The effects of birth weight and postnatal growth patterns on fat depth and plasma leptin concentrations in juvenile and adult pigs

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Low birth weight is associated with altered adipose tissue deposition and regulation of leptin production. This study determined the effects of naturally occurring variations in birth weight in pigs on postnatal growth patterns, body fat depth and plasma leptin and other hormone concentrations. Low (< 1.47 kg) and high (> 1.53 kg) birth weight piglets were studied at 3 months (juvenile; $n = 47$) and 12 months of age (young adult; $n = 17$). At each age, arterial and venous catheters were inserted under general anaesthesia. Plasma leptin, cortisol, glucose, insulin and catecholamine concentrations were determined in basal blood samples. Body fat depth was measured by ultrasound at 12 months of age. Overall, adult fat depth was greater in low compared to high birth weight pigs and increased fat depth was associated with thinness at birth and poor early growth rates. These effects were strongest in females. Fat depth was related to current weight only in males. Compared to high birth weight pigs, plasma leptin concentrations were reduced in low birth weight females at 3 months and in low birth weight males at 12 months of age. This study demonstrates sex-specific effects of low birth weight on postnatal growth and body fatness and on plasma leptin concentrations in pigs.

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Obesity is a major health problem that is linked with other metabolic disorders, such as glucose intolerance and non-insulin-dependent diabetes mellitus (NIDDM). Worldwide, its incidence is increasing rapidly, not only in middle to old age, but also in young adults, children and even infants (World Health Organization, 1998). While obesity and its related disorders are commonly attributed to lifestyle and dietary factors in adult life, there is increasing evidence to suggest that their prevalence is determined, at least in part, by the environment during early life (Breier *et al.* 2001). Epidemiological studies have revealed that poor pre- and postnatal growth is linked with adult obesity: low birth weight (BW) and low weight at 1 year of age is associated with an increased waist to hip ratio, indicative of abdominal fat deposition, in 50- to 60-year-old men (Law *et al.* 1992). Furthermore, increased body mass index (BMI) and body fat has been observed in children as early as 5 years of age that were small and thin at birth but showed catch-up growth in the first 2 years of life (Ong *et al.* 2000). Low BW and small size at birth are also associated with an increased risk of adult-onset degenerative diseases such as cardiovascular disease, glucose intolerance and NIDDM (Barker *et al.* 1989; Hales *et al.* 1991; Phillips *et al.* 1994), which are exacerbated by adult obesity (Hales *et al.* 1991; Fall *et al.* 1995).

Leptin is synthesized and secreted primarily by adipocytes and correlates with BMI and adiposity in adults and newborn infants (Maffei *et al.* 1995; Considine *et al.* 1996). However, for any given level of obesity in adulthood, leptin concentrations increase as BW is reduced (Phillips *et al.* 1999), suggesting that low BW individuals have relatively more body fat as adults. Experimental studies in animals have confirmed that impaired fetal growth has long-term effects on postnatal body composition, hormonal and metabolic homeostasis. Manipulation of fetal development by restriction of maternal dietary intake has been shown to induce changes in postnatal body fat and adipocyte abnormalities in rats (Jones & Friedman, 1982; Anguita *et al.* 1993; Vickers *et al.* 2000) and in sheep (Greenwood *et al.* 1998) and in adipocyte leptin production in pigs (Ekert *et al.* 2000).

In species such as pigs, there is a naturally occurring 2- to 3-fold variation in BW amongst littermates from normally fed sows due to differences in placental size and

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functional capacity (Bauer *et al.* 1998). There is evidence in pigs that small and disproportionate size at birth, with associated effects on postnatal growth, is associated with altered glucose tolerance, insulin sensitivity, and cardiovascular and endocrine function (Poore *et al.* 2002; Poore & Fowden, 2002*b*, 2003, 2004). The aim of the current study was to characterize the effect of naturally occurring low BW in male and female pigs on body fat depth and plasma leptin concentrations at 3 (juvenile) and 12 (adult) months of age. In addition, hormones involved in energy balance were measured. Some of these data have been previously published in preliminary format (Poore & Fowden, 2002*a*).

Methods

All procedures were carried out in accordance with the regulations of the UK Home Office Animals (Scientific Procedures) Act, 1986.

Animals

Pure-bred Large White pigs were obtained from sows mated to a single boar and which were allowed to farrow normally at term (115 ± 2 days). Piglets from 15 litters (average litter size of 11 ± 1) from 9 sows were used in this study. Sows were fed a standard diet (15% protein; 12.6 MJ kg^{-1} digestible energy; ABN, Peterborough, UK) at least 4 weeks prior to conception (2 kg day^{-1}) and during gestation and lactation (2.5–3 kg day^{-1}) such that nutritional requirements were satisfied according to standard guidelines (Agricultural and Food Research Council, 1990). Water was provided *ad libitum*. Piglets were kept indoors and provided with straw bedding and infrared heat lamps from birth until weaning at 4–5 weeks of age. Weaner piglets were housed in groups in open barns and fed *ad libitum* on a standard pig diet (creep feed; 20% protein; H & C Beart Ltd, Kings Lynn, UK) until the first studies were performed at 3 months of age. During this first study period, pigs were housed individually indoors adjacent to their siblings and fed according to their size (Agricultural and Food Research Council, 1990) until at the completion of these studies, pigs were returned to group housing (*ad libitum* feeding of 20% protein pig creep). At 4–5 months of age, prior to puberty, pigs were again housed individually and fed on the adult 15% protein diet according to size for the remainder of the study.

At birth, all piglets in each litter were weighed and a set of morphometric measurements were made: head length (snout to between ears), crown–rump length (between ears to base of tail; CRL) and abdominal circumference (AC). The average BW of all piglets born to all litters was 1.50 ± 0.02 kg ($n = 170$) and the 95% confidence interval of the mean was 1.47–1.53 kg. Piglets whose BWs fell within the confidence interval of the mean were excluded from the study. Forty-seven piglets remained and were assigned to one of two groups: those with BW lower than the 95% confidence interval of the mean were defined as 'low BW' pigs (< 1.47 kg at birth) and those higher than the 95% confidence interval of the mean BW were defined as 'high BW' pigs (> 1.53 kg at birth). The range of birth weights in the low BW group was 0.80–1.40 kg ($n = 22$) and in the high BW group was 1.65–2.40 kg ($n = 25$). Approximately similar numbers of each sex were selected (low BW females, $n = 15$; low BW males, $n = 7$, high BW females, $n = 13$; high BW males, $n = 12$). Selected pigs were weighed and measured again at 1, 3 and 10–12 months of age. Fat depth at 10–12 months of age was measured by ultrasound in five locations: the maximum depth in a sweep between the shoulder blades (SH); mid-back, at the head of the last rib (MB); loin, 8–10 cm from the top of the tail (LN); and at two sites over the last rib, 4–5 cm and 8 cm from the spine (P1 and P2, respectively). The sum of fat depth in all these areas was used as an index of total fat depth. It was not possible to obtain all data from all animals: the number of observations for each experimental data set is indicated in the legend of each table.

At 9–10 weeks of age, before the morning feed, selected pigs were tranquilized (azaperone (Janssen Pharmaceuticals Ltd, Oxford, UK; 5 mg kg^{-1} I.M. for pigs > 20 kg) or diazepam (Phoenix Pharmaceuticals Ltd, Gloucester, UK; 2 mg kg^{-1} I.M. for pigs < 20 kg), each in combination with ketamine (Fort Dodge Animal Health Ltd, Southampton, UK; 10 mg kg^{-1} I.M.)) and anaesthetized with halothane (3–6% in O_2). Catheters were inserted into the dorsal aorta and vena cava via the femoral vessels and were exteriorized via a small incision on the animal's back. Pigs were kept in protective coats made of elastic tubing (Tubigrip, Seton Healthcare Group, Oldham, UK) to protect the catheters. Post-operative recovery was monitored, without analgesia, and pigs were seen to restore normal feeding patterns and behaviour immediately after recovery from surgery. Antibiotic treatment was administered I.M. on the day of surgery (Depocillin (procaine benzylpenicillin, 15 mg kg^{-1}; Depocillin Mycofarm Ltd, Cambridge, UK) and Duphatrim (trimethoprim, 2.5 mg kg^{-1} with sulfadiazine, 12.5 mg kg^{-1}; Fort Dodge Animal Health Ltd)) and then Duphatrim was administered alone (I.V.) for 3 days following surgery and every 2–3 days thereafter (Duphatrim alone, I.V.). During the experimental period,

feeding patterns, behaviour and weight gain were monitored to ensure continued animal wellbeing.

Seventeen pigs had their catheters and coats removed at the completion of the experiments at 3 months of age and were then studied again at 10–12 months of age (low BW females, $n = 5$; low BW males, $n = 4$; high BW females, $n = 4$; high BW males, $n = 4$). Femoral artery and vein catheters were inserted in the previously unoperated leg under general anaesthesia (sodium pentobarbitone (Rhône Mérieux Ltd, Harlow, UK); 20 mg kg^{-1} I.V.) following tranquilization with azaperone (5 mg kg^{-1} I.M.).

Experimental protocol

All animals were allowed at least 2 days for recovery from surgery. For measurement of basal hormone concentrations, 1–2 arterial blood samples were collected in the morning from each animal, at least 2 days apart, during baseline, fasted conditions, and the results were averaged. Blood samples were collected (2 ml into chilled EDTA tubes) for analysis of plasma leptin, glucose, insulin and cortisol concentrations. A further blood sample (1 ml) was collected into chilled heparinized tubes containing EGTA (5 μmol (ml blood)$^{-1}$ and glutathione (40 μmol (ml blood)$^{-1}$) for analysis of catecholamine concentrations. All blood samples were centrifuged immediately for 5 min at 4°C and the plasma was stored at −80°C (samples for catecholamine analysis) or at −20°C (all others).

Biochemical analyses

Plasma leptin concentrations were measured in a single assay using a commercially available radioimmunoassay kit (Linco Research Inc., St Charles, MO, USA). The intra- and interassay coefficients of variation of human leptin within the range of values observed in the pigs were 3.5% and 7.8%, respectively.

Total plasma cortisol concentrations were measured by radioimmunoassay as previously described (Silver *et al.* 1983). Tritiated cortisol (TRK 407) was purchased from Amersham Biosciences UK Ltd (Little Chalfont, UK) and the cortisol antibody (Pink 72) was a generous gift from the Tenovus Institute for Cancer Research (University of Wales, College of Medicine, Cardiff, UK). The intra-assay coefficients of variation for the cortisol assay were 5.0% at the level of 27.4 ± 0.4 ng ml^{-1} and 13.6% at the level of 5.6 ± 0.2 ng ml^{-1}. The interassay coefficient of variation for the cortisol assay was 10% at the level of 23.6 ± 0.3 ng ml^{-1} and the minimum detectable dose was 0.4 ng ml^{-1}.

Plasma glucose concentrations were measured using an automated analyser (Yellow Springs 2300 Stat Plus Glucose/Lactate analyser; YSI, Farnborough, UK) and plasma insulin concentrations by a commercially available radioimmunoassay kit (INSIK-5; Diasorin Ltd, Wokingham, UK). The interassay and intra-assay coefficients of variation for the insulin assay were 10% and 8%, respectively.

Plasma catecholamine (noradrenaline and adrenaline) concentrations were determined by HPLC using electrochemical detection (Silver *et al.* 1982). Samples were prepared by absorption of 250 μl of plasma onto acid-washed alumina and 20 μl aliquots of the 100 μl perchloric acid elutes were injected onto the column. Dihydroxybenzylamine was added as the internal standard to each plasma sample before absorption. Recovery ranged from 63 to 97% and all catecholamine values were corrected for their respective recovery. The interassay coefficients of variation for noradrenaline and adrenaline were 6.2% and 7.3%, respectively, and the minimum detectable dose was 10 pg ml^{-1}.

Statistics

All results are expressed as mean ± standard error of the mean (S.E.M.). The relationships between two factors were tested using linear regression analysis. Student's unpaired *t* tests were used to identify differences between two factors. For all statistical tests, significance was accepted when $P < 0.05$.

Results

Postnatal growth

Morphometric parameters measured at birth, 3 and 12 months of age in low and high BW pigs are presented in Tables 1 and 2. At birth, there were significant ($P < 0.01$) differences in BMI (body weight × CRL^{-2}), head length:BW ratio, CRL and AC between low and high BW pigs overall, and in males and females separately (Tables 1 and 2). Overall, these differences persisted until 3 but not 12 months of age (Table 1). However, when considered separately, current weight (CW) in males was not significantly different between low and high BW pigs at 3 or 12 months of age (Table 2), and there were no significant relationships between CW at either postnatal age and BW. In females, CW at both postnatal ages studied was significantly ($P < 0.001$) correlated with BW (3 months: $r = +0.53$, $n = 28$; 12 months: $r = +0.73$, $n = 9$), and the mean CW of low BW pigs was significantly different from high BW pigs at 3 months ($P < 0.005$) and

Table 1. Body weights, morphometry (at birth and at 3 and 12 months of age) and postnatal growth rates in low and high BW pigs

	Low BW	High BW
At birth:		
BW (kg)	1.13 ± 0.04^{d}	1.90 ± 0.04
CRL (cm)	25.2 ± 0.4^{d}	29.7 ± 0.3
AC (cm)	21.8 ± 0.5^{d}	26.8 ± 0.4
BMI (kg m^{-2})	17.7 ± 0.31^{d}	21.4 ± 0.51
Head length:BW (cm kg^{-1})	8.78 ± 0.36^{d}	5.73 ± 0.14
At 3 months:		
CW (kg)	23.5 ± 1.9^{d}	37.2 ± 2.5
CRL (cm)	75.2 ± 2.6^{a}	84.9 ± 2.8
AC (cm)	63.2 ± 2.7^{c}	80.2 ± 3.9
BMI (kg m^{-2})	40.9 ± 2.4^{a}	51.6 ± 2.7
Head length:CW (cm kg^{-1})	0.99 ± 0.09^{a}	0.63 ± 0.08
At 12 months:		
CW (kg)	152.8 ± 8.1	169.4 ± 6.9
CRL (cm)	145.9 ± 4.9	146.0 ± 6.2
AC (cm)	141.7 ± 5.4	135.2 ± 4.8
BMI (kg m^{-2})	81.5 ± 3.8	82.0 ± 5.9
Head length:CW (cm kg^{-1})	0.223 ± 0.016	0.260 ± 0.032
Total fat depth (mm)	116 ± 11	86 ± 7
SH fat depth (mm)	38 ± 3	32 ± 1
MB fat depth (mm)	21 ± 2	15 ± 2
LN fat depth (mm)	19 ± 3	14 ± 2
P1 fat depth (mm)	18 ± 2^{a}	12 ± 1
P2 fat depth (mm)	19 ± 2^{a}	13 ± 2
GR 0–1 months (kg day^{-1})	0.237 ± 0.012^{d}	0.350 ± 0.014
GR 0–3 months (kg day^{-1})	0.284 ± 0.020^{d}	0.452 ± 0.030
GR 0–12 months (kg day^{-1})	0.476 ± 0.034	0.541 ± 0.025
FGR 0–1 months (kg day^{-1} kg^{-1})	0.175 ± 0.008^{b}	0.144 ± 0.009
FGR 1–3 months (kg day^{-1} kg^{-1})	0.046 ± 0.003	0.056 ± 0.003
FGR 3–12 months (kg day^{-1} kg^{-1})	0.026 ± 0.002^{c}	0.016 ± 0.002

Values are means ± S.E.M. AC, abdominal circumference; BMI, body mass index; BW, birth weight; CRL, crown–rump length; CW, current weight; FGR, fractional growth rate; GR, growth rate; LN, loin; MB, midback; P1 and P2, two sites over the last rib, 4–5 cm and 8 cm from the spine, respectively; SH, shoulder. Low BW *versus* high BW (unpaired *t* test): $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.005$, $^{d}P < 0.001$. At 3 months: low BW, $n = 22$; high BW, $n = 25$. At 12 months: low BW, $n = 9$; high BW, $n = 8$; except for fat depths: low BW, $n = 5$; high BW, $n = 5$.

12 months of age ($P < 0.05$; Table 2). The head length:body weight ratio at 3 months, but not 12 months of age, was also significantly ($P < 0.05$) positively related to that measured at birth overall ($r = +0.45$, $n = 47$) and in females ($r = +0.54$, $n = 28$), but not males ($r = +0.60$, $n = 19$).

Absolute postnatal growth rates (GR; kg gained (day)$^{-1}$) from birth to 1 month and from birth to 3 months of age were significantly ($P < 0.001$) less in low than in high BW pigs overall, and in males and females separately (Tables 1 and 2). During suckling (0–1 month), the fractional GR (kg gained (day)$^{-1}$ (starting kg)$^{-1}$) in low BW pigs overall and in males was significantly ($P < 0.05$) greater than in high BW pigs (Tables 1 and 2). During the postweaning period of the study (1–3 months) the relative increase in body weight in low BW pigs was not different to that seen in high BW pigs (Tables 1 and 2). Between 3 and 12 months of age, the fractional GR in low BW pigs was significantly ($P < 0.05$) greater than in high BW pigs, an effect that was only observed in females when analysed separately (Tables 1 and 2).

Overall, postnatal growth rates in the first month and the first 3 months of life were significantly ($P < 0.005$) determined by BW ($r = +0.71$ and $+0.50$, respectively; $n = 47$). However, low BW significantly ($P < 0.01$) predicted high fractional GR in the first month and between 3 and 12 months of age ($r = +0.71$, $n = 47$ and $r = +0.50$, $n = 17$, respectively). Within each sex, BW in males was significantly ($P < 0.01$) related to GR from birth to 1 month ($r = +0.59$, $n = 19$) and fractional GR in the same period ($r = -0.60$, $n = 19$). In females, BW significantly ($P < 0.05$) determined absolute GRs at all ages, from birth to 1 month ($r = +0.73$, $n = 28$), 3 months ($r = +0.54$, $n = 28$) and 12 months ($r = +0.68$, $n = 9$) and negatively predicted the fractional GR from 3 to 12 months ($r = -0.81$, $n = 9$). Fractional GR (0–1 month) in females tended ($P = 0.07$) to be negatively correlated to BW.

Fat depth in adult pigs

At 12 months of age, fat depths in the P1 and P2 regions were significantly ($P < 0.05$) greater in low BW compared to high BW pigs (Table 1). There were strong tendencies ($P \leq 0.06$) for similar increases in total, SH and MB fat depths in low BW pigs (Table 1). Small group numbers prevented statistical comparisons between low and high BW within each sex. Fat depth (total, SH, MB, P1 and P2) in adult pigs was significantly ($P < 0.05$) determined by poor GR in the first month of life (Table 3). Low body weight at 1 and 3 months of age also significantly ($P < 0.05$) predicted high fat depth at 12 months of age in all regions (total, SH, MB, LN, P1 and P2; Table 3). SH fat depth was significantly ($P < 0.05$) higher in those pigs that were thin (low BMI) at birth (Table 3). High fat depth (total, SH, MB, LN, P1 and P2) was also significantly ($P < 0.05$) predicted by high fractional GR from 3 to 12 months of age (Table 3).

Regression analysis within each sex revealed that in males, fat depth (total, SH, MB and LN) was significantly ($P < 0.05$) determined by CW (Table 3). High fractional

Table 2. Body weights, morphometry (at birth and at 3 and 12 months of age) and postnatal growth rates in low and high BW male and female pigs

	Males		Females	
	Low BW	High BW	Low BW	High BW
At birth:				
BW (kg)	1.18 ± 0.05^{d}	1.93 ± 0.05	1.10 ± 0.05^{d}	1.86 ± 0.05
CRL (cm)	25.4 ± 0.7^{d}	29.8 ± 0.4	25.2 ± 0.5^{d}	29.6 ± 0.5
AC (cm)	22.4 ± 0.8^{c}	26.9 ± 0.8	21.6 ± 0.6^{d}	26.7 ± 0.5
BMI (kg m^{-2})	18.26 ± 0.67^{b}	21.83 ± 0.79	17.30 ± 0.31^{d}	21.45 ± 0.68
Head length:BW (cm kg^{-1})	8.21 ± 0.63^{d}	5.67 ± 0.23	8.97 ± 0.44^{d}	5.65 ± 0.17
At 3 months:				
CW (kg)	25.6 ± 4.6	36.3 ± 3.0	22.6 ± 1.8^{c}	38.2 ± 4.1
CRL (cm)	72.0 ± 11.0	83.6 ± 2.6	75.9 ± 2.1	86.3 ± 5.3
AC (cm)	62.0 ± 12.0	77.9 ± 3.7	64.9 ± 2.5^{a}	82.6 ± 7.1
BMI (kg m^{-2})	39.20 ± 12.33	49.39 ± 3.79	42.69 ± 1.86	51.60 ± 4.25
Head length:CW (cm kg^{-1})	1.17 ± 0.53	0.68 ± 0.07	0.91 ± 0.05	0.63 ± 0.15
At 12 months:				
CW (kg)	168.8 ± 13.9	176.3 ± 9.9	140.0 ± 5.5^{a}	162.5 ± 6.3
CRL (cm)	150.3 ± 11.3	140.0 ± 2.0	142.4 ± 2.1	150.0 ± 10.4
AC (cm)	139.5 ± 12.5	135.0 ± 5.0	143.4 ± 3.0	135.3 ± 8.2
BMI (kg m^{-2})	84.13 ± 6.85	94.52 ± 5.25	79.39 ± 4.51	73.66 ± 6.57
Head length:CW (cm kg^{-1})	0.23 ± 0.03	0.21 ± 0.01	0.218 ± 0.023	0.295 ± 0.050
GR 0–1 months (kg day^{-1})	0.272 ± 0.024^{d}	0.354 ± 0.017	0.224 ± 0.011^{d}	0.338 ± 0.023
GR 0–3 months (kg day^{-1})	0.308 ± 0.046^{d}	0.455 ± 0.038	0.278 ± 0.020^{c}	0.445 ± 0.046
GR 0–12 months (kg day^{-1})	0.535 ± 0.046	0.550 ± 0.039	0.429 ± 0.042	0.532 ± 0.038
FGR 0–1 months (kg day^{-1} kg^{-1})	0.195 ± 0.017^{a}	0.146 ± 0.010	0.169 ± 0.009	0.141 ± 0.016
FGR 1–3 months (kg day^{-1} kg^{-1})	0.042 ± 0.008	0.051 ± 0.006	0.047 ± 0.003	0.056 ± 0.004
FGR 3–12 months (kg day^{-1} kg^{-1})	0.022 ± 0.002	0.017 ± 0.002	0.029 ± 0.003^{a}	0.016 ± 0.002

Values are means ± S.E.M. Abbreviations as in Table 1. Low BW *versus* high BW within each sex (unpaired *t* test): $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.005$, $^{d}P < 0.001$. At 3 months: low BW males, $n = 7$; high BW males, $n = 12$; low BW females, $n = 15$; high BW females, $n = 13$. At 12 months: low BW males, $n = 4$; high BW males, $n = 4$; low BW females, $n = 5$; high BW females, $n = 4$.

GR from 3 to 12 months of age was also significantly ($P < 0.05$) related to total, SH, MB and P2 fat depth in males (Table 3). In females, pigs that were light (BW), thin (BMI), short (CRL) and of disproportionate body shape (head:BW) at birth had significantly ($P < 0.05$) increased P1 fat depth at 12 months of age (Table 3). Poor GR in the first month and low body weight at 1 and 3 months of age also significantly ($P < 0.05$) predicted high P1 fat depth in females (Table 3). MB, P1 and P2 fat depth was significantly ($P < 0.05$) associated with high fractional GR from 3 to 12 months of age in females (Table 3).

Plasma leptin concentrations

Overall, plasma leptin concentrations were not different between low and high BW pigs at 3 or 12 months of age (Table 4) and were not related to body weights or morphometric measurements at birth or either postnatal age. However, when analysed within each sex, plasma leptin concentrations were significantly ($P < 0.05$) reduced in low BW compared to high BW pigs in 3-month-old females and in 12 month-old males (Table 4). Reduced plasma leptin concentrations in 3-month-old females were significantly ($P < 0.05$) associated with low BW, thinness (BMI) at birth and high fractional GR between birth and 1 month of age ($r = +0.43, +0.55$ and -0.42, respectively; $n = 23$). At 12 months of age, plasma leptin concentrations were lowest ($P < 0.05$) in male, but not female, pigs that were light (BW), thin (BMI) and short (CRL) at birth ($r = +0.87$, $+0.74$ and $+0.83$, respectively; $n = 8$). In females, plasma leptin concentrations at 12 months of age were significantly ($P < 0.05$) positively associated with current abdominal circumference ($r = +0.72$, $n = 5$) and were weakly related to low leptin concentrations at 3 months of age ($r = -0.60$, $P = 0.09$, $n = 9$).

Overall, plasma leptin concentrations in adult pigs were not associated with CW or fat depth in any region. In females, plasma leptin concentrations at 12 months of age were significantly ($P < 0.05$) negatively associated with LN fat depth at this age ($r = -0.89$).

Table 3. Correlation coefficients (*r*) between fat depth at 12 months of age and body weights, morphometry (at birth and at 1, 3 and 12 months of age) and postnatal growth rates

A.	Total fat depth			SH fat depth			MB fat depth		
	All	Males	Females	All	Males	Females	All	Males	Females
At birth:									
BW	−0.55	−0.45	−0.65	−0.62	−0.50	−0.66	−0.54	−0.54	−0.62
CRL	−0.52	−0.37	−0.77	−0.55	−0.36	−0.67	−0.52	−0.45	−0.89
AC	−0.52	+0.04	−0.57	−0.33	+0.13	−0.54	−0.28	−0.08	−0.57
BMI	−0.60	−0.45	−0.84	−0.73[a]	−0.55	−0.84	−0.57	−0.54	−0.83
Head length:BW	+0.35	+0.17	+0.73	+0.41	−0.13	+0.72	+0.36	−0.07	+0.73
At 1 month:									
CW	−0.67[a]	−0.24	−0.88	−0.71[a]	−0.34	−0.70	−0.65[a]	−0.25	−0.89
At 3 months:									
CW	−0.81	+0.82	−0.82	−0.73[a]	−0.85	−0.51	−0.82[d]	−0.76	−0.92[a]
At 12 months:									
CW	+0.08	+0.88[a]	−0.39	+0.11	+0.99[d]	−0.36	+0.04	+0.93[a]	−0.41
CRL	+0.13	+0.52	−0.32	+0.04	+0.43	−0.30	+0.17	+0.63	−0.41
AC	+0.49	+0.69	+0.51	+0.49	+0.71	+0.49	+0.51	+0.75	−0.59
BMI	−0.47	−0.47	−0.48	−0.46	−0.36	−0.54	−0.47	−0.58	−0.32
Head length:CW	−0.37	−0.49	−0.57	−0.51	−0.59	−0.57	−0.26	−0.37	−0.62
GR 0−1 months	−0.69[a]	−0.49	−0.79	−0.79[b]	−0.66	−0.82	−0.63[a]	−0.45	−0.75
GR 0−3 months	−0.61	−0.14	−0.61	−0.57	−0.15	−0.67	−0.62	−0.12	−0.52
GR 0−12 months	−0.01	+0.72	−0.51	−0.10	+0.65	−0.54	+0.04	+0.80	−0.47
FGR 0−1 months	−0.26	+0.22	−0.65	−0.30	+0.12	−0.67	−0.17	+0.37	−0.56
FGR 1−3 months	+0.31	+0.49	+0.88	+0.54	+0.69	−0.77	+0.22	+0.49	+0.95[a]
FGR 3−12 months	+0.84[c]	−0.94[a]	+0.92	+0.81[c]	+0.89[a]	+0.86	+0.83[c]	+0.97[b]	+0.97[b]
B.	LN fat depth			P1 fat depth			P2 fat depth		
	All	Males	Females	All	Males	Females	All	Males	Females
At birth:									
BW	−0.37	−0.30	−0.32	−0.57	−0.40	−0.91[a]	−0.57	−0.47	−0.75
CRL	−0.36	−0.24	−0.68	−0.57	−0.37	−0.95[a]	−0.30	−0.40	−0.77
AC	−0.13	+0.10	−0.23	−0.33	+0.05	−0.87	+0.33	+0.71	−0.69
BMI	−0.47	−0.32	−0.61	−0.56	−0.37	−0.96[b]	−0.57	−0.45	−0.87
Head length:BW	+0.22	−0.31	+0.44	+0.32	−0.20	+0.95[a]	−0.56	−0.14	+0.80
At 1 month:									
CW	−0.59	−0.01	−0.43	−0.68[a]	−0.06	−0.99[c]	−0.66[a]	−0.08	−0.85[a]
At 3 months:									
CW	−0.78[b]	−0.57	−0.46	−0.78[b]	−0.73	−0.83[a]	−0.79[c]	−0.73	−0.65
At 12 months:									
CW	+0.05	+0.88[a]	−0.08	+0.10	−0.22	−0.76	+0.08	−0.17	−0.53
CRL	+0.08	+0.44	−0.20	+0.14	+0.51	−0.51	+0.20	+0.54	−0.22
AC	+0.36	+0.55	+0.41	+0.53	+0.65	+0.67	+0.50	+0.70	+0.44
BMI	−0.47	−0.39	−0.59	−0.39	−0.46	−0.17	−0.49	−0.49	−0.52
Head length:CW	+0.35	−0.51	−0.45	−0.36	−0.48	−0.67	−0.28	−0.47	−0.48
GR 0−1 months	−0.56	−0.31	−0.52	−0.68[a]	−0.47	−0.95[a]	−0.69[a]	−0.52	−0.86
GR 0−3 months	−0.54	−0.08	−0.28	−0.61	−0.22	−0.81	−0.62	−0.20	−0.71
GR 0−12 months	+0.04	+0.73	−0.22	−0.07	+0.66	−0.73	−0.03	+0.67	−0.69
FGR 0−1 months	0.39	+0.15	−0.85	−0.14	+0.17	−0.22	−0.20	+0.22	−0.52
FGR 1−3 months	+0.22	+0.32	+0.90	+0.24	+0.44	+0.75	+0.26	+0.51	+0.80
FGR 3−12 months	+0.82[c]	+0.88	+0.82	+0.76[a]	+0.92	−0.92[a]	+0.78[c]	+0.94[a]	+0.88[a]

Abbreviations as in Table 1. [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.005$. Males, $n = 5$; females, $n = 5$.

Table 4. Plasma hormone concentrations at 3 and 12 months of age in low and high BW pigs

	All		Males		Females	
	Low BW	High BW	Low BW	High BW	Low BW	High BW
At 3 months:						
Plasma leptin (ng ml^{-1})	1.4 ± 0.1	1.6 ± 0.1	1.4 ± 0.2	1.5 ± 0.1	1.4 ± 0.1[b]	1.8 ± 0.1
Plasma glucose (nmol l^{-1})	5.4 ± 0.2	5.4 ± 0.2	5.5 ± 0.3	5.5 ± 0.3	5.3 ± 0.3	5.3 ± 0.3
Plasma insulin (μU ml^{-1})	15.7 ± 3.0	15.8 ± 2.0	11.3 ± 3.2	15.4 ± 2.3	18.0 ± 4.3	16.3 ± 3.7
Plasma cortisol (ng ml^{-1})	37.3 ± 5.1	30.8 ± 2.6	25.8 ± 3.5	28.4 ± 2.4	44.0 ± 7.2	34.6 ± 5.4
Plasma noradrenaline (pg ml^{-1})	297 ± 50[a]	184 ± 19	270 ± 76	187 ± 20	314 ± 69	179 ± 42
Plasma adrenaline (pg ml^{-1})	148 ± 20	144 ± 31	132 ± 33	169 ± 48	158 ± 27	102 ± 19
At 12 months:						
Plasma leptin (ng ml^{-1})	1.7 ± 0.1	1.8 ± 0.1	1.5 ± 0.1[c]	1.9 ± 0.1	1.8 ± 0.2	1.6 ± 0.2
Plasma glucose (nmol l^{-1})	4.2 ± 0.1[a]	4.8 ± 0.2	4.1 ± 0.1[a]	5.0 ± 0.2	4.3 ± 0.2	4.6 ± 0.2
Plasma insulin (μU ml^{-1})	10.5 ± 1.5[a]	15.6 ± 1.4	8.3 ± 1.4[a]	15.2 ± 1.6	12.3 ± 2.2	16.0 ± 2.6
Plasma cortisol (ng ml^{-1})	18.9 ± 3.0	29.6 ± 4.4	20.3 ± 5.4	36.4 ± 7.5	17.7 ± 3.7	22.8 ± 1.7
Plasma noradrenaline (pg ml^{-1})	79 ± 19	122 ± 25	96 ± 20	132 ± 48	65 ± 31	113 ± 23
Plasma adrenaline (pg ml^{-1})	111 ± 28	154 ± 32	175 ± 41	112 ± 23	59 ± 18[a]	196 ± 57

Values are means ± S.E.M. BW, birth weight. Low BW *versus* high BW within each sex (unpaired *t* test): [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$. Plasma leptin, glucose, insulin and cortisol at 3 months: low BW males, $n = 7$; high BW males, $n = 12$; low BW females, $n = 13$; high BW females, $n = 9$. Plasma noradrenaline and adrenaline at 3 months: low BW males, $n = 7$; high BW males, $n = 10$; low BW females, $n = 11$; high BW females, $n = 3$. At 12 months: low BW males, $n = 4$; high BW males, $n = 4$; low BW females, $n = 5$; high BW females, $n = 4$.

Fasting plasma glucose and insulin concentrations

At 3 months of age, there were no significant effects of BW on fasting plasma glucose concentrations or insulin concentrations overall or in male or female pigs (Table 4). At 12 months of age, plasma glucose and insulin concentrations were significantly reduced in low BW compared to high BW pigs (Table 4). Analysis within each sex revealed that this effect was only observed in males (Table 4). In 12-month-old pigs, fasting plasma insulin concentrations were significantly ($P < 0.05$) positively related to plasma leptin concentrations overall, and in male and female pigs separately ($r = +0.74$, $n = 17$, $r = +0.95$, $n = 8$, and $r = +0.68$, $n = 9$, respectively). Fasting glucose levels in adult male but not female pigs were significantly ($P < 0.05$) associated with plasma leptin concentrations ($r = +0.80$, $n = 8$). There were significant negative associations between plasma insulin concentrations at 12 months of age and SH fat depth overall ($r = -0.64$, $n = 11$, $P < 0.05$) and in females ($r = -0.89$, $n = 5$, $P < 0.05$) but this relationship just failed to reach statistical significance in males ($r = -0.87$, $n = 5$, $P = 0.055$).

Basal plasma cortisol and catecholamine concentrations

There were no significant effects of BW on basal plasma cortisol concentrations in either male or female pigs when combined or when considered separately at 3 or at 12 months of age (Table 4). At 3 months of age, plasma noradrenaline concentrations were significantly ($P < 0.05$) increased in low BW compared to high BW pigs overall, but adrenaline concentrations were unaffected by BW (Table 4). By 12 months of age, BW had no effect on plasma noradrenaline or adrenaline concentrations overall (Table 4). Within each sex, there was no effect of BW on 3 month old plasma adrenaline concentrations or noradrenaline concentrations (Table 4). At 12 months of age, plasma adrenaline concentrations were significantly ($P < 0.05$) reduced in low BW females but not males (Table 4), but there were no effects of BW on plasma noradrenaline concentrations in males or females (Table 4).

Overall, plasma cortisol concentrations at 12 months of age were significantly ($P < 0.05$) negatively related to total, MB, LN and P2 fat depths ($r = -0.66$, -0.73, -0.73 and -0.64, respectively). Plasma noradrenaline concentrations at 12 months were also negatively associated with MB, LN, P1 and P2 fat depths ($r = -0.71$, -0.71, -0.75 and -0.72, respectively; $n = 11$). Within each sex, plasma cortisol concentrations at 12 months were related to LN fat depth in females only ($r = -0.89$, $n = 6$, $P < 0.05$). Elevated basal adrenaline concentrations at 3 months of age were significantly ($P < 0.05$) associated with increased fat depth (total, SH and LN) in adult females ($r = 0.98$, 0.98 and 0.98, respectively).

Discussion

This study has characterized the effects of BW in pigs on growth rates and body proportions and composition

during the first postnatal year. Low BW, small stature and disproportionate body shape at birth had a lasting effect on postnatal weight gain and body proportions in pigs. Overall, low BW pigs underwent a period of catch-up growth in the first month of life, as indicated by a high fractional GR, although this was not maintained to the same extent after weaning and these pigs remained smaller, thinner and shorter at 3 months of age when compared to high BW pigs. A second period of catch-up growth between juvenile and young adult life allowed full recovery of body weight and size; however, by 12 months of age, body fat depth was greater in low BW pigs than in the high BW group. Indeed, increased fat depth at 12 months of age was associated with thinness at birth and poor growth rates in very early life. Persistently low body weight at the time of weaning (1 month) and at 3 months of age, followed by catch-up growth between 3 and 12 months of age were also determinants of adult fat depth. This study therefore supports those in humans which have demonstrated that low BW, poor infant growth (Law *et al.* 1992) and infant catch-up growth (Ong *et al.* 2000) are associated with increased adult adiposity.

However, the effects of birth size on postnatal growth patterns and later body size differed in males and females. When analysed within each sex, catch-up growth during suckling was evident most strongly in male low BW pigs and by as early as 3 months of age there were no longer any differences between low and high BW male pigs in body weight or proportions. Adult body fat depth in males was directly related to current body weight rather than body proportions measured at birth or to early growth patterns. Despite this, leptin concentrations at 12 months of age in low BW males were reduced, and, across the entire BW range, adult leptin concentrations were lower in small, thin and short newborn male piglets. The lack of a relationship between leptin concentrations and current weight or BMI suggests that low BW male pigs may have a deficiency in adipocyte leptin production as adults, although they were not underweight. Low leptin levels, for a given fat mass, may predispose to later obesity since these animals may exist in a state of perceived energy deficit, which would be consistent with their low fasting glucose and insulin concentrations. In human population studies, low leptin levels also predispose pre-obese Pima Indians to weight gain (Ravussin *et al.* 1997).

The effect of low BW persisted for much longer into adult life in female than in male pigs. In fact, low BW females remained smaller and thinner than high BW pigs at both 3 and 12 months of age. In further contrast to males, increased fat depth in adult female pigs was directly related to low BW, thinness, short stature and disproportionate body shape at birth, as well as poor early growth and persistently reduced body weights in juvenile life. Like the males, the effect of BW on plasma leptin concentrations was dependent on age in females, but in a different manner. In 3-month-old females, plasma leptin was directly related to weight and thinness at birth and was reduced in the low BW, compared to high BW, group, presumably reflecting their persistent thinness and reduced adiposity at this age, although body fatness was not directly measured at 3 months of age. By 12 months, however, plasma leptin concentrations were no longer reduced in low BW female pigs but were related to current size, as measured by abdominal circumference. These results suggest that low BW in female pigs, with its associated disproportionate body shape and thinness, is linked with increased body fat depth in adulthood. The increased fractional growth rate between 3 and 12 months of age in these animals, whilst not achieving full catch-up in body weight, is partly due to the deposition of body fat. However, the effect of low BW and postnatal growth on plasma leptin concentrations in females remains unclear. Small numbers prevented direct comparisons of body fat depth in low and high BW pigs within each sex. Taken together, these results indicate that low BW in female, but not male pigs, is associated with increased body fat, particularly in relation to their reduced body weight.

There are a number of possible mechanisms by which poor early growth in pigs may lead to altered fat deposition and leptin production in later life. Appetite may have been programmed during early development, resulting in hyperphagia, as occurs in other species after impaired intrauterine growth (Greenwood *et al.* 1998; Vickers *et al.* 2000). Hyperphagia could not occur in the low BW group in the current study as the adult pigs were fed to standard guidelines with a fixed ration each day. However, this ration may have exceeded the requirements of the low BW group, thus promoting increased fat accumulation. Alternatively, poor early growth may have programmed a 'thrifty' phenotype with an increased efficiency of fat storage. Certainly, increased fat pad mass in the absence of any increase in food intake has also been observed in young adult female rats following maternal under-nutrition during pregnancy (Anguita *et al.* 1993). The current finding of increased adult fatness with reduced body weight in low BW female pigs compared to high BW animals indicates that there may have been a redistribution of body mass between lean and adipose tissue after impaired early growth in female pigs.

Another possible cause of increased fat accumulation after poor early growth is changes in the endocrine

environment. In adults, adiposity and leptin secretion are regulated by hormones known to be sensitive to intrauterine programming, such as insulin, cortisol and the catecholamines (Poore *et al.* 2002; Poore & Fowden, 2002*b*, 2003). Indeed, the positive correlation observed between the plasma concentrations of insulin and leptin and the inverse correlations between adult fat depth and the concentrations of cortisol and noradrenaline indicate that endocrine status may play an important role in linking early growth to fat deposition in adult pigs. The latter correlations also suggest that fat accumulation was greatest in the animals that were least stressed as adults. However, increased stress responsiveness in earlier life may play a part in the predisposition of low BW pigs to later fat accumulation, since these pigs have elevated cortisol responses to insulin-induced hypoglycaemia and ACTH administration at 3 months of age (Poore & Fowden, 2003). In addition, increased fat depth in female adult pigs was predicted by high basal adrenaline concentrations in juvenile life.

In male low BW pigs, the low plasma leptin concentrations at 12 months of age were also associated with low glucose concentrations, which may reflect the reduced hepatic glucogenic capacity of these animals (Poore & Fowden, 2002*b*). There was no association between plasma leptin and fat depth in any of the regions examined in the current study. Taken together, these observations suggest that, in pigs at 12 months of age, leptin may be signalling energy availability more generally than adiposity *per se*. If food was available *ad libitum* to the male low BW pigs, they too may show excessive fat accumulation in an attempt to normalize their leptin concentrations. Indeed, the full effects of low BW on adult adiposity may not be evident at 12 months as, although postpubertal, this it still early in adult life in the pig.

In conclusion, this study has demonstrated sex-specific effects of size at birth on adult fatness and plasma leptin concentrations. In females, thinness at birth and poor early growth was associated with increased body fat in young adult life. In males, however, low BW or body shape at birth did not predict fatness as adults but was associated with low plasma leptin concentrations. The mechanisms regulating the deposition and utilization of fat stores also appeared to differ in males and females. These results have important implications for the intrauterine programming of adult obesity and may provide an explanation for the links between pre- and postnatal patterns of growth and the incidence of obesity-related diseases, such as NIDDM and cardiovascular disease.

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