# Impact of Intrauterine Growth Retardation and Early Protein Intake on Growth, Adipose Tissue, and the Insulin-Like Growth Factor System in Piglets

ANNE MORISE, BERNARD SÈVE, KATHERINE MACÉ, CORINNE MAGLIOLA, ISABELLE LE HUËROU-LURON,
AND ISABELLE LOUVEAU

*INRA [A.M., B.S., I.L.L., I.L.], Agrocampus Rennes [A.M., B.S., I.L.L., I.L.], UMR 1079, F-35000 Rennes, France; Nestlé Research Center
[K.M., C.M.], CH-1000 Lausanne 26, Switzerland*

**ABSTRACT:** Small birth weight and excess of early protein intake are suspected to enhance later adiposity. The present study was undertaken to determine the impact of diets differing in protein content on short-term growth, adipose tissue development, and the insulin-like growth factor (IGF) system in piglets. Normal (NW) and small (SW) birth weight piglets were fed milk-replacers formulated to provide an adequate (AP) or a high protein (HP) supply between 7 and 28 d of age. The fractional growth rate was higher ($p < 0.01$) in SW than in NW piglets. At 7 d of age, the lower ($p < 0.05$) weight of perirenal adipose tissue relative to body mass in SW than in NW piglets did not involve significant changes in plasma IGF-I, leptin, or insulin-like growth factor binding protein levels, but involved differences ($p < 0.05$) in the expression of IGF-I and leptin in adipose tissue. Growth rates did not differ between AP and HP piglets. At 28 d of age, HP piglets had lower ($p < 0.001$) relative perirenal adipose tissue weight but did not differ clearly from AP piglets with regard to the IGF system. It remains to be determined whether piglets fed such a high protein intake will stay subsequently with a low adiposity. (*Pediatr Res* **65:** 45–50, 2009)

Among the different factors that may contribute to the rise in the prevalence of overweight and obesity in the world, early nutrition is receiving increasing attention. According to the concept of "metabolic programming," alteration of nutrition at a critical period of development in early life affects the subsequent pattern of growth and development of tissues and organs and may predispose individuals to several disorders in later life (1–3). It is especially considered that such a risk is quite high for neonates born with small birth weights. These neonates are often fed formulae enriched in protein to promote catch-up growth and brain development. In this context, some authors have raised the hypothesis that a high protein intake during early life may increase the risk of subsequent obesity, possibly by affecting regulatory axes (4,5). However, whereas some epidemiologic studies tend to show a positive correlation between dietary protein to energy ratio in early life and body mass index in childhood (5,6), other studies fail to show such a relationship (7,8).

Animal models are useful to clarify this issue, which is of great interest for nutrition (9). The few available data in rodents are based on the modulation of protein intake of the dam and not of the offspring (10–12). The pig may be an appropriate model to design experiments dealing with both birth weight and neonatal nutrition. There can be up to a 3-fold difference in body weight among littermates in normally fed sows, thus providing a natural form of intrauterine growth retardation (IUGR; 13,14). In addition, this model allows artificial rearing and, therefore, it enables modulation and control of food intake during the neonatal period. Therefore, the present study was undertaken to evaluate the short-term effects of a high protein intake between 7 and 28 d of age on growth and adipose tissue development in normal and small birth weight piglets. It was also examined whether this diet induced changes in the endocrine system and especially in the insulin-like growth factor (IGF) system that is known to play an important role in the processes that link nutrition and growth (5,15).

## MATERIALS AND METHODS

*Animals and experimental design.* The care and use of animals were performed in compliance with the guidelines of the French Ministry of Agriculture and Fisheries (certificate of authorization to experiment on living animals no 7676). Crossbred [Pietrain × (Large White × Landrace)] piglets from 15 litters were obtained from the experimental herd of INRA (Saint-Gilles, France). All piglets were weighed at birth. Piglets with a weight close to the average birth weight of the herd (1.40 kg) were identified as normal birth weight (NW) piglets and those with a 30% lower weight were defined as small birth weight (SW) piglets. The range of birth weights in the NW group was 1.28–1.66 kg ($n = 28$) and in the SW group was 0.74–1.10 kg ($n = 28$). In each litter, one pair of piglets of each weight group, at least, was selected. Piglets were allowed to suckle the dam naturally up to 7 d of age. At this stage, NW and SW piglets ($n = 8$/group) were slaughtered as initial controls. Other selected piglets were randomly assigned to one of the two dietary groups. They were separated from their dam and were fed milk-replacers formulated to provide an adequate (AP) or a high protein (HP) supply. It was chosen to start the nutritional manipulation at 7 d of age because the fat mass of piglets of this age (approximately 8% of body weight; 16) is similar to that of human newborn babies (17).

*Animal feeding.* Piglets fed formulae were individually housed in stainless steel metabolism cages in a temperature-controlled room (30 ± 0.5°C) from 7 to 28 d of age. They were fed with an automatic milk feeder (Fig. 1)

**Abbreviations: A-FABP,** adipocyte (A)-type fatty acid binding protein; **AP,** adequate protein formula; **CPT-I,** carnitine-palmitoyl transferase (CPT)-I; **FAS,** fatty acid synthase; **GLUT4,** glucose transporter 4; **HP,** high protein formula; **NW,** normal birth weight; **SW,** small birth weight; **PPAR-γ,** peroxisome proliferator-activated receptor gamma
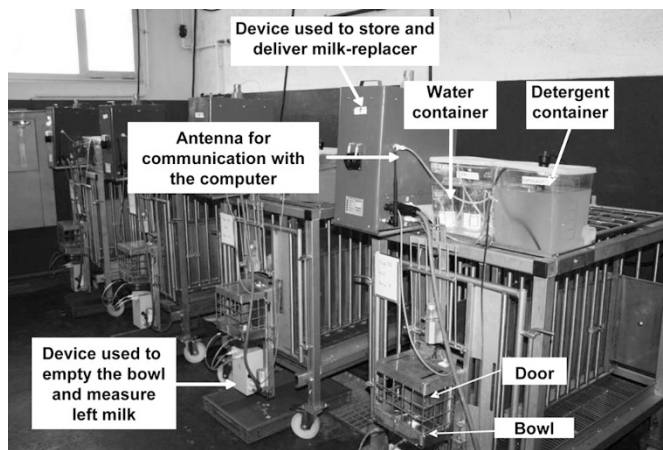
**Figure 1.** Automatic milk feeder.

designed and assembled in our laboratory to deliver predetermined quantity of diet and to record piglet consumption. Formulae were prepared daily, continuously stirred and maintained at 4°C in individual refrigerated boxes. At each meal, formulae were warmed up to avoid piglet digestive disturbances. The daily formula rations were calculated in net energy (NE) according to a predefined feeding table based on milk production studies (18). The daily NE allowance of piglets fed formulae was 1300 kJ/kg body weight$^{0.75}$ from 7 to 15 d of age, progressively decreasing to 940 kJ/kg body weight$^{0.75}$ between 15 and 28 d of age. Body weights were recorded weekly. The ration was partitioned into eight meals automatically distributed between 0600 and 2130. Piglets had free access to water only at night.

The AP diet was formulated to match the protein, amino acid, fat, and carbohydrate composition as well as the ratio of casein to soluble whey protein (46:54) in sow's milk solids. Mean sow's milk composition as assessed between 7 and 22 d of lactation was used as reference (Table 1, 19). The HP powder was formulated to provide more amino acids per unit of NE than the AP powder. The protein supplement was incorporated in partial substitution for nonprotein ingredients, keeping constant both the casein to soluble whey protein and the fat to carbohydrate ratios. Consequently, the ratio of protein to NE was 46.5% higher in the HP than in the AP powder (Table 1).

*Sample collection.* At 14 d of age, catheters were inserted into one external jugular vein of each animal under general anesthesia. A solution of heparin

**Table 1.** *Composition of sow milk and formula*

| | Sow milk (literature)* | Sow milk (analyzed)† | AP formula | HP formula |
|---|---|---|---|---|
| Protein (g/L) | 50 | 53 | 50.5 | 74.7 |
| Lipid (g/L) | 80 | 90 | 80 | 73 |
| Lactose (g/L) | 51 | 44 | 51 | 46 |
| Net energy (NE), kcal/L | 855 | 944 | 850 | 850 |
| Protein/NE (g/100 kcal) | 5.85 | 5.60 | 5.91 | 8.78 |

* See Ref. 19.

† Samples taken during the experiment.

(50 UI/mL) in saline was flushed every other day through the catheter to keep it patent throughout the experimental period. Blood samples were collected 1 h after a meal every third day between 17 and 28 d of age. At 28 d of age, piglets were slaughtered 3 h after the last meal in the experimental slaughterhouse by electrical stunning and exsanguination. Adipose tissue samples were collected within 15 min. For histologic analysis, tissue samples were restrained on flat sticks and frozen in 2-methylbutane cooled by liquid nitrogen. For other measurements, tissues cut into small pieces were frozen in liquid nitrogen. All tissue samples were stored at −75°C and EDTA-plasma samples were stored at −20°C for later analyses.

*Determination of adipocyte diameter.* Adipocyte diameters were determined in four serial cross-sections (10-$\mu$m thick at 40-$\mu$m interval) of frozen tissues, cut with a cryostat (2800 Frigocut Reichert-Jung, Francheville, France). Cross-sections were fixed for 10 min in 100 mM phosphate buffer (pH 7.4) containing 2.5% glutaraldehyde, and stained for 4 min in isopropanol containing 0.5% Oil red O. Individual adipocyte areas were measured using an image analysis system (Optimas 6.5, Media Cybernetics, Silver Spring, MD). Cells with a diameter below 10 $\mu$m were not considered. Results were the mean of determinations performed on the four sections and were expressed as diameter ($\mu$m) of visible adipocytes. The coefficient of variation for cell diameter between the four successive sections was 9.9%.

*Hormone assays.* All samples were analyzed within a single assay. Concentrations of plasma IGF-I were determined using a validated radioimmunoassay (20) that used recombinant human IGF-I (GroPep, Adelaïde, Australia). The intra-assay and interassay coefficients of variation for plasma samples with a mean IGF-I concentration of 28 ng/mL were 12% and 18%, respectively. Concentrations of plasma leptin were measured using the multispecies radioimmunoassay kit (LINCO Research, St. Charles, MO) previously validated for use in porcine plasma (21). Modification of the assay included the doubling of the plasma sample. The intra-assay and interassay coefficients of variation for a porcine plasma sample with a mean leptin concentration of 1.82 ng/mL were 5% and 14%, respectively.

*Western ligand blot analysis of plasma IGFBPs.* The assay was performed on samples collected at slaughter. Plasma samples (2 $\mu$L) were subjected to SDS/PAGE under nonreducing conditions using a 12.5% resolving gel. Proteins were then transferred onto nitrocellulose membranes (22). Briefly, the blots were washed and then incubated with 90,000 cpm/mL of $^{125}$I-IGF-I for 2 h at room temperature. After extensive washings, the blots were dried and were exposed to a Kodak X-Omat AR film for 6–7 d at −75°C. Autoradiograms were scanned and the relative levels of insulin-like growth factor binding proteins (IGFBPs) were quantified with an image processor (Quantity One, V4, Bio-Rad, CA). To prevent gel-to-gel variation in IGFBP evaluation, the different treatment groups were represented on each gel.

*Real-time RT-PCR.* Total RNA was extracted from adipose tissues using Trizol reagent (Invitrogen, Cergy-Pontoise, France). RNA integrity was then checked on an ethidium bromide-stained agarose gel. Treated-DNAse total RNA (2 $\mu$g) was reverse-transcribed using random hexamer primers. Primers for selected genes (Table 2) were designed using Primer Express software (Version 2.0, Applied Biosystems, Courtaboeuf, France). Real-time quantitative PCR analyses were performed in a final volume of 12.5 $\mu$L starting with 5 ng of reverse-transcribed RNA using SYBR® Green I PCR core reagents in an ABI PRISM 7000 Sequence Detection System instrument (Applied Biosystems). Forty cycles of amplification were performed, with each cycle consisting of denaturation at 95°C for 15 s, and annealing and extension at 59°C for 1 min. Cycle threshold ($C_T$) values are means of triplicate measurements. Negative controls were included on each 96-well plate. Endogenous 18S ribosomal RNA amplifications were used for each sample to normalize the expression of the selected genes. A cDNA sample obtained from a pool of total RNA isolated from adipose tissues was used as an interplate calibrator for each gene. Because PCR

**Table 2.** *Primers used for analysis of gene expression by real-time RT-PCR*

| Gene | Accession no. | Forward primer (5′→3′) | Reverse primer (5′→3′) |
|---|---|---|---|
| IGF-I | M31175 | GCTGGACCTGAGACCCTCTGT | TACCCTGTGGGCTTGTTGAAAT |
| IGF-II | X56094 | AGGGCATCCAAACCACAAAC | GGGGTTCAATTTTTGGTATGTAACTTG |
| IGF-I R* | U58370 | CAACCTCCGGCCTTTTACTTT | CAGGAATGTCATCTGCTCCTTCT |
| Leptin | AF102856 | GTTGAAGCCGTGCCCATCT | CTGATCCTGGTGACAATCGTCTT |
| Insulin R* | AF102858 | CAGCGATGTATTTCCATGTTCTGT | GCGTTTCCCTCGTACACCAT |
| PPAR-γ | AF103946 | ATTCCCGAGAGCTGATCCAA | TGGAACCCCGAGGCTTTAT |
| A-FABP | AJ416020 | GGAAAGTCAAGAGCACCATAACCT | TTCCACCACCAACTTATCATCTACTATTT |
| CPT-I | AY181062 | CACTGTCTGGGCAAACCAAA | GCCACCTGGTAGGAACTCTCAAT |
| FAS | AY183428 | AGCCTAACTCCTCGCTGCAAT | TCCTTGGAACCGTCTGTGTTC |
| GLUT4 | DT324033 | GGCAGCCCCTCATCATTG | TCGAAGATGCTGGTTGAATAGTAGAA |

* R, receptor.
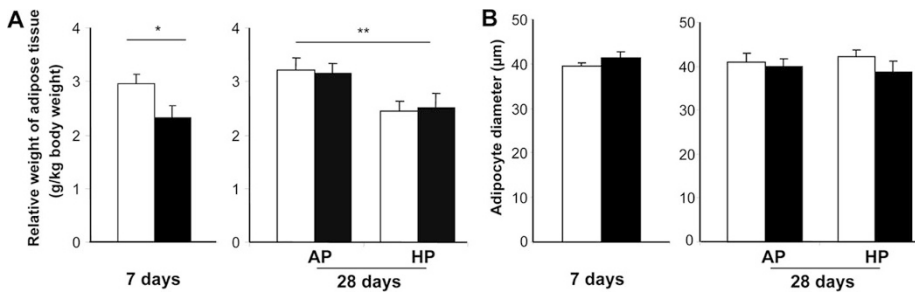
**Figure 2.** Features of perirenal adipose tissue in NW (□) and SW (■) piglets before (7 d of age) and at the end of the experimental period (28 d of age). (A) Relative weight of adipose tissue. (B) Diameter of adipocytes. Values are means ± SEM ($n = 8–10$ per group). *$p < 0.05$ NW *vs* SW; **$p < 0.001$ AP *vs* HP.

efficiencies for target genes and 18S gene were close to 1, the relative expression of a target gene was calculated as follows (23):

$$\text{Ratio} = (2^{-\Delta C_T \text{ target(sample } - \text{ calibrator)}})/(2^{-\Delta C_T \text{ 18S(sample } - \text{ calibrator)}}).$$

*Statistical analysis.* Data were analyzed using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). For piglets slaughtered at 7 d of age, the model included the effects of litter and birth weight that were tested against the residual mean square error between pairs of piglets. For piglets fed formulae and slaughtered at 28 d of age, the model allowed testing in addition diet and diet by birth weight interaction. For hormone concentrations, means for each piglet was considered in the analysis because there was no significant effect of sampling day on plasma concentrations whatever the group considered. All data are presented as means ± SEM. Differences were considered significant at $p < 0.05$.

## RESULTS

*Growth and feed intake.* Absolute growth rate (weight gain/d) during the experimental period was lower ($p < 0.01$) in SW piglets than in their NW counterparts (Table 3). The fractional growth rate (weight gain/d/kg mean body weight during the experimental period) was, however, higher ($p < 0.01$) in SW than in NW piglets. In addition, the absolute and fractional growth rates did not differ between AP and HP piglets. Food intake was lower ($p < 0.001$) in SW than in NW

piglets, but it was similar when expressed per kg mean body weight. Whatever its expression, food intake did not differ between AP and HP piglets.

*Adipose tissue.* The relative weight of perirenal adipose tissue, expressed per kg body weight, was lower ($p < 0.05$) in SW than in NW piglets at 7 d of age but did not differ between the two birth weight groups at 28 d of age (Fig. 2A). These data reflected an increase in adipose tissue weight between 7 and 28 d that was higher in SW than in NW piglets (+200% *versus* +138% in AP piglets; +142% *versus* +90% in HP piglets). At 28 d of age, piglets fed the HP diet exhibited a lower proportion ($p < 0.001$) of perirenal adipose tissue compared with piglets fed the AP diet. Adipocyte diameters (Fig. 2B) in this tissue or in s.c. adipose tissue (data not shown) varied neither with birth weight nor with diet.

*Plasma hormones.* In 7-d-old piglets, concentrations of plasma IGF-I (27.7 ± 3.1 *versus* 25.7 ± 3.4 ng/mL in NW and SW, respectively) and leptin (2.52 ± 0.19 *versus* 2.45 ± 0.13 ng/mL in NW and SW, respectively) were not affected by birth weight. The diet did not influence the plasma concentration of these hormones in 28-d-old piglets (Table 4). With

**Table 3.** *Growth and consumption of NW and SW piglets fed formulae between 7 and 28 d of age*

| | AP formula | | HP formula | | Effects | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | NW | SW | NW | SW | Diet | BW | I |
| Birth weight (kg) | 1.39 ± 0.02 | 1.00 ± 0.02 | 1.39 ± 0.04 | 1.00 ± 0.02 | NS | <0.001 | NS |
| Initial weight at 7 d (kg) | 2.82 ± 0.12 | 1.97 ± 0.13 | 2.82 ± 0.17 | 2.01 ± 0.14 | NS | <0.001 | NS |
| Final weight (kg) | 6.46 ± 0.31 | 5.14 ± 0.37 | 6.51 ± 0.29 | 4.91 ± 0.46 | NS | <0.001 | NS |
| Absolute growth rate (g/d) | 177 ± 12 | 152 ± 13 | 185 ± 12 | 139 ± 13 | NS | <0.01 | NS |
| Fractional growth rate (g/d/kg MBW) | 38.7 ± 1.3 | 43.7 ± 1.4 | 40.0 ± 1.6 | 41.1 ± 1.4 | NS | <0.01 | NS |
| Daily dry matter intake (g/d) | 166.29 ± 10.2 | 137.7 ± 8.9 | 169.1 ± 6.1 | 129.4 ± 12.7 | NS | <0.001 | NS |
| Daily dry matter intake (g/d/kg MBW) | 36.6 ± 1.2 | 40.0 ± 0.8 | 37.7 ± 1.6 | 38.6 ± 1.9 | NS | NS | NS |

Values are means ± SEM ($n = 8–10$ per group).
BW, birth weight; I, interaction between diet and BW; MBW, mean body weight during the experimental period; NS, not significant.

**Table 4.** *Concentrations of IGF-I and leptin and relative levels of IGFBPs in plasma from NW and SW piglets fed formulae between 7 and 28 days of age*

| | AP formula | | HP formula | | Effects | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | NW | SW | NW | SW | BW | Diet | I |
| IGF-I (ng/mL) | 21.7 ± 1.2 | 23.5 ± 2.0 | 20.1 ± 1.5 | 21.4 ± 2.5 | NS | NS | NS |
| Leptin (ng/mL) | 1.72 ± 0.09 | 1.61 ± 0.09 | 1.65 ± 0.09 | 1.56 ± 0.09 | NS | NS | NS |
| IGFBPs (arbitrary units) | | | | | | | |
| 43–39 kD | 1.25 ± 0.14 | 1.07 ± 0.04 | 1.05 ± 0.04 | 1.16 ± 0.10 | NS | NS | NS |
| 34 kD | 0.91 ± 0.07 | 0.99 ± 0.02 | 0.89 ± 0.10 | 0.90 ± 0.13 | NS | NS | NS |
| 29 kD | 0.96 ± 0.05 | 1.01 ± 0.02 | 0.92 ± 0.10 | 0.94 ± 0.09 | NS | NS | NS |
| 24 kD | 1.00 ± 0.08 | 0.99 ± 0.02 | 1.13 ± 0.15 | 0.97 ± 0.04 | NS | NS | NS |

Values are means ± SEM ($n = 8–10$ per group).
BW, birth weight; I, interaction between diet and BW; NS, not significant.

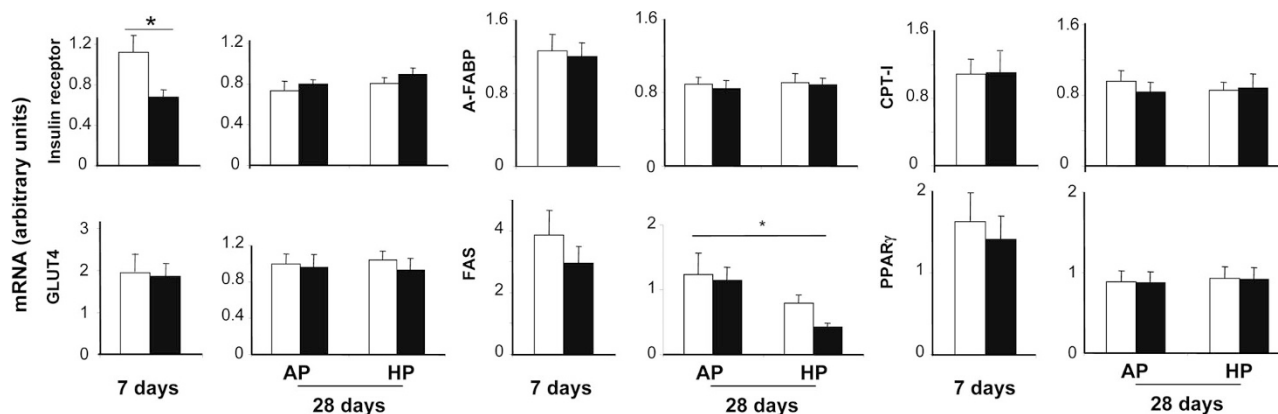**Figure 3.** Relative levels of insulin receptor, GLUT4, A-FABP, FAS, CPT-I, and PPARγ mRNA in perirenal adipose tissue in NW (□) and SW (■) piglets before (7 d of age) and at the end of the experimental period (28 d of age). Values are means ± SEM ($n = 8$–10 per group). *$p < 0.05$ NW *vs* SW or AP *vs* HP.

regard to IGFBPs, relative levels in plasma were not affected by birth weight in 7-d-old piglets (data not shown) or by birth weight or diet in 28-d-old piglets (Table 4).

***Expression of genes in adipose tissues.*** In the perirenal adipose tissue of 7-d-old piglets, the expression of the insulin receptor gene was lower ($p < 0.05$) in SW than in NW piglets, whereas the expression of genes coding for GLUT4, A-FABP, FAS, CPT-I, and PPARγ did not differ between the two birth weight groups (Fig. 3). At 28 d of age, these genes were influenced neither by birth weight nor by diet except the FAS gene, which exhibited a lower ($p < 0.05$) expression in HP than in AP piglets. In 7-d-old piglets, the expressions of genes coding for IGF-I and leptin were lower ($p < 0.05$) in perirenal adipose tissue of SW than in that of NW piglets (Fig. 4). These differences were not observed in s.c. adipose tissue (data not shown). The relative levels of IGF-II, IGF-I receptor, and leptin mRNA were similar in SW and NW piglets. At 28 d of age, the relative levels of IGF-I, IGF-II, IGF-I receptor, and leptin mRNA in perirenal (Fig. 4) and s.c. (data not shown) adipose tissues were influenced neither by birth weight nor by diet.

## DISCUSSION

To our knowledge, the present study is the first to evaluate the short-term effects of protein intake and the possible inter-

action between early protein nutrition and birth weight on growth and adipose tissue development in neonatal animals reared in a well-controlled environment. The very few other experimental studies performed in rodents have examined the impact of maternal feeding during the suckling period and not the impact of milk protein content (10–12). We showed that birth weight influenced piglet growth, perirenal adipose tissue development, and some components of the endocrine system and that a high protein intake reduced perirenal adipose tissue mass without any clear effect on the regulatory axes or on the expression of genes related to lipid metabolism with the exception of the FAS gene.

In agreement with previous reports (14,24,25), SW piglets were lighter than their counterparts at 7 and 28 d of age. Nevertheless, the fractional growth rate was higher in SW than in NW piglets. With regard to perirenal adipose tissue, our findings are consistent with a compensatory development of this tissue in SW piglets. Indeed, the proportion of perirenal adipose tissue was similar in both birth weight groups in 28-d-old piglets, whereas it was lower in SW than in NW piglets at 7 d of age. This difference agrees with data showing that SW piglets have less fat and protein and more water than their littermates at birth (26). At the cellular level, the rise in adipose tissue mass may involve a high rate of proliferation or
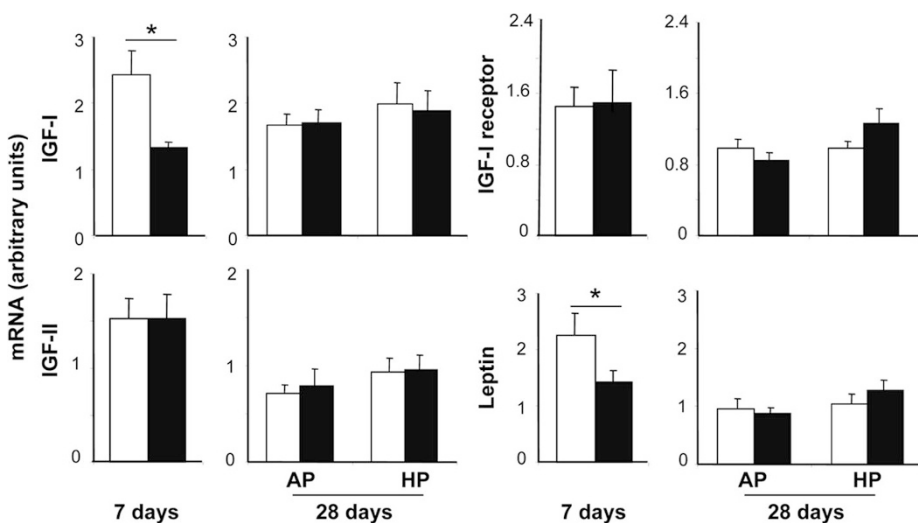


**Figure 4.** Relative levels of IGF-I, IGF-II, IGF-I receptor (IGF-I R), and leptin mRNA in perirenal adipose tissues in NW (□) and SW (■) piglets before (7 d of age) and at the end of the experimental period (28 d of age). Values are means ± SEM ($n = 8$–10 per group). *$p < 0.05$ NW *vs* SW.

differentiation of precursor cells and not an increase in cell volume, because adipocyte diameters were similar in 7- and 28-d-old piglets. This may increase the potential to develop adipose tissue mass and lead subsequently to higher adiposity as observed previously in IUGR pigs (14,25) and in human individuals born small for gestational age. Even though small for gestational age babies exhibit a reduced proportion of fat mass at birth (27), other literature observations indicate that these infants retain excess calories as fat, while protein retention in the form of muscle remain low (28).

The impact of birth weight on leptin and the IGF system was also evaluated. Leptin plays an important role in the regulation of energy partitioning and its plasma concentration is often considered as a good marker of adiposity in adults (29). However, ours findings are not consistent with such a role in the neonatal period. Indeed, differences in perirenal adipose tissue weight between NW and SW piglets were not associated with differences in plasma leptin concentration at 7 d of age. One cannot exclude the hypothesis that adipose tissues other than perirenal adipose tissue were not affected by birth weight in a similar manner. Nevertheless, previous reported data in older pigs (25) do not support this hypothesis. It is rather possible that a leptin surge occurs in the neonatal pig as reported in other species (30). Altogether, these observations suggest that plasma leptin cannot be considered as a signal of fat mass in piglets. With respect to the IGF system, whereas plasma concentration of IGF-I has been reported to be lower in SW than in NW piglets at birth (31,32), the present study indicates that there was no difference between the two birth weight groups at 7 or 28 d of age. This rapid normalization of plasma IGF-I levels that agrees with previous observations in suckling piglets (33–35) may reflect an adequate nutritional status. The expressions of IGF-I, leptin, and insulin receptor genes in perirenal adipose tissue differed between NW and SW piglets with a lower expression of these genes in 7-d-old SW than in NW piglets, whereas no differences were detected in s.c. adipose tissue. Taken together, these observations further document the differences between adipose depots and suggest some disturbances in the ontogeny of the regulatory system in perirenal adipose tissue of SW piglets.

The use of artificially reared piglets has allowed us to evaluate the impact of early protein intake. Because piglets fed the AP and HP diets consumed the same quantity of energy, the differences observed between piglets fed the two diets were due to variation in the protein/energy ratio. Our results indicate that AP and HP piglets from both birth weight groups displayed a similar growth rate between 7 and 28 d of age. Even though, it is often considered that a high protein intake may induce a catch-up growth in low birth weight individuals, we found no evidence of such an effect in the period examined. As suggested by Davis *et al.* (31), it is possible that SW piglets cannot exhibit a catch-up growth because they may have been undernourished throughout gestation. It is also conceivable that the investigated period is too short. Indeed, an increase in protein intake for a longer duration (from 1.8 to 15 kg body weight) has been shown to increase weight gain in pigs (24).

Another important finding of the present study is that the high protein intake induced a reduction of adiposity in both birth weight groups, at least at the perirenal level. In agreement with our findings, another study has shown that pigs receiving a high protein diet between 1.8 and 15 kg live weight had also a lower body fat content at 15 kg live weight compared with animals fed an adequate protein diet (24). However, after being fed the same high energy intake between 15 and 75 kg, pigs of these two groups exhibited a similar body fat content at 75 kg live weight. This indicates that a high protein intake in early life may reduce adiposity only temporarily. Therefore, it cannot be excluded that HP piglets may be fatter subsequently. According to the hypothesis raised in human (4,5), change in adiposity may involve modification in the IGF system. However, the lower perirenal adipose tissue mass of HP compared with AP piglets was not associated with any clear differences in the IGF system in the current study. The reasons for this are not clear. One can suppose that the difference in the protein content between the two diets was not sufficient to induce a clear increase in IGF-I gene expression. Alternatively, the sensitivity of the IGF system to protein intake during this early period might be low (36). A recent study in human further supports the existence of a critical period (37). According to these authors, there was no association between protein intake before 6 mo and later adiposity, whereas a high protein intake during the period of complementary feeding (6–12 mo) was associated with later adiposity.

In summary, the present work shows that birth weight affected the development of perirenal adipose tissue and supports a compensatory development of this tissue in SW piglets. It also indicates that a high protein intake reduced adiposity in both birth weight groups, at least at the perirenal level during the neonatal period. Further investigations are needed to assess whether piglets fed such a diet during the early period of life will stay subsequently with reduced body fat mass or not.

## REFERENCES

1. Dauncey MJ 1997 From early nutrition and later development to underlying mechanisms and optimal health. Br J Nutr 78:S113–S123
2. Hales CN, Barker DJ 1992 Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia 35:595–601
3. McMillen IC, Robinson JS 2005 Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiol Rev 85:571–633
4. Koletzko B 2006 Long-term consequences of early feeding on later obesity risk. In: Rigo J, Ziegler EE (eds) Protein and Energy Requirements in Infancy and Childhood. Nestlé Nutr Workshop Ser Pediatr Program, Vol 58, pp 1–18
5. Rolland-Cachera MF, Deheeger M, Akrout M, Bellisle F 1995 Influence of macronutrients on adiposity development: a follow up study of nutrition and growth from 10 months to 8 years of age. Int J Obes Relat Metab Disord 19:573–578
6. Scaglioni S, Agostoni C, Notaris RD, Radaelli G, Radice N, Valenti M, Giovannini M, Riva E 2000 Early macronutrient intake and overweight at five years of age. Int J Obes Relat Metab Disord 24:777–781

7. Dorosty AR, Emmett PM, Cowin S, Reilly JJ 2000 Factors associated with early adiposity rebound. ALSPAC Study Team. Pediatrics 105:1115–1118
8. Hoppe C, Molgaard C, Thomsen BL, Juul A, Michaelsen KF 2004 Protein intake at 9 mo of age is associated with body size but not with body fat in 10-y-old Danish children. Am J Clin Nutr 79:494–501
9. Spurlock ME, Gabler NK 2008 The development of porcine models of obesity and the metabolic syndrome. J Nutr 138:397–402
10. Daenzer M, Ortmann S, Klaus S, Metges CC 2002 Prenatal high protein exposure decreases energy expenditure and increases adiposity in young rats. J Nutr 132:142–144
11. Shepherd PR, Crowther NJ, Desai M, Hales CN, Ozanne SE 1997 Altered adipocyte properties in the offspring of protein malnourished rats. Br J Nutr 78:121–129
12. Zambrano E, Bautista CJ, Deás M, Martínez-Samayoa PM, González-Zamorano M, Ledesma H, Morales J, Larrea F, Nathanielsz PW 2006 A low maternal protein diet during pregnancy and lactation has sex- and window of exposure-specific effects on offspring growth and food intake, glucose metabolism and serum leptin in the rat. J Physiol 571:221–230
13. Morise A, Louveau I, Le Huërou-Luron I 2008 Growth and development of adipose tissue and gut and related endocrine status during early growth in the pig: impact on low birth weight. Animal 2:73–83
14. Poore KR, Fowden AL 2004 The effects of birth weight and postnatal growth patterns on fat depth and plasma leptin concentrations in juvenile and adult pigs. J Physiol 558:295–304
15. Thissen JP, Ketelslegers JM, Underwood LE 1994 Nutritional regulation of the insulin-like growth factors. Endocr Rev 15:80–101
16. Seerley RW, Poole DR 1974 Effect of prolonged fasting on carcass composition and blood fatty acids and glucose in neonatal swine. J Nutr 104:210–217
17. Butte NF, Hopkinson JM, Wong WW, Smith EO, Ellis KJ 2000 Body composition during the first 2 years of life: an updated reference. Pediatr Res 47:578–585
18. Noblet J, Etienne M 1989 Estimation of sow milk nutrient output. J Anim Sci 67:3352–3359
19. Dourmad JY, Noblet J, Etienne M 1998 Effect of protein and lysine supply on performance, nitrogen balance, and body composition changes of sows during lactation. J Anim Sci 76:542–550
20. Louveau I, Bonneau M 1996 Effect of a growth hormone infusion on plasma insulin-like growth factor-I in Meishan and large white pigs. Reprod Nutr Dev 36:301–310
21. Qian H, Barb CR, Compton MM, Hausman GJ, Azain MJ, Kraeling RR, Baile CA 1999 Leptin mRNA expression and serum leptin concentrations as influenced by age, weight, and estradiol in pigs. Domest Anim Endocrinol 16:135–143
22. Hossenlopp P, Seurin D, Segovia-Quinson B, Binoux M 1986 Identification of an insulin-like growth factor binding protein in human cerebrospinal fluid with a selective affinity for IGF-II. FEBS Lett 208:439–444
23. Pfaffl MW 2001 A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29:e45
24. Campbell RG, Dunkin AC 1983 The influence of protein nutrition in early life on growth and development of the pig. 1. Effects on growth performance and body composition. Br J Nutr 50:605–617
25. Gondret F, Lefaucheur L, Juin H, Louveau I, Lebret B 2006 Low birth weight is associated with enlarged muscle fiber area and impaired meat tenderness of the longissimus muscle in pigs. J Anim Sci 84:93–103
26. Rehfeldt C, Khun G 2006 Consequences of birth weight for postnatal growth performance and carcass quality in pigs as related to myogenesis. J Anim Sci 84:E113–E123
27. Petersen S, Gotfredsen A, Knudsen FU 1988 Lean body mass in small for gestational age and appropriate for gestational age infants. J Pediatr 113:886–889
28. Hediger ML, Overpeck MD, Kuczmarski RJ, McGlynn A, Maurer KR, Davis WW 1998 Muscularity and fatness of infants and young children born small- or large-for-gestational-age. Pediatrics 102:E60
29. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, Kern PA, Friedman JM 1995 Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat Med 1:1155–1161
30. Bouret SG, Simerly RB 2006 Developmental programming of hypothalamic feeding circuits. Clin Genet 70:295–301
31. Davis TA, Fiorotto ML, Burrin DG, Pond WG, Nguyen HV 1997 Intrauterine growth restriction does not alter response of protein synthesis to feeding in newborn pigs. Am J Physiol 272:E877–E884
32. Ritacco G, Radecki SV, Schoknecht PA 1997 Compensatory growth in runt pigs is not mediated by insulin-like growth factor-I. J Anim Sci 75:1237–1243
33. Dauncey MJ, Burton KA, Tivey DR 1994 Nutritional modulation of insulin-like growth factor-I expression in early postnatal piglets. Pediatr Res 36:77–84
34. Mostyn A, Litten JC, Perkins KS, Euden PJ, Corson AM, Symonds ME, Clarke L 2005 Influence of size at birth on the endocrine profiles and expression of uncoupling proteins in subcutaneous adipose tissue, lung, and muscle of neonatal pigs. Am J Physiol Regul Integr Comp Physiol 288:R1536–R1542
35. Schoknecht PA, Ebner S, Skottner A, Burrin DG, Davis TA, Ellis K, Pond WG 1997 Exogenous insulin-like growth factor-I increases weight gain in intrauterine growth-retarded neonatal pigs. Pediatr Res 42:201–207
36. Davis TA, Burrin DG, Fiorotto ML, Nguyen HV 1996 Protein synthesis in skeletal muscle and jejunum is more responsive to feeding in 7- than in 26-day-old pigs. Am J Physiol 270:E802–E809
37. Günther AL, Remer T, Kroke A, Buyken AE 2007 Protein intake during the period of complementary feeding and early childhood and the association with body mass index and percentage body fat at 7 y of age. Am J Clin Nutr 85:1626–1633