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

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Short title: Insulin sensitivity in Iberian and Landrace pigs

Abstract

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 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) compared with 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 ($P < 0.05$) and lactate ($P < 0.1$), and 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 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.

Keywords: insulin resistance, metabolism, obesity, *fatty pigs, lean pigs*

Implications

The pig has evolved as a complementary alternative research model to laboratory animals for obesity studies in humans. Obesity is characterized by an over-accumulation of body fat and is often linked to insulin resistance. The

Iberian pig, a breed very much appreciated for their expensive cured products, is a candidate swine model with elevated capacity of fat deposition. We have shown that growing Iberian pigs also show features compatible with an installation of early insulin resistance

Introduction

The Iberian pig is a slow growing native breed of the Mediterranean basin with much greater whole body fat content than lean-type pigs (Nieto *et al.*, 2002) and may be, considered as an obese pig breed (Barea *et al.*, 2007; Torres-Rovira *et al.*, 2011, 2012). Compared with conventional breeds, Iberian pigs show a lower efficiency of energy utilisation for protein deposition in the growing period (Barea *et al.*, 2007). The greater relative viscera weight (Rivera-Ferre *et al.*, 2005) and total heat production (González-Valero *et al.*, 2016) associated in part with the greater rate of muscle protein turnover (Rivera-Ferre *et al.*, 2005) in Iberian compared with lean-type pigs help to explain the low energy efficiency for growth. In fact, Rivera-Ferre *et al*. (2005) showed that muscle protein degradation was increased in Iberian pigs resulting in decreased muscle protein accretion compared with Landrace pigs. Interestingly, insulin resistance at the muscle level could explain an increased protein degradation (Wang *et al.*, 2006) affecting overall protein accretion. In a previous study using balanced or lysine deficient diets at two CP levels (12% and 16% CP), Iberian pigs had greater fasting serum insulin concentration than Landrace pigs (Fernández-Fígares *et al.*, 2007), suggesting the possibility of insulin resistance in Iberian pigs. We hypothesised that compared with modern lean breeds Iberian pigs have decreased insulin sensitivity, which could explain differences on growth, body

76 composition and metabolic characteristics compared with modern breeds. The
77 objective of the present study was to evaluate differences on insulin sensitivity
78 between Iberian and Landrace pigs using an intra-arterial glucose tolerance test
79 (IAGTT).
80
81 **Material and methods**
82 *Animals*
83 All procedures used in this study were approved by the Bioethical Committee of
84 the Spanish National Research Council (CSIC, Spain), and the animals were
85 cared for in accordance with the Royal Decree No. 1201/2005 (Spain). The
86 experiment was performed with five Landrace and four Iberian (*Silvela* strain)
87 barrows supplied by Granja El Arenal (Córdoba, Spain) and Sánchez Romero
88 Carvajal (Jabugo S.A., Puerto de Santa María, Cádiz, Spain), respectively. The
89 pigs were allowed *ad libitum* access to a standard diet (160 g CP/kg; 14 MJ
90 metabolizable energy/kg DM) with free access to water in a controlled-
91 environment room (21 ± 1.5°C). After acclimatization, each animal was
92 surgically fitted with a chronic catheter in carotid artery following the procedure
93 described previously (Rodríguez-López *et al.*, 2013). After surgery, the pigs
94 were fed at 2.4 × metabolizable energy for maintenance (444 kJ/kg$^{0.75}$ BW;
95 National Research Council (NRC) 1998) with the same standard diet. On the
96 day of the experiment, pigs (46.0 ± 3.0 and 47.8 ± 3.6 kg BW, Iberian and
97 Landrace, respectively, that is about 18 weeks and 14 weeks, respectively)
98 were given an intra-arterial bolus (500 mg/kg BW) of glucose (50% sterile
99 dextrose; glucosado 50% Braun, B. Braun Medical S.A., Rubi, Barcelona,
100 Spain) over one min period after an overnight fast. The catheter was

immediately flushed with 5 mL of sterile saline solution. Blood samples (5 mL) were collected at -10, 0 (20-30 seconds after the bolus of glucose and the saline solution), 5, 10, 15, 20, 25, 30, 45, 60, 90, 120 and 180 min following glucose infusion.

Biochemical analysis and calculations

Plasma was obtained by centrifugation (4ºC, 1820 x g for 30 min; Eppendorf 5810 R, Hamburg, Germany) and stored in aliquots at -20ºC until insulin and metabolites (glucose, lactate, triglycerides, cholesterol, creatinine, albumin and urea) were analysed. All samples were assayed in duplicate except for insulin which was assayed in triplicate.

Insulin was measured using commercially-available radioimmuno assay kit following the directions of the manufacturer (Millipore porcine insulin radioimmuno assay kit; Cat. PI-12K). Radioactivity in samples was measured using a gamma counter (Behring 1612; Nuclear Enterprises Ltd, Edinburgh, Scotland). Human insulin was used as standard, and the assay was validated for use in porcine plasma samples (Fernández-Fígares *et al.*, 2007). The intra- and inter-assay CVs for plasma insulin were 4.4 and 9.1%, respectively.

Plasma glucose, lactate, triglycerides, cholesterol, creatinine, albumin and urea were measured colorimetrically using an automated Cobas Integra 400® analyser (Roche Diagnostics GmbH, Mannheim, Germany).

Responses of plasma insulin, glucose and lactate were evaluated separately by computing total area under the response curve (AUC) determined using trapezoidal geometry (GraphPad Prism, Version 5.02. San Diego, CA) for the time period indicated following intra-arterial glucose infusion (e.g. AUC0-5

stands for the integrated area between 0-5 min post-infusion, AUC0-10 between 0-10 min post-infusion, and so on, until AUC0-180). Basal levels per breed (at time -10 min) were used to calculate the corresponding AUC per metabolite. The rates of decline in plasma insulin and glucose concentrations for both breeds were calculated based on the slope in the linear portion of the response curve from 0 to 30 min after IAGTT challenge (Christoffersen *et al.*, 2009). Results were then expressed as a fractional rate constant determined from the slope of the natural logarithm of plasma concentrations *vs.* time (Shipley and Clark, 1972; cited by (Gopinath and Etherton, 1989). The fractional turnover rates (*k),* or disappearance rates, of plasma insulin and glucose (%/min) were calculated using the relationship (Kaneko *et al.*, 2008):

k = (Ln1 - Ln2)/(T2 - T1)

where Ln1 and Ln2 are the natural logarithms of plasma insulin concentration, (µU/mL, (or glucose concentration, mM) concentrations at times T1 (0 min) and T2 (30 min), respectively.

From the *k* value, the half-life, $T_{½}$ (min), may be calculated as:

$T_{½} = 100 \times 0.693/k$

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

The so-called homeostasis model assessment (HOMA; Matthews *et al.*, 1985) was calculated for estimating insulin resistance (HOMA-IR) and β-cell function (HOMA-%B) at fasting conditions, as follows:

HOMA-IR: fasting plasma insulin (µU/mL) × fasting plasma glucose (mM)/22.5

HOMA-%B: (20 × fasting plasma insulin (µU/mL))/(fasting plasma glucose (mM) - 3.5)

150 It is assumed that non-insulin-resistant individuals have 100% β-cell function
151 and an insulin resistance of 1.
152 The quantitative insulin sensitivity check index (QUICKI; Katz *et al.*, 2000)
153 was computed as:
154 $1/[Ln(I_0) + Ln(G_0)]$
155 where I_0 is the fasting insulin (μU/mL), and G_0 is the fasting glucose (mg/dl).
156 Finally, the insulin sensitivity index (CSI; Tura *et al.*, 2010) was calculated as:
157 $CSI = K_G/(\Delta AUC_{INS}/T)$
158 where K_G is the slope of Ln glucose in the linear portion of the response curve,
159 ΔAUC_{INS} is the AUC of insulin above basal value, and T is the time interval
160 (between 0 and 30 min) when K_G and ΔAUC_{INS} are calculated.
161
162 *Statistical analyses*
163 The number of animals was calculated using the G*Power software (Heinrich-
164 Heine-Universität Düsseldorf; (Faul *et al*., 2007). Accepting an alpha risk of 0.05
165 and a beta risk of 0.2 in a two-sided test, 5 subjects are necessary in first group
166 and 5 in the second to recognize as statistically significant a difference greater
167 than or equal to 12 μU/ml on insulin concentration and a common standard
168 deviation of 6.3 μU/ml based on previous studies (Fernández-Fígares *et al*.,
169 2007). A total of 5 pigs per treatment was also used by others (e.g. Stoll *et al.*,
170 1999). However, one Iberian pig lost the arterial catheter during the recovery
171 period after surgery and only four Iberian pigs could be used.
172 Plasma metabolites were evaluated using a mixed ANOVA with repeated
173 measures (Version 9.4; PROC MIXED, SAS Institute Inc., Cary, NC, USA) with
174 the fixed effects of breed, time of sampling and their interaction in the model

175 statement. The pig was considered a random effect. First-order ante
176 dependence covariance ANTE(1) was used, which allows unequal variances
177 over time and unequal correlations and covariance among different pairs of
178 measurements. Plasma concentration differences between breeds at each
179 sampling time were analysed by the pdiff (piecewise differentiable) option.
180 Assumptions that are required for an ANOVA were tested following the protocol
181 from Zuur *et al*. (2010). Homogeneity of variance was assured by applying the
182 Levene's-Test. No transformation was required. Least square means and
183 pooled SEM are presented. Differences were considered significant at $P < 0.05$
184 and trends approaching significance were considered for $0.05 < P < 0.10$.
185
186 **Results**
187 Fasting plasma insulin was greater in Iberian compared with Landrace pigs
188 (15.6 *and* 8.10 µU/mL, respectively; sem=1.55, $P < 0.05$) whereas fasting
189 plasma glucose was similar for both breeds (4.68 and 5.85 mmol/L for Iberian
190 and Landrace pigs, respectively; sem=0.92, $P > 0.10$). No differences between
191 breeds were found in fasting plasma albumin (Iberian 0.50 and Landrace 0.54
192 µM), urea (Iberian 3.3 and Landrace 3.0 mM), cholesterol (Iberian 1.46 and
193 Landrace 1.79 mM) and triglycerides (Iberian 0.28 and Landrace 0.22 mM). On
194 the other hand plasma fasting creatinine was lower in Iberian compared to
195 Landrace (54 and 102 µM, respectively; $P < 0.01$).
196 Average plasma metabolites and insulin concentrations after the IAGTT are
197 shown in Table 1. Mean plasma glucose, cholesterol and creatinine
198 concentrations were lower in Iberian (14, 22 and 68%, respectively; $P < 0.05$)
199 compared with Landrace pigs. However, mean plasma insulin, lactate,

triglycerides and urea concentrations were greater in Iberian (50, 35, 18 and 23%, respectively; $0.01 < P < 0.001$) than in Landrace pigs. No differences ($P > 0.1$) were found between breeds for albumin levels.

Only plasma insulin (Figure 1), glucose (Figure 2) and lactate (Figure 3) concentrations changed throughout time ($P < 0.001$; Table1) after the IAGTT.

An interaction between breed and time was found for plasma insulin, such that concentration of insulin was greater in Iberian pigs from -10 to 15 min and from 90-180 min ($P < 0.05$, with $P < 0.1$ at times 0 and 90 min) and lower at 25 min ($P < 0.1$; Figure 1). In both breeds, plasma insulin levels increased 7-fold, reaching a peak concentration 10 and 15 min after glucose infusion for Iberian (113.6 ± 7.1 µU/mL) and Landrace (55.7 ± 6.4 µU/mL) pigs, respectively. Insulin remained well above fasting levels until 20 and 45 min after glucose infusion for Iberian and Landrace pigs, respectively; thereafter insulin levels rapidly decreased until fasting levels were attained. Fractional turnover rate tended to increase in Iberian compared with Landrace pigs (6.2 and 4.7 ± 0.46%/min, respectively; $0.05 < P < 0.1$) while half-life tended to decrease (11.3 and 15.2 ± 1.30 min for Iberian and Landrace pigs, respectively; $0.05 < P < 0.1$).

Glucose peaked (Figure 2) immediately after glucose infusion reaching a value of 19.6 mmol/L and 21.2 mmol/L for Iberian and Landrace pigs, respectively. Subsequently, glucose concentration gradually decreased to values below fasting levels after 25 min and 30 min, respectively for Iberian and Landrace pigs. The lowest plasma glucose concentration (glucose nadir) was found at 45 min (2.95 mmol/L and 3.70 mmol/L for Iberian and Landrace pigs, respectively). After glucose nadir, glucose concentration gradually increased again to reach values comparable to fasting levels at 180 min. No differences

225 were found between breeds for glucose fractional turnover rate (4.1 and 3.3 ±
226 0.37%/min for Iberian and Landrace pigs, respectively; $P > 0.10$) or glucose
227 half-life (17.1 and 22.1 ± 2.67 min for Iberian and Landrace pigs, respectively; P
228 > 0.10).

229 Lactate increased after the IAGTT, peaked at 20 min for both breeds and
230 declined progressively until reaching basal concentrations at 180 min (Figure 3).

231 The AUC values for each sampling time of insulin, glucose and lactate are
232 shown in Figure 4, 5 and 6, respectively. Insulin AUC was greater ($P < 0.05$) for
233 Iberian compared with Landrace pigs at all times. Conversely, glucose AUC
234 between 0-15, 0-20, 0-25, 0-30, 0-45 and 0-60 min were lower ($P < 0.05$) for
235 Iberian than Landrace pigs. Glucose AUC tended to be lower ($0.05 < P < 0.10$)
236 between 0-10, 0-90 and 0-120 min. No differences ($P < 0.05$) in glucose AUC
237 was found between 0-5, 0-150 and 0-180 min. Plasma lactate AUC was greater
238 ($P < 0.05$) for Iberian pigs for 0-10 and 0-15 min and tended to be greater (0.05
239 $< P < 0.10$) for 0-5, 0-20, 0-25, 0-90, 0-120, 0-150 and 0-180 min after glucose
240 infusion.

241 Indices of insulin sensitivity are shown in Table 2. The QUICKI index
242 decreased ($P < 0.05$) while HOMA-%B index increased ($P < 0.01$) in Iberian
243 compared with Landrace pigs. No differences ($P > 0.10$) were found for HOMA-
244 IR and CSI.

245

246 **Discussion**

247 The IAGTT method allowed the comparison of the insulin responsiveness of an
248 obese (Iberian) and a lean (Landrace) pig breed. It has previously been shown
249 that Iberian pigs are fatty pigs with slow growth rate compared to modern

250 breeds (Nieto *et al*., 2012). As arterial blood represents the metabolites
251 concentration to which the tissues are exposed (Brouns *et al.*, 2005), chronic
252 catheters were inserted in carotid artery for glucose infusion and blood
253 sampling.

254 In the current study we have shown increased fasting plasma insulin and
255 insulin response (higher plasma concentrations and AUC) after glucose infusion
256 in Iberian compared with Landrace pigs (18 and 14 weeks of age, respectively).
257 Greater postprandial serum levels of insulin have been described in 20 kg
258 Iberian (11 weeks of age) compared to Landrace after glucose infusion
259 (Fernández-Fígares *et al*., 2007), and in 11kg Ossabaw (obese; 10 weeks of
260 age) compared to 16.5 kg Yorkshire (10 weeks of age) pigs (Wangsness *et al.*,
261 1981). However, other comparative studies using a standard diet found
262 increased insulin secretion in 75-120 kg Large White boars than in 40-75 kg
263 Meishan boars (obese breed) at 20 and 52 weeks of age, respectively (Weiler
264 *et al.*, 1998). The limited growth and development of slow growing pigs could
265 result at least partly from disturbances in insulin secretion and/or in insulin
266 binding, leading to insulin sensitivity, because most cells of the body require
267 insulin for adequate uptake of glucose and amino acids (Claus and Weiler,
268 1994). If the concentration of insulin is compared among animals of different
269 breeds, the sensitivity of each breed to insulin should be considered. In this
270 study we were also interested in other key metabolites which could provide
271 additional information concerning insulin sensitivity in Iberian pigs.

272 After glucose infusion, glucose plasma concentration rapidly returns to
273 preprandial values as shown in the present experiment, which indicates that
274 exogenous glucose was efficiently metabolized, stored as glycogen, or both. As

275 expected, when glucose was infused, plasma glucose levels were rapidly
276 increased and a subsequent insulin response was observed. The elevated
277 insulin lowered plasma glucose below fasting values within 20 and 25 min for
278 Iberian and Landrace pigs, respectively, and insulin levels returned to baseline
279 as plasma glucose declined. In our study, glucose concentration and glucose
280 AUC during the IAGTT were lower in Iberian compared with Landrace pigs, with
281 no differences in fasting plasma glucose, maybe due to the limited number of
282 pigs. When interpreting the individual glucose curves, a monophasic pattern
283 was identified for both breeds. The lower glucose AUC of Iberian pigs (-19% on
284 average) may be related to the greater insulin AUC (+33% on average), a
285 common pattern in many models of obesity (Kay *et al*., 2001). However, the
286 reasons for the unequal physiological response between breeds are not well
287 understood and must be discussed.

288 As it has been proved that the energy needs of portal-drained viscera are
289 fulfilled by the oxidation of glucose, glutamate, and glutamine in pigs (Stoll *et*
290 *al.*, 1999), a larger gastrointestinal tract of Iberian pigs compared to Landrace
291 (Rivera-Ferre *et al.*, 2005) is in line with the decreased AUC of glucose reported
292 in our experiment.

293 However, despite the larger size of the gastrointestinal tract and lower portal
294 blood flow (González-Valero *et al.*, 2016) of Iberian compared with Landrace
295 pigs, no differences on net portal flux of glucose after ingestion of the same diet
296 were found (Rodríguez-López *et al.*, 2013). Differences on insulin stimulated
297 glucose transport at portal-drained viscera level may help to explain these
298 results. Iberian have lower glucose concentrations than Landrace pigs after an
299 intravenous adrenaline challenge (Fernández-Fígares *et al.*, 2016), suggesting

a decreased response of Iberian pigs to sympathetic nervous system stimuli which is in line with the lower glucose AUC reported here.

Lactate appearance after an intravenous glucose test is positively associated with insulin sensitivity in humans (Lovejoy *et al.*, 1992), as it is related to lactate production by insulin sensitive tissues (mainly muscle and fat). Because only limited amounts of lactate are produced by muscle after glucose loading (Ykijarvinen *et al.*, 1990), the source of lactate appearance should predominantly be adipose tissue (Lovejoy *et al.*, 1992), with a large capacity to convert glucose to lactate (Marin *et al.*, 1987). We report here a delay of 20 min in plasma lactate elevation relative to glucose peak following IAGTT, which may reflect the time lag in adipose tissue uptake of glucose and subsequent lactate production under the stimulation of insulin. Compared with Landrace, the increased lactate AUC in Iberian pigs after the IAGTT could therefore be a consequence of a larger adipose tissue (Nieto *et al.*, 2002) instead of greater insulin sensitivity. On the other hand, insulin resistance was associated with elevated basal lactate levels in obese humans (Lovejoy *et al.*, 1990), so increased basal lactate concentrations in Iberian pigs (1.040 vs. 0.730 mmol/L; sem=0.063) could also indicate insulin resistance or reduced insulin sensitivity. Although inhibition of insulin action on glycogenolysis in fasting conditions may lead to increased glucose release from glycogen and subsequent conversion of glucose to lactate, there is no direct evidence of this. There is indirect evidence, though, that elevated lactate levels reflect a glucose sparing effect (decreased glucose utilisation) in muscle (Pearce and Connett, 1980).

Obesity is frequently associated with different degrees of dyslipidemia manifested as increased triglyceridemia and low HDL-cholesterol. In our

325 experiment, we found lower plasma cholesterol but greater plasma triglycerides
326 concentration in Iberian compared with Landrace pigs, although we did not
327 separate LDL and HDL fractions. Reduced total cholesterol concentration could
328 be due to reduced hepatic insulin sensitivity as insulin stimulates cholesterol
329 synthesis (Nelson and Cox, 2017). In any case the cholesterolemia for both
330 breeds in the present experiment was in the lower range of published values
331 (Fernández-Fígares *et al*., 2007) and it cannot be considered that Landrace
332 pigs were hypercholesterolemic.

333 Previous studies in our lab have shown the low genetic potential of growing
334 Iberian pigs for muscle protein deposition in comparison to lean breeds (Nieto *et*
335 *al*., 2002), possibly due to the greater muscle protein degradation and turnover
336 of the former (Rivera-Ferre *et al.*, 2005). In line with this, plasma urea level (an
337 indirect protein degradation indicator) was in the present study 23% greater in
338 Iberian compared with Landrace pigs. Differences on circulating insulin or the
339 capacity of insulin release between breeds may explain differences in lean
340 tissue deposition, as insulin has an important role in skeletal muscle metabolism
341 (Wang *et al.*, 2006). In obese db/db mice (a model of insulin deficiency) higher
342 muscle protein degradation in comparison with control mice (normal plasma
343 insulin concentration) was reported; the authors concluded that insulin
344 resistance was associated with accelerated muscle protein degradation (Wang
345 *et al.*, 2006). The elevated protein degradation reported in Iberian compared
346 with Landrace pigs (Rivera-Ferre *et al.*, 2005) suggests the possibility of insulin
347 resistance at this level. The lower plasma creatinine level (indicator of muscle
348 mass) found in this study for Iberian pigs, is in accordance with previous studies
349 (Fernández-Fígares *et al.*, 2007) and also with the low muscle protein

350 deposition and muscle size described previously (Nieto *et al.*, 2002; Rivera-
351 Ferre *et al.*, 2005). As insulin resistance is associated with decreased muscle
352 mass, plasma creatinine levels can also be used as an indicator of insulin
353 signalling disorders as reported by Kashima *et al*. (2017) in humans. Further
354 research regarding amino acid concentration after an IAGTT may help to
355 explain differences in the effect of insulin on muscle protein metabolism
356 between breeds.

357 When insulin sensitivity indices used in human medicine were applied to the
358 conditions of the present experiment, QUICKI and HOMA-%B were more
359 sensitive detecting differences between breeds. Indeed, QUICKI index
360 decreased in Iberian compared with Landrace pigs, pointing out an incipient
361 insulin sensitivity impairment in fasting Iberian pigs. Similarly, reduced QUICKI
362 index (0.5 *vs.* 0.6) was found in Bama miniature pigs fed a high sucrose high fat
363 diet compared with a control diet, respectively (Liu *et al.*, 2017). The QUICKI
364 index has been shown to provide reasonable approximations of insulin
365 efficiency in minipigs (Christoffersen *et al.*, 2009).

366 When we used the homeostasis model assessment (HOMA), differences on
367 hepatic insulin resistance (HOMA-IR index) were negligible between breeds
368 (3.3 and 2.3 for Iberian and Landrace, respectively; $P>0.10$). However, Iberian
369 had improved β-cell function compared with Landrace pigs according to HOMA-
370 %B index (267 and 100 for Iberian and Landrace, respectively; $P<0.01$), which
371 may be due to enhanced sensitivity of the β-cells to glucose during the fasting
372 period. As a consequence, β-cell insulin synthesis in Iberian pigs increased in
373 accordance with the increased insulin release after the glucose tolerance test
374 and the elevated basal insulin concentrations reported for Iberian pigs. This is

consistent with decreased quantitative insulin sensitivity check index in Iberian pigs compared to Landrace (0.31 and 0.33 for Iberian and Landrace, respectively; $P<0.05$).

Previous studies from our lab indicate that growing Iberian pigs are prone to insulin resistance compared with modern breeds as denoted by increased hepatic gluconeogenesis (González-Valero *et al.*, 2014), greater plasma free fatty acid concentration (Fernández-Fígares *et al.*, 2016) and lower plasma creatinine and QUICKI index (Fernández-Fígares *et al.*, 2007). Additionally, in this experiment we show greater HOMA-%B and increased plasma insulin and lactate concentrations after an IAGTT. The increased plasma insulin AUC after an IAGTT suggests insulin resistance in comparison to values obtained for lean pigs, although the concentration of glucose remained low which could indicate the absence of a peripheral insulin resistance. Although Iberian pigs may be considered an obese breed in terms of body composition (Nieto *et al.*, 2002; Barea *et al.*, 2007), insulin resistance mechanisms have not yet been fully established at the development stage of the pigs in this experiment. Insulin resistance and impaired glucose tolerance has been shown in Iberian sows (2.5 years old) *ad libitum* fed a saturated fat enriched diet for three months (Torres-Rovira *et al.*, 2012). Although our results support the existence of an insulin resistance or a decreased insulin sensitivity in growing Iberian pigs, caution should be taken because of the reduced number of pigs used. The utilization of the hyperinsulinemic euglycemic clamp, the most definitive approach to determine whole-body insulin action should provide conclusive evidence regarding the establishment of insulin resistance in growing Iberian pigs.

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Declaration of interest

There are no conflicts of interest.

Ethics statement

The procedures used in this study were approved by the Bioethical Committee of the Spanish National Research Council (CSIC, Spain), and the animals were cared for in accordance with the Royal Decree No. 1201/2005 (Spain).

Software and data repository resources

Data and models are not deposited in an official repository.

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Table 1 *Average plasma metabolites and insulin concentrations in Iberian (n = 4) and Landrace (n = 5) pigs during an intra-arterial glucose tolerance test (500 mg/kg BW, 0-180 min)*

	Breed			*P*-value[1]		
	Iberian	Landrace	SEM	Breed	Time	Breed × Time
Insulin (µU/mL)	41	27	1.9	***	***	***
Glucose (mmol/L)	6.8	7.7	0.26	**	***	0.257
Lactate (mmol/L)	1.3	1.0	0.039	***	***	0.989
Triglycerides (mmol/L)	0.28	0.24	0.009	**	0.474	0.954
Cholesterol (mmol/L)	1.5	1.8	0.033	***	0.934	0.999
Creatinine (µmol/L)	54	90	1.2	**	0.722	0.955
Albumin (mmol/L)	0.48	0.50	0.009	0.131	0.992	0.999
Urea (mmol/L)	3.0	2.4	0.102	***	0.779	0.999

[1]ns = non-significant; **$P < 0.01$; ***$P < 0.001$.

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Table 2 *Indices of glucose tolerance and insulin sensitivity in Iberian (n = 4) and Landrace (n = 5) pigs subjected to an intra-arterial glucose tolerance test (500 mg/kg BW)*[1]

	Iberian	Landrace	SEM	*P*-value[2]
QUICKI	0.31	0.33	0.007	*
HOMA-IR	3.3	2.3	0.58	0.205
HOMA-%B	267	100	25.6	**
CSI ($\times 10^{-4}$)	-12	-13	1.8	0.845

[1]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.

[2]ns = non-significant; *$P < 0.05$; **$P < 0.01$.

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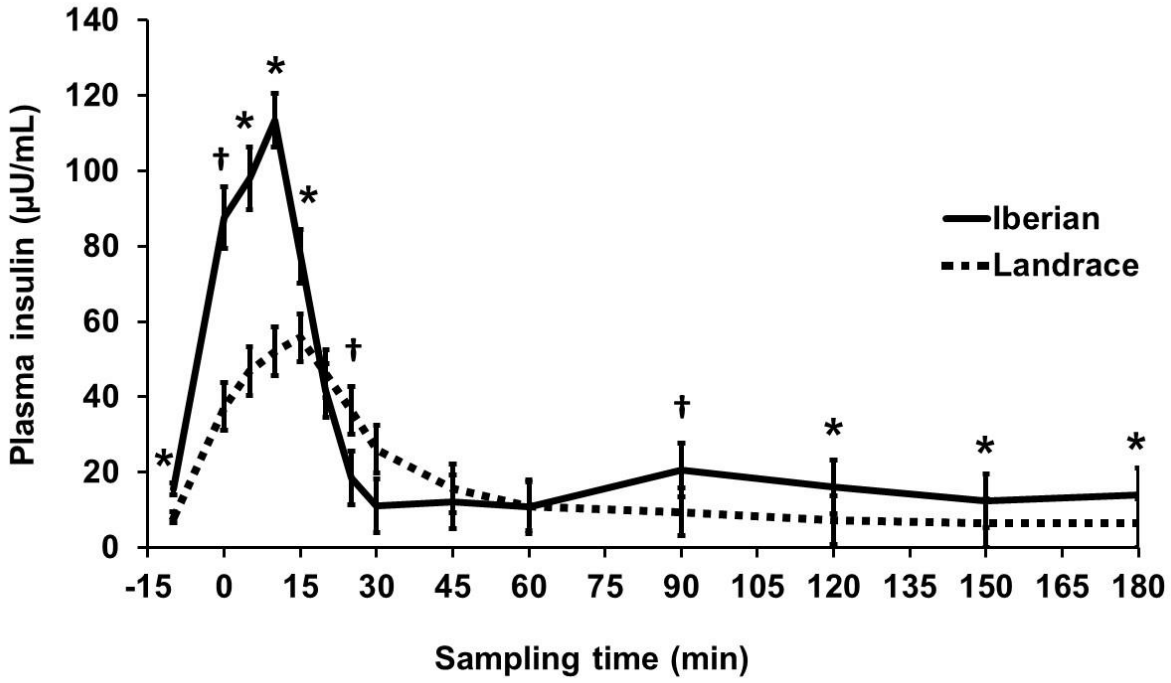
549 **Figure 1** Plasma insulin concentrations before and after intra-arterial glucose
550 tolerance test (500 mg/kg BW) in growing Iberian (n = 4) and Landrace (n = 5)
551 pigs[1, 2].



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553 [1]Significant differences in plasma insulin concentration for each time point expressed
554 as: †$0.05 < P < 0.1$, *$P < 0.05$.

555 [2]Concentrations at time 0 correspond to 20-30 s after glucose bolus injection.

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564 **Figure 2** Plasma glucose concentration before and after intra-arterial glucose
565 tolerance test (500 mg/kg BW) in growing Iberian (n = 4) and Landrace (n = 5)
566 pigs[1, 2].

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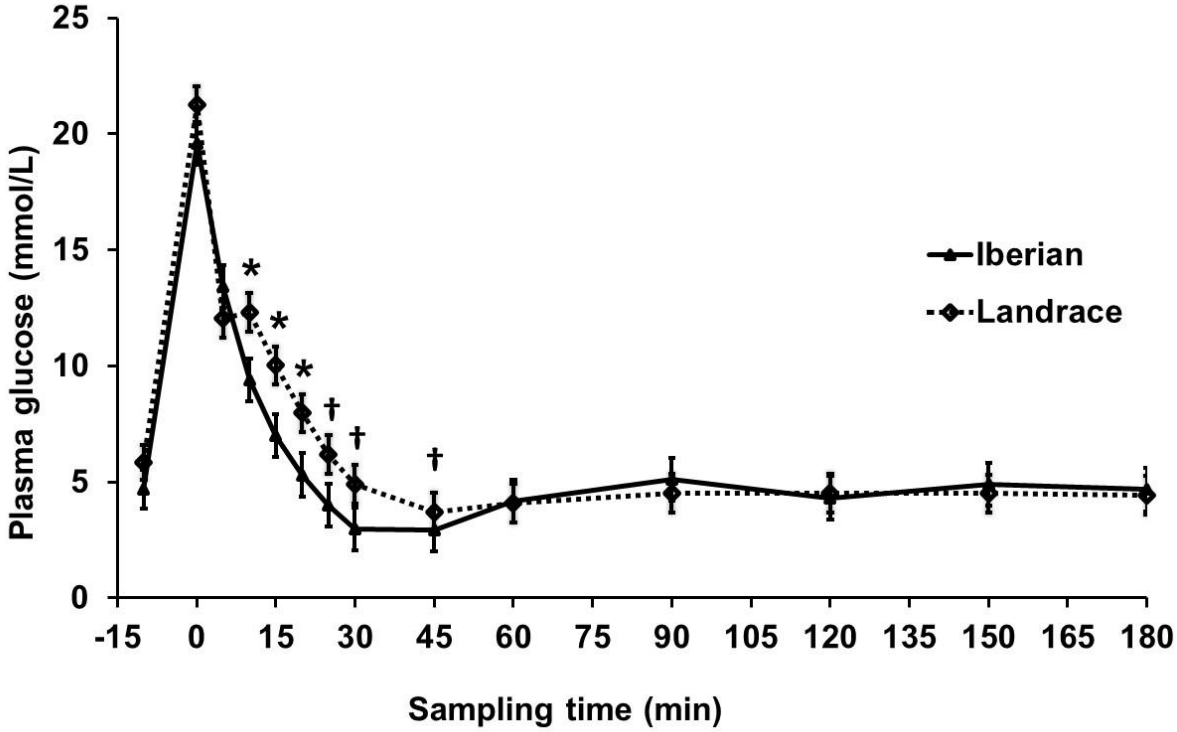

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569 [1]Significant differences in plasma glucose concentration for each time point expressed
570 as: [†]$0.05 < P < 0.1$, *$P < 0.05$.

571 [2]Concentrations at time 0 correspond to 20-30 s after glucose bolus injection.

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581 **Figure 3** Plasma lactate concentration before and after intra-arterial glucose

582 tolerance test (500 mg/kg BW) in growing Iberian (n = 4) and Landrace (n = 5)

583 pigs[1, 2].

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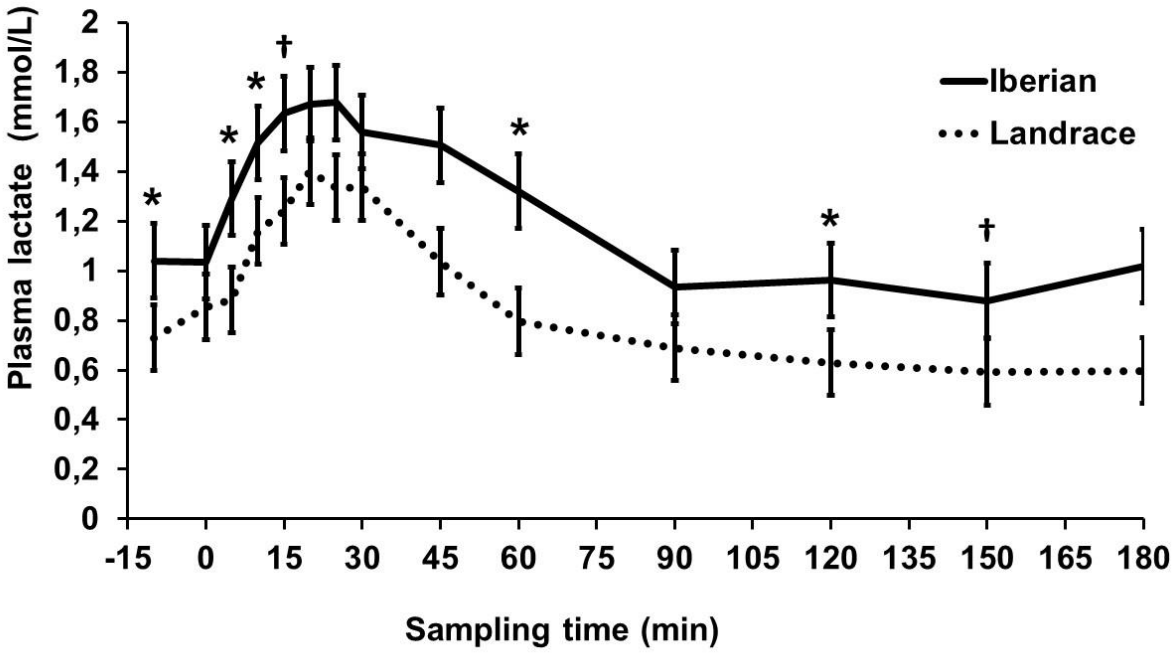

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586 [1]Significant differences in plasma lactate concentration for each time point expressed

587 as: [†]0.05 < *P* < 0.1, **P* < 0.05.

588 [2]Concentrations at time 0 correspond to 20-30 s after glucose bolus injection.

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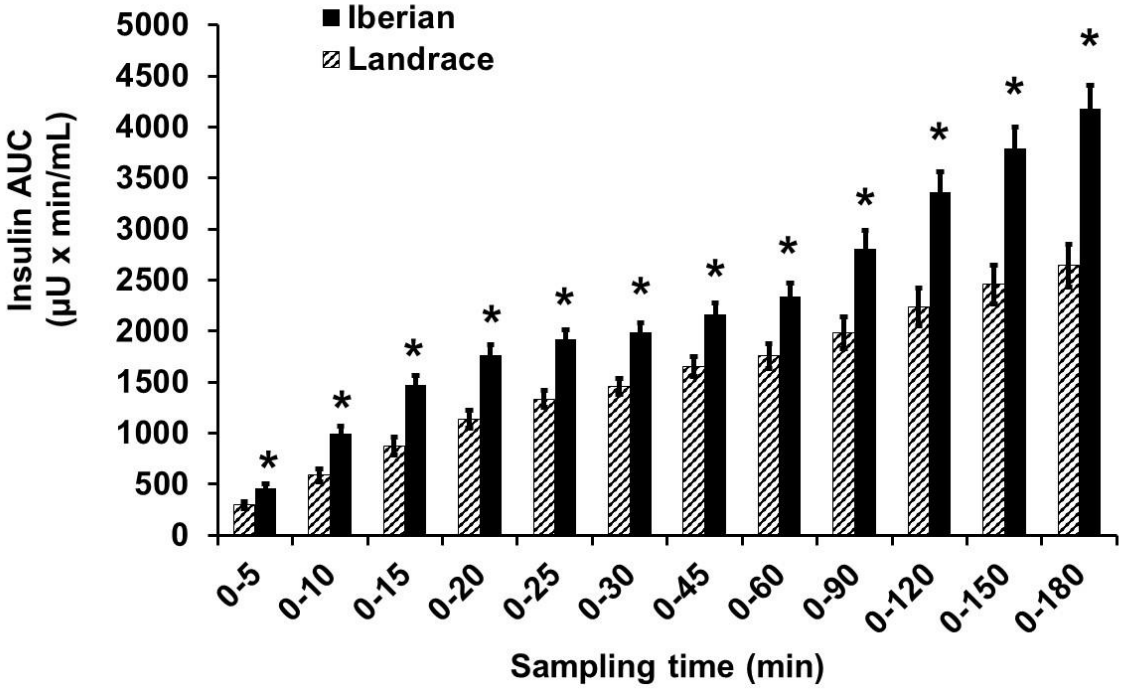
Figure 4 Area under the curve (AUC) of plasma insulin concentration during intra-arterial glucose tolerance test (500 mg/kg BW) between minute 0 and the time points indicated in growing Iberian (n = 4) and Landrace (n = 5) pigs[1].



[1]Differences between breeds: *$P < 0.05$.

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612 **Figure 5** Areas under the curve (AUC) of plasma glucose concentration during
613 intra-arterial glucose tolerance test (500 mg/kg BW) between minute 0 and the
614 time points indicated in growing Iberian (n = 4) and Landrace (n = 5) pigs[1].

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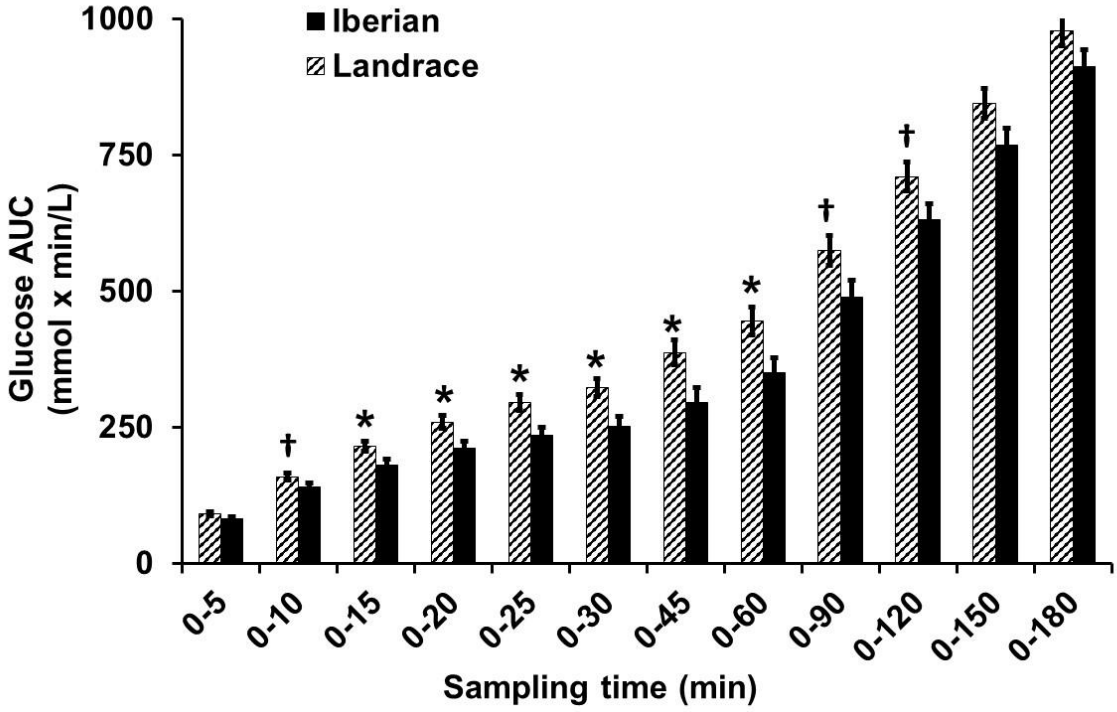

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618 [1]Differences between breeds: †$0.05 < P < 0.1$, *$P < 0.05$.

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626 **Figure 6** Area under the curve (AUC) of plasma lactate concentration during an
627 intra-arterial glucose tolerance test (500 mg/kg BW) between minute 0 and the
628 time points indicated in growing Iberian (n = 4) and Landrace (n = 5) pigs[1].

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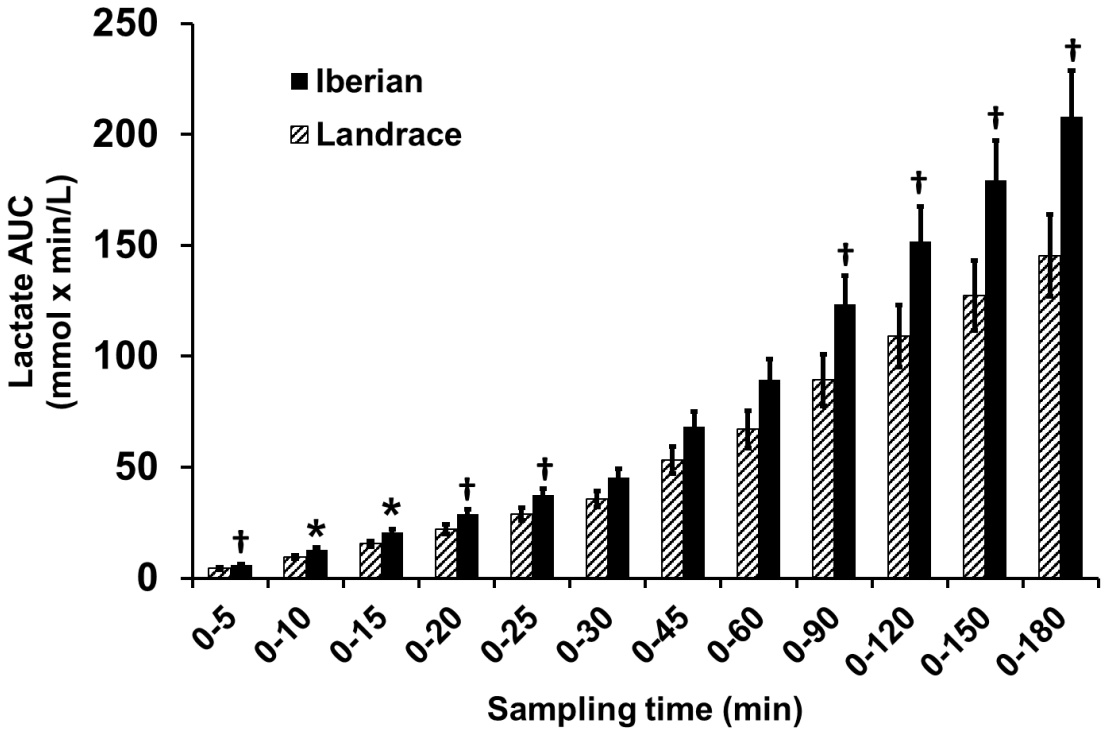

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632 [1]Differences between breeds: †$0.05 < P < 0.1$, *$P < 0.05$.