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Leptin levels in lines of mice developed by long-term divergent selection on fat content

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Summary

The mouse lines were developed by long-term selection for fatness, after which the fat line (F) had about a 5-fold (23% vs 4%) higher fat percentage than the lean (L) line at 14 weeks; but the lines differed little in fat-free body weight. To assess the contribution of genetic changes in leptin hormone level to the selection response, plasma leptin levels were assayed in these lines in generation 60 and in an unselected control (C) from the same base population. With access to food prior to assay, the F, C and L lines had 16·5, 0·91 and 0·26 ng/ml leptin, respectively. In fasted animals these levels were much lower: 2·98, 0·171 and 0·0087 ng/ml, respectively. Thus the leptin levels differ greatly between the lines, with the fattest mice showing the highest level: almost 20 times higher than the control and 60–300 times higher than the L line. These correlated selection effects are an order of magnitude greater than the direct selection response, and believed to be much larger than seen for any hormonal or other trait. Correlations between leptin level and fat amount were high (over 0·86) in fed or fasted animals of the F line, indicative of leptin resistance.

1. Introduction

An understanding of the genetics and physiology of leanness and obesity is important in both animal production and human medicine. Progress towards such an understanding has come from laboratory animal studies, at both the quantitative and individual gene level. There are several well-defined genetic models for obesity in rodents: in mice there are at least six different single gene mutations on five chromosomes producing obesity–diabetes syndromes: obese (*Lep* [formerly *ob*], Chr 6), diabetes (*Lepr* [formerly *db*], Chr 4), tubby (*tub*, Chr 7), *Cpe* (*Cpe* [formerly *fat*], Chr 8), adult obesity (*Ad*, Chr 7) and the dominant yellow mutations at the agouti coat colours locus (A^Y, A^{vy}, Chr 2) (reviewed by Doolittle *et al.*, 1996). Zhang *et al.* (1994) identified the recessive gene responsible for obesity in the *Lep*/*Lep* mouse, which has a mutation at the structural gene that leads to insufficient leptin production. This started a cascade of experiments using these new insights into the molecular genetic basis and the factors important in the metabolic pathways leading to obesity (reviewed by Caro *et al.*, 1996).

Leptin administration reduces body weight and fatness in some forms of obesity, such as in the *Lep*/*Lep* mouse. But because leptin is generally overexpressed in most obese rodent models other than *Lep*/*Lep* mice and in obese humans (Maffei *et al.*, 1995), insensitivity to leptin rather than insufficient leptin production, as found in the *Lepr*/*Lepr* mouse, may be a common cause of obesity. A role for leptin deficiency in human obesity has been considered but no pathogenic mutation in the human *Lep* gene was found until recently, when Montague *et al.* (1997) provided a few cases of a recessive mutation of the *Lep* gene in humans. Thus it becomes clear that leptin is an important, but not the only, player in the control of body fat stores and energy balance and that an unknown number of genes together with environmental factors are involved (e.g. Andersson, 1996; Bouchard, 1996; Chua & Leibel, 1997).

In this laboratory we have lines of mice that have been selected for high and low body fat content from

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the same base population for *c*. 60 generations. At the age of selection, 14 weeks, the fat line has about a 5-fold higher fat percentage of body weight than the lean line, with this ratio increasing with age (Hastings *et al*., 1991). The lines differ in fat-free body weight, however (Hastings & Hill, 1989). They therefore provide very suitable material for experiments to investigate the role of specific metabolic pathways and candidate genes and to check to what extent changes in the endogenous leptin level and in some other blood constituents contributed to the selection response. We have previously shown that the fat mice show very variable response to leptin administration (Bünger & Hill, 1997), indicative of differences in sensitivity to the hormone. If the fat mice, or many of them, are leptin-insensitive an up-regulation of their plasma leptin concentrations compared with the control and lean mice would be expected. To test this hypothesis, leptin levels were analysed in non-fasted mice. Then, having observed that the plasma leptin concentration in the obese mice was markedly elevated, we sought to examine whether leptin secretion was still responsive to dietary intake by comparing plasma leptin concentrations after fasting.

2. Material and methods

(i) *Selection lines*

Selection lines were founded by divergent selection from a three-way cross of two inbred (CBA, JU) and one outbred (CFLP) line (Sharp *et al*., 1984). For the first 20 generations, selection was on the ratio of gonadal fat pad weight (GFPW) to body weight (BW) at 10 weeks of age in males with three replicate lines in each direction. Subsequently the replicates were crossed and selection continued in a single replicate using the ratio of dry carcass weight to body weight in males at 14 weeks as an indicator of fatness (Hastings & Hill, 1989; Hastings *et al*., 1991). Animals from generation 60 of the selection lines were used in this study. Selection had been suspended from generation 53 to 59 while all mouse stocks were transferred to a new mouse house by embryo transfer.

(ii) *General management*

Mice were fed *ad libitum* Rat and Mouse no. 3 diet (digestible crude (dc) oil, 3·9%; dc protein, 20·9%; starches, 27·3%; sugars, 11·2%; digestible energy 12·1 MJ/kg) from weaning onwards (Special Diet Services, Witham, Essex, UK) and maintained with controlled lighting (12 h light) at a temperature of 21 ± 1 °C. The animals were kept after weaning in groups of 3–8 animals in plastic cages (MB1, Kents Plastics). The lids of all experimental cages were covered inside with metal plates to restrict lid-climbing activity and thereby facilitate fat aggregation (L. Bünger, unpublished).

(iii) *Experimental procedures*

(a) *Animals*

Fourteen-week-old male mice from the Fat (F, $n = 29$), Lean (L, $n = 32$) and from an unselected Control line (C, $n = 32$) were used in the study. The control line was derived from the same base population, but in this sample had a somewhat lower body weight than both selection lines.

(b) *Treatment and bleeding*

About half the animals (see Table 1) were fasted (fst) for 18–20 h (water was provided *ad libitum*) and the other half remained fed (non-fasted = nfst) before blood was obtained between 11:00 and 14:00 hours by cardiac puncture whilst under anaesthesia. The anaesthetic was a combination of Rompun 2% Stock Solution (Bayer) and Vetalar (100 mg/ml ketamine; Parke-Davis). The working solution was made up as 0·5 part Rompun, 1 part Vetalar and 1·5 parts sterile water (Sinden, 1996) and given as an intramuscular injection into the inside muscle of the leg at 2·5 μl/g body weight.

(c) *Assays*

Plasma leptin levels were determined by an enzyme immunoassay as described by Hotta *et al*. (1996). Forty-four samples from among the three lines, mostly from non-fasted animals, were assayed twice, allowing the estimation of a correlation coefficient between duplicate measurements. The correlation coefficient (95% confidence interval) was 0·985 (0·972, 0·992). Other plasma chemistries were assayed in a Hitachi 717 Chemistry Analyser.

(d) *Fat percentage*

All animals were killed by cervical dislocation after bleeding. The digestive tract (stomach, intestine) from the non-fasted animals was removed before recording body weight to eliminate a possible effect of gut fill on the fat content prediction, because the formula used (see below) was derived from data on fasted animals. The prepared carcasses were freeze-dried to determine the individual dry weight (DW).

Prediction of individual fat content was by regression on dry matter content (DW/BW) using an

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equation given by Hastings & Hill (1989) for fat content at 10 weeks: Fat content (%) = DW/BW × 113 − 30·2. The fat-free body weight (ffBW) was estimated as body weight less estimated fat content.

(iv) *Statistical methods*

Data on blood constituents were analysed using the following model:

$$Y = M + S + G + T + G \times T + e,$$

where M is an overall mean, T is the effect due to the treatment (fasted/non-fasted), G is a genotype (line) effect (1–3), $G \times T$ are interactions between genotype and treatment, and e is residual error. All effects except e were fitted as fixed. ANOVA was undertaken with GLM using the SAS System for Windows Release 6·08 (SAS Institute, Cary NC 27513, USA). The analysis was undertaken on non-transformed data and, for traits where there was a clear relation between mean and standard deviation (e.g. leptin levels), also on log-transformed data.

The sensitivity threshold of the leptin assay was 0·1 ng/ml. In total, 15 of 93 samples fell below this threshold of the assay: 12 of 18 samples from fasted lean line animals and 3 of 16 from fasted control animals. Therefore, these fasted groups provided data that were recorded as at the boundary, but can be expected to be lower than this threshold. The data of these two groups were therefore pre-processed by the procedure CENSOR in the Genstat package (Genstat 5 Release 3, Statistics Department, Rothamsted Experimental Station, AFRC Institute of Arable Crops Research, Harpenden, Herts AL5 2JQ, UK), which predicts values for the data at boundary in the range under the boundary (Taylor, 1973). Thereafter the whole data set (including the predicted values instead of boundary value) was analysed using the GLM procedure in the SAS system.

3. Results

(i) *Body weight and composition of test animals*

Body weight and body composition of the animals used for the leptin study are summarized in Table 1. The F line animals have a predicted fat content (23 %) almost 5 times that of the L line animals (4·8 %), or a difference of about 5 phenotypic standard deviations. These are typical of other records taken on these lines following transfer to the new animal house. In addition to being much fatter, the F line animals were significantly heavier than L line animals but they differed little in fat-free body weight, indicating that the body weight difference was due to fat content. The weights of animals from this particular control line sample were unexpectedly low.

The predicted fat content, using the regression equation determined for non-bled, fasted animals, in all fasted groups was 1·6- to 3-fold lower than for their corresponding non-fasted group, which was at least partly caused by the different preparation of the carcasses rather than by the 18–20 h fasting. Therefore, statistical analyses to compare lines were made only within the fasted and non-fasted groups (Table 1).

(ii) *Assays*

(a) *Leptin*

The test of statistical significance for leptin values was conducted on log-transformed data (Table 2), but the back-transformed group means are presented in Fig. 1 for clarity. In the fed state the leptin levels differed greatly between the lines; the highest levels were found in the F line – some 18-fold higher than control and 64-fold higher than lean animals (Fig. 1 *a*). The ratios of the mean leptin levels to the mean fat amount (cf. Table 1) were 1·9, 0·33 and 0·20 ng/ml per gram fat in the F, C and L lines, respectively.

The leptin levels in fasted mice were much lower in all lines (Fig. 1 *b*). Again the highest values were found in the F line, being 17-fold higher than in the control and 340-fold higher than in the L line. Averaged over lines the fasting caused an approximately 90 % reduction in leptin level (Table 2). The lines reacted differently to fasting, as shown by the significance (even under log transformation) of the line × treatment interaction ($P < 0{\cdot}001$), the leptin levels in the F and C lines decreasing by about 80 % and in the L line by about 97 %.

(b) *Cholesterol*

Cholesterol values in the non-fasted state are highest in the F line (108 mg/dl) followed by the C line (90 mg/dl) and L line (78 mg/dl) (Table 2). They dropped by fasting on average by 13 %, but the lines reacted significantly differently ($P < 0{\cdot}05$).

(c) *Triglycerides*

The levels of triglycerides were highest in the fed F line animals. They were 47 % and 71 % higher than in the C line and the L line respectively (Table 2). Fasting caused the triglyceride levels to drop by about 50 % on average. Again the line × treatment interaction was significant.

(iii) *Correlations*

Phenotypic correlations between the log-transformed leptin level and body composition and blood

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Table 1. *Body composition of non-fasted and fasted animals from the fat line (F), lean line (L) and control (C)*

Trait	*n*	Non-fasted* F ($n = 14$)	Non-fasted* C ($n = 16$)	Non-fasted* L ($n = 14$)	Fasted* F ($n = 15$)	Fasted* C ($n = 16$)	Fasted* L ($n = 18$)
Wet weight (g)†	Mean	$38{\cdot}2^{a}$	$28{\cdot}3^{c}$	$31{\cdot}8^{b}$	$35{\cdot}7^{a}$	$28{\cdot}1^{c}$	$31{\cdot}6^{b}$
	SE	1·16	0·75	0·56	1·11	0·95	0·47
Dry matter (%)	Mean	$46{\cdot}9^{a}$	$35{\cdot}1^{b}$	$31{\cdot}0^{c}$	$39{\cdot}1^{a}$	$32{\cdot}1^{b}$	$28{\cdot}1^{c}$
	SE	0·76	0·44	0·15	0·87	0·49	0·12
Predicted fat (%)	Mean	$22{\cdot}8^{a}$	$9{\cdot}5^{b}$	$4{\cdot}8^{c}$	$14{\cdot}0^{a}$	$6{\cdot}1^{b}$	$1{\cdot}5^{c}$
	SE	0·86	0·50	0·17	0·98	0·56	0·13
Predicted fat (g)	Mean	$8{\cdot}83^{a}$	$2{\cdot}70^{b}$	$1{\cdot}53^{c}$	$5{\cdot}14^{a}$	$1{\cdot}74^{b}$	$0{\cdot}49^{c}$
	SE	0·57	0·17	0·059	0·50	0·20	0·043
Predicted ffBW (g)	Mean	$29{\cdot}4^{a}$	$25{\cdot}6^{b}$	$30{\cdot}2^{a}$	$30{\cdot}6^{a}$	$26{\cdot}3^{b}$	$31{\cdot}1^{a}$
	SE	0·64	0·66	0·54	0·68	0·85	0·46

Predicted: predicted from dry matter content as described in Material and methods; ffBW: fat-free body weight.
* Means within fasted or non-fasted groups sharing a common superscript are not significantly different (Welch test, two-tailed, inhomogeneous variances, $P > 0{\cdot}05$). Comparisons were not undertaken between fasted and non-fasted groups as other systematic effects are included (different preparation of the carcasses: see Material and Methods).
† Wet weight = whole body minus blood sample (about 0·5–1·8 ml) in fasted groups and whole body minus blood sample, stomach and intestine in non-fasted groups.

Table 2. *Blood plasma constituents (least square means ± SE)*

Traits	*n*	Non-fasted (nfst) F	Non-fasted (nfst) C	Non-fasted (nfst) L	Fasted (fst) F	Fasted (fst) C	Fasted (fst) L	Treatment* nfst	Treatment* fst
Leptin (ng/ml)†		16·48	0·91	0·26	2·98	0·171‡	0·0087‡	1·57	0·16
Log (leptin)	Mean	$1{\cdot}22^{a}$	$-0{\cdot}041^{b}$	$-0{\cdot}59^{c}$	$0{\cdot}47^{a}$	$-0{\cdot}77^{b}$	$-2{\cdot}06^{c}$	0·196	−0·78
	SE	0·146	0·136	0·146	0·141	0·136	0·129	0·082	0·078
Cholesterol (mg/dl)	Mean	$108{\cdot}0^{a}$	$89{\cdot}6^{b}$	$78{\cdot}4^{b}$	$84{\cdot}5^{a}$	$87{\cdot}0^{a}$	$69{\cdot}9^{b}$	92·0	80·5
	SE	4·15	3·88	4·15	4·01	3·88	3·66	2·35	2·22
Triglycerides (mg/dl)	Mean	$120{\cdot}0^{a}$	$81{\cdot}5^{b}$	$70{\cdot}3^{b}$	$55{\cdot}8^{a}$	$52{\cdot}0^{a}$	$21{\cdot}1^{b}$	90·6	43·0
	SE	5·9	5·5	5·9	5·7	5·5	5·2	3·3	3·2

Significance of the Genotype (G) and Treatment (T) effects and their interaction (G × T) was tested by ANOVA. All G and T effects and their interaction were highly significant ($P < 0{\cdot}01$) for all traits, except G × T for cholesterol where $P < 0{\cdot}05$. The results of a pair-wise comparison of line means (Welch test, using the SE for each line to calculate the SE for the difference) within the fst and nfst groups are coded by superscripts: means with same superscripts are not significantly different ($P > 0{\cdot}05$).
* T: treatment = non-fasted or fasted. Means are averaged over lines and were significantly different for all traits ($P < 0{\cdot}001$).
† Back-transformed values.
‡ These means were derived using the estimated instead of the censored data (see Material and methods). Using values at the detection limits as such, the means were C_{fst}: 0·197 and L_{fst}: 0·0232.

parameters are given in Table 3. The correlation between the log leptin levels and fat content (%) and fat amount (g) in the non-fasted and fasted F animals were very high: between 0·81 and 0·96. Slightly smaller but significant correlations were found in non-fasted control animals, but the correlations were not significant when the animals were fasted. No significant correlations were found in the L line, but a high proportion of animals were at the threshold.

Most correlations between leptin and cholesterol or triglycerides were not significant. High and significant correlations were found in the F line, especially when non-fasted.

4. Discussion

Long-term selection on fat content had brought about a high divergence between lines – about 23% fat in the fat (F) line and 4% in the lean (L) line (L. Bünger & W. G. Hill, unpublished) – which corresponds well with the observed divergence in this experiment (Table 1). Changes in leptin level were proportionately larger. The plasma leptin concentrations in the F line mice (16 ng/ml) were some 18-fold higher than in the control and 64-fold higher than in L line animals in the fed state (Fig. 1*a*). The magnitude of the relative difference between obese and normal individuals

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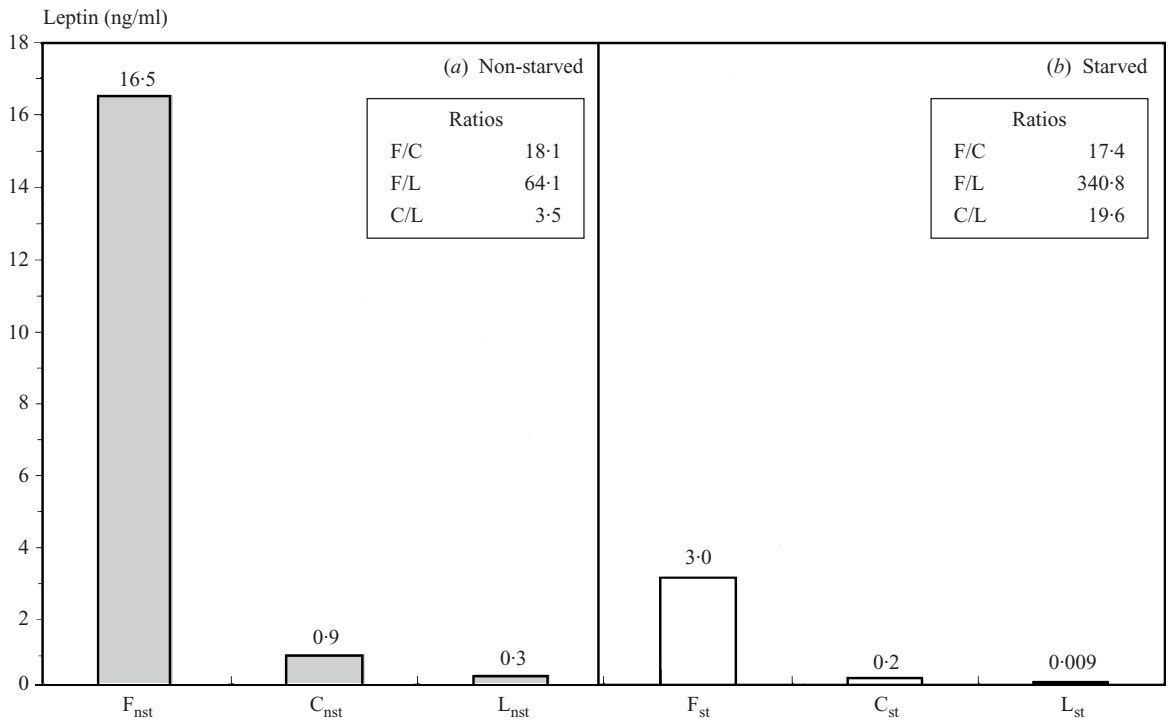

Fig. 1. Leptin levels of animals from the fat (F), control (C) and lean (L) lines in non-fasted (*a*) and fasted state (*b*). Leptin data were analysed on a log scale (Table 2). Values shown are back-transformed least square means.

Table 3. *Correlation coefficients between leptin level* (*log values*) *and body composition and some blood data among fasted* (*fst*) *and non-fasted* (*nfst*) *animals*

	F line		Control		L line	
	nfst	fst	nfst	fst*	ns	fst*
Wet weight (g)	0·89	0·81	0·18	0·32	0·21	0·10
Predicted fat content (%)	0·86	0·96	0·60	0·32	−0·01	−0·37
Fat amount (g)	0·90	0·96	0·55	0·38	0·10	−0·34
Fat-free body weight (g)	0·81	0·63	0·06	0·27	0·21	0·13
Cholesterol (mg/dl)	0·71	0·22	−0·35	−0·16	0·11	0·23
Triglycerides (mg/dl)	0·73	0·62	0·16	0·05	0·25	0·35
Critical level, $P < 0{\cdot}05$	0·53	0·51	0·50	0·50	0·53	0·47

* Correlations were estimated using estimated values (censor method) for those at the boundary of detection in the fasted L and C groups.

found here corresponds very well with other observations in rodents and humans (e.g. Maffei *et al.*, 1995), but ratios as high as those between the obese and the extreme lean line have not been found.

The leptin levels in the fasted state were much lower in all lines (Fig. 1*b*), but with the largest decrease in the L line, where under fasting 66% of all values were under the limit of detection (0·1 ng/ml). The leptin level in fasted F animals was nearly 3 ng/ml, which was 17-fold higher than in the control and 340-fold higher than in the L line. The magnitude of the relative difference between the F and L lines found in the fasted state is much higher than reported elsewhere in rodents (e.g. Frederich *et al.*, 1995; Maffei *et al.*, 1995) or humans (e.g. Hosoda *et al.*, 1996; McGregor *et al.*, 1996; Sinha *et al.*, 1996). This seems to be due mainly to the inclusion in this study of the L line, which reacted very strongly to fasting.

The correlated selection response in the leptin levels, a divergence between the selection lines by a factor of about 60–340, is an order of magnitude greater than the direct selection response, the di-

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vergence between F and L line in their fat content (factor 5–6). In selection experiments correlated responses are usually smaller than the direct response (Falconer & Mackkay, 1996), as were those for the other plasma constituents (Table 2). Cholesterol and triglycerides, some of the conventional indicators for the degree of obesity (e.g. Deshaies *et al*., 1986), were increased by 'only' 38 % and 71 % respectively. The latter is of similar order to the 2- to 2·5-fold difference in insulin reported by Asante (1988) for the same lines.

As far as we know, such a high correlated response in relation to the direct response as reported here for leptin has not been found before. To prove the plausibility of such a large change in a correlated character requires a complete set of genetic parameters, which is not yet available, but some relevant but approximate calculations can be made using results from this study and from that of Sharp *et al*. (1984) for variances and realized heritabilities of fat levels. The asymmetry in response in fat and leptin levels to high and low selection is largely removed by transformation to logarithms. On the $\log_{10}$ scale the phenotypic standard deviation of (predicted) fat content is $\sigma_{P_f} = 0{\cdot}129$, and the divergence in response between the F and L lines is $R_f = 0{\cdot}760 = 5{\cdot}87\sigma_{P_f}$. For leptin levels the corresponding figures are $\sigma_{P_l} = 0{\cdot}546$, and $R_l = 1{\cdot}81 = 3{\cdot}31\sigma_{P_l}$. The ratio of correlated to direct response is an estimate of $(R_l/\sigma_{P_l})/(R_f/\sigma_{P_f}) = r_A h_l/h_f$, where r_A is the genetic correlation between fat and leptin levels (Falconer & Mackay, 1996). Taking $h_f^2 = 0{\cdot}5$, then $r_A h_l = 0{\cdot}40$. Thus, because of the very high coefficient of variation of leptin levels (over 100 %), a response of the magnitude obtained is plausible (i.e. $r_A h_l < 1$), but implies that $r_A > 0{\cdot}4$ and $h_l^2 > 0{\cdot}16$. If, for example, $h_l^2 = 0{\cdot}50$, then $r_A = 0{\cdot}56$.

Taken over lines, the phenotypic correlation between fat and leptin levels would be very high, but a more meaningful estimate can be obtained within lines. The correlations between leptin and fat amount (g) and fat content (%) in fed animals were high and positive in the F line ($r = 0{\cdot}86$–$0{\cdot}96$) but decreased in the C and L lines. These high correlations between leptin concentrations and the fat percentage between the lines and within the F line suggest most obese individuals are relatively insensitive to endogenous leptin, which is in agreement with previous findings (Bünger & Hill, 1997). Their 'set point' seems to be shifted up. This could indicate that sensitivity rather than leptin production has changed by selection.

In theory, leanness could result from increased leptin levels and their negative consequences on food intake, but the leptin level in lean animals was decreased by selection (Table 1). In addition, a recent comparison of the food intake of the F and L lines shows that the food intake of the L lines is even higher than that of the F line; also when the food intake is related to metabolic body size (Bünger, in preparation).

The mean levels of leptin relative to fat amount (cf. Table 1) in the fed state were highest in the F and lowest in the L line, which could reflect a higher leptin production per gram fat weight in the F line and eventually a lower clearance rate and vice versa in the L line.

Plasma leptin levels were also found to be highly correlated with traits characterizing fat content (body mass index, etc.) in other studies on mice, mostly observed in the fed state. Maffei *et al*. (1995) found a high positive correlation between a lean mass index (BMI) and log leptin level ($r = 0{\cdot}77$), pooling data from different fat and lean groups. When they excluded the *Lepr*/*Lepr* mice, the correlation increased to 0·9. Frederich *et al*. (1995) found a strong positive correlation between total body lipid, determined by carcass analysis, and the circulating leptin level in serum of fed mice ($r = 0{\cdot}99$ in females and $r = 0{\cdot}95$ in males). These correlations were, however, calculated from group means, not from individual data, and so may be biased upwards.

In humans, strong positive relations have been reported between leptin levels and parameters of fatness (Maffei *et al*., 1995; McGregor *et al*., 1996; Considine *et al*., 1996; Caprio *et al*., 1996). Hickey *et al*. (1996) found a high correlation (0·92) between serum leptin and fat mass even in relatively lean men (male distance runners), whereas on fed L line mice, where leptin values were very low but still above detection limit, the correlation was only 0·1. Altogether these high correlations in humans and rodents between leptin concentrations and fat content indicate a high insensitivity to endogenous leptin in obese individuals.

In a previous experiment on the same mouse lines the sensitivity to leptin was tested by administering leptin to F line and C line mice twice daily by intraperitoneal injections of 5 mg/kg leptin from 91 to 105 days of age (Bünger & Hill, 1997). Treated (T) compared with untreated (U, injected with saline) animals of both lines had significantly lower mean body weight, food intake and fatness at the end of the test (mean fat content (%): CT 3·0 %, CU 7·4 %, FT 14·9 %, FU 21·1 %). A wide range of fatness among FT animals (3–29 %) was found, much higher than in FU (15–31 %), CT (0·7–6·4 %) and CU (2–15 %). These results indicate that sensitivity to exogenous leptin remained in the F line even after such a long time of selection, but response appears to vary among animals at the dose level used.

Attempts were also made to estimate insulin levels in this study, but they were difficult to analyse and to interpret because only 17 of 93 samples were above the detection limit of 0·2 ng/ml. Of these, 12 (of 14 sampled) were from the non-fasted F lines and only 1

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or 2 from the other five line × fasting groups. This agrees with observations at earlier generations of insulin values in the F line that were about 2·5-fold higher at 5 weeks and about 2-fold higher at 10 weeks of age than in the L line (Asante, 1988). These results indicate some degree of hyperinsulinaemia in the F line.

Further studies could be directed at testing the degree of the apparent resistance to leptin in the F line using a higher dosage and a longer treatment, involving leptin and leptin receptor assays on these animals. Molecular genetic techniques and breeding experiments should be employed to test for variation at candidate loci and for mapping relevant loci. By repeated backcrosses of known mutated or knocked-out genes into both selection lines certain metabolic pathways could be eliminated to elucidate their contribution to the observed line differences.

The results suggest that circulating leptin levels could be used effectively as a predictor of leanness in selection programmes in livestock, since the estimate of accuracy to selection, $r_A h_l$, is high. Such a prediction would have to be investigated in the relevant species, and in any case might not be useful because leanness is easy to record on the live animal using, for example, ultrasonic equipment. There is, however, an important cautionary note here: administration of exogenous leptin to increase circulating levels leads to a reduction in obesity; whereas our results suggest that effective selection for leanness would be by selecting to decrease levels of endogenous circulating leptin!

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