Determining insulin sensitivity from glucose tolerance tests in Iberian and landrace pigs

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As insulin sensitivity may help to explain divergences in growth and body composition between native and modern breeds, metabolic responses to glucose infusion were measured using an intra-arterial glucose tolerance test (IAGTT). Iberian (n = 4) and Landrace (n = 5) barrows (47.0 ± 1.2 kg body weight (BW)), fitted with a permanent carotid artery catheter were injected with glucose (500 mg/kg BW) and blood samples collected at -10, 0, 5, 10, 15, 20, 25, 30, 45, 60, 90, 120 and 180 min following glucose infusion. Plasma samples were analysed for insulin, glucose, lactate, triglycerides, cholesterol, creatinine, albumin and urea. Insulin sensitivity indices were calculated and analysed. Mean plasma glucose, creatinine and cholesterol concentrations were lower ($P <$ 0.01) in Iberian (14, 68 and 22%, respectively) than in Landrace pigs during the IAGTT. However, mean plasma insulin, lactate, triglycerides and urea concentrations were greater ($P < 0.001$) in Iberian (50, 35, 18 and 23%, respectively) than in Landrace pigs. Iberian pigs had larger area under the curve (AUC) of insulin ($P < 0.05$) or tended to a greater AUC of lactate ($P < 0.10$), and a smaller ($P < 0.05$) AUC for glucose 0-60 min compared with Landrace pigs. Indices for estimating insulin sensitivity in fasting conditions indicated improved β-cell function in Iberian compared with Landrace pigs, but no difference ($P >$ 0.10) in calculated insulin sensitivity index was found after IAGTT between breeds. A time response ($P < 0.05$) was obtained for insulin, glucose and lactate so that maximum concentration was achieved at 10 and 15 min post-infusion for insulin (Iberian and Landrace pigs, respectively), immediately post-infusion for glucose, and 20 min post-infusion for lactate, decreasing thereafter until basal levels. There was no time effect for the rest of metabolites evaluated. In conclusion, growing Iberian pigs challenged with an IAGTT showed changes in biochemical parameters and insulin response that may indicate an early stage of insulin resistance.

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3 **Abstract**

4 As insulin sensitivity may help to explain divergences in growth and body composition between

5 native and modern breeds, metabolic responses to glucose infusion were measured using an

6 intra-arterial glucose tolerance test (IAGTT). Iberian (n = 4) and Landrace (n = 5) barrows (47.0

7 ± 1.2 kg body weight (BW)), fitted with a permanent carotid artery catheter were injected with

8 glucose (500 mg/kg BW) and blood samples collected at -10, 0, 5, 10, 15, 20, 25, 30, 45, 60, 90,

9   120 and 180 min following glucose infusion. Plasma samples were analysed for insulin, glucose,

10 lactate, triglycerides, cholesterol, creatinine, albumin and urea. Insulin sensitivity indices were

11 calculated and analysed. Mean plasma glucose, creatinine and cholesterol concentrations were

12 lower ($P < 0.01$) in Iberian (14, 68 and 22%, respectively) than in Landrace pigs during the

13 IAGTT. However, mean plasma insulin, lactate, triglycerides and urea concentrations were

14 greater ($P < 0.001$) in Iberian (50, 35, 18 and 23%, respectively) than in Landrace pigs. Iberian

15 pigs had larger area under the curve (AUC) of insulin ($P < 0.05$) or tended to a greater AUC of

16 lactate ($P < 0.10$), and a smaller ($P < 0.05$) AUC for glucose 0-60 min compared with Landrace

17 pigs. Indices for estimating insulin sensitivity in fasting conditions indicated improved β-cell

18 function in Iberian compared with Landrace pigs, but no difference ($P > 0.10$) in calculated

19 insulin sensitivity index was found after IAGTT between breeds. A time response ($P < 0.05$) was

20 obtained for insulin, glucose and lactate so that maximum concentration was achieved at 10 and

21 15 min post-infusion for insulin (Iberian and Landrace pigs, respectively), immediately post-

22 infusion for glucose, and 20 min post-infusion for lactate, decreasing thereafter until basal levels.

23 There was no time effect for the rest of metabolites evaluated. In conclusion, growing Iberian

24 pigs challenged with an IAGTT showed changes in biochemical parameters and insulin response

25 that may indicate an early stage of insulin resistance.

27 Introduction

28 The Iberian pig is a slow growing native breed of the Mediterranean basin with much greater

29 whole body fat content than lean-type pigs (Nieto et al., 2002). Compared with conventional

30 breeds, Iberian pigs show a lower efficiency of energy utilisation for protein deposition in the

31 growing period (Barea et al., 2007). The greater relative viscera weight (Rivera-Ferre et al.,

32   2005) and total heat production (González-Valero et al., 2016) associated in part with the greater

33   rate of muscle protein turnover (Rivera-Ferre et al., 2005) in Iberian compared with lean-type

34   pigs help to explain the low energy efficiency for growth. In fact, Rivera-Ferre et al. (2005)

35   showed that muscle protein degradation was increased in Iberian pigs resulting in decreased

36   muscle protein accretion compared with Landrace pigs. Interestingly, insulin resistance at the

37   muscle level could explain an increased protein degradation (Wang et al., 2006) affecting overall

38   protein accretion. In a previous study using balanced or lysine deficient diets at two crude protein

39   levels, Iberian had greater fasting serum insulin concentration than Landrace pigs (Fernández-

40   Fígares et al., 2007), suggesting the possibility of insulin resistance in Iberian pigs. We

41   hypothesised that Iberian pigs have decreased insulin sensitivity, which could explain differences

42   on growth, body composition and metabolic characteristics compared with modern breeds. The

43   objective of the present study was to evaluate differences on insulin sensitivity between Iberian

44   and Landrace pigs using an intra-arterial glucose tolerance test (IAGTT).

45

46   Materials & Methods

47   Animals and experimental design

48   All procedures used in this study were approved by the Bioethical Committee of the Spanish

49   Council for Scientific Research (RD 53/2013; CSIC, Spain; project reference RECUPERA 2020,

50   FEDER funding) and the animals were cared for in accordance with the Royal Decree No.

51   1201/2005 (Spain). The experiment was performed with five Landrace and four Iberian (Silvela

52   strain) barrows supplied by Granja El Arenal (Córdoba, Spain) and Sánchez Romero Carvajal

53   (Jabugo S.A., Puerto de Santa María, Cádiz, Spain), respectively.

54   The pigs were group housed in a controlled-environment room (20 m²; 21 ± 1.5°C) with *ad*

55   *libitum* access to a standard barley-soybean meal diet (160 g crude protein/kg; 14 MJ

56   metabolizable energy/kg dry matter) and water. During acclimatization, the pigs were adapted to

57   close contact with the personnel involved in the study to facilitate pig handling without stress.

58   After acclimatization and to subsequently avoid the stress of repeated blood sampling, each

59   animal was surgically fitted with a chronic catheter (Tygon, i.d. 1.02 mm, o.d. 1.78 mm; Cole-

60   Parmer, Vernon Hills, IL, USA) in the carotid artery following a procedure described previously

61   (Rodríguez-López et al., 2013). In brief, Tygon rings were attached to the catheter to mark the

62   extent of introduction into the vessel. The day before surgery, pigs were placed in individual

63   pens (2 m²), where nose and eye contact was possible, in a controlled environment room (21 ±

64   1.5 ºC) and feed and water removed. General anaesthesia was induced using an intamuscular

65   (i.m.) combination of Ketamine (15 mg/kg BW; Imalgene 1000, Merial, Barcelona,

66   Spain)/Azaperone (2 mg/kg BW; Stresnil, Steve, Barcelona, Spain) and maintained with

67   isoflurane (0.5-2%; Isoflo; Laboratorios Esteve S.A., Barcelona, Spain) and O₂ (22-44 mL/kg

68   BW/min) through a face mask. N-butyl hyoscine bromide + Sodium metamizol (Buscapina

69   Compositum; Boehringer Ingelheim Spain S.A., Barcelona, Spain) was administered as analgesic

70   (5 mL i.m.). Strict aseptic and sterile conditions were applied along the whole surgical

71   procedure. An incision (8-10 cm) was done along the jugular furrow and to expose the carotid

72   artery. Catheter was introduced 12 cm toward the aorta arch and fixed by non-absorbable suture.

73   The catheter was secured directly in place with a purse-string suture where the artery was not

74   occluded. The incision was sutured. A patch (10 × 10 cm) was glued to the skin together with the

75   catheter close to the exteriorization point (down and caudal from the ear), guided to the shoulder,

76   fixed again and kept coiled with a second patch-pocket. Following surgery, pigs returned to their

77   individual pens under heat lamps to provide additional warmth during anaesthesia recovery.

78   After that, pigs were fed with free access to water. Feed, water intake and body temperature were

79   monitored during a couple of days. Then, pigs were group housed again until the blood sampling

80   and fed at 2.4 × metabolizable energy for maintenance (444 kJ/kg$^{0.75}$ body weight (BW)/day;

81   National Research Council (NRC), 1998). Wound from surgery and catheter exteriorization site

82   were kept clean and sprayed with antibiotic (Veterin Tenicol; Lab. Intervet S.A., Salamanca,

83   Spain) to prevent infection. Pigs were injected i.m. with a broad spectrum antibiotic (Duphapen

84   Strep; Fort Dodge Vet. S.A., Gerona, Spain) during 5 days (5-10 mg/kg BW/day). After 10 days,

85   stitches were removed and pigs were ready for the blood sampling. Patency of catheter was

86   checked weekly, cleaned with alcohol and flushed with sterile heparinized (Fragmin, 5000

87   IU/0.2 mL; Pharmacia Spain S.A., Barcelona, Spain) saline (250 IU/mL).

88   The day before the experiment, pigs were randomly accommodated in the individual pens

89   for easier blood sampling and fed normally. On the day of the experiment, all pigs (46.0 ± 3.0

90   and 47.8 ± 3.6 kg BW for Iberian and Landrace pigs, respectively; that is about 18 and 14 weeks

91   of age, respectively) were given an intra-arterial bolus (500 mg/kg BW) of glucose (50% sterile

92   dextrose; glucosado 50% Braun, B. Braun Medical S.A., Rubi, Barcelona, Spain) over one min

93   period after an overnight fast. The catheter was immediately flushed with 5 mL of sterile saline

94   solution. Blood samples (5 mL) were collected at -10, 0 (20-30 seconds after the bolus of glucose

95   and the saline solution), 5, 10, 15, 20, 25, 30, 45, 60, 90, 120 and 180 min following glucose

96   infusion. At the end of the study, pigs were slaughtered -in accordance with the Royal Decree

97   No. 1201/2005 (Spain)- by electrical stunning.

98   The staff involved in the experiment was aware of the group allocation at the different stages

99   of the experiment.

100

Biochemical analysis and calculations

102 Plasma was obtained by centrifugation (4ºC, 1820 × g for 30 min; Eppendorf 5810 R, Hamburg,

103 Germany) and stored in aliquots at -20ºC until insulin and metabolites (glucose, lactate,

104 triglycerides, cholesterol, creatinine, albumin and urea) were analysed. All samples were assayed

105 in duplicate except for insulin which was assayed in triplicate.

106 Insulin was measured using commercially-available radioimmuno assay kit following the

107 directions of the manufacturer (Millipore porcine insulin radioimmuno assay kit; Cat. PI-12K).

108 Radioactivity in samples was measured using a gamma counter (Behring 1612; Nuclear

109 Enterprises Ltd, Edinburgh, Scotland). Human insulin was used as standard, and the assay was

110 validated for use in porcine plasma samples (Fernández-Fígares et al., 2007). The intra- and

111 inter-assay coefficient of variation for plasma insulin were 4.4 and 9.1%, respectively. The

112 lowest level of insulin that can be detected by this assay is 1.611 μU/mL when using a 100 μL

113 sample size.

114 Plasma glucose, lactate, triglycerides, cholesterol, creatinine, albumin and urea were

115 measured colorimetrically using an automated Cobas Integra 400® analyser (Roche Diagnostics

116 GmbH, Mannheim, Germany). Analyses were performed in a single run where intra-assays

117 coefficients of variation were 1.3, 0.92, 1.6, 0.81, 3.1, 1.2 and 2.3% for glucose, lactate,

118 triglycerides, cholesterol, creatinine, albumin and urea, respectively.

119 Responses of plasma insulin, glucose and lactate were evaluated separately by computing

120 total area under the response curve (AUC) determined using trapezoidal geometry (GraphPad

121 Prism, Version 5.02. San Diego, CA) for the time period indicated following intra-arterial

122 glucose infusion (e.g. AUC_{0-5} stands for the integrated area between 0-5 min post-infusion,

123   AUC0-10 between 0-10 min post-infusion, and so on, until AUC0-180). Basal levels per breed

124   (at time -10 min) were used to calculate the corresponding AUC per metabolite. The rates of

125   decline in plasma insulin and glucose concentrations for both breeds were calculated based on

126   the slope in the linear portion of the response curve from 0 to 30 min after IAGTT challenge

127   (Christoffersen et al., 2009). Results were then expressed as a fractional rate constant determined

128   from the slope of the natural logarithm of plasma concentrations vs. time (Shipley and Clark,

129   1972 (cited by Gopinath and Etherton, 1989)). The fractional turnover rates (k), or disappearance

130   rates, of plasma insulin and glucose (%/min) were calculated using the relationship (Kaneko et

131   al., 2008):

132    $k = (Ln1 - Ln2)/(T_2 - T_1)$

133   where Ln1 and Ln2 are the natural logarithms of plasma insulin ($\mu U/mL$) or glucose (Mm)

134   concentrations at times T_1 (0 min) and T_2 (30 min), respectively.

135   From the k value, the half-life, $T_{1/2}$ (min), may be calculated as:

136    $T_{1/2} = 100 \times 0.693/k$

137   For insulin sensitivity, indices used in human medicine were used.

138   The so-called homeostasis model assessment (HOMA; Matthews et al., 1985) was

139   calculated for estimating insulin resistance (HOMA-IR) and β-cell function (HOMA-%B) at

140   fasting conditions, as follows:

141   HOMA-IR = fasting plasma insulin ($\mu U/mL$) $\times$ fasting plasma glucose (mM)/22.5

142   HOMA-%B = (20 $\times$ fasting plasma insulin ($\mu U/mL$))/(fasting plasma glucose (mM) - 3.5)

143   It is assumed that non-insulin-resistant individuals have 100% β-cell function and an insulin

144   resistance of 1.

145   The quantitative insulin sensitivity check index (QUICKI; Katz et al., 2000) was computed

146   as:

147    $QUICKI = 1/[Ln(I_0) + Ln(G_0)]$

148   where I_0 is the fasting insulin (µU/mL), and G_0 is the fasting glucose (mg/dl).

149   Finally, the insulin sensitivity index (CSI; Tura et al., 2010) was calculated as:

150    $CSI = K_G/(\Delta AUC_{INS}/T)$

151   where K_G is the slope of Ln glucose in the linear portion of the response curve, ΔAUC_{INS} is the

152   AUC of insulin above basal value, and T is the time interval (between 0 and 30 min) when K_G

153   and ΔAUC_{INS} are calculated.

154

155   **Statistical analyses**

156   The number of animals was *a priori* calculated using the G*Power software (Heinrich-Heine-

157   Universität Düsseldorf (Faul et al., 2007)). Accepting an alpha risk of 0.05 and a beta risk of 0.2

158   in a two-sided test, five subjects are necessary in first group and five in the second to recognize

159   as statistically significant a difference greater than or equal to 12 µU/mL on insulin

160   concentration and a common standard deviation of 6.3 µU/mL based on previous studies

161   (Fernández-Fígares et al., 2007). A total of five pigs per treatment was also used by others (e.g.

162   Stoll et al., 1999). However, one Iberian pig lost the arterial catheter during the recovery period

163   after surgery and only four Iberian pigs could be used.

164   Plasma metabolites were evaluated using a mixed ANOVA with repeated measures (Version

165   9.4; PROC MIXED, SAS Institute Inc., Cary, NC, USA) with the fixed effects of breed, time of

166   sampling and their interaction in the model statement. The pig was considered the experimental

167   unit and a random effect. First-order ante dependence covariance ANTE(1) was used, which

168 allows unequal variances over time and unequal correlations and covariance among different

169 pairs of measurements. Plasma concentration differences between breeds at each sampling time

170 were analysed by the pdiff (piecewise differentiable) option.

171 Assumptions that are required for an ANOVA were tested following the protocol from Zuur

172 et al. (2010). Homogeneity of variance was assured by applying the Levene's-Test. No

173 transformation was required. Least square means and pooled standard error of mean (SEM) are

174 presented. Outliers were identified and removed when the absolute studentized residues

175 exceeded 3. Differences were considered significant at $P < 0.05$ and trends approaching

176 significance were considered for $0.05 < P < 0.10$.

177

178 **Results**

179 Average plasma metabolites and insulin concentrations after the IAGTT are shown in Table 1.

180 Mean plasma glucose, cholesterol and creatinine concentrations were lower in Iberian (14, 22

181 and 68%, respectively; $P < 0.05$) compared with Landrace pigs. However, mean plasma insulin,

182 lactate, triglycerides and urea concentrations were greater in Iberian (50, 35, 18 and 23%,

183 respectively; $0.01 < P < 0.001$) than in Landrace pigs. No differences ($P > 0.10$) were found

184 between breeds for albumin levels.

185 Fasting plasma insulin was greater in Iberian compared with Landrace pigs ($P < 0.05$; Table

186 2) whereas fasting plasma glucose was similar for both breeds ($P > 0.10$; Table 2). No

187 differences between breeds were found in fasting plasma albumin (Iberian 0.50 and Landrace

188 0.54 μM), urea (Iberian 3.3 and Landrace 3.0 mM), cholesterol (Iberian 1.46 and Landrace 1.79

189 mM) and triglycerides (Iberian 0.28 and Landrace 0.22 mM). On the other hand plasma fasting

190 creatinine was lower in Iberian pigs compared to Landrace (54 and 102 µM, SEM = 8.18,

191 respectively; $P < 0.01$).

192   Only plasma insulin (Fig. 1), glucose (Fig. 2) and lactate (Fig. 3) concentrations changed

193 throughout time ($P < 0.001$; Table 1) after the IAGTT.

194   An interaction between breed and time was found for plasma insulin, such that concentration

195 of insulin was greater in Iberian pigs from -10-15 min and from 90-180 min ($P < 0.05$, with $P <$

196 0.10 at times 0 and 90 min) and lower at 25 min ($P < 0.10$; Fig. 1). In both breeds, plasma

197 insulin levels increased 7-fold, reaching a peak concentration at 10 and 15 min after glucose

198 infusion for Iberian (113.6 ± 7.1 µU/mL) and Landrace (55.7 ± 6.4 µU/mL) pigs, respectively.

199 Insulin remained well above fasting levels until 20 and 45 min after glucose infusion for Iberian

200 and Landrace pigs, respectively; thereafter insulin levels rapidly decreased until fasting levels

201 were attained. Insulin disappearance rate tended to increase in Iberian compared with Landrace

202 pigs ($0.05 < P < 0.10$) while insulin half-life tended to decrease ($0.05 < P < 0.10$; Table 2).

203   Glucose peaked (Fig. 2) immediately after glucose infusion reaching a value of 19.6 and

204 21.2 mmol/L for Iberian and Landrace pigs, respectively. Subsequently, glucose concentration

205 gradually decreased to values below fasting levels after 25 and 30 min, respectively for Iberian

206 and Landrace pigs. The lowest plasma glucose concentration (glucose nadir) was found at 45

207 min (2.95 and 3.70 mmol/L for Iberian and Landrace pigs, respectively). After glucose nadir,

208 glucose concentration gradually increased again to reach values comparable to fasting levels at

209 180 min. No differences were found between breeds for glucose disappearance rate ($P > 0.10$;

210 Table 2) or glucose half-life ($P > 0.10$; Table 2).

211   Lactate increased after the IAGTT, peaked at 20 min for both breeds and declined

212 progressively until reaching basal concentrations at 180 min (Fig. 3).

213   The AUC values for each sampling time of insulin, glucose and lactate are shown in Table 3,

214   4 and 5, respectively. Insulin AUC was greater ($P < 0.05$) for Iberian compared with Landrace

215   pigs at all times.

216   Conversely, glucose AUC at 0-15, 0-20, 0-25, 0-30, 0-45 and 0-60 min were lower ($P <$

217   0.05) for Iberian than Landrace pigs. Aditionally, glucose AUC tended to be lower ($0.05 < P <$

218   0.10) at 0-10, 0-90 and 0-120 min. Plasma lactate AUC was greater ($P < 0.05$) for Iberian pigs at

219   0-10 and 0-15 min and tended to be greater ($0.05 < P < 0.10$) at 0-5, 0-20, 0-25, 0-90, 0-120, 0-

220   150 and 0-180 min after glucose infusion.

221   Indices of insulin sensitivity are shown in Table 2. The QUICKI index decreased ($P < 0.05$)

222   while HOMA-%B index increased ($P < 0.01$) in Iberian compared with Landrace pigs. No

223   differences ($P > 0.10$) were found for HOMA-IR and CSI.

224

Discussion

226   The IAGTT method allowed the comparison of the insulin responsiveness of an obese (Iberian)

227   and a lean (Landrace) pig breed. It is well established that Iberian pigs have a much greater

228   capacity of lipid deposition in comparison to lean swine breeds (Nieto et al., 2002; Ovilo et al.,

229   2005; Muñoz et al., 2009) and has since been proposed as a pig model for obesity studies

230   (Rodriguez Rodriguez et al., 2020). For the reason that important differences in for instance

231   protein turnover may take place during development (Lobley, 1993), the study of animal breeds

232   with disparate growth capability is not a simple issue. Thus, it is desirable that animals are

233   comparable for age or physiological state. As the developmental age of the animals may vary, a

234   decision was made regarding the use pigs of the same BW considering that age difference at this

235   early state was acceptable.

236   As arterial blood represents the metabolites concentration to which the tissues are exposed

237   (Brouns et al., 2005), chronic catheters were inserted in carotid artery for glucose infusion and

238   blood sampling.

239   In the current study we have shown that despite the higher fasting plasma insulin, Iberian

240   pigs produce a higher insulin response after glucose infusion when compared to Landrace pigs

241   (18 and 14 weeks of age, respectively). Greater postprandial serum levels of insulin have been

242   described in 20 kg BW Iberian (11 weeks of age) compared to Landrace pigs after glucose

243   infusion (Fernández-Fígares et al., 2007), and in 11 kg BW Ossabaw (obese; 10 weeks of age)

244   compared to 16.5 kg Yorkshire (10 weeks of age) pigs (Wangsness et al., 1981). However, other

245   comparative studies using a standard diet found increased insulin secretion in 75-120 kg BW

246   Large White boars than in 40-75 kg BW Meishan boars (obese breed) at 20 and 52 weeks of age,

247   respectively (Weiler et al., 1998). The limited growth and development of slow growing pigs

248   could result at least partly from disturbances in insulin secretion and/or in insulin binding,

249   leading to insulin sensitivity, because most cells of the body require insulin for adequate uptake

250   of glucose and amino acids (Claus and Weiler, 1994). If the concentration of insulin is compared

251   among animals of different breeds, the sensitivity of each breed to insulin should be considered.

252   In this study we were also interested in other key metabolites which could provide additional

253   information concerning reduced insulin sensitivity in Iberian pigs. After glucose infusion,

254   glucose plasma concentration rapidly returned to preprandial values in the present experiment,

255   which indicates that exogenous glucose was efficiently metabolized, stored as glycogen, or both.

256   As expected, when glucose was infused, plasma glucose levels were rapidly increased and a

257   subsequent insulin response was observed. The elevated insulin lowered plasma glucose below

258   fasting values within 20 and 25 min for Iberian and Landrace pigs, respectively, and insulin

259   levels returned to baseline as plasma glucose declined. In our study, glucose concentration and

260   glucose AUC during the IAGTT were lower in Iberian compared with Landrace pigs, with no

261   differences in fasting plasma glucose, maybe due to the limited number of pigs. When

262   interpreting the individual glucose curves, a monophasic pattern was identified for both breeds.

263   The lower glucose AUC of Iberian pigs (-19% on average) may be related to the greater insulin

264   AUC (+33% on average), a common pattern in many models of obesity (Kay et al., 2001).

265   However, the reasons for the unequal physiological response between breeds are not well

266   understood and must be discussed.

267   As it has been proved that the energy needs of portal-drained viscera are fulfilled by the

268   oxidation of glucose, glutamate, and glutamine in pigs (Stoll et al., 1999), a larger

269   gastrointestinal tract of Iberian pigs compared to Landrace (Rivera-Ferre et al., 2005) is in line

270   with the decreased AUC of glucose reported in our experiment.

271   However, despite the larger size of the gastrointestinal tract and lower portal blood flow

272   (González-Valero et al., 2016) of Iberian compared with Landrace pigs, no differences on net

273   portal flux of glucose after ingestion of the same diet were found (Rodríguez-López et al., 2013).

274   Differences on insulin stimulated glucose transport at portal-drained viscera level may help to

275   explain these results. Iberian have lower glucose concentrations than Landrace pigs after an

276   intravenous adrenaline challenge (Fernández-Fígares et al., 2016), suggesting a decreased

277   response of Iberian pigs to sympathetic nervous system stimuli which is in line with the lower

278   glucose AUC reported here.

279   When insulin sensitivity indices used in human medicine were applied to the conditions of

280   the present experiment, QUICKI and HOMA-%B were more sensitive detecting differences

281   between breeds. Indeed, QUICKI index decreased in Iberian compared with Landrace pigs,

282   pointing out an incipient insulin sensitivity impairment in fasting Iberian pigs. Similarly, reduced

283   QUICKI index (0.5 vs. 0.6) was found in Bama miniature pigs fed a high sucrose and fat diet

284   compared with a control diet, respectively (Liu et al., 2017). The QUICKI index has been shown

285   to provide reasonable approximations of insulin efficiency in minipigs (Christoffersen et al.,

286   2009).

287   When we used the HOMA, differences on hepatic HOMA-IR were negligible between

288   breeds (3.3 and 2.3 for Iberian and Landrace pigs, respectively; $P > 0.10$). However, Iberian had

289   improved β-cell function compared with Landrace pigs according to HOMA-%B (267 and 100,

290   respectively; $P < 0.01$), which may be due to enhanced sensitivity of the β-cells to glucose

291   during the fasting period. As a consequence, β-cell insulin synthesis in Iberian pigs increased in

292   accordance with the increased insulin release after the glucose tolerance test and the elevated

293   basal insulin concentrations reported for Iberian pigs. This is consistent with decreased QUICKI

294   in Iberian pigs compared to Landrace (0.31 and 0.33, respectively; $P < 0.05$).

295   Lactate appearance after an intravenous glucose test is positively associated with insulin

296   sensitivity in humans (Lovejoy et al., 1992), as it is related to lactate production by insulin

297   sensitive tissues (mainly muscle and fat). Because only limited amounts of lactate are produced

298   by muscle after glucose loading (Ykijarvinen et al., 1990), the source of lactate appearance

299   should predominantly be adipose tissue (Lovejoy et al., 1992), with a large capacity to convert

300   glucose to lactate (Marin et al., 1987). We report here a delay of 20 min in plasma lactate

301   elevation relative to glucose peak following IAGTT, which may reflect the time lag in adipose

302   tissue uptake of glucose and subsequent lactate production under the stimulation of insulin.

303   Compared with Landrace, the increased lactate AUC in Iberian pigs after the IAGTT could

304   therefore be a consequence of the greater adipose tissue availability (Nieto et al., 2002) instead

305   of greater insulin sensitivity. On the other hand, insulin resistance was associated with elevated

306   basal lactate levels in obese humans (Lovejoy et al., 1990), so increased basal lactate

307   concentrations in Iberian pigs (1.040 vs. 0.730 mmol/L; SEM = 0.063) could also indicate

308   insulin resistance or reduced insulin sensitivity. Although inhibition of insulin action on

309   glycogenolysis in fasting conditions may lead to increased glucose release from glycogen and

310   subsequent conversion of glucose to lactate, there is no direct evidence of this. There is indirect

311   evidence, though, that elevated lactate levels could be a consequence of greater adipose tissue

312   availability and may also reflect a glucose sparing effect (decreased glucose utilisation) in

313   muscle (Pearce and Connett, 1980).

314   Obesity is frequently associated with different degrees of dyslipidemia manifested as

315   increased triglyceridemia and low HDL-cholesterol. In our experiment, we found lower plasma

316   total cholesterol but greater plasma triglycerides concentration in Iberian compared with

317   Landrace pigs. Although we did not separate LDL and HDL fractions, total cholesterol

318   concentration are phenotypically related with LDL and HDL cholesterol concentrations in pigs

319   (Rauw et al., 2007). Reduced total cholesterol concentration could be due to reduced hepatic

320   insulin sensitivity as insulin stimulates cholesterol synthesis (Nelson and Cox, 2017). In any case

321   the cholesterolemia for both breeds in the present experiment was in the lower range of

322   published values (Fernández-Fígares et al., 2007). Indeed, the pigs in this study were still very

323   young and so a greater level should be expected at a later stage of development (Rauw et al.,

324   2007).

325   Previous studies in our lab have shown the low genetic potential of growing Iberian pigs for

326   muscle protein deposition in comparison to lean breeds (Nieto et al., 2002), possibly due to the

327   greater muscle protein degradation and turnover of the former (Rivera-Ferre et al., 2005). In line

328   with this, plasma urea level (an indirect protein degradation indicator) was in the present study

329   23% greater in Iberian compared with Landrace pigs. Differences on circulating insulin or the

330   capacity of insulin release between breeds may explain differences in lean tissue deposition, as

331   insulin has an important role in skeletal muscle metabolism (Wang et al., 2006). In obese db/db

332   mice (a model of insulin deficiency) higher muscle protein degradation in comparison with

333   control mice (normal plasma insulin concentration) was reported; the authors concluded that

334   insulin resistance was associated with accelerated muscle protein degradation (Wang et al.,

335   2006). The elevated protein degradation reported in Iberian compared with Landrace pigs

336   (Rivera-Ferre et al., 2005) suggests the possibility of insulin resistance at this level. The lower

337   plasma creatinine level (indicator of muscle mass) found in this study for Iberian pigs is in

338   accordance with previous studies (Fernández-Fígares et al., 2007) and also with the low muscle

339   protein deposition and muscle size described previously (Nieto et al., 2002; Rivera-Ferre et al.,

340   2005). As insulin resistance is associated with decreased muscle mass, plasma creatinine levels

341   can also be used as an indicator of insulin signalling disorders as reported by Kashima et al.

342   (2017) in humans. Further research regarding amino acids concentration after an IAGTT may

343   help to explain differences in the effect of insulin on muscle protein metabolism between breeds.

344   Previous studies from our lab indicate that growing Iberian pigs are prone to insulin

345   resistance compared with modern breeds as denoted by increased hepatic gluconeogenesis

346   (González-Valero et al., 2014), greater plasma free fatty acid concentration (Fernández-Fígares

347   et al., 2016) and lower plasma creatinine and QUICKI (Fernández-Fígares et al., 2007).

348   Additionally, in this experiment we show greater HOMA-%B and increased plasma insulin and

349   lactate concentrations after an IAGTT. The increased plasma insulin AUC after an IAGTT

350   suggests insulin resistance in comparison to the values obtained for lean pigs, although the

351 concentration of glucose remained low which could indicate the absence of a peripheral insulin

352 resistance. Although Iberian pigs may be considered an obese breed in terms of body

353 composition (Nieto et al., 2002; Barea et al., 2007), insulin resistance mechanisms have not yet

354 been fully established at the development stage of the pigs in this experiment. Insulin resistance

355 and impaired glucose tolerance has been shown in Iberian sows (2.5 years old) *ad libitum* fed a

356 saturated fat enriched diet for three months (Torres-Rovira et al., 2012).

357

358 **Conclusions**

359 Although our results support the existence of an insulin resistance or a decreased insulin

360 sensitivity in growing Iberian pigs, caution should be taken because of the reduced number of

361 pigs used. The utilization of the hyperinsulinemic euglycemic clamp, the most definitive

362 approach to determine whole-body insulin action should provide conclusive evidence regarding

363 the establishment of insulin resistance in growing Iberian pigs.

364

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503

Average plasma metabolites and insulin concentrations in Iberian (n = 4) and Landrace (n = 5) pigs during an intra-arterial glucose challenge (IAGTT; 500 mg/kg BW, 0-180 min).[a]

[a] Average (n = 9) basal level of each metabolite: Glucose = 5.33 mM, Insulin = 11.43 μU/mL, Lactate = 0.87 mM, Triglycerides = 0.25 mM, Albumin = 0.46 mM, Cholesterol = 1.63 mM, Creatinine = 76.2 μM, Urea = 2.92 mM.

[b] ns = non-significant, ** $P < 0.01$, *** $P < 0.001$.

[c] Standard error of mean.

	Breed			P-value[b]		
	Iberian	Landrace	SEM[c]	Breed	Time	Breed × Time
Insulin (μU/mL)	41	27	1.9	***	***	***
Glucose (mmol/L)	6.8	7.7	0.26	**	***	ns
Lactate (mmol/L)	1.3	1.0	0.039	***	***	ns
Triglycerides (mmol/L)	0.28	0.24	0.009	**	ns	ns
Cholesterol (mmol/L)	1.5	1.8	0.033	***	ns	ns
Creatinine (μmol/L)	54	90	1.2	**	ns	ns
Albumin (mmol/L)	0.48	0.50	0.009	ns	ns	ns
Urea (mmol/L)	3.0	2.4	0.102	***	ns	ns

Indices of glucose tolerance and insulin sensitivity in Iberian (n = 4) and Landrace (n = 5) pigs subjected to an intra-arterial glucose tolerance test.[a]

[a] QUICKI, quantitative insulin sensitivity check index; HOMA-IR, homeostasis model assessment for estimating insulin resistance; HOMA-%B, homeostasis model assessment for estimating β-cell function; CSI, calculated insulin sensitivity index.

[b] Standard error of mean.

[c] ns = non-significant, [†] $0.05 < P < 0.10$, * $P < 0.05$, ** $P < 0.01$.

	Breed		SEM[b]	*P*-value[c]
	Iberian	Landrace		
Fasting insulin (μU/mL)	16	8	1.6	*
Fasting glucose (mmol/L)	4.7	5.9	0.92	ns
Insulin disappearance rate (%/min)	7.2	3.8	1.01	†
Glucose disappearance rate (%/min)	5.9	3.9	0.77	†
Insulin half-live (min)	10	21	3.6	†
Glucose half-live (min)	22	12	4.8	ns
QUICKI	0.31	0.33	0.007	*
HOMA-IR	3.3	2.3	0.58	ns
HOMA-%B	267	100	26	**
CSI ($\times 10^{-4}$)	-12	-13	1.8	ns

Area under the curve (AUC, µU × min/mL) of plasma insulin during intra-arterial glucose tolerance test between minute 0 and indicated time post-challenge in Iberian (n = 4) and Landrace (n = 5) pigs.

[a] Standard error of mean.

[b] * $P < 0.05$, ** $P < 0.01$.

	Iberian	Landrace	SEM[a]	P-value[b]
AUC 0-5 min	464	295	40.7	*
AUC 0-10 min	993	587	72.6	**
AUC 0-15 min	1470	873	93.3	**
AUC 0-20 min	1768	1135	95.1	**
AUC 0-25 min	1919	1335	92.5	**
AUC 0-30 min	1993	1461	89.0	**
AUC 0-45 min	2169	1652	106.3	**
AUC 0-60 min	2340	1757	135.1	*
AUC 0-90 min	2809	1985	176.3	*
AUC 0-120 min	3358	2239	206.5	**
AUC 0-150 min	3787	2459	214.3	**
AUC 0-180 min	4183	2642	229.1	**

Area under the curve (AUC, mmol × min/L) of plasma glucose during intra-arterial glucose tolerance test between minute 0 and indicated time post-challenge in Iberian (n = 4) and Landrace (n = 5) pigs.

[a] Standard error of mean.

[b] ns = non-significant, [†] 0.05< P < 0.10, * P < 0.05.

	Iberian	Landrace	SEM[a]	*P*-value[b]
AUC 0-5 min	82.6	90.8	3.94	ns
AUC 0-10 min	140	159	7.6	†
AUC 0-15 min	181	215	10.4	*
AUC 0-20 min	211	260	12.9	*
AUC 0-25 min	235	295	15.7	*
AUC 0-30 min	252	323	18.7	*
AUC 0-45 min	297	388	25.7	*
AUC 0-60 min	350	446	28.3	*
AUC 0-90 min	489	575	30.4	†
AUC 0-120 min	631	710	30.0	†
AUC 0-150 min	769	845	30.2	ns
AUC 0-180 min	913	979	31.7	ns

Area under the curve (AUC, mmol × min/L) of plasma lactate during intra-arterial glucose tolerance test between minute 0 and indicated time post-challenge in Iberian (n = 4) and Landrace (n = 5) pigs.

[a] Standard error of mean.

[b] ns = non-significant, [†] $0.05 < P < 0.10$, * $P < 0.05$.

	Iberian	Landrace	SEM[a]	P-value[b]
AUC 0-5 min	5.81	4.34	0.473	†
AUC 0-10 min	12.8	9.4	0.91	*
AUC 0-15 min	20.7	15.5	1.45	*
AUC 0-20 min	29.0	22.1	2.23	†
AUC 0-25 min	37.3	28.9	3.09	†
AUC 0-30 min	45.4	35.6	4.00	ns
AUC 0-45 min	68.4	53.3	6.87	ns
AUC 0-60 min	89.6	67.1	9.29	ns
AUC 0-90 min	123	89.4	13.01	†
AUC 0-120 min	152	109	15.7	†
AUC 0-150 min	180	128	17.6	†
AUC 0-180 min	208	145	20.7	†

Figure 1

Plasma insulin concentration during intra-arterial glucose challenge test (500 mg/kg BW; 180 min sampling) in growing Iberian (n = 4) and Landrace (n = 5) pigs.

† 0.05 < P < 0.10, * P < 0.05, *** P < 0.001.
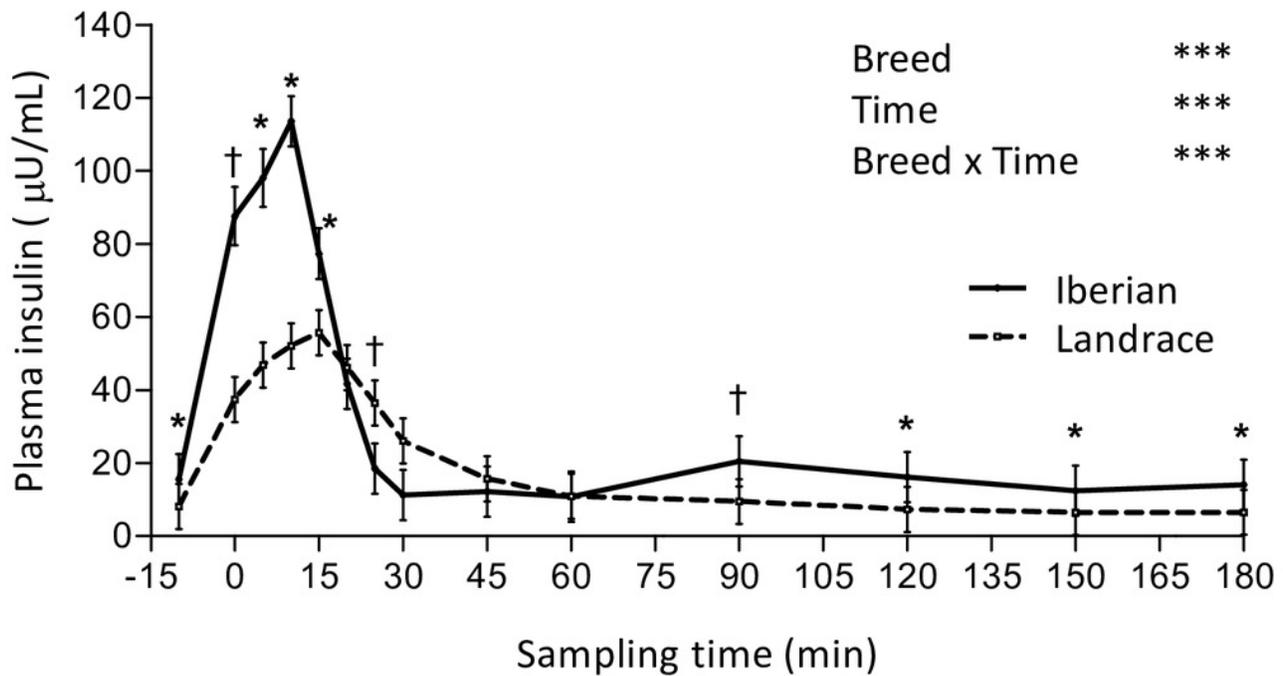
Figure 2

Plasma glucose concentration during intra-arterial glucose challenge test (500 mg/kg BW; 180 min sampling) in growing Iberian (n = 4) and Landrace (n = 5) pigs.

ns, not significant ($P > 0.10$); † $0.05 < P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
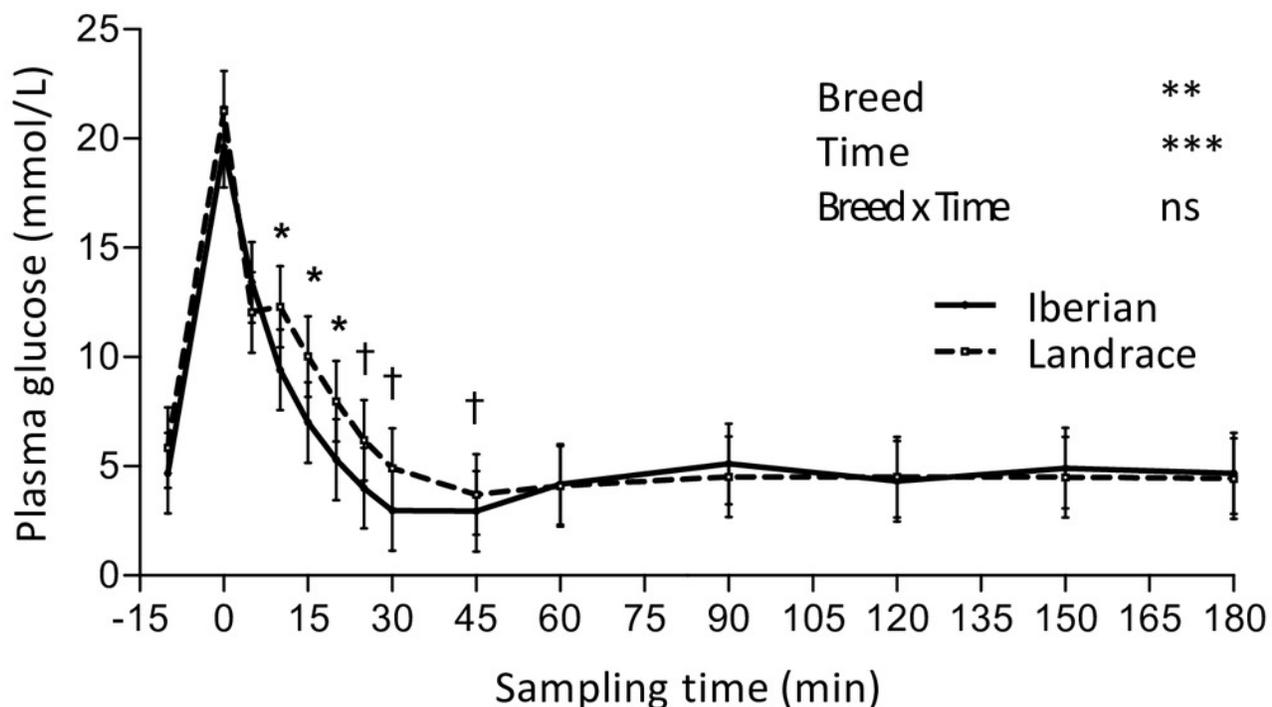
Figure 3

Plasma lactate concentration during intra-arterial glucose tolerance test (500 mg/kg BW; 180 min sampling) in growing Iberian (n = 4) and Landrace (n = 5) pigs.

ns, not significant ($P > 0.10$); † $0.05 < P < 0.10$, * $P < 0.05$, *** $P < 0.001$.