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Original article

GH and IGF-I binding sites in adipose tissue, liver, skeletal muscle and ovaries of feed-restricted gilts

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Abstract — The influence of feed restriction on GH and IGF-I binding was examined in the cyclic gilt. IGF-I, IGFBPs, insulin and leptin levels in plasma were also measured. Twenty-four gilts whose oestrous cycle was synchronised were used. From day 3 to day 12, (with day 0 being the first day of oestrus) feed allowance was 240% and 80% of the energy requirements for maintenance for well-fed (H) and restricted gilts (L) respectively. Six gilts in each group were treated with an antagonist of GnRH but the treatment had no effect on any reported measurements. Blood and tissue samples were collected on days 12 and 13 respectively. L gilts lost live weight whereas H gilts gained weight. Plasma IGF-I, insulin and leptin concentrations were lower in L than in H gilts whereas plasma IGFBP levels were not affected by feed restriction. The specific binding of ^{125}I-bGH to adipose tissue, liver, skeletal muscle and ovary membranes did not differ significantly between H and L gilts. Specific binding of ^{125}I-IGF-I to hepatic membranes was higher in L than in H gilts whereas it did not differ between the two groups in the other tissue membranes.

gilt / food restriction / GH / IGF-I / binding

Résumé — Liaison de la GH et de l'IGF-I dans le tissu adipeux, le foie, le muscle squelettique et l'ovaire de la truie cyclique rationnée. L'objectif de cette étude est de déterminer l'influence du statut nutritionnel sur les sites de liaison de la GH et de l'IGF-I chez la truie cyclique. Les niveaux plasmatiques d'IGF-I, d'IGFBPs, d'insuline et de leptine sont aussi mesurés. Du 3^{e} au 12^{e} jour du cycle œstral (J0 = début de l'œstrus), 24 truies reçoivent deux niveaux alimentaires correspondant à 240 (H) ou 80 % (L) de leurs besoins énergétiques d'entretien. Six truies de chaque groupe sont traitées avec un antagoniste de GnRH mais ce traitement n'a aucun effet sur les mesures réalisées. Des prélèvements de sang sont réalisés à J12 et les tissus sont prélevés à J13. Les truies L ont perdu du poids alors que les truies H ont pris du poids. Les concentrations plasmatiques d'IGF-I, d'insuline et de leptine sont plus faibles chez les truies L que chez les truies H. Par contre, le rationnement alimentaire ne modifie pas les niveaux plasmatiques d'IGFBPs. Dans le foie, le muscle squelettique, l'ovaire et le tissu adipeux, la liaison spécifique de ^{125}I-bGH ne diffère pas significativement entre les deux lots. La liaison spécifique de ^{125}I-IGF-I est plus élevée dans le foie des truies L que dans celui des truies H mais ne diffère pas entre les deux lots dans les autres tissus.

truie / rationnement alimentaire / GH / IGF-I / liaison

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1. INTRODUCTION

The GH/IGF-I axis is known to play an important role in mediating the interactions between nutrition, growth and reproduction [23, 33]. Numerous studies have clearly demonstrated that plasma IGF-I concentrations decrease in response to fasting or undernutrition and that circulating IGFBPs are altered by nutritional status [36]. Furthermore, it was shown that the response of the IGF system to malnutrition varies with the stage of development [10, 22]. Although receptors may represent important levels of regulation of GH and IGF-I actions, very little is known about the effects of altered nutrition on GH and IGF-I receptors in various tissues especially in pubertal animals. Therefore, this experiment was designed to examine the influence of nutritional deficiency on GH and IGF-I receptors in adipose tissue, liver, skeletal muscle and ovaries of cyclic gilts. Changes in IGF-I, IGFBPs, insulin and leptin levels in plasma were also evaluated. In an attempt to get a better understanding of the relationship between somatotrophic, gonadotrophic axes, nutrition and the ovarian function, the effects of feed restriction were also evaluated in gilts treated with an antagonist of GnRH [28].

2. MATERIALS AND METHODS

2.1. Animals and experimental design

Twenty-four crossbred Landrace × Large White gilts, which averaged 219 ± 8 days of age (mean ± SD) had their oestrous cycles synchronised by daily feeding of 20 mg·d^{-1} altrenogest (Regumate®, Roussel-Uclaf, Romainville, France) for 18 days. The first day of oestrus behaviour was designed as day 0. During the whole experiment, the gilts received a diet containing 3.04 Mcal metabolisable energy per kg, 18.1% crude protein and 0.96% lysine twice daily, at 08.30 and 13.40 h. From the beginning of the altrenogest treatment until day 2, feed allowance for all gilts was calculated to meet 240% of the energy requirements for maintenance. Maintenance requirements were calculated for individual gilts on the basis of their metabolic body weight (0.24 Mcal metabolisable energy per kg of body weight$^{0.60}$) [21]. At day 2, the gilts averaged 158 ± 14 kg body weight (mean ± SD). From day 3 to day 12, feed allowance was 240% of the energy requirements for maintenance for well-fed (H, n = 12) and 80% for restricted gilts (L, n = 12). In the L group, there were no feed refusals. In the H group, feed refusals were rarely observed and were not measured. All gilts were slaughtered on day 13 after a 16–17 h fast. The animals were reared in compliance with national regulations for the human care and the use of animals in research.

This experiment included a study on the influence of feed restriction on ovarian development and a comparison with the influence of gonadotrophin deprivation. In this context, six gilts in each feeding group were treated with an antagonist of GnRH, Antarelix, from day 3 to day 12. A full description was reported previously [28].

2.2. Sample collection

A catheter was inserted into the jugular vein of the gilts under general anaesthesia during the last week of altrenogest administration. The animals were deprived of feed on the day of surgery and then refed. Catheters were rinsed daily with physiological serum containing sodium heparin (190 IU·mL^{-1}) and antibiotics. Blood samples were collected every 15 min from 10.00 to 16.00 h on day 12. Samples were collected in heparinized tubes, kept on ice and immediately centrifuged for removal of plasma. Plasma samples were stored at –20 °C until analysis. At slaughter, the ovaries, liver, skeletal muscle (*longissimus*) and subcutaneous adipose tissue samples were collected within 15 min after death and were stored at –20 °C.

2.3. Insulin, IGF-I and leptin assays

Plasma concentrations of insulin were measured every 15 min using a previously described RIA [24]. Concentrations of IGF-I were determined in plasma samples taken at 16.00 h using a double antibody RIA after an acid-ethanol extraction [14]. Concentrations of leptin were measured in plasma taken at 13.15 h using the multi-species double-antibody kit assay (Linco Research, St. Charles, MO, USA) previously validated in the pig [25]. All samples were analysed in duplicate within a single assay. The intra-assay CV was 6.3% at 167 $\mu IU \cdot mL^{-1}$ for insulin, 8.8% at 41 $ng \cdot mL^{-1}$ for IGF-I and 6.9% at 2.94 $ng \cdot mL^{-1}$ HE (human equivalent) for leptin. Average sensitivities, estimated as 90% of total binding, were 2 $\mu IU \cdot mL^{-1}$ for insulin, 0.08 $ng \cdot mL^{-1}$ for IGF-I and 1 $ng \cdot mL^{-1}$ HE for leptin.

2.4. SDS-PAGE and Western ligand blot analysis of plasma IGFBPs

Plasma samples (2 µL) collected at 16.00 h were subjected to SDS/polyacrylamide gel electrophoresis under non-reducing conditions, using a 4% stacking gel and a 12.5% resolving gel [13]. The proteins were then transferred to a nitrocellulose membrane (BA85, 0.45 µm; Schleicher and Schuell, Dassel, Germany) using a Bio-Rad electrophoresis transfer unit. The nitrocellulose membrane sheets were treated following a previously described procedure [11]. Briefly, the blots were washed and then incubated with 90 000 $cpm \cdot mL^{-1}$ of ^{125}I-IGF-I for 2 h at 20 °C. After extensive washings [11], the blots were dried and then exposed to a Kodak X-Omat AR film with two intensifying screens for 7 days at –70 °C. The relative levels of IGFBPs were quantified using a phosphorImager (STORM, Molecular Dynamics, Bondoufle, France) and the IMAGEQUANT software. To prevent gel to gel variation in IGFBP evaluation, the two treatments were represented on each gel.

2.5. Membrane preparation and binding studies

Microsomal membranes were prepared as previously described [20] with modification for adipose tissue [34]. In brief, frozen tissues were individually cut into small pieces and homogenised in ice-cold 50 mM Tris-HCl buffer (pH 7.4) containing 250 mM sucrose, 1 mM EDTA, 0.02% NaN_3 and 1 mM phenylmethylsulfonyl fluoride using a Polytron tissue grinder. After the final centrifugation (100 000 × g, 60 min), the pellet, which contained the microsomal membranes, was resuspended in 50 mM Tris-HCl (pH 7.4) and stored at –20 °C until use. The protein content was estimated using the bicinchoninic acid assay (Pierce, Rockford, IL, USA) with BSA as a standard.

All GH [32] and IGF-I [15] binding assays were carried out in triplicate as previously described. Microsomal membrane proteins (400 µg for adipose tissue, 500 µg for liver and muscle and 800 µg per tube for ovaries) were incubated with the ^{125}I-ligand (30 000 cpm or 0.3 ng per tube for GH and 60 000 cpm or 0.2 ng per tube for IGF-I) in binding buffer (25 mM Tris-HCl (pH 7.4), 10 mM $CaCl_2$, 0.5% BSA, 0.02% NaN_3 for GH; 50 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 0.5% BSA, 0.02% NaN_3 for IGF-I) in the absence or presence of an excess of unlabelled bGH or IGF-I. After incubation for 48 h at room temperature (GH) or for 24 h at 4 °C (IGF-I), 2 mL ice-cold binding buffer was added to stop the reaction. Bound and free hormones were separated by centrifugation.

2.6. Statistical analyses

All data were analysed by analysis of variance using the GLM procedure of SAS [31]. All models included feeding level, Antarelix treatment and the interaction between these two factors. For insulin concentrations, split-plot models were used that

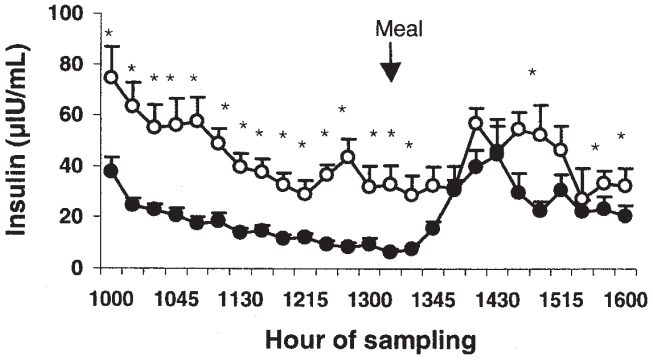

Figure 1. Profile of plasma insulin concentrations in well-fed (H, ❍) and feed-restricted (L, ●) gilts. Meals were distributed at 08.30 and 13.40 h. Values are means ± SEM, $n = 12$ per group. *$P < 0.05$.

also included gilt nested within feeding level × Antarelix treatment, sampling time, time × feeding level and time × Antarelix treatment. The effects of feeding level, Antarelix treatment and feeding level × Antarelix treatment were tested using gilt within feeding level × Antarelix treatment as the error term. The effect of time, time × feeding level and time × Antarelix treatment were tested using the residual error term. When the time × feeding level interaction was significant, comparisons between groups were performed for each sampling time.

3. RESULTS

The treatment by the GnRH antagonist Antarelix had no influence on any of measured parameters ($P > 0.1$). Moreover, there was no interaction between this treatment and the level of feeding. Therefore, data presented here took into account all gilts (H, $n = 12$ and L, $n = 12$), without distinction between Antarelix-treated and non-treated gilts.

During the experimental period, L gilts lost live weight whereas H gilts gained weight (–2.4 ± 0.9 versus +12.9 ± 0.8 kg, mean ± SD, $P < 0.001$). Plasma concentrations of insulin were lower in L than in H gilts before the 13.40 h meal (Fig. 1, $P < 0.05$). After the meal, concentrations differed between the two groups at 15.00, 15.45 and 16.00 h ($P < 0.05$). Plasma concentrations of leptin, measured just before the 13.40 h meal, were lower in L than in H gilts (Fig. 2, $P < 0.001$). Plasma IGF-I concentrations, measured at 16.00 h, were lower (Fig. 2, $P < 0.001$) in L than in H gilts. Ligand blot analysis revealed the presence of five IGFBP bands with apparent molecular masses of 43, 39, 34, 29 and 24 kDa (Fig. 3). The 43–39 kDa IGFBP was predominant. The relative quantification showed no significant variations in plasma 43–39 IGFBP (Fig. 3, $P > 0.1$) and in other IGFBP levels between females (data not shown).

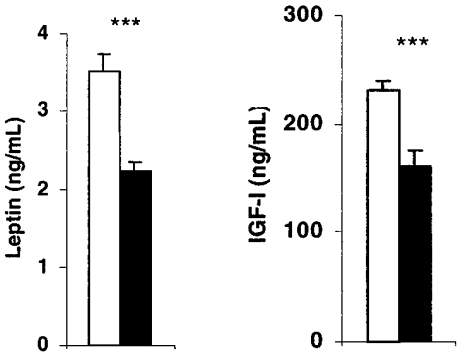

Figure 2. Plasma leptin and IGF-I concentrations in well-fed (H, □) and feed-restricted (L, ■) gilts. Plasma concentrations were measured just before the 13.40 h meal for leptin and at 16.00 h for IGF-I. Values are means ± SEM, $n = 12$ per group. ***$P < 0.001$.

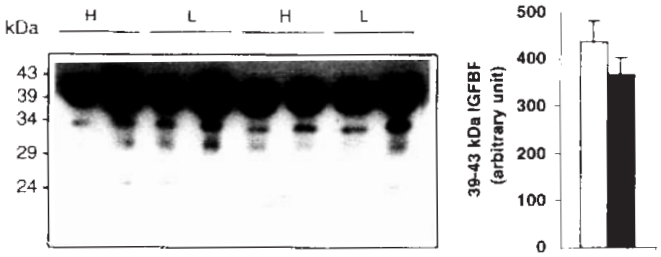

Figure 3. Plasma IGFBPs in well-fed (H, □) and feed-restricted (L, ■) gilts. IGFBP levels were evaluated using ligand blot analysis as described in Materials and Methods. Samples of plasma were collected at 16.00 h. The left-hand panel shows a representative autoradiogram (7-day exposure) from ligand blot analysis of IGFBPs in plasma samples (2 μL) of representative gilts per experimental group. The right-hand panel shows relative levels of plasma 39–43 kDa IGFBP in well-fed (H, □) and feed-restricted (L, ■) gilts. Values are means ± SEM, n = 12 per group.

The effect of feed restriction on ^{125}I-bGH specific binding to the liver, skeletal muscle, subcutaneous adipose tissue and ovary membranes was not significant (Fig. 4, $P > 0.05$). In adipose tissue, however, binding tended to be lower ($P = 0.086$) in L than in H gilts. Specific binding of ^{125}I-IGF-I to hepatic membranes was higher ($P < 0.01$) in L than in H gilts (Fig. 5). In the ovaries, it tended to be higher ($P = 0.098$) in L than in H gilts. No significant differences ($P > 0.1$) between the two groups were observed for skeletal muscle and subcutaneous adipose tissue.

4. DISCUSSION

The results of the present experiment indicate that GH (GHR) and IGF-I (IGF-IR) receptors in adipose tissue, liver, skeletal muscle and ovaries are poorly affected by a reduction of feed intake despite significant changes in body weight gain and hormonal status. The decline in plasma insulin concentrations which is consistent with previous studies in prepubertal gilts [3, 6] and in lactating sows [12, 27, 38], probably allows the restricted gilts to maintain their glycemia. The decrease in plasma leptin concentrations in response to feed restriction

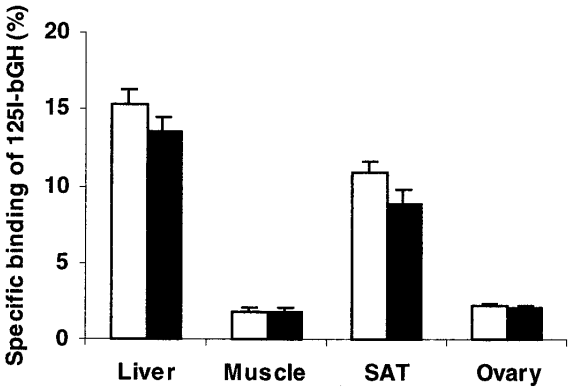

Figure 4. Specific binding of ^{125}I-bGH to the liver, muscle, subcutaneous adipose tissue (SAT) and ovaries in well-fed (H, □) and feed-restricted (L, ■) gilts. Microsomal membranes were incubated with ^{125}I-bGH in the absence or the presence of an excess of unlabelled bGH for 48 h at room temperature as described in Materials and Methods. Measurements were made after a 16–17 h fast. Values are means ± SEM, n = 12 per group.

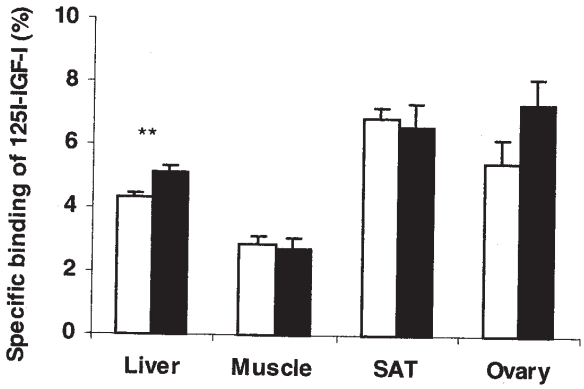

Figure 5. Specific binding of ^{125}I-IGF-I to the liver, muscle, subcutaneous adipose tissue (SAT) and ovaries in well-fed (H, □) and feed-restricted (L, ■) gilts. Microsomal membranes were incubated with ^{125}I-IGF-I in the absence or the presence of an excess of unlabelled IGF-I for 24 h at 4 °C as described in Materials and Methods. Measurements were made after a 16–17 h fast. Values are means ± SEM, n = 12 per group. $^{**}P < 0.01$.

has also been described by Mao et al. [18]. The finding of a lower plasma IGF-I concentration in feed-restricted gilts is consistent with the response to feed deprivation or restriction previously reported in young growing pigs [5, 8, 9], prepubertal and mature gilts [1, 6] and lactating sows [18, 27]. Because the liver is considered as one of the major sites of IGF-I production, it has often been indicated that the low concentration of IGF-I results from a decrease in GH binding. In the current study, the decrease did not arise from a reduction of this receptor. Nevertheless, a decrease in total hepatic GHR cannot be ruled out. Indeed, the total number of GHR was likely to be lower in restricted gilts since their liver was 30% lighter than in well-fed gilts (data not shown).

Reduction of circulating IGF-I during malnutrition has been shown to be associated with changes in the plasma IGFBP profile in growing pigs [8, 19]. However, the present study failed to show a significant difference in IGFBP levels between restricted and well-fed gilts. This finding was in agreement with the lack of effect of a 48 h food deprivation in prepubertal and mature gilts [1]. This suggests that the sensitivity of plasma IGFBPs to feed restriction differs between pubertal and younger animals.

There is little information regarding the influence of feed restriction on IGF-IR. The finding of slightly higher levels of IGF-I binding in the liver of feed-restricted gilts is consistent with the increase in IGF-IR in some tissues in response to fasting or protein deprivation [16, 35] and with data showing that IGF-I down-regulates its own receptor in vitro [29, 30]. The lack of alteration of IGF-I binding in skeletal muscle and adipose tissue despite changes in plasma IGF-I concentration does not corroborate this relationship. The influence of local factors like locally produced IGF-I cannot be excluded. Altogether, our findings showed the existence of a tissue-specific regulation that may represent a way to regulate the effect of feed restriction according to organs and/or tissues.

The observation that feed restriction did not alter GH binding in the liver is not consistent with previously reported data. A reduction of GH binding in liver has been observed in rats [17, 37], cattle [4] and sheep [2]. It has also been reported that feed restriction decreases GHR mRNA levels in the liver of young pigs [9], whereas it increases these levels in peripubertal gilts [7]. There is little data regarding the influence of feed restriction on GHR levels in tissues other than the liver. The present study failed to show a significant effect on GHR in skeletal muscle as reported in peripubertal gilts [7] whereas an increase in the GHR mRNA content has been observed in young pigs [9]. It is likely that the differences

observed in the response of GHR to malnutrition are related to the developmental stages. In rats, it has been reported that the regulation of hepatic GHR and/or plasma IGF-I by nutritional status is age-dependent and that the young have a greater sensitivity to undernutrition [10, 22].

Feed-restriction, which significantly decreased ovarian weight but did not alter the number of medium and large follicles (≥ 2mm, [28]), had no significant influence on GH and IGF-I binding to ovarian membranes. However, cell-specific alterations cannot be excluded since GH and IGF-I binding sites are widely distributed within porcine ovaries [26].

In conclusion, the present study indicates that feed restriction has a small effect on GH and IGF-I receptors despite changes in plasma concentrations of insulin, leptin and IGF-I in cyclic gilts. Our findings associated with previous observations are consistent with the suggestion that the response of the GH/IGF-I axis to undernutrition varies with developmental age.

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