar absorption, low repeatability and poor sensitivity of this test or insulin assay used.[39]

The OSTT is the recommended dynamic test to diagnose ID in horses[8]; however, based on the results of this study, the OGTT appears to better evaluate the postprandial glucose and insulin response in this species. Additional studies evaluating the effects of diet, season, age, other dynamic protocols (eg in-feed OGTT), test
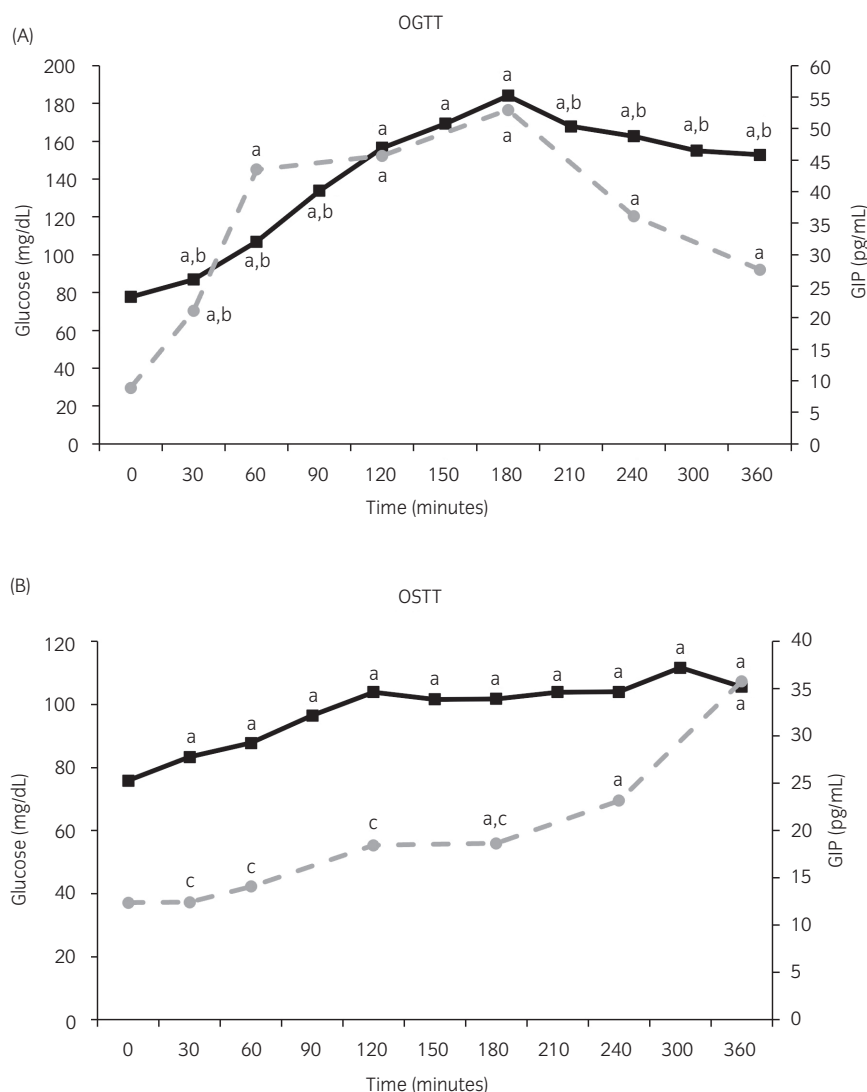
FIGURE 2 Plasma glucose (continuous black line) and glucose-dependent insulinotropic polypeptide (GIP, dashed grey line) concentrations after oral glucose (A, OGTT) and oral sugar (B, OSTT) tolerance tests in healthy adult Andalusian female donkeys (n = 6). [a]$P < .05$ vs baseline; [b]$P < .05$ vs 180 min; [c]$P < .05$ vs. 360 min

repeatability, different carbohydrate doses and comparing healthy and ID donkeys will be needed to further understand endocrine regulation and dysregulation in donkeys. In addition, caution must be taken with cut-off values, since they could differ depending on the assay (radioimmunoassay, chemiluminescent assay or enzyme-linked immunosorbent assay) and analyser.[40]

Baseline plasma GIP concentrations in the donkeys of this study for OGTT were similar to values in ponies using the same ELISA,[17,18] but lower than values reported in ponies and horses using a porcine radioimmunoassay but similar glucose dose (1 g/kg).[41,42] Moreover, the plasma GIP peak occurred later in our study (180 minutes) compared with other equids (120-150 minutes).[41,42] An opposite response to that seen in donkeys was shown in healthy newborn foals after enteral glucose or lactose administration (1 g/kg), where GIP decreased from 5 to 180 minutes post-administration.[15]

Baseline plasma aGLP-1 concentrations for the OGTT in donkeys were higher than values reported for ponies and horses,[17,18,27,43] but similar to other reports,[38,44] all of them using similar ELISA kits. Differences could be attributed to breed, age, diet, body condition, geography or experimental conditions.

Information on GIP changes in response to OSTT in equids is not available. However, GIP concentrations after an in-feed OGTT containing 0.75 g/kg of glucose in 200 g of wheat bran was assessed in ponies.[18] Of interest, the donkeys of our study were administered a higher glucose dose (1 g/kg), but GIP increase was lower and peak occurred later compared with ponies (360 minutes vs 120 minutes).[18] In healthy newborn foals using 0.3, 0.5 or 1 g/kg of oral glucose, a decrease in GIP concentration was observed.[15]

Plasma aGLP-1 concentrations increased after OSTT in the donkeys of this study, with a secretion pattern similar to values reported for horses using 0.15 mL/kg of corn syrup,[27,38] in ponies with the in-feed OGTT[17,18,45] and after feeding in horses and ponies.[43,44] The aGLP-1 response to the OGTT was higher and peaked later than for the OSTT (240% baseline and 240 minutes vs 150% baseline ad 120 minutes). This difference could be due to a higher glucose concentration in the intestinal lumen after intragastric compared with oral carbohydrate administration,[46] which may trigger stronger and longer effect on GLP-1 intestinal receptors. A higher and a delayed aGLP-1 peak in response to OSTT has been related to EMS/laminitis predisposition in horses and ponies and ID diagnosis in

FIGURE 3 Plasma glucose (continuous black line) and active glucagon-like peptide-1 (aGLP-1, dashed grey line) concentrations after oral glucose (A, OGTT) and oral sugar (B, OSTT) tolerance tests in healthy adult Andalusian female donkeys (n = 6). [a]$P < .05$ vs baseline; [b]$P < .05$ vs 180 min; [c]$P < .05$ vs 240 min
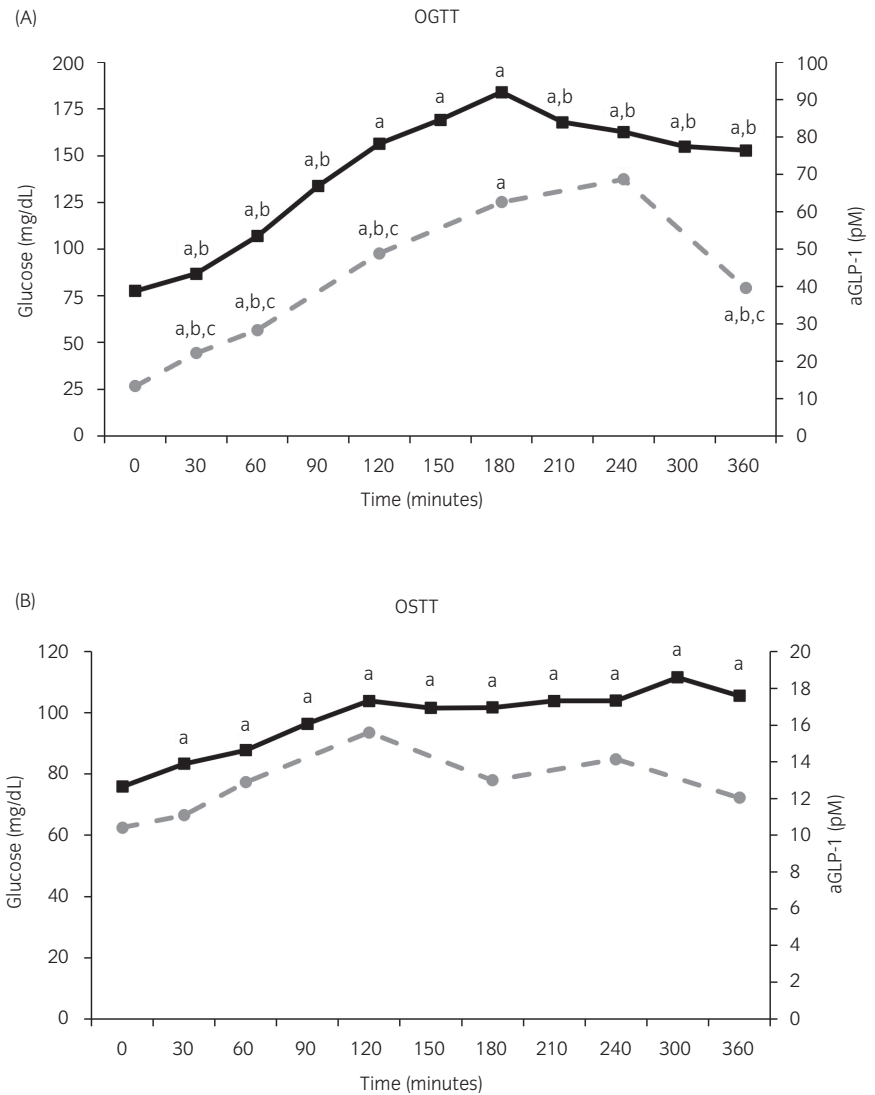

TABLE 2 Analysis of the OGTT and OSTT curves for incretins

Parameter	Total GIP (n = 6)		aGLP-1 (n = 6)	
	OGTT	OSTT	OGTT	OSTT
PPD (min)	360 (0)	360 (0)	360 (0)	360 (0)
PGCR (mg/dL/min)	0.05 (0.04)	0.01 (0.01)	0.01 (0.03)	0.007 (0.01)
NPD (min)	–	–	–	–
SNI (min)	–	–	–	–
Nadir (mg/dL)	–	–	–	–
VD (min)	–	–	–	–
NGCR (mg/dL/min)	–	–	–	–
VBI (min)	–	–	–	–
AUC (×10³ mg/dL/min)	9.1 (14.3)	6.6 (4.3)	19.3 (8.2)	5.1 (3.9)

Abbreviations: aGLP-1, active glucagon-like peptide-1; AUC, area under the curve; GIP, glucose-dependent insulinotropic polypeptide; NGCR, negative glucose clearance rate; NPD, negative phase duration; OGTT, oral glucose tolerance test; OSTT, oral sugar tolerance test; PGCR, positive glucose clearance rate; PPD, positive phase duration; SNI, start-to-nadir interval; VBI, valley-to-baseline interval; VD, valley duration.

horses.[17,18,27,43] Contrary results have been also reported in horses and ponies.[38,44] Donkeys in this study were healthy, with no history or clinical signs of recurrent laminitis, and the same donkey breed was included, thus these variables could be discarded.

The AUC for GIP and aGLP-1 was larger in the OGTT than in the OSTT, mirroring glucose peak and AUC. These findings indicate luminal glucose is a strong stimulating factor for incretin release in donkeys. The distribution and density of GIP and GLP-1 receptors as well as feed adaptations could be related to these findings.

Our results also indicate that insulin secretion is influenced by incretins, being higher with the OGTT. Whether this response is different between healthy and ID donkeys remains to be elucidated. The insulin curve was mirrored better by the aGLP-1 curve (in both the OGTT and the OSTT) than by the GIP curve. This finding could indicate that either aGLP-1 is the main incretin in donkeys or the OGTT is a better method to activate the enteroinsular axis in this species.

The differences observed between either tests for glucose, insulin or incretin concentrations could have also been in part due to mechanisms bypassed with the OGTT (deglutition or mouth sweet taste receptors) activating neural pathways that influence gastrointestinal and pancreatic secretion, an effect of the glucose amount administered on gastric emptying rate, diet composition adaptations and/or gut transit times (small vs large intestine), etc.[47-49] These factors need to be further elucidated in future studies.

Evaluating the effect of feed was not a goal in this study, however, feed composition (high fat, high nonstructural carbohydrate or amino acids content), supplementation and grazing have been reported to alter GIP and aGLP-1 curves in healthy and ID ponies.[17,45] In this study, donkeys were fed the same batch of alfalfa hay from 1 month prior to the challenges and they were not supplemented. Since hay composition analysis was not performed and donkeys were not fed during the tests, further studies evaluating the effect of these factors on incretin response are needed.

A multitude of factors could have influenced discrepancies in insulin and incretins between our results and information published for horses and ponies, including species, breed, age, season, sex, sample size, diet and immunoassays.[26,33,43] Since we tested a small number of Andalusian donkeys, studies with a larger population of healthy and ID donkeys of different breeds would be valuable to further dissect differences between donkeys and horses as well as the clinical value of this information. Donkeys in this study had a narrow age range and tests were performed in February and March, discarding the effects of age and season. However, these tests should not be performed for ID diagnosis until cut-off values and glucose/sugar concentration validation in ID donkeys. The addition of protease inhibitors (eg dipeptidyl peptidase 4 inhibitor) to blood tubes to reduce incretin degradation is recommended by some authors[17,45]; however, one equine study did not find incretin differences when samples were kept on ice and processed rapidly.[18] Although blood samples in our study were chilled on ice and centrifuged and stored to −20°C within 15 minutes post-collection, this variable should be taken into consideration.

In conclusion, donkeys have a functional enteroinsular axis that is activated by enteral carbohydrates. Results from this study further demonstrate endocrine differences between donkeys and horses, supporting the validation of the OSTT and OGTT to assess insulin sensitivity in this species to avoid extrapolation from horses, with the potential risk of misdiagnosis.

CONFLICT OF INTERESTS

No competing interests have been declared.

AUTHOR CONTRIBUTIONS

A. Buzon-Cuevas contributed to study design and study execution. A. Perez-Ecija, R. Toribio and F. Mendoza contributed to study design, data analysis and interpretation, and preparation of the manuscript. All authors gave their final approval to the manuscript.

ETHICAL ANIMAL RESEARCH

This study received approval from Welfare Committees (2015PI/04 and 19-03-15-214).

INFORMED CONSENT

Not applicable.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Francisco J. Mendoza https://orcid.org/0000-0002-7725-8080
Ramiro E. Toribio https://orcid.org/0000-0002-9063-540X
Alejandro Perez-Ecija https://orcid.org/0000-0002-4086-0940

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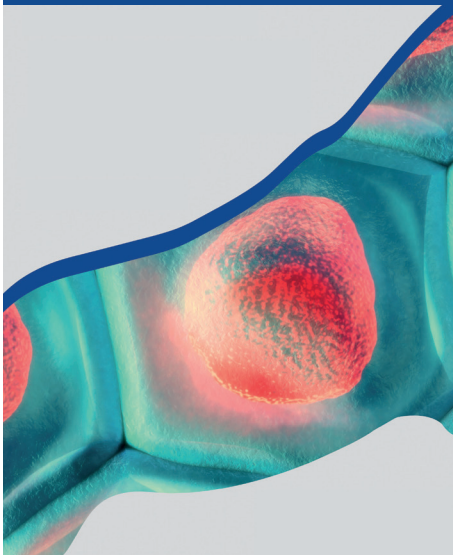
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