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

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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 challenge (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, 2, 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 (0.01 < *P*

< 0.001) 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 (0.01 <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 (0.1 < P < 0.05), and smaller (P < 0.05) AUC for glucose 0-60 min compared with Landrace pigs. QUICKI index decreased while HOMA-%B index increased indicating improved β-cell function in Iberian compared with Landrace pigs, but no difference (P > 0.10) in calculated insulin sensitivity index was found 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, 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, *Sus scrofa domestica, Sus scrofa mediterraneus*

Implications

Intense selective breeding and selection for increased growth rate and leanness has favourable consequences for the health of consumers and, from an economic point of view, for the livestock industry. Nevertheless, the metabolic repercussion of this selection may involve differences on insulin -a central hormone regulator of nutrient

uptake and metabolism- sensitivity between modern and local breeds. The Iberian pig is an autochthonous breed showing insulin resistance during the growing phase.

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). 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, Iberian 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 composition and metabolic characteristics compared with modern breeds. The objective of the present study was to evaluate differences on insulin sensitivity between Iberian and Landrace pigs using an intra-arterial glucose tolerance test (IAGTT).

Material and methods

Animals

All procedures used in this study were approved by the Bioethical Committee of the Spanish National Research Council (CSIC, Spain), and the animals cared for in accordance with the Royal Decree No. 1201/2005 (Spain). The experiment was performed with five Landrace and four Iberian (*Silvela* strain) barrows supplied by Granja El Arenal (Córdoba, Spain) and Sánchez Romero Carvajal (Jabugo S.A., Puerto de Santa María, Cádiz, Spain), respectively. The pigs were allowed *ad libitum* access to a standard diet (160 g CP/kg; 14 MJ metabolizable energy/kg DM) with free access to water in a controlled-environment room (21 ± 1.5°C). After acclimatization, each animal was surgically fitted with a chronic catheter in carotid artery following the procedure described previously (Rodríguez-López et al., 2013). After surgery, the pigs were fed at 2.4 × metabolizable energy for maintenance (444 kJ/kg$^{0.75}$ BW; National Research Council (NRC) 1998). On the day of the experiment, pigs (47.0 ± 1.2 kg BW) were given an intra-arterial bolus (500 mg/kg BW) of glucose (50% sterile dextrose; glucosado 50% Braun, B. Braun Medical S.A., Rubi, Barcelona, Spain) over a 1 min period after an overnight fast. The catheter was immediately flushed with 5 ml of sterile saline. Blood samples (5 ml) were collected at -10, 0, 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 (RIA) kit following the directions of the manufacturer (Millipore porcine insulin RIA 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 for the time period indicated following intra-arterial glucose infusion (e.g. AUC5 stands for the integrated area between 0-5 min post-infusion, AUC10 between 0-10 min post-infusion, etc.).

The rates of decline in plasma insulin and glucose concentrations were calculated based on the slope in the linear portion of the response curve (0 to 30 min post-challenge) and expressed as a fractional rate constant determined from the slope of the natural logarithm of plasma concentrations *vs.* time (Shipley and Clark, 1972). 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 (or glucose) 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 utilized.

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 × fasting plasma glucose/22.5

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

It is assumed that non-insulin-resistant individuals have 100% β-cell function and an insulin resistance of 1.

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

$1/[Ln(I_0) + Ln(G_0)]$

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

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

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

where K_G is the slope of Ln glucose in the linear portion of the response curve, ΔAUC_{INS} is the AUC of insulin above basal value, and T is the time interval (between 0 and 30 min) when K_G and ΔAUC_{INS} are calculated.

Statistical analyses

Plasma metabolites were evaluated using a mixed ANOVA with repeated measures (Version 9.4; PROC MIXED, SAS Institute Inc., Cary, NC, USA) with the fixed effects

of breed, time of sampling and their interaction in the model statement. The experimental unit was the pig. The covariance-structure used was the autoregressive(1). To compare differences among the means, the pdiff (piecewise differentiable) option was used. Assumptions that are required for a ANOVA were tested following the protocol from Zuur et al. (2010).Homogenity of variance was assured by applying the Levene's-Test. No transformation was required.

Fractional turnover rates, indices of insulin resistance and AUC corrected for baseline (0 to 180 min or fractions) were analysed using the GLM procedure of SAS with the pig as the experimental unit. Least square means and pooled SEM are presented. Differences were considered significant at $P < 0.05$ and trends approaching significance were considered for $0.05 < P < 0.10$.

Results

Fasting plasma insulin was greater in Iberian compared with Landrace pigs (15.6 *and* 8.10 µU/ml, respectively; sem=1.55, $P < 0.05$) whereas fasting plasma glucose was similar for both breeds (4.68 and 5.85 mmol/l for Iberian and Landrace, respectively; sem=0.92, $P > 0.10$).

Average plasma metabolites and insulin concentrations after the IAGTT are shown in Table 1. Mean plasma glucose, cholesterol and creatinine concentrations were lower in Iberian (14, 22 and 68%, respectively; $0.01 < P < 0.001$) 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.

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

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 and lower between 25-45 min (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 (288% on average (n = 9) compared with mean basal levels) and thereafter gradually decreased to values below fasting levels after 20 min for Iberian and 25 min for Landrace pigs. After glucose nadir (3.3 ± 0.6 mM), glucose concentration gradually increased again to reach values comparable to fasting levels at 180 min. No differences were found between breeds for glucose fractional turnover rate (4.1 and 3.3 ± 0.37%/min for Iberian and Landrace pigs, respectively; $P > 0.10$) or glucose half-life (17.1 and 22.1 ± 2.67 min for Iberian and Landrace pigs, respectively; $P > 0.10$).

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

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

Discussion

The IAGTT method allowed the comparison of the insulin responsiveness of an obese (Iberian) and a lean (Landrace) pig breed. It has previously been shown that Iberian pigs are fatty pigs with slow growth rate compared to modern breeds (Nieto et al., 2012). As arterial blood represents the metabolites concentration to which the tissues are exposed (Brouns *et al.*, 2005), chronic catheters were inserted in carotid artery for glucose infusion and blood sampling.

In the current study we have shown increased fasting plasma insulin and insulin response (higher plasma concentrations and AUC) after glucose infusion in Iberian compared with Landrace pigs. Greater postprandial serum levels of insulin have been described in Iberian (Fernández-Fígares *et al*., 2007) and Ossabaw (Wangsness *et al.*, 1981) pigs (both obese pigs) compared with lean pigs after ingestion of feed or glucose infusion. However, other comparative studies found increased insulin secretion in

Large White boars than in Meishan (Weiler *et al.*, 1998) boars (obese breed). The limited growth and development of slow growing pigs could result at least partly from lower concentrations of plasma insulin, because most cells of the body require insulin for adequate uptake of glucose and amino acids (Claus and Weiler, 1994). Nevertheless, decreased insulin concentration in blood does not seem to explain the poor growth performance of Iberian compared to Landrace pigs. If the concentration of insulin is compared among animals of different breeds, the sensitivity of each breed to insulin should be considered.

After glucose ingestion, blood concentration rapidly returns to preprandial values as shown in the present experiment, which indicates that exogenous glucose was efficiently metabolized, stored as glycogen, or both. As expected, when glucose was infused, plasma glucose levels were rapidly increased and a subsequent insulin response was observed. The elevated insulin lowered plasma glucose below fasting values within 20 min and insulin levels returned to baseline as plasma glucose declined. In our study, glucose concentration and glucose AUC during the glucose challenge were lower in Iberian compared with Landrace pigs, with no differences in fasting plasma glucose, may be due to the limited number of pigs. When interpreting the individual glucose curves, a monophasic pattern was identified for both breeds. The lower glucose AUC of Iberian pigs (-19% on average) may be related to the greater insulin AUC (+33% on average), a common pattern in many models of obesity. However, the reasons for the unequal physiological response between breeds are not well understood and must be discussed.

As it has been proved that the energy needs of portal-drained viscera are fulfilled by the oxidation of glucose, glutamate, and glutamine in pigs (Stoll *et al.*, 1999), a

larger gastrointestinal tract of Iberian pigs compared to Landrace (Rivera-Ferre *et al.*, 2005) is in line with the decreased AUC of glucose in the present experiment.

However, despite the larger size of the gastrointestinal tract and lower portal blood flow (González-Valero *et al.*, 2016) of Iberian compared with Landrace pigs, no differences on net portal flux of glucose after ingestion of the same diet were found (Rodríguez-López *et al.*, 2013). Differences on insulin stimulated glucose transport at portal-drained viscera level may help to explain these results. Iberian have lower glucose concentrations than Landrace pigs after an 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 to 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 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 to different degrees of dyslipidemia manifested as increased triglyceridemia and low HDL-cholesterol. In our experiment, we found lower plasma cholesterol but greater plasma triglycerides concentration in Iberian compared with Landrace pigs, although we did not separate LDL and HDL fractions. Reduced total cholesterol concentration could be due to reduced hepatic insulin sensitivity as insulin stimulates cholesterol synthesis (Nelson and Cox, 2017).

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

for Iberian pigs, is in accordance with previous studies (Fernández-Fígares *et al.*, 2007) and also with the low muscle protein deposition and muscle size described previously (Nieto *et al.*, 2002; Rivera-Ferre *et al.*, 2005). As insulin resistance is associated with decreased muscle mass, plasma creatinine levels can also be used as an indicator of insulin signalling disorders as reported by Kashima *et al*. (2017) in humans. Further research regarding amino acid concentration after an IAGTT may help to explain differences in the effect of insulin on muscle protein metabolism between breeds.

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

When we used the homeostasis model assessment (HOMA), differences on hepatic insulin resistance (HOMA-IR index) were negligible between breeds. However, Iberian had improved β-cell function compared with Landrace pigs according to HOMA-%B index, which may be due to enhanced sensitivity of the β-cells to glucose during the fasting period, consistent with QUICKI index. As a consequence, Iberian β-cell insulin synthesis increases in accordance with the increased insulin release after the glucose challenge and the elevated basal insulin concentrations reported for Iberian pigs.

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), at the development stage of this experiment, insulin resistance has not yet been fully established. Nevertheless, 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). The utilization of the hyperinsulinemic euglycemic clamp, the most definitive approach to determine whole-body insulin action, may 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 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 challenge (IAGTT; 500 mg/kg BW, 0-180 min)*[1]

	Breed			*P*-value[2]		
	Iberian	Landrace	SEM	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

[1]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.

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

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*[1]

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

[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; *$P < 0.05$; **$P < 0.01$.

Figure 1 Plasma insulin concentrations during intra-arterial glucose challenge (500 mg/kg BW) in growing Iberian (n = 4) and Landrace (n = 5) pigs. Comparisons *versus* basal or control treatment: †0.05 < *P* < 0.1, **P* < 0.05, ****P* < 0.001. Average basal level of insulin 11.43 µU/ml.

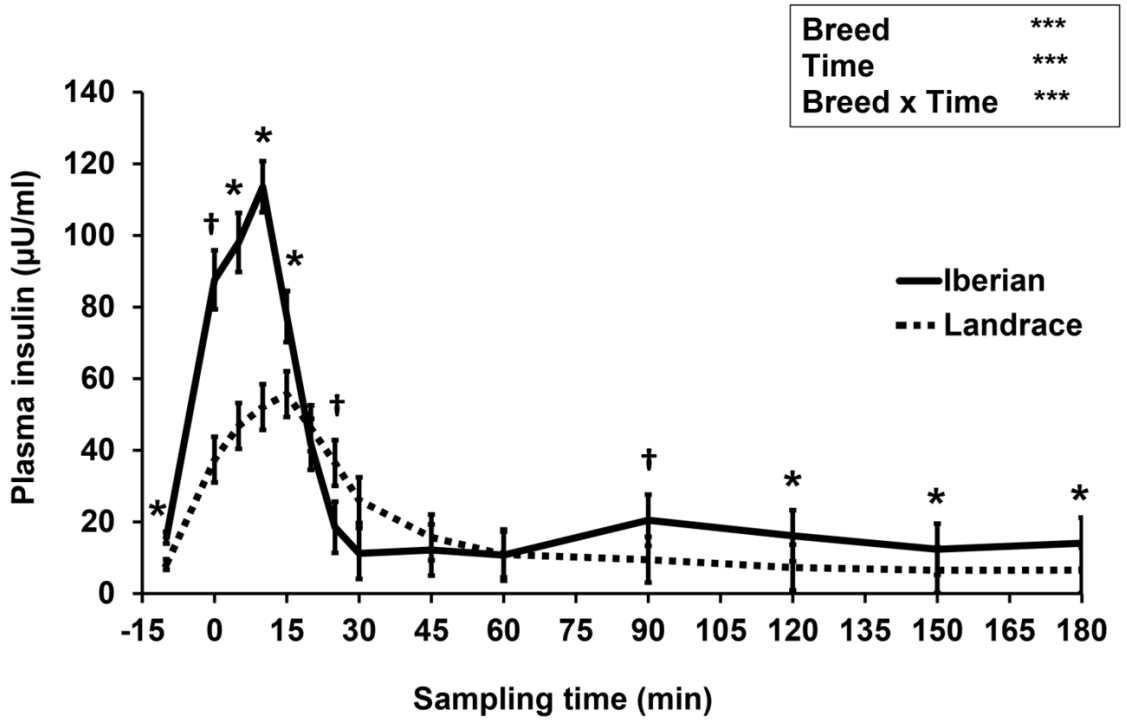

Figure 2 Plasma glucose concentration during intra-arterial glucose challenge (500 mg/kg BW) in growing Iberian (n = 4) and Landrace (n = 5) pigs. Comparisons *versus* basal or control treatment: †0.05 < *P* < 0.1, **P* < 0.05, ***P* < 0.01, *** *P* < 0.001; ns, not significant (*P* > 0.10). Average basal level of glucose 5.33 mM.

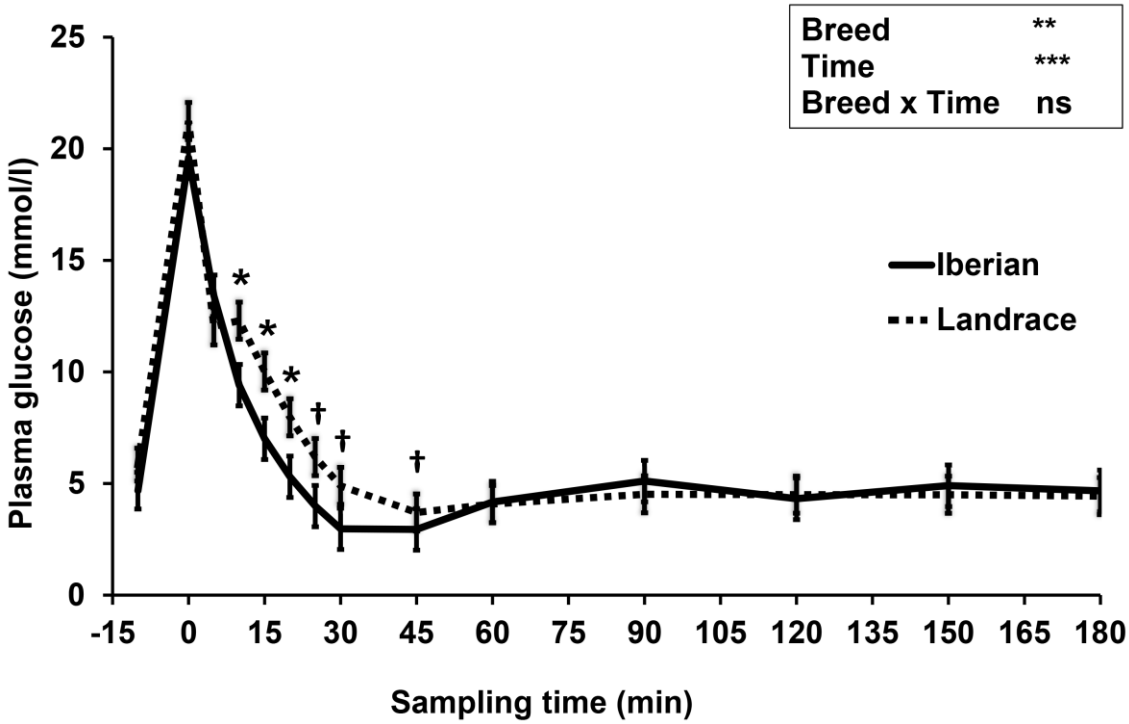

Figure 3 Plasma lactate concentration during intra-arterial glucose challenge (500 mg/kg BW) in growing Iberian (n = 4) and Landrace (n = 5) pigs. Comparisons *versus* basal or control treatment: †0.05 < *P* < 0.1, **P* < 0.05, ****P* < 0.001; ns, not significant (*P* > 0.10). Average basal level of lactate 0.87 mM.

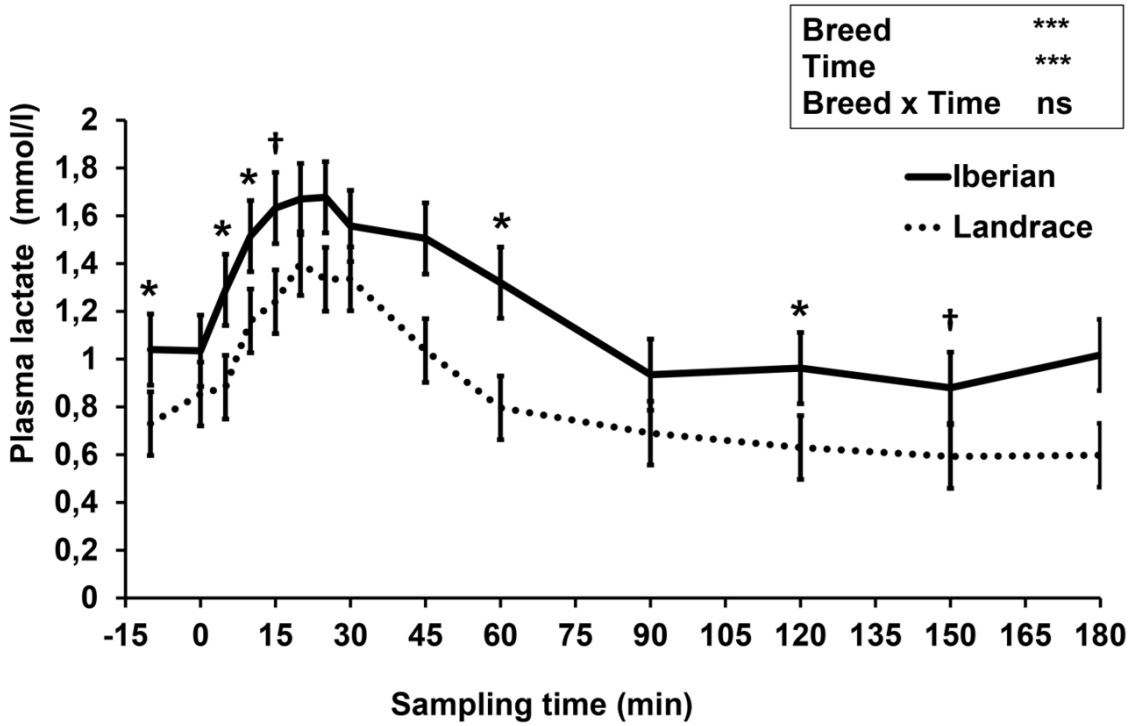

Figure 4 Area under the curve (AUC) of plasma insulin concentration during intra-arterial glucose challenge (500 mg/kg BW) between minute 0 and the time points indicated in growing Iberian (n = 4) and Landrace (n = 5) pigs. Differences between breeds: * *P* < 0.05.

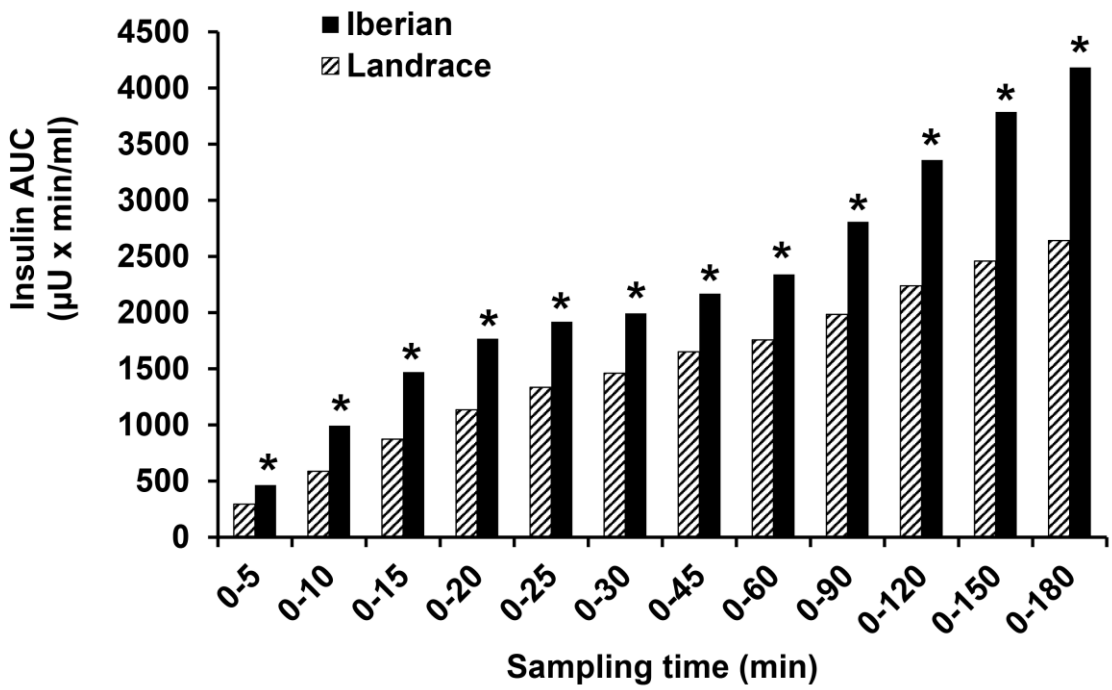

Figure 5 Areas under the curve (AUC) of plasma glucose concentration during intra-arterial glucose challenge (500 mg/kg BW) between minute 0 and the time points indicated in growing Iberian (n = 4) and Landrace (n = 5) pigs. Differences between breeds:†0.05 < *P* < 0.1, **P* < 0.05.

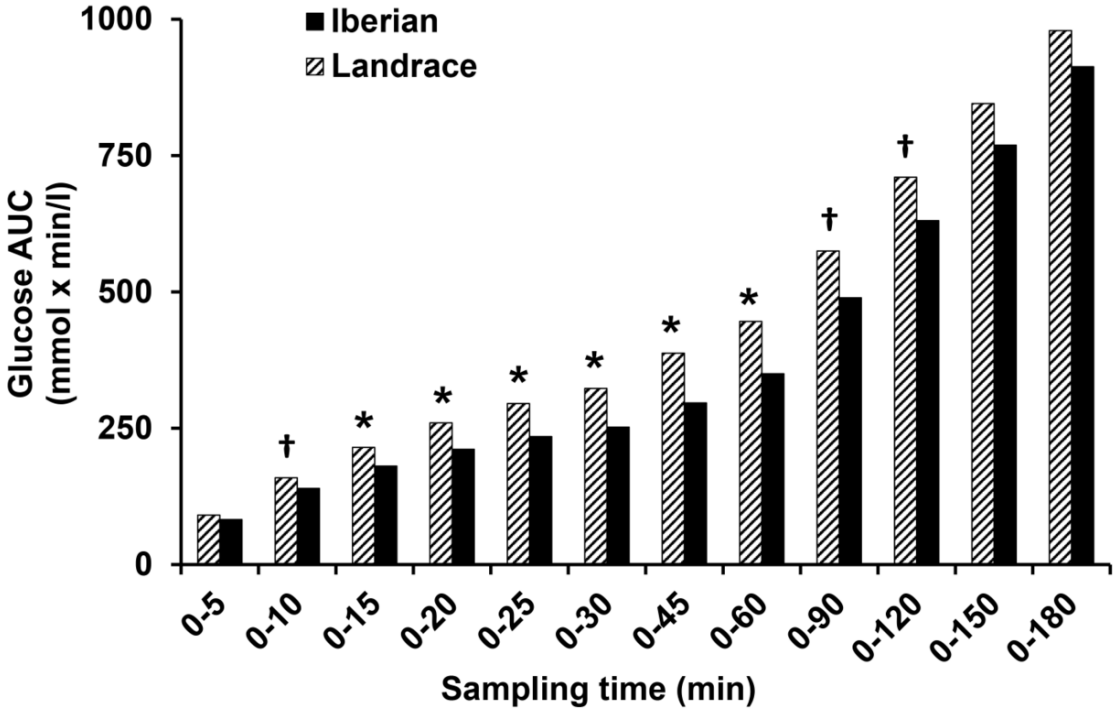
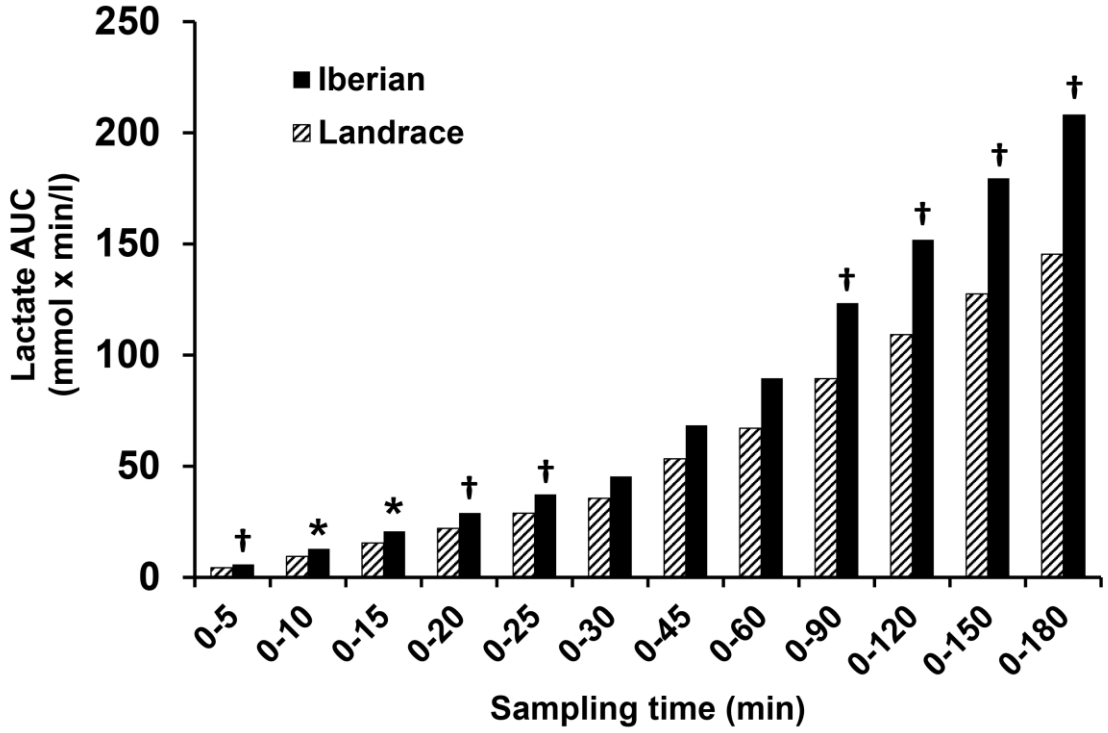
Figure 6 Area under the curve (AUC) of plasma lactate concentration during an intra-arterial glucose challenge (500 mg/kg BW) between minute 0 and the time points indicated in growing Iberian (n = 4) and Landrace (n = 5) pigs. Differences between breeds:†0.05 < *P* < 0.1, **P* < 0.05.