THE ROLE OF LEPTIN PRODUCTION AND RECEPTION FOR THE DIVERGENCE OF MOUSE LINES SELECTED ON HIGH AND LOW FATNESS - INTROGRESSION OF *Lep^ob* AND *Lepr^db*

L. Bünger[1], S. Horvat[2], J.R. Speakman[3] and W.G. Hill[1]

[1]Institute of Cell, Animal and Population Biology, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JT, UK,
[2]Biotechnical Faculty, Zootechnical Department, University of Ljubljana, Groblje 3, 1230 Domzale, Slovenia,
[3]Department of Zoology, University of Aberdeen, Aberdeen, AB24 2TZ, and Division of Appetite and Energy Balance, Rowett Research Institute, Bucksburn, Aberdeen, AB21 9BS, UK,

INTRODUCTION

Obesity is a common multifactorial disorder and is a major disease risk in man. The problem of excess fat in livestock and poultry is ubiquitous (Eisen, 1989) and causes high financial losses. Although remarkable progress has been made in identifying and characterising the genes mutated in monogenic mouse strains, efforts to identify genes in humans and farm animals with major effects on body fatness have been only partially successful. The cloning of the Lep^{ob} gene in mice (Zhang *et al*., 1994) opened a new area of research into the function of leptin as a 'starvation' factor. Recently, the lipostatic interpretation of its role as a 'starvation signal' has been questioned, suggesting that other independent regulatory systems may be important in controlling body fatness (Fruhbeck and Gomez-Ambrosi, 2001).
Here we investigate the role of leptin production (LepProd) and reception (LepRec) in lean (L) and fat (F) selected lines of mice in which we introgressed the two recessive mutations Lep^{ob} and $Lepr^{db.}$ These lines were produced by over 60 generations of divergent selection on fat content and differ four-fold in amount of body fat. A genome-wide quantitative trait locus (QTL) analysis of a cross between the F and L lines revealed four major QTLs (Horvat *et al*., 2000), but none mapped to regions of known single gene obesity mutations, including Lep^{ob} and $Lepr^{db}$. A study on sensitivity to leptin on the F-line males (Bünger and Hill, 1997) revealed that sensitivity to leptin remained in the F line, suggesting that the selection had altered a separate pathway for regulatory control over body fatness. To further investigate this possibility we analysed F and L lines into which we had introgressed the recessive mutations Lep^{ob} and $Lepr^{db}$ (formerly named *ob* and *db*) that cause LepProd and LepRec deficiency, respectively.

MATERIAL AND METHODS

Mice. Divergent selection on fatness at 70 and later at 98 days of age (d) was initiated in 1980 (Sharp *et al*., 1984). It gradually generated (heritability = 0.5) two highly divergent lines : F with 22% and L with 4% total body fat in males (Bünger and Hill, 1999), which were then inbred. Here we introgressed the recessive mutations Lep^{ob} and $Lepr^{db}$ separately into the inbred F and L lines by repeated backcrossing, and made intercrosses to generate families segregating for all three genotypes. Thus we had available F-line mice, designated F*ob/ob*,

F*ob/+*, F*db/db*, F*db/+* which were homozygous or heterozygous for the respective mutation, and wildtype (wt) F+/+ mice. The same groups were available for the L line. Analysis of overall body fatness, food intake, circulating leptin and energy budgets were assessed in these segregating litters and results reported elsewhere (Bünger *et al*., 2002, submitted). In this paper the focus is on the body weight and a major fat depot, the gonadal fat depot.

Carcass dissection and analysis. 291 animals (table 1) were killed by cervical dislocation at 106 ± 7d (sd). Body weight was recorded and the gonadal fat pads were weighed and later on added to obtain total body fat. The dry matter weight (DM) of the remaining carcass was determined by freeze-drying. Fat percentage (fat%) was predicted from the regression equation of fat% on DM derived previously (Bünger and Hill, 1997).

RESULTS AND DISCUSSION

Body weight development. Prior to 30d, line and genotype effects on body weight were small, but afterwards became increasingly clear (figure 1). Heterozygous effects were relatively small, although significant at some age points, showing that both mutations are not truly recessive.

At 106d the female F controls (F +/+) were about 10g heavier (35.5g vs.25.1g) than the L+/+, which is mainly fat (table 1) and the result of the selection applied. When the LepProd or LepRec is deficient, the body weights of all lines, but especially of F*ob/ob* and F*db/db* females, reached very high and similar weights of 65 to 68g.

Table 1. Body composition at 106d

Males

Traits	F *ob/ob*	F *ob/+*	F *db/db*	F *db/+*	F +/+	L *ob/ob*	L *ob/+*	L *db/db*	L *db/+*	L +/+	sd§
n	24	24	5	22	35	14	17	13	13	21	
Fat (g)	18.8^{c}	9.32^{ef}	17.2^{c}	10.8^{de}	8.50^{fg}	18.0^{c}	3.53^{h}	12.7^{d}	2.40^{h}	1.25^{h}	3.6
n	17	16	5	17	21	14	12	13	9	14	
GFPW (g)	2.82^{b}	1.72^{cd}	1.91^{c}	1.73^{cd}	1.56^{d}	2.10^{c}	0.34$_{ef}$	1.55^{d}	0.19^{f}	0.13^{f}	0.19
GFPW/BW (%)¶	5.08^{b}	3.91^{c}	4.26^{bc}	4.05^{c}	3.80^{c}	4.30^{c}	1.01^{f}	3.65^{cd}	0.68^{f}	0.55^{f}	1.21
GFPW/Fat (%)*	13.9^{c}	19.3^{ab}	11.7^{cd}	17.0^{b}	19.6^{ab}	12.3^{cd}	9.8^{d}	12.3^{cd}	8.3^{d}	9.6^{d}	5.1

Females

Traits	F *ob/ob*	F *ob/+*	F *db/db*	F *db/+*	F +/+	L *ob/ob*	L *ob/+*	L *db/db*	L *db/+*	L +/+	sd§
n	17	14	2	9	15	8	8	9	11	10	
Fat (g)	27.4^{a}	7.73^{g}	24.1^{ab}	9.08^{ef}	6.25^{g}	23.4^{b}	2.85^{h}	18.7^{c}	2.19^{h}	2.36^{h}	3.8
n	9	4	2	2	5	7	3	7	3	5	
GFPW (g)	4.46^{a}	1.31^{d}	4.60^{a}	1.14^{d}	1.75^{cd}	2.95^{b}	1.09^{de}	4.04^{a}	0.10^{f}	0.65^{ef}	0.36
GFPW/BW (%)¶	7.04^{a}	3.36^{cd}	7.32^{a}	3.61^{cd}	4.54^{bc}	5.22^{b}	2.53^{de}	8.49^{a}	0.87^{ef}	1.47^{ef}	1.27
GFPW/Fat (%)*	16.0^{bc}	17.1^{bc}	20.2^{ab}	13.3^{bc}	24.3^{a}	11.1^{cd}	17.0^{b}	20.0^{ab}	6.4^{d}	10.4^{cd}	5.2

§sd averaged over groups; GFPW gonadal fat pad in (%)¶of body weight and *total body fat. Means sharing a common character in their superscript are not significantly different ($P > 0.05$); pairwise comparison were made involving all 20 groups- compare corresponding row from males and females

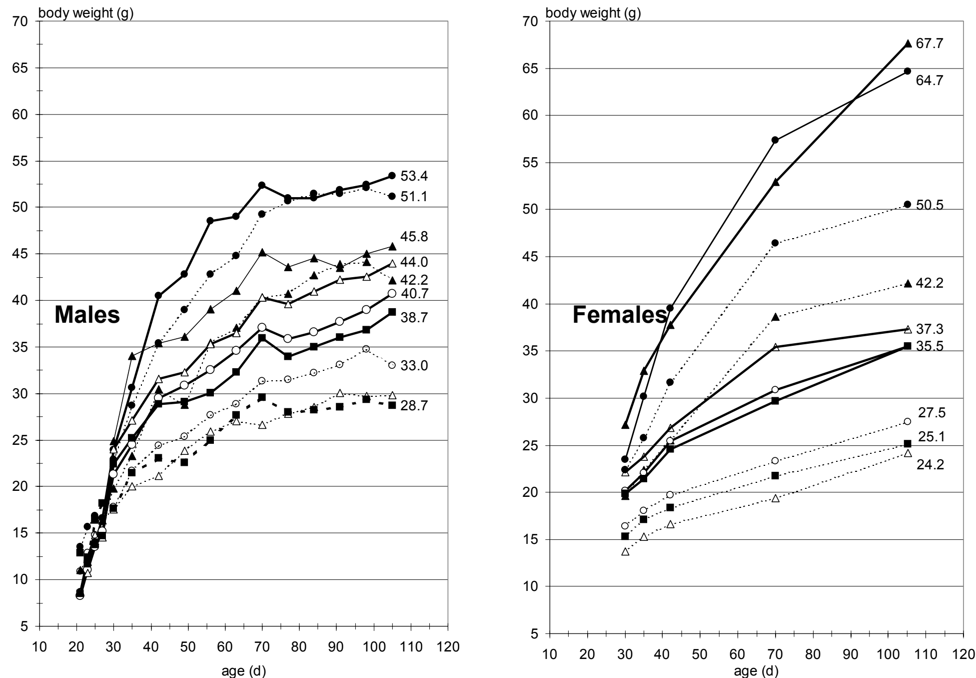

Figure 1. Least square means for body weights from 21 (males) or 30 (females) to 106d, from records on 18 males and 13 females on average per age and genotype

L*ob/ob* and L*db/db* females were also much heavier than L+/+ and clearly heavier than F+/+, but the L- background seemed to prevent the excessive weights reached in both homozygous L groups. In L females the LepRec deficiency (L*db/db*) was less severe than LepProd deficiency (L*ob/ob*). Similar group differences but of lower magnitude were seen in males. However, both homozygous L groups in males reached similar final weights to their F counterparts, but their body weights before 70-80d were well below the homozygous F groups, indicating a later onset of obesity. The LepRec deficiency in males was less severe than LepProd deficiency in both lines. A substantial sex x genotype interaction resulted from the much stronger reaction of females.

Fat content and its distribution. The female wild types of F and L differed in body fatness at 105d on average by 3.9g and the males by 7.2g (table 1). When LepRec was inactivated (F*db/db* and L*db/db*), the body fat contents of both lines increased dramatically, but the difference between the lines was preserved (5.4g in females and 4.5g in males). LepProd deficient mice (F*ob/ob* and L*ob/ob*) were also very fat compared with wild type mice. The difference in fat amount between lines was preserved in females (F*ob/ob* 4.0g higher than

L*ob/ob*) but not in males (F*ob/ob* only 0.8g higher than L*ob/ob*). Heterozygotes for LepProd or LepRec (F*ob*/+, Fdb/+, L*ob*/+ and L*db*/+) had normal body fat contents, relative to wild-type, and the differences between lines were also preserved in each of these cases. The differences between F and L lines were 4.9 and 5.8g for *ob*/+ females and males respectively, and 5.4 and 4.5g for *db*/+ females and males, respectively. Therefore, of 8 comparisons between the two introgressed genotypes in the L and F lines in both sexes, the difference between F and L genotypic classes was preserved in 7 cases. These results are discussed in more detail by Bünger *et al*. (2002, submitted).

The gonadal fat pad (GFP) is one of the biggest single fat depots, comprising 0.6 to 8.5% of the body weight or about 6 to 24% of the total body fat (table 1) with substantial line difference (F+/+ 20-24%, L+/+ 10%). This is probably due to the selection history of the lines, as selection for the first 20 generations was on GFPW/BW in males and afterwards on total body fat predicted from DM%. The GFPW of F++ males (1.6g) is 1.4g or 12-fold higher than of L++ males (0.13g) (and 3-fold in females). The differences between F and L homozygous mutant groups, is much smaller, 0.4 and 0.7g in males, due to a larger increase of GFPW in homozygous L males. It is of note that GFPW/total body fat (%) has increased in LepRec and LepProd deficient L males, but decreased in homozygous F males. A similar picture emerges in females, but is less clear as fewer females were dissected. Conclusions drawn from one fat depot GFPW, about body fatness could be misleading as line differences in weight of total body fat are mostly conserved independently of the introgression of both mutations. This is somewhat unexpected as the overall correlation between GFPW and fat (g) ($r = 0.88$, $n=182$ over all groups) and the within group correlations are relatively high (L line: $r = 0.60$ in the mutant homozygotes and 0.69 in others; F line: $r = 0.85$ and 0.79, respectively). This indicates a different reaction of different fat depots in response to both deficiencies: fat depots other than GFP are more favoured in F animals deficient in LepProd and LepRec, whereas animals from the L line deposit more fat as GFP.

In conclusion, the difference between F and L lines in total body fat was maintained after introgression of the mutations that knocked out the action of leptin. This suggests that genes responsible for the line divergence act independently of the leptin regulatory system and that multiple pathways regulate fatness, perhaps independently responsive to intervention.

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