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Serum leptin concentration in pigs selected for high or low daily food intake

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Summary

Selection for high or low daily food intake (DFI) in Large White pigs resulted in higher serum leptin concentration, fat deposition and food intake in the high DFI line. The response in serum leptin concentration indicated that the higher fat deposition of the high DFI line was not due to insufficient leptin production, as in the Lep^{ob}/Lep^{ob} mouse. Serum leptin was more highly correlated with fat deposition than with food intake indicating that the response in serum leptin was primarily due to increased fat deposition rather than to higher energy intake *per se*. The low correlations between serum leptin measured at 30 kg and performance test traits indicate that serum leptin would not be efficient for selection of animals prior to performance test. However, the consistent positive correlations between serum leptin and a measure of fat deposition suggest that serum leptin could usefully be incorporated in selection criteria for genetic improvement of carcass lean content in pigs.

1. Introduction

Leptin is synthesized and secreted from the adipocytes into the blood stream and transported to the brain, where it acts to cause a release of factors, such as neuropeptide Y, which results in reduced food intake (Houseknecht *et al*., 1998). However, leptin has also been associated with stimulated metabolic rate and reproductive function (Hossner, 1998). A recessive gene (Lep^{ob}) has been identified in the mouse with a mutation leading to insufficient leptin production and subsequent obesity (Zhang *et al*., 1994). Compensation for insufficient leptin production by administration of leptin to Lep^{ob}/Lep^{ob} mice (Maffei *et al*., 1995) has resulted in reduced food intake. Suppression of food intake in pigs was also achieved by administration of leptin (Barb *et al*., 1998). In contrast, divergent selection for body fat content in mice was associated with a positive response in serum leptin concentration (Bünger *et al*., 1999). However, Bünger & Hill (1997) reported substantial between-animal variation in response to leptin administration, which indicated that high leptin concentration did not always result from insensitivity to leptin. The recessive mutation in mice ($Lepr^{db}$) also results in elevated leptin concentrations in diabetic $Lepr^{db}/Lepr^{db}$ mice due to reduced sensitivity to leptin (Zhang *et al*., 1996). In the mouse selection lines of Bünger *et al*. (1999), the leptin response was indicative of reduced sensitivity to leptin, to a lesser extent than in diabetic $Lepr^{db}/Lepr^{db}$ mice, rather than insufficient leptin production of Lep^{ob}/Lep^{ob} mice (Maffei *et al*., 1995).

The difference between insufficient leptin production and insensitivity to leptin of Lep^{ob}/Lep^{ob} and $Lepr^{db}/Lepr^{db}$ mice has important implications for pig breeding programmes. For example, genetic improvement of carcass lean content may require selection for high serum leptin in a pig population analogous to a mouse population with a high incidence of Lep^{ob}/Lep^{ob} mice, but selection for low serum leptin in a population analogous to a mouse population with a high incidence of $Lepr^{db}/Lepr^{db}$ mice. In the Edinburgh lean growth pig experiment (Cameron, 1994), selection for high and low daily food intake (DFI) has been practised for seven generations in a Large White herd. The current study measured the correlated responses in serum leptin and determined

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whether the correlated response in fat deposition was consistent with insufficient leptin production or with insensitivity to leptin.

2. Materials and methods

(i) *Animals and performance test measurements*

Details on establishment of the Large White population and seven generations of divergent selection for DFI were given by Cameron (1994). For the current study, 20 pigs from each of the high and low DFI selection lines were penned individually and fed *ad-libitum*, using the diet-choice procedure (Bradford & Gous, 1991) to reduce nutritional constraints on animals' genetic merit for growth. Pigs were offered two isoenergetic (14·0 MJ DE/kg) diets differing in lysine: energy (0·69 and 1·12 g: MJ DE) throughout the fixed-weight test period of 30 ± 3 kg to 90 ± 5 kg. Blood samples were taken at the start and end of test and at 50 ± 4 kg and 75 ± 5 kg, when ultrasonic backfat and muscle depths at the last rib, liveweight and food intake were also measured. In practice, the mean weights that animals were measured were 29·0, 49·5, 73·1 and 88·2 kg, with no difference between the selection lines. Standard deviations for liveweights at each blood sample (1·8, 2·2, 2·3 and 2·4 kg) were consistent with the range permitted in the experimental design.

(ii) *Serum leptin measurements*

On each sampling occasion, 10 ml blood samples were taken from the vena cava and 24 h later, samples were centrifuged for 30 min at 4 °C. The red blood cell-free serum was extracted and frozen at −20 °C until required for assay. Serum leptin concentrations, expressed as nanograms per millilitre human equivalent (HE), were determined in duplicate by a commercially available radio-immunoassay procedure (Linco Research, Missouri) using an antibody raised against human leptin which displayed 67% cross-reactivity to porcine leptin (Linco Research, Missouri) due to high homology between the human and porcine amino acid sequences (Bidwell *et al.*, 1997). The detection limit of the serum leptin assay was 1 ng/ml HE (Linco Research, Missouri) and in the current study the assay had a repeatability of 0·87 (SE 0·16).

(iii) *Statistical methods*

Differences between the high and low DFI selection lines and the interaction with stage of performance test were estimated using residual maximum likelihood (REML) analysis, using the REML algorithm of the Genstat package (Genstat Committee, 1989). The model contained the two-way interaction of selection line and sampling time, sex and laboratory assay as fixed effects, with litter and animal fitted as random effects. Log transformation of serum leptin concentration was not required, as there was no evidence of non-normality within each selection line.

3. Results

Serum leptin concentrations in the high DFI line increased with liveweight but remained constant in the low DFI line, such that serum leptin was significantly

Table 1. *Serum leptin and performance of the high and low DFI lines*

		Liveweight at measurement (kg)				
Trait	Line	30	50	75	90	SED
Serum leptin (ng/ml HE)	High DFI	2·48	2·55	2·93	3·38	
	Low DFI	2·21	2·23	2·30	2·34	0·20
Backfat depth (mm)	High DFI	7·8	11·5	14·9	17·3	
	Low DFI	6·6	8·8	10·6	12·0	0·74
Muscle depth (mm)	High DFI	32·2	41·4	48·8	51·4	
	Low DFI	34·0	42·1	50·4	56·3	1·2
		Between liveweights (kg)				
Trait	Line	30–50	50–75	75–90		SED
Daily food intake (g)	High DFI	1862	2362	2797		
	Low DFI	1611	1996	2394		182
Grwoth rate (g/day)	High DFI	867	933	902		
	Low DFI	766	840	845		49

DFI, daily food intake; HE, human equivalent.

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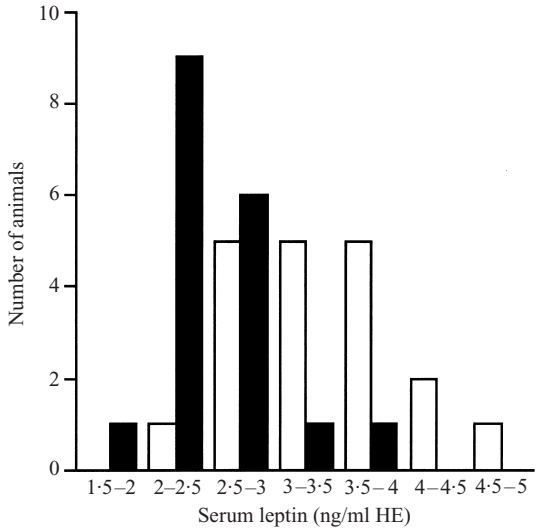

Fig. 1. Serum leptin concentration at 90 kg liveweight of pigs from the high (open columns) and low (filled columns) daily food intake (DFI) selection lines.

higher in the high DFI line from 75 kg liveweight (Table 1 and Fig. 1). Ultrasonic backfat depth and DFI were significantly higher in the high DFI line, from 50 kg, with the difference in backfat depth increasing with liveweight. However, at each of the four liveweights, the sums of backfat and muscle depths were similar in the two selection lines. Although growth rate was higher in the high DFI line, the difference was not statistically significant. Carcass fat content was not measured in the current study, but in the previous generation (Cameron *et al*., 1999) carcass fat contents of the high and low DFI lines were 249 and 190 g/kg (SED 7).

Throughout the performance test, the chosen diet of the low DFI line contained 0·78 g/g of the low lysine food with an effective dietary total lysine content of 11·0 g/kg. In contrast, the contribution of the low lysine food to the chosen diet of the high DFI line increased over the three weight ranges (0·68, 0·80 and 0·85 g/g, SED 0·06) with a reduction in effective dietary total lysine content (11·6, 10·9 and 10·6 g/kg, SED 0·4). Although lysine intakes of the high DFI line were greater than those of the low line, during each of the three weight ranges (22·2 vs 17·8, 26·2 vs 21·5 and 29·6 vs 26·4 g/day, SED 2·4), the differences were primarily due to higher daily food intakes (Table 1) rather than to differences in effective dietary total lysine content. The higher daily food intake (2241 vs 1911 g/day, SED 79) combined with fewer days on test (68·3 vs 73·4, SED 3·1) for the high DFI line, compared with the low DFI line, still resulted in greater total food intake (153·2 vs 139·6 kg, SED 6·7).

The repeatability of leptin concentrations when measured at adjacent liveweights was consistently 0·5 (Table 2). Leptin concentration was significantly, positively correlated with ultrasonic backfat depth at each liveweight. Ultrasonic muscle depth was negatively correlated with leptin, although not significantly. Correlations between ultrasonic backfat and muscle depths were low (−0·16), such that the correlation between leptin concentration and muscle depth was not just a consequence of the correlation between leptin concentration and backfat depth. Correlations for leptin concentration with growth rate, DFI and total food intake were generally not significantly different from zero for each of the three test periods and the total test. Correlations between serum leptin

Table 2. *Correlations* (*x100*) *between serum leptin concentrations and performance traits*

	Liveweight at measurement (kg)			
Trait	30	50	75	90
Leptin at 50 kg (ng/ml HE)	50	—	—	—
Leptin at 75 kg (ng/ml HE)	21	49	—	—
Leptin at 90 kg (ng/ml HE)	26	37	54	—
Backfat depth (mm)	33	41	44	53
Muscle depth (mm)	−14	−23	−20	−28
	Between liveweights (kg)			
	30–50[a]	50–75[b]	75–90[c]	30–90[c]
Growth rate (g/day)	27	−3	−6	13
Daily food intake (g)	22	22	5	27
Total food ntake (kg)	0	42	18	18

* SE of correlations = 0·16.
[a] Serum leptin concentration at 50 kg.
[b] Serum leptin concentration at 75 kg.
[c] Serum leptin concentration at 90 kg.

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concentration at 30 kg and traits measured at the end of test were not significantly different from zero (backfat depth, 0·16; muscle depth, −0·03; growth rate, 0·19; daily food intake, 0·21; total food intake, 0·09).

4. Discussion

Selection for high or low daily food intake (DFI) in Large White pigs resulted in higher serum leptin concentration, fat deposition and food intake in the high DFI line. The response in serum leptin concentration indicated that the higher fat deposition of the high DFI line was not due to insufficient leptin production, as in the Lep^{ob}/Lep^{ob} mouse. Serum leptin was more highly correlated with fat deposition than with food intake, indicating that the response in serum leptin was primarily due to increased fat deposition rather than to higher energy intake *per se*. The low correlations between serum leptin measured at 30 kg and performance test traits indicate that serum leptin would not be efficient for selection of animals prior to performance test. However, the consistent positive correlations between serum leptin and a measure of fat deposition suggest that serum leptin could usefully be incorporated in selection criteria for genetic improvement of carcass lean content in pigs.

The direct response in DFI to divergent selection on DFI after seven generations of selection was 16% of the mean, while the correlated responses in serum leptin and ultrasonic backfat depth increased from 14% of the mean, when measured at 30 kg, to 36% when measured at 90 kg. The larger indirect response in serum leptin relative to the direct response in DFI is indicative of a combination of a high heritability for serum leptin and a high genetic correlation between serum leptin and daily food intake. Currently, sufficient animals have not been measured for serum leptin to obtain reliable estimates of the genetic parameters with confidence. However, if the ratio of the correlated (CR_L) and direct responses (R_{DFI}) are expressed, in terms of their standard deviations, as $(CR_L/\sigma_L)/(R_{DFI}/\sigma_{DFI}) = r_A h_L/h_{DFI}$, where r_A is the genetic correlation between serum leptin and DFI, with heritabilities h^2_L and h^2_{DFI}, respectively, then an estimate of $r_A h_L$ can be determined, as described by Bünger *et al.* (1999). The value of $r_A h_L$ must be less than unity as the maximum value of both parameters is one. Using a heritability for DFI of 0·4 (Cameron & Curran, 1994), then the estimate of $r_A h_L$ is 0·78 for serum leptin measured at 90 kg. The ratio of responses implies a heritability of serum leptin of at least 0·61 and that r_A is at least 0·78. Given the observed correlation between serum leptin at 90 kg and DFI of 0·27, the proposal of similarity between phenotypic and genetic correlations (Cheverud, 1988) and the high value of $r_A h_L$, then the response in serum leptin would appear to be higher than expected.

The corresponding calculation of $r_A h_B$, where h^2_B is the heritability of backfat depth, resulted in a value of 1·08, which indicated that the direct response in DFI was not consistent with the correlated response in backfat depth. Given the heritability for backfat depth of 0·43 and the genetic correlation with DFI of 0·40 in the DFI selection lines (Cameron & Curran, 1994) and the correlated response in backfat, the expected value of R_{DFI}/σ_{DFI} would be 5·4, rather than the observed value of 1·3. The direct response and standard deviation of DFI were consistent with previous studies (Cameron & Curran, 1994) as was the standard deviation for backfat depth. Therefore, both the correlated responses in ultrasonic backfat depth and serum leptin concentration were higher than expected from the direct response in DFI.

There was some evidence that use of the diet choice procedure for performance testing animals from the high and low DFI selection lines had reduced nutritional constraints on growth. Based on ultrasonic backfat depths, a higher rate of fat deposition in the high DFI line relative to the low DFI line was consistent with a greater reduction in effective dietary lysine content as the performance test progressed. However, the effect of dietary lysine content on fat deposition was less than that of DFI, as indicated by the greater increase in ultrasonic backfat depth of the high DFI line during the 50–75 kg weight range, when the effective dietary lysine contents of the two lines were similar.

The positive response in serum leptin concentration and a measure of fat deposition was consistent with the responses to divergent selection for body fat content in mice (Bünger *et al.*, 1999). In studies of lean and fat pigs (Ramsay *et al.*, 1998; Robert *et al.*, 1998), leptin mRNA levels were higher in fat than in lean pigs indicating elevated serum leptin concentrations, but the difference in leptin may not be due solely to carcass fat content, as the lean and fat pigs were derived from different genetic populations. The mouse selection lines (Bünger *et al.*, 1999), the pig selection lines in the current study and the lean/fat pig studies (Ramsay *et al.*, 1998; Robert *et al.*, 1998) indicate that increased fat content was not a result of reduced leptin production, as in the Lep^{ob}/Lep^{ob} mouse (Maffei *et al.*, 1995). Therefore, selection for increased carcass lean content in pigs would require selection for low serum leptin, rather than for high serum leptin as indicated by the studies using administration of leptin in diabetic Lep^{ob}/Lep^{ob} mice (Maffei *et al.*, 1995) and in pigs (Barb *et al.*, 1998). Further, the pair of high and low DFI selection lines could be considered as a porcine model for the human condition of obesity, given the association between leptin and fatness in humans (Maffei *et al.*, 1995; McGregor *et al.*, 1996).

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