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Effects of leptin administration on long-term selected fat mice

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Summary

To assess the role of genetic changes in sensitivity to leptin hormone in contributing to responses to long-term selection for fatness, leptin was administered to a long-term fat selected (F) and a control line (C) of mice. These lines differ almost three fold in their percentage of fat (fat%) at about 15 weeks of age. Treated (T) animals received twice-daily intraperitoneal injections of 5 mg/kg leptin from 91 to 105 days of age; untreated (U) animals received equivolume injections of phosphate-buffered saline. Treated compared with untreated animals in both lines had significantly ($P < 0{\cdot}05$) lower mean body weight, food intake and fatness at the end of test (fat%: CT 3%, CU 7·4%, FT 14·9%, FU 21·1%). The differences in response between the lines [(CT − CU) − (FT − FU)] were all non-significant ($P > 0{\cdot}05$), however. There was a very wide range of fatness (estimated from dry matter content) among FT animals (3–29%), much higher than in FU (15–31%), CT (0·7–6·4%) and CU (2–15%) animals. While sensitivity to leptin remains in the fat line, response appears to vary among animals at the dose level used.

1. Introduction

Divergent long-term selection for fat content in mice in our laboratory has produced genetic change in each direction, resulting in a very high divergence between the lines. After 50 generations the mean estimated percentage of fat (fat%) in 14-week-old males was about 23% in the fat line (F) and 4% in the lean line (L), compared with about 10% in the base population. To understand these responses to selection it is necessary to know which genes and which metabolic pathways are involved.

Recent work has shed new light on the control of fatness. Zhang *et al.* (1994) identified the gene responsible for obesity in one of the most intensively studied genetic rodent models of this metabolic disorder: the *ob*/*ob* obese mouse. In the C57BL/6J strain this introduces a stop codon into the *ob* mRNA, so that despite a 20-fold overexpression of the mRNA in white fat, these mice can not produce normal *OB* protein. A second mutation is found in *ob*2J/*ob*2J mice; in this case the mutation occurs in the 5′ region of the *ob* gene and prevents the synthesis of any *ob* mRNA.

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Soon after this publication, three laboratories reported simultaneously that the administration of recombinant *OB* protein to *ob*/*ob* mice reduced both their hyperphagia and their body weight and led also to a normalization of their metabolic status and obesity (Pelleymounter *et al.*, 1995; Halaas *et al.*, 1995; Campfield *et al.*, 1995). It was proposed that this protein, named leptin (Halaas *et al.*, 1995), serves as a feed back regulatory of satiety (Zhang *et al.*, 1994) or, as suggested by Collins & Surwit (1996), as a sensor of fat cell hypertrophy. Leptin can be detected immunologically in plasma of normal mice and in lean humans, but not in plasma and adipose tissue from *ob*/*ob* mice. Leptin reduces food intake and the body weight and corrects the hyperglycaemia, hyperinsulinaemia, hypometabolism and hypoactivity in *ob*/*ob* mice, but can also reduce body fat and fat content in lean mice (Pelleymounter *et al.*, 1995; Halaas *et al.*, 1995; Campfield *et al.*, 1995; Stephens *et al.*, 1995) and in mice with a diet-induced obesity (Campfield *et al.*, 1995).

Leptin is, however, overexpressed (7 times) in plasma and adipose tissue of another form of genetically obese mice – homozygotes for the diabetic gene (*db*) (Zhang *et al.*, 1996) – and is ineffective at reducing body weight and food intake in these animals.

Parabiosis studies with *ob*/*ob* and *db*/*db* mice had indicated that *db*/*db* mice may be defective in the reception of the *ob* gene product signal (Coleman 1973), so it was speculated that the *db* gene may encode the receptor for leptin. Tartaglia *et al*. (1995) identified and cloned a leptin-binding receptor, *OB*-R, and mapped it to the 5 cM interval that contains the *db* locus, suggesting that the *db* gene encodes the *OB*-R. Chua *et al*. (1996) and Lee *et al*. (1996) found that the *OB-Rb* is allelic with *db* and that it has at least six alternatively spliced forms. The mutant protein lacks the cytoplasmic region, and is likely to be defective in signal transduction. Phillips *et al*. (1996) conclude that a variety of leptin receptor defects may result in obesity in rats and mice.

As leptin injections reduce body weight and fatness in some forms of obesity, such as in the *ob*/*ob* mouse that has insufficient leptin production, obesity may be treatable by replacement therapy. If however, leptin is generally overexpressed in obese humans and most rodents other than *ob*/*ob* mice, insensitivity to leptin rather than insufficient leptin production may be a common impairment and efficacy of leptin would be limited (Maffei *et al*., 1995).

It is likely that many loci have contributed to the selection response in fatness in our long-term selected mouse populations, for many factors influencing growth, food intake, thermoregulation, locomotor activity and energy partition are likely to be involved. Our selected fat line therefore comprises a polygenic model of obesity. Nevertheless certain major genes may have contributed to the response, including non-severe variants at the *ob* or *db* locus or alleles at other loci involved in the production of responsiveness to leptin. The aim of this study was to assess the role of leptin in the selection response by administering the hormone to the fat and control lines, and testing whether its effects on body weight, food intake and fatness differed between the lines.

2. Materials and methods

(i) *Selection lines*

These selection lines were founded by divergent selection from a three-way cross of two inbred lines and one outbred line (Sharp *et al*., 1984). For the first 20 generations, selection was practised 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 BW in males at 14 weeks as an indicator of fatness (Hastings & Hill, 1989; Hastings *et al*., 1991).

Mice from the fat selected line (F) at generations 58 and 59 were used. Selection had been suspended from generation 53 to 59 while all mouse stocks were transferred to a new mouse house by embryo transfer. An unselected control line (C) from the same base population was also used.

(ii) *Experiment I*

Experiment I was a preliminary experiment. Only males from the F line were included, using a leptin dosage known to be effective in *ob*/*ob* mice (Halaas *et al*., 1995; Pelleymounter *et al*., 1995). Ten mice were injected with leptin and 10 untreated, one mouse being assigned to each group from 10 full-sib families.

Treated (FT) mice were kept in groups until 75 days (d) of age and thereafter kept singly in plastic cages (M3, Kents Plastics) equipped with special food hoppers for the measurement of food intake. Body weight and food intake (FI) were measured daily throughout a 3 day preinjection period and the 21 day injection period. From 78 to 98 d leptin (biologically active form of recombinant mouse *OB* protein from an *Escherichia coli* expression system, kindly supplied by AMGEN, Thousand Oaks, CA) was administered by one daily (at about 1000 hours) intraperitoneal injection of 0·2 mg leptin dissolved in 2 ml phosphate-buffered saline (PBS) (pH 7·4). The mean initial body weight was 38·3 g. The mean dose was 5·2 mg/kg, ranging from 4·5 to 6·2 mg/kg.

Untreated (FU) mice were treated the same as group FT, but the mice received equivolume (2 ml per animal daily) injections of PBS without leptin.

(iii) *Experiment II*

In experiment II a total of 60 males from the F line and 60 males from the C line were used. The experiments in these lines were conducted 4 weeks apart, due to a time shift between the schedules for line maintenance, but the critical comparisons are between treated and untreated animals within lines. The animals were kept in groups of 3 to 8 (from weaning onwards, males from two litters were grouped together). They were weighed at 70 d and at the beginning of the 7 d preinjection period when the mice were 84 d ($\pm 1{\cdot}3$ = SD) old.

Each line × treatment set comprised 10 cages each of 3 males. To reduce distress caused by re-grouping 84-d-old males and by single caging, 3 males from the groups established at weaning were caged together in plastic cages (MB1, Kents Plastics) equipped with three food hoppers for the measurement of group food intake. The mice from the F line were sampled from 20 different litters; 18 of them were split between treatment groups, with not more than 3 mice from any litter in each. In the C line up to 3 animals per litter from 21 litters were sampled. Because of an undesirable age structure in this generation of this line only one litter could be split between treatment groups; therefore most cage groups consisted of full sibs.

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The lids of the experimental cages were covered inside with metal plates to reduce lid climbing and to maximize fatness in the F line (L. Bünger & W. G. Hill, unpublished data). Body weights at the beginning and end of the 7 d preinjection period (84–91 d) and food intake during this week were measured, to obtain a baseline for both traits. At 91 d ± 1·3 (= test day 0) the groups were treated as follows:

Treated (FT, CT): Leptin was administered by two daily (0900–1100 hours and 2100–2300 hours) intraperitoneal injections of 5 mg/kg each to every mouse. The protein was dissolved in PBS (pH 7·4) at a leptin concentration of 13 mg/ml. The mice were treated over 15 d (test day 0 to 14). On the last day, day 105, they received only the morning injection (0900–1100 hours) and in late afternoon starvation was initiated. During the treatment period, body weight and group food intake were measured daily. Food intake was expressed on an individual basis by dividing group food intake by the number of mice per cage (normally 3, but less if an animal died). (Of 120 animals, 6 died during experiment II: 1 in the FT and 5 in the CT group. None died in experiment I.)

Untreated (FU, CU): These mice were treated as groups FT and CT except that they received twice-daily equivolume injections of PBS without leptin.

(iv) *General management and measurement of fat traits*

Mice were fed Rat and Mouse no. 3 diet (Special Diet Services) *ad libitum* and maintained with controlled lighting (12L:12D).

All animals were killed by cervical dislocation at 99 d in experiment I or at an age of 105·5 d ± 1·4 in experiment II after starvation for about 18 h. The gonadal fat pad weight was taken and the dry matter weight (DM) of the whole body was determined by freeze-drying the prepared carcass.

In experiment II the dried carcasses of 3 (minimum) to 5 (usual) mice were pooled together in one sample. The resulting six samples per experimental group were subjected to chemical fat determined by Soxlet extraction. Prediction of individual fat% values was by regression on dry matter content (DM/BW). The regression of fat% (analytical values) on dry matter content was similar for the four groups and the pooled equation was: $\text{Fat\%} = \text{DM/BW} \times 140 - 39{\cdot}41$ ($r = 0{\cdot}998$). (This prediction is similar to that obtained by Hastings & Hill (1989) for fat% at 10 weeks: $\text{Fat\%} = \text{DM/BW} \times 113 - 30{\cdot}2$. However, the latter would overestimate low and underestimate high values in our data set.)

(v) *Statistical methods*

Results are presented as simple means with standard errors computed for that group, because there was substantial heterogeneity in variance between lines and treatments. To test for significance between the groups in a line the Welch test (two-tailed, inhomogeneous variances) was used. To test in experiment II the different reaction of the lines to the treatment (linear combination of all four means: $(\text{CT} - \text{CU}) - (\text{FT} - \text{FU})$) the Welch test was used, but influences were checked using the following model: $Y = \text{M} + \text{T} + \text{L} + \text{TL} + \text{F(L)} + \text{C(L} \times \text{T)} + \text{e}$, where M is an overall mean, T is the fixed effect of the treatment, L is a fixed line effect, TL is the interaction, F(L) is a random family within-line effect, C(L × T) is a random cage within-line × treatment effect and was used therefore to test the interaction (T × L) and e is residual error. Data from chemical analysis were available only on pooled samples, so cage and family effects were not fitted. Food intake was recorded per cage of 3 animals and most cages in the selection line and all in the control comprised a full-sib group, so family was not fitted and cage is the error term. ANOVA was undertaken with GLM using the SAS System for Windows release 6.08 (SAS Institute, Cary, NC).

3. Results

(i) *Experiment I*

In experiment I the treated (FT) animals were slightly leaner than the untreated (FU) animals but there was no significant difference ($P > 0{\cdot}05$) between these groups for any trait (Table 1). Body weights diverged increasingly during the treatment, the FU animals being about 2 g heavier at the end. Although the difference did not reach significance at any age (Fig. 1), the linear regression of the divergence on days (0·07 g/d) was significant ($P < 0{\cdot}05$).

Food intake was lower (but not significantly, $P > 0{\cdot}05$) in the treated animals (Fig. 1), the total intake differing by 4 g, corresponding to almost one day's food (Table 1).

As numbers of mice were small and the dosage of leptin was low (5 mg/kg once daily), the main experiment was conducted with higher numbers and dosage.

(ii) *Experiment II*

Results of experiment II are summarized in Table 2 and Figs. 2 and 3.

(a) *Body weight*

Body weights of the treated and untreated F mice did not differ initially but increasingly diverged after the start of treatment at 91 d, the difference increasing to 4 g ($P < 0{\cdot}05$) at the end of the experiment (Fig. 2*a*).

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Table 1. *Means for leptin treated (FT) and untreated (FU) fat line mice and their differences with standard errors in experiment I*

	FT		FU		FT − FU	
Trait	Mean	SE	Mean	SE	Diff[a]	SE
BW 78 (g)	38·3	1·29	38·8	1·71	−0·5	2·1
BW 98 (g)	38·4	1·62	40·4	1·58	−2·0	2·3
gain 78–98 (g)	0·16	0·63	1·65	0·62	−1·5	0·9
ave FI 78–98 (g/d)	5·2	0·17	5·4	0·86	−0·2	0·9
GFPW/BW (mg/g)	32·4	4·3	36·5	3·7	−0·41	5·7
pred Fat (%)	18·0	2·25	19·2	1·61	−1·2	2·8

BW xx, body weight at xx days; FI xx, food intake between xx and yy days; ave, average; GFPW, gonadal fat pad weight at 99 days; pred, predicted from dry matter.

[a] All differences in the means were non-significant ($P > 0{\cdot}05$).

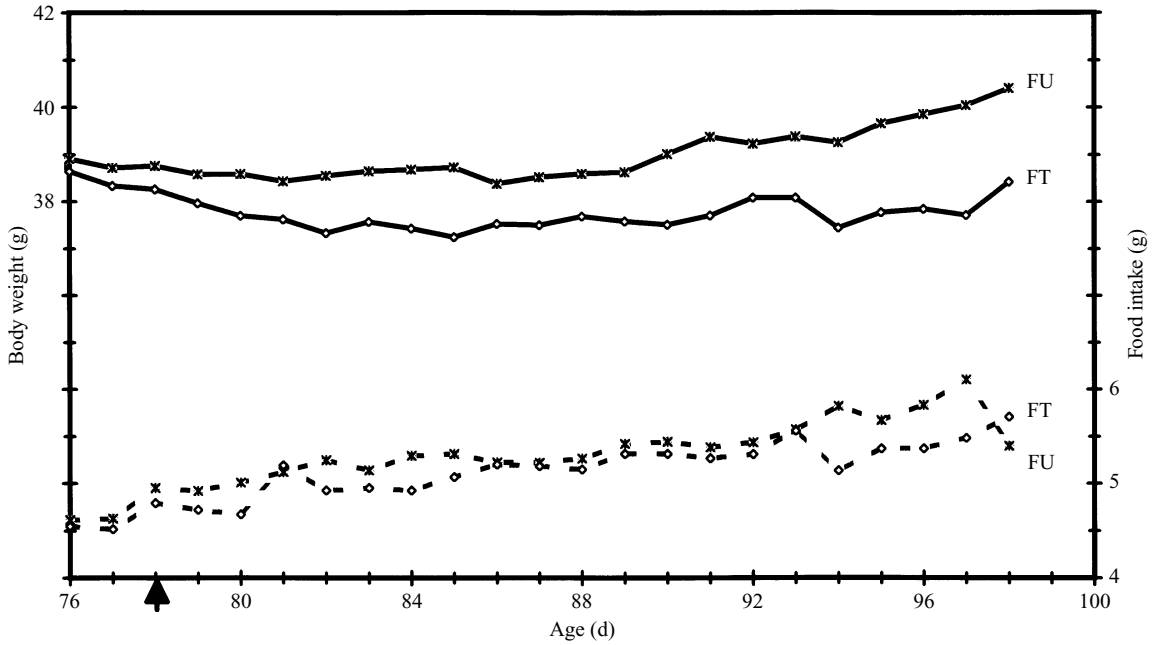

Fig. 1. Development of average body weight (continuous line) and daily food intake (dashed line) in experiment I. The start of treatment is marked by an arrow.

The change in body weight from the start of the test (91 d) differed significantly between the groups from day 4 onwards and amounted to about 9 % at the end (Fig. 4).

In the C line, by chance the treated group was heavier on average at the start. During the injection period the leptin-treated animals lost weight and became lighter than the untreated (Fig. 2*b*), but not significantly so. The deviations from initial weight (Fig. 4) show that the CU animals increased in weight, while the CT animals decreased in weight by about 6 %. From day 1 onwards the divergence in weight gain for the deviations from the baseline between leptin-treated and untreated animals in both lines is significant. The pattern of change in weight is that for the C line the divergence in weight increased, but stabilized, whereas for the F line it continued to increase (cf. Fig. 2*a* and *b*).

(b) *Food intake*

The food intake in both lines decreased as a result of leptin administration (Fig. 3a, b). The difference between treated and untreated F line animals amounts to about 1 g, significant ($P < 0{\cdot}05$) on most days, but there was an unexplained increase in food intake in the FT group at days 10 and 11. The food intake in the treated C line was significantly lower from day 3, but it began to increase at day 7 and thereafter reached a level similar to that in the untreated group. The pattern therefore differs: a continued reduction in the F line and a temporary reduction in the C line.

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Table 2. *Means for leptin-treated (T) and untreated (U) fat line (F) and control (C) mice, their differences and standard errors in experiment II*

	Control line (C)						Fat selected line (F)						Effect in F vs in C	
	CT		CU		CT−CU		FT		FU		FT−FU		(CT−CU)−(FT−FU)	
Trait	Mean	SE	Mean	SE	$Diff_C$	SE	Mean	SE	Mean	SE	$Diff_F$	SE	$Diff_{C-F}$	SE
BW 91 (g)	31·1	0·51	29·1	0·63	1·97*	0·81	42·8	0·97	42·8	1·11	−0·01	1·48	1·98	1·68
BW 105 (g)	29·6	0·50	30·7	0·62	−1·06	0·80	39·1	1·15	43·0	1·15	−3·90*	1·62	2·84	1·81
gain (g)	−1·50	0·15	1·53	0·20	−3·03*	0·26	−3·69	0·30	0·20	0·31	−3·89*	0·43	0·86	0·50
BW 105/BW 91 (%)	95·2	0·50	105·4	0·74	−10·2*	0·89	91·05	0·85	100·5	0·72	−9·45*	1·11	−0·76	1·43
BW 106 (g)	25·9	0·44	27·3	0·58	−1·34	0·73	36·5	1·02	39·9	1·06	−3·41*	1·47	2·06	1·64
cum FI 91–105 (g)	53·2	1·37	59·8	0·98	−6·66*	1·69	62·0	3·86	71·0	2·07	−8·99	4·38	2·33	4·69
ave FI 91–105 (g/d)	3·8	0·10	4·3	0·07	−0·48*	0·12	4·4	0·28	5·1	0·15	−0·64	0·31	0·17	0·34
GFPW (mg)	60	12	360	28	−300*	30	1090	102	1630	92	−540*	137	240	141
GFPW (mg/g)	2·7	0·4	13·1	0·9	−10·4*	0·97	28·4	2·3	40·0	1·4	−11·7*	2·70	1·2	2·9
chem Fat (g)	0·77	0·07	2·04	0·30	−1·27*	0·31	5·54	0·79	8·48	0·51	−2·94*	0·95	1·67	1·00
chem Fat (%)	3·00	0·29	7·37	0·81	−4·37*	0·86	14·94	1·66	21·15	0·71	−6·20*	1·81	1·83	2·00
chem ffBW (g)	25·1	0·58	25·2	0·8	−0·09	0·98	30·8	0·88	31·5	0·71	−0·7	1·13	0·6	1·50

Sample sizes: $n = 25, 29, 30$ in CT, CU, FT and FU for all BW and GFPW; $n = 10$ for FI traits ad $n = 6$ for chem Fat.
cum, cumulative; chem, data based on chemical analysis; ffBW, fat-free body weight; other abbreviations as in Table 1.
* Significant differences ($P < 0·05$).

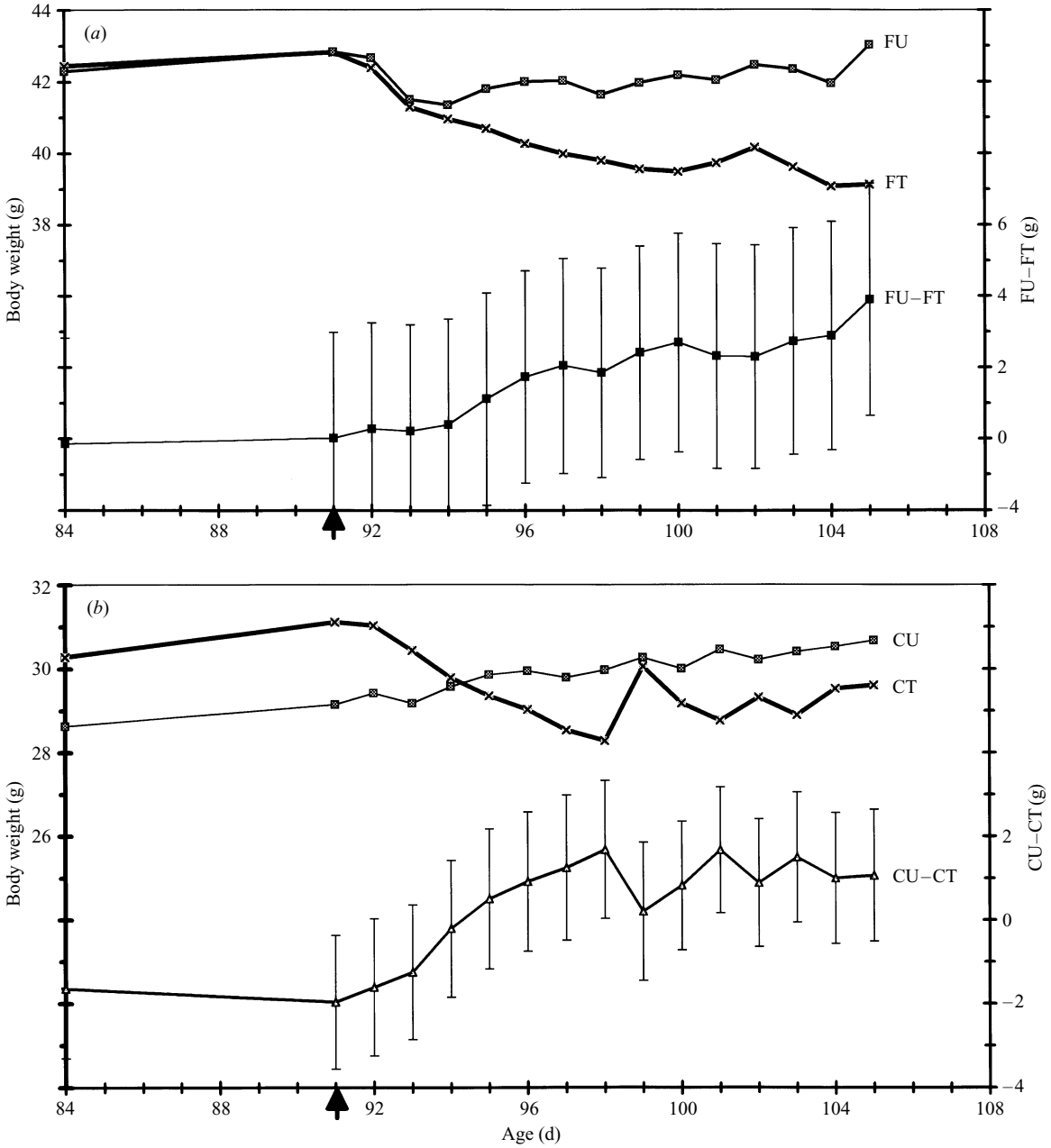

Fig. 2. Development of average body weight in the fat line (*a*) and control line (*b*) in experiment II. The difference FU − FT or CU − CT is given with the 95% confidence interval. The start of treatment is marked by an arrow.

Fat content

Leptin treatment significantly reduced fat content in each line (Table 2). Results of analyses of gonadal fat pad give broadly similar results to those from dry matter content and chemical analysis, except that in the leptin-treated control group (experiment II, Table 2), gonadal fat pad weight is much less than 15–20% of total fat weight. Fat-free body weight has been little affected and the loss in body weight during the treatment has come from loss in fat. The weight of fat has been reduced by leptin administration from 8·5 g (21%) to 5·5 g (15%) in the selected lines and from 2·0 g (7%) to 0·8 g (3%) in the control line (experiment II). There has therefore been a greater absolute but smaller proportional loss in the F line.

Fat% estimated from DM is given for individual mice at the end of the experiment. In the C line (Fig. 5*a*) the range in leptin-treated mice is somewhat smaller than in the untreated animals, the means ± SD being 3·29% ± 1·5% and 7·23% ± 3·7% respectively. In contrast in the F line (Fig. 5*b*) the range of estimated fat% in the treated animals (3–29%) is much greater than in the untreated (15–31%), with the corresponding means ± SD being 14·8% ± 6·7% and 21·3% ± 4·1%. This indicates some mice responded little or not at all to the leptin.

4. Discussion

In both experiments there was a response to leptin in the F line, but it was small in experiment I, presumably

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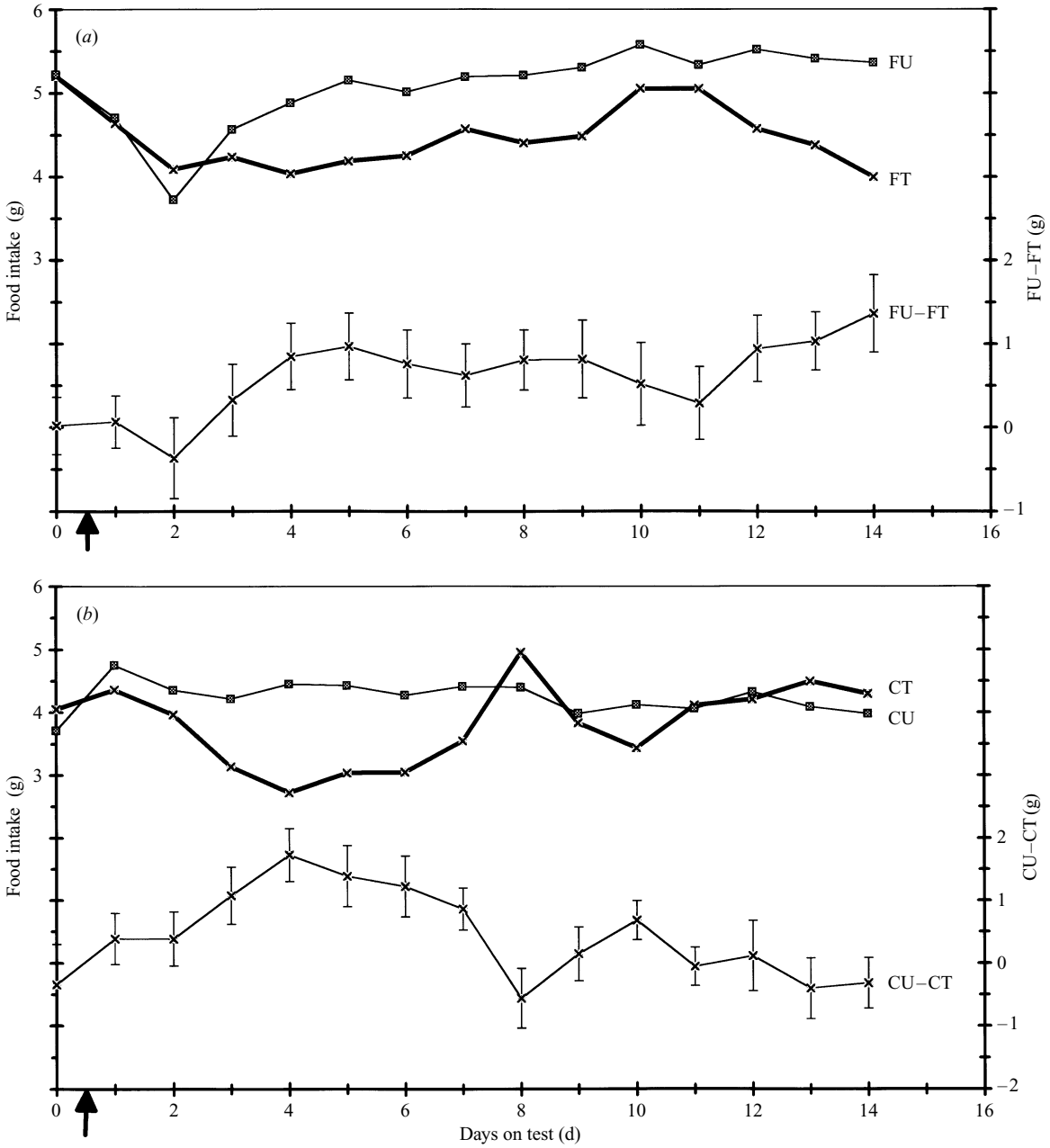

Fig. 3. As Fig. 2 but for daily average food intake.

due to an insufficient dose. Both the F and C lines responded to the leptin treatment with a decrease of body weight, fat content and food intake, indicating that selection has not blocked all or perhaps any receptors to leptin. There is an indication that most of these effects occurred in the C line within a few days, but continued in the F line over the test period, judging by the growth and food intake graphs (Figs. 2, 3).

(i) *Pattern of body weight development*

There is a clear asymptotic development of body weight decrease in the CT group, which could be due to the exhaustion of the fat reserves, an average of only 0·8 g (3·3 %) remaining at the end of treatment. The development of body weight in the FT group tends also to be non-linear, but in the light of the extreme variation of fat content in the FT group (Fig. 5*b*) the development of an average body weight in this group seems a little doubtful. Some animals may have a similar pattern to that in the control: body weight loss until 'exhaustion' of the fat reserves. This would take longer in F animals because they have 'more to lose' and the development in them would be nearly linear, at least for the duration of our experiment.

To check the pattern of change an exponential model $[y = \alpha - (\alpha - \gamma)\exp((-\beta t)/(\alpha - \gamma))]$ was fitted to the body weights (y) on days on test (t) for all

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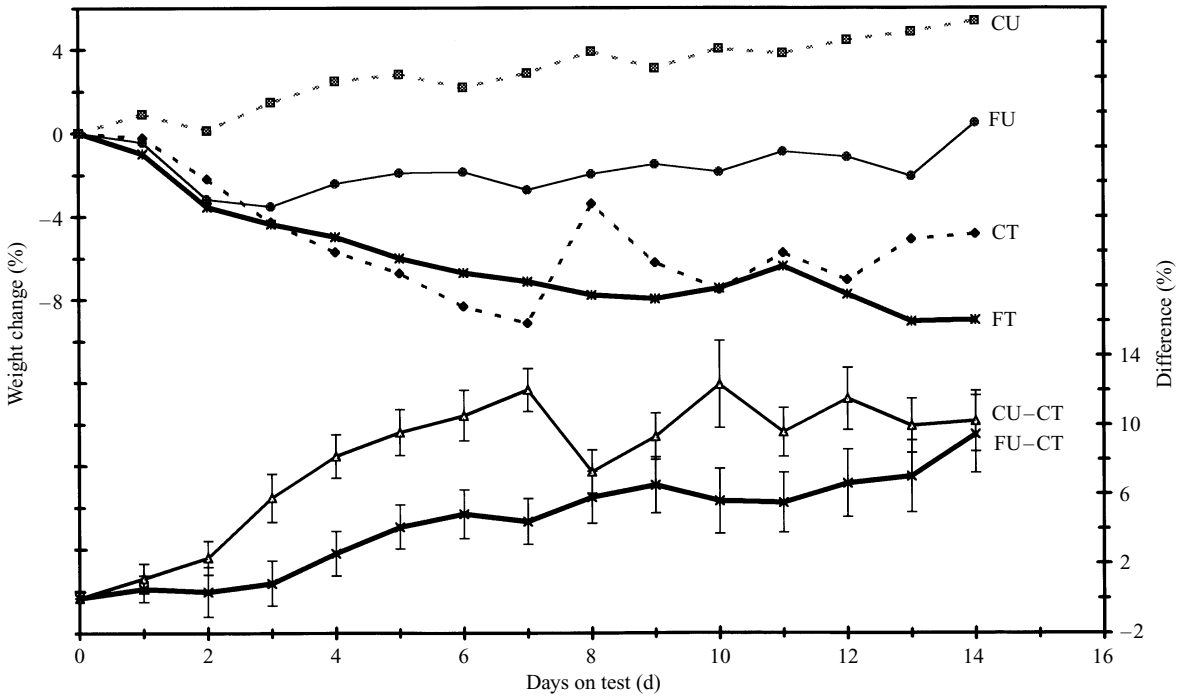

Fig. 4. Effects of leptin treatment on body weight in the fat and control lines, expressed as change (%) from the body weight at 91 days of age (= test day 0) and their differences, with 95% confidence interval.

animals in the leptin-treated groups during the injection period (total number of observations: $n_{FT} = 435$, $n_{CT} = 375$). The exponential model fitted the body weight better than the linear model in both cases, significantly so only in CT animals, indicating a clear asymptotic development of body weight in the CT group and a tendency for a diminishing body weight decrease in the FT group. The fit of an individual exponential curve to each group was not significantly better than a four-parameter model including only a proportion factor f to distinguish between the groups ($\alpha_{FT} = f\,\alpha_{CT}$, $\beta_{FT} = f\,\beta_{CT}$, $\gamma_{FT} = f\,\gamma_{CT}$). This implies that leptin has a similar proportional effect in the treated C and F lines, but about 1·4 times greater effect in the F line. Therefore these fitted values are presented together with the observed values as curve (*a*) in Fig. 6. Assuming the ratio 'body weight after 18 h starvation/body weight fed animal' (0·896 for the CT group and 0·924 for the FT group estimated at the end of the experiment) is constant during the test period, the curve for fed animals can be transformed into a curve (*b*) describing the weight development for starved animals (Fig. 6).

(ii) *Prediction of initial fat% values*

Initial (91 d) values for fat content for the experimental groups were not available, but would shed some more light on the situation. Unfortunately there is no reliable method available for measuring the fat content in individual live mice and, because of the high within-litter variation, measurement of full sibs seems also of doubtful value. Some information may, however, be gathered from the change in body weight of treated animals during the experiment.

If, because animals were mature, it is assumed that the fat-free body weight (ffBW) does not change very much during the test period from 91 to 105 days, the area between curves (*b*) and (*c*) (= ffBW estimated using 106 d data) in Fig. 6 reflects the amount of fat. The average initial weight of fat in the FT and CT groups thus estimated would be about 8·8 g and 3·0 g, respectively, which agrees roughly with the weights of fat in the untreated groups at the end (8·5 g and 2 g), noting that the CT animals were by chance heavier (Fig. 2*b*) and perhaps fatter than the CU animals at the beginning of treatment. The mean body weight depressions in the FT and CT groups, predicted from the fitted curves for starved animals, were 3·2 g and 2·5 g and should represent the fat loss. The fat losses from the comparison FU − FT and CU − CT were 3 g and 1·1 g respectively, which correspond with these values reasonably well but again are a slight underestimate in the C groups, perhaps because of lower initial weights.

There were significant ($P < 0{\cdot}05$) positive correlations between the change in body weight (g) and fat (g) at 106 d in both the FT group ($r = 0{\cdot}50$) and CT group ($r = 0{\cdot}49$), the regression coefficients being about 1 g and 0·3 g fat per 1 g body weight, respectively. The regression coefficient of about 1 g/g in the FT group implies that the body weight loss is nearly equal to the loss in weight of fat, so if this applies to both treated and untreated F groups, an approximate individual fat content for the start of the

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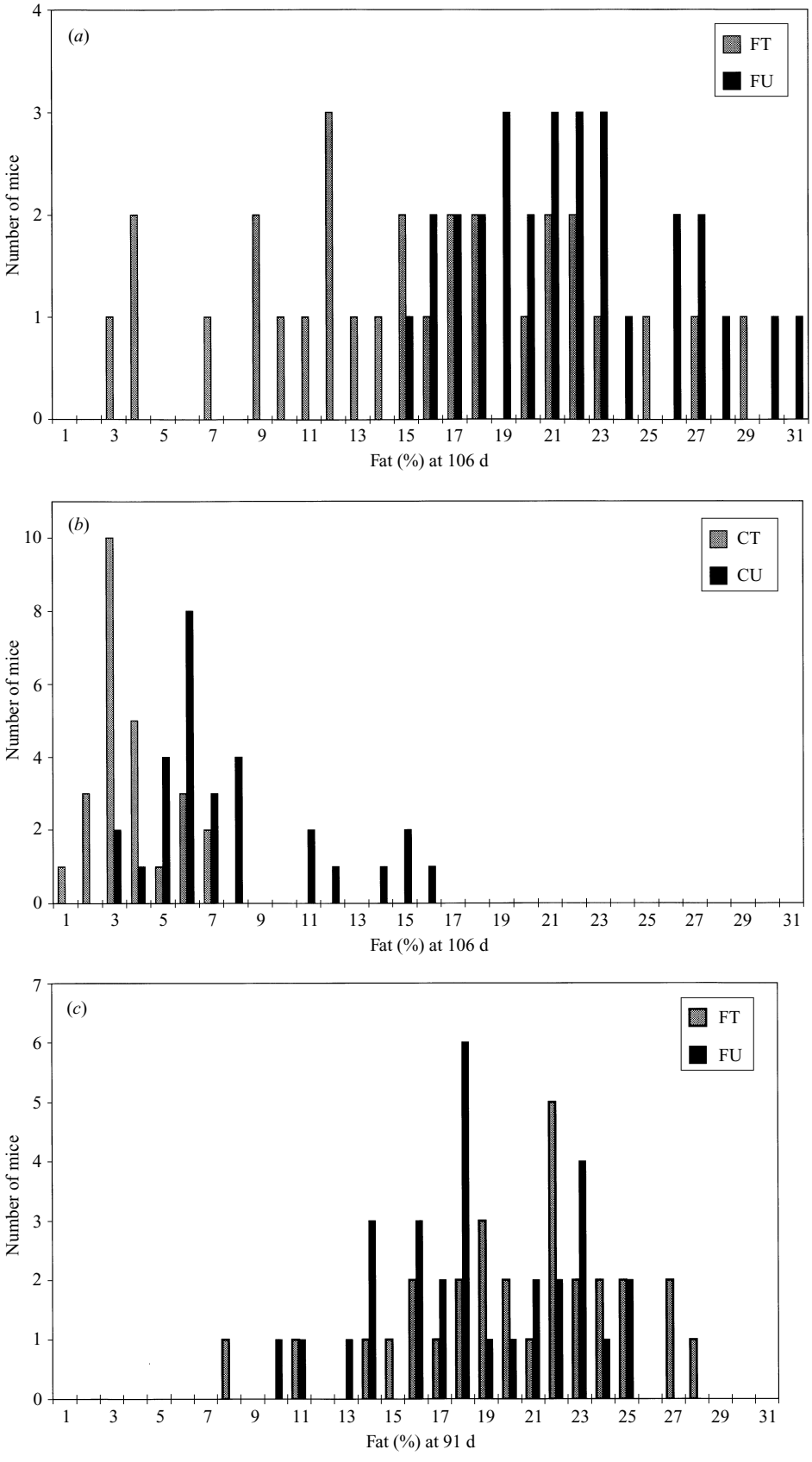

Fig. 5. Frequency distribution for fat percentage of individual mice predicted from dry matter content. (*a*) Fat line at the end of the experiment (106 d). (*b*) Control line at the end of the experiment (106 d). (*c*) Fat line at the start of the experiment (91 d) computed as: fat weight$_{106}$ − body weight gain$_{91\text{to}105}$.

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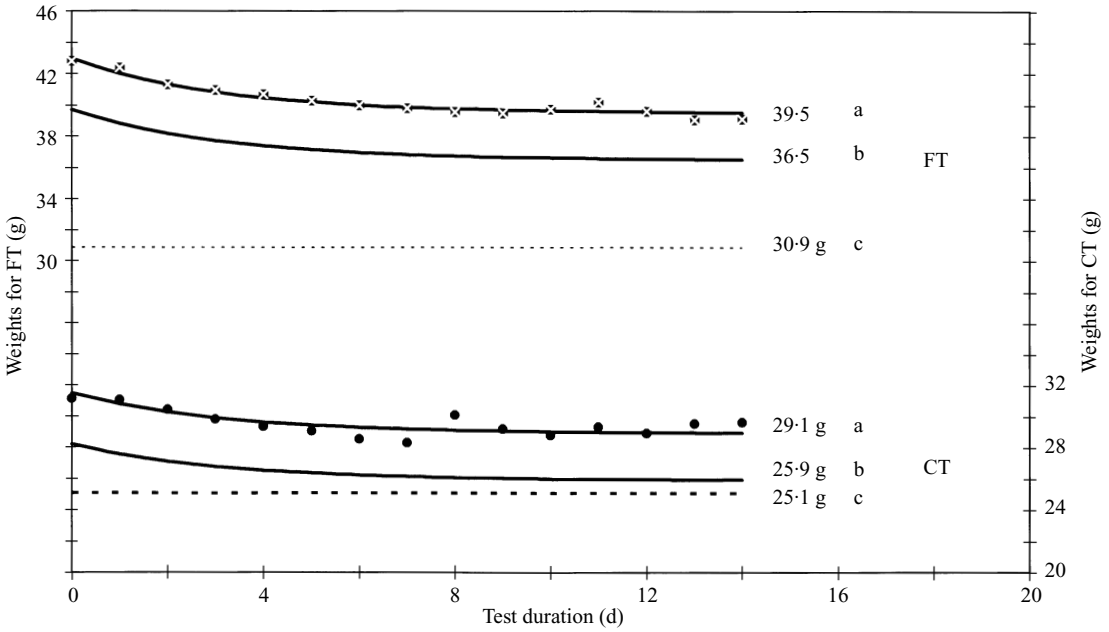

Fig. 6. Body weight development in the leptin-treated groups during the injection period. (*a*) Observed means for body weights (*y*, including filled digestive tract) and the fitted line (exponential model). (*b*) 'Empty' body weight, assuming the ratio 'body weight after 18 h starvation/body weight fed animal' (0·896 for CT and 0·924 for FT animals; estimated at the end of the experiment) is constant. (*c*) Fat-free body weight estimated from the end of the experiment and assumed to be constant over the test. The difference between (*b*) and (*c*) should reflect the mean fat amount.

experiment can be obtained from the difference between the fat amount at 106 d and the observed body weight change. This would predict an initial fat content (±SE) of 9·1 g±0·5 (20 %±0·9) and 8·5 g± 0·5 (18 %±0·7) in FT and FU groups, respectively (not significantly different from each other, $P > 0{\cdot}05$). Such an approach seems to be appropriate for the FT group but the fat amount in the FU group could be somewhat underestimated. Whereas all animals from the FT group lost body weight, some FU animals gained throughout the experimental period (Table 2). As a previous study has shown that mice of about 100 d are still increasing slowly in fat-free body mass (Hastings *et al.*, 1991), the regression coefficient for the FU animals should be lower than 1 g/g. Thus the predicted fat for the FU animals with a positive gain may be underestimated.

These predicted initial fat values provide a check of a possible relation between the initial fat weight and the susceptibility to leptin, as measured by weight loss. This correlation in the FT group was equal to zero, suggesting independence of the resistance/susceptibility to leptin and the initial fat amount.

(iii) *Frequency distribution for fat%*

The predicted values of initial fat can be used to elucidate whether the high variation for fat% in the treated F animals is produced by the leptin treatment or was already higher at the beginning of the experiment. In Fig. 5 it was shown that the range for fat% at the end of the experiment in F animals is very strongly increased by the leptin treatment: the leanest mice have 3 % fat and the fattest 29 %, whereas the range is 15–31 % in the untreated mice. There were still 8 animals (of 30) in the FT group with over 20 % fat, suggesting they responded little or not at all to the leptin treatment. Continued treatment could have led to further reduction of the fat content in the 'still fat' mice, but this has yet to be tested. Some animals could have a lower susceptibility to leptin, as suggested by the histogram for fat% (Fig. 5*b*).

FT animals lost 3·7 g body weight on average during the treatment, whereas there was almost no change in the FU group. The variance for these gains in the treated F group suggests that the treated animals which still had a high fat content may also have responded, at least a little. Otherwise we could expect a much higher variance for the gain in the FT compared with the FU group, but the standard deviations are nearly the same (cf. standard errors in Table 2).

To shed some light on this increase in variance of fat% at the end of the treatment in the F line (Fig. 5*b*) the distribution of fat% at the start of the experiment, estimated from final fat and gain, is presented in Fig. 5*c*. Although fat% in FU animals is probably somewhat underestimated, the predicted distributions are, as expected, very similar, suggesting that the enormous increase in the variance is produced by the leptin treatment. Therefore some animals must have reacted strongly to the treatment whereas other animals seem to have been relatively resistant. It is, perhaps, surprising to find so much variation after

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more than 50 generations of selection for fatness, which would, in turn, put selection pressure on leptin sensitivity. The question arises as to whether this variation could be exploited by selection for 'leptin responsiveness' and for 'leptin resistance' in the F line, from which could be expected good models for human obesity. Analysis of variance, however, indicated that most of the variance for fat% in leptin-treated animals was within litters, indicating either a high degree of environmental variance or segregation of genes which could not be fixed by selection. Leptin treatment might therefore be useful in a quantitative trait loci analysis in this line, with loci for resistance and susceptibility against leptin mapped more efficiently than without treatment.

One important question remains: How would the selected animals, and in particular the 'more resistant' animals, react to a longer and/or stronger treatment? The observed losses of animals, in particular in the CT group, as a negative side effect of leptin treatment, indicate a certain danger of an overdose of leptin in probably susceptible and (already) relative lean animals. Whereas in the untreated control group all animals survived, five of the treated animals died during the injection period. One of them died on the second day, and is excluded from the following consideration. Four other animals died after 6–11 days. All had lost weight: the mean gain, estimated from their final live weight, was $-3{\cdot}5$ g (over a shorter period), and range $-1{\cdot}9$ g to $-5{\cdot}9$ g. The mean gain of all surviving animals averaged $-1{\cdot}5$ g over 14 d, range $+0{\cdot}2$ g to $-3{\cdot}3$ g, suggesting that the animals which died during the treatment showed a strong reaction to leptin. These animals did not differ in their initial mean weight at 91 d (31·0 g) from the mean of the other 25 animals (31·1 g). An attempt to predict mean fat% from day matter content for these animals gives very low fat% values, but this is imprecise because biological processes may have influenced dry matter content before the bodies were found ($\leqslant$ 12 h) and stored in the freezer.

Future experiments on the mouse lines used in this study, and in addition on the lean line (see Section 1), should involve an analysis of levels of endogenous circulating leptin, from which further elucidation of resistance/susceptibility to leptin and its contribution to the selection response can be expected.

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