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Fat storage capacity in growth-selected and control mouse lines is associated with line- specific gene expression and plasma hormone levels

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OBJECTIVE: For a detailed understanding of the complex traits growth and fat storage, a dissection into single genetic entities is mandatory. Therefore, blood plasma concentrations of hormones and the expression of selected genes were measured in extremely differentiated mouse lines. Genes were selected as candidates which might influence the complex traits body weight and fat accumulation, and which are located in chromosomal regions recently identified to affect trait differences between the lines.

SUBJECTS AND MEASUREMENTS: The mouse lines were selected for high body weight (DU6), high carcass protein content (DU6P) and unselected controls (DUKs). In the selected lines DU6 and DU6P, mean body weights at the age of six weeks were about twice as high as the DUKs, whereas total fat weight was increased 2.2-fold in DU6 mice compared to DU6P and 3.2-fold in comparison to DUKs. Blood plasma concentrations of insulin-like growth factor 1 (IGF-1), growth hormone (GH), insulin and leptin, were measured in all lines at three weeks and at six weeks of age. Expression patterns of the genes encoding growth hormone (*Gh*), insulin-like growth factor 1 (*IGF-1*), lipoprotein lipase (*Lpl*), glycerolphosphate dehydrogenase 1 (*GDC-1*), and adipocyte protein 2 (*Ap2*) were analyzed by Northern blot hybridization.

RESULTS: In line DU6, highly significant increased concentrations of insulin and leptin were observed at six weeks of age; at this stage, IGF-1 concentrations were elevated in the two selected lines compared to controls with maximal concentrations of IGF-1 and GH in DU6P. The amount of mRNA for GH in the pituitary gland, for IGF-1 in the liver and for LPL in epididymal fat tissue was significantly elevated in the two selected lines compared to controls at the age of three weeks, but not at six weeks. IGF-1 and GDC-1 mRNA concentrations were significantly higher in the DU6 mice than in the DU6P ($P \sim 0.01$) and the DUKs ($P < 0.001$) mice examined at both ages.

CONCLUSIONS: The results prove line-specific concentrations of the analyzed hormones and the transcription amounts of *Gh, IGF-1, GDC-1* and *Lpl*. The measured differences are either direct genetic effects or secondary changes, resulting from different food consumption.

Keywords: growth hormone; insulin-like growth factor-I; leptin; insulin; glycerolphosphate dehydrogenase 1; lipo-protein lipase

Introduction

Growth and fat storage in mammals are complex traits, and the genetic contribution to the continuous phenotypic variability is, in general, controlled by multiple loci. In many studies on lines selected for growth and/or fat content, correlated responses in body composition, enzymes and hormones have been measured.[1-3] For a detailed understanding of the genetic bases of these phenomena, further research, employing different experimental models, is mandatory. One promising approach is based on directed selection in an initially diverse population, which results in increased frequencies of those alleles which favour the desired phenotype. Thus, selected populations provide a possibility to assess the genetic background underlying phenotypic changes. For fat accumulation, involvement of the genes influencing hormonal status, cellular energy balance, response to endocrine and nutritional stimuli, and the lipogenic and storage capacity in adipose tissue can be considered.[4-8] A summary of single gene defects in rodent models of obesity is given by Bray and Bouchard.[9] The mode1 mouse lines used in our investigation were established by long-term selection for high body weight in line DU6 and for high carcass protein amount in line DU6P.[10] Both selected lines have twice as high body weights, accompanied by enhanced protein mass, in comparison to the random-mated control line (DUKs); fat accumulation is strongly increased in the DU6 line selected for high body-weight. By means of marker segregation analysis in crosses between these lines, significant differences were detected in body weight between the genotype groups in several chromosome regions, in particular, on chromosomes 3, 8, 10, 11, and 15.[11-13] On some of these chromosomes, loci affecting fat accumulation were mapped in crosses of different inbred lines.[14]

Several genes which map to the identified chromosomal regions are plausible factors affecting function and development of adipose tissue. Among these candidate genes putatively infuencing the complex quantitative traits are those encoding lipoprotein lipase (*Lpl*, chr 8), glycerolphosphate dehydrogenase 1 (*GDC-l*, chr 15), and adipocyte protein 2 (*Ap2*, chr 3), which contro1 crucial steps in fatty acids uptake in adipocytes, triglyceride synthesis and intracellular lipid storage. These genes have expression patterns characteristic for cell maturity and metabolic condition, and are subject to a complex regulation by hormones and second messengers, in particular, by insulin, glucocorticoids and cAMP, as shown with cultured cells[15,16] and for in vivo models.[17] If these genes contribute to obesity, this is due to a DNA sequence variation affecting expression or function. On the endocrine level, growth hormone (GH) and insulin-like growth factor 1 (IGF-1, which is partially controlled by GH), insulin and leptin are among the most important agents which promote linear growth, preadipocyte proliferation and induce expression of the adipocyte-specific genes including *GDC-1, Lpl,* and *Ap2*.[18-20]

In our analyses, we initially characterized the selected lines DU6 and DU6P, and the control line, DUKs, for their body composition and the blood plasma concentrations of some hormones which have a known relationship with body composition, that is, GH, IGF-1, insulin and leptin. Secondly, we compared the transcript levels for *Gh* and *Igf-1* genes in the pituitary gland and the liver, respectively, in all three lines, in order to analyze their association with the selection response as candidate genes which map to chromosoma1 regions responsible for trait differences between the mouse lines. In the present study, we analyzed two developmental stages: weaning at three weeks and the end of the juvenile phase at six weeks, the age of selection decision.

Subjects and methods

Animals

The study was carried out on mouse lines, differentially selected for high growth parameters in the Research Institute for the Biology of Farm Animals, Dummerstorf, Germany. The initial population was derived from an original crossing of four base (NMRI orig., Han:NMRI, CFW, CF1) and four inbred (CBA/Bln, AB/Bln, C57BL/Bln, XVII/Bln) populations. Selection was performed on more

than 80 generations for the high body weight line DU6 and for the high carcass protein content line DU6P, at the age of six weeks.[10] For the determination of the body protein content, two test males from every litter were randomly chosen at the age of 10 d, and killed at the age of 42d. The standard Kjeldahl-procedure[10] was performed to determine nitrogen in the carcass (without coat, head, digestive tract and legs) from which the protein weight was then estimated. For generating the next generation, full siblings were selected from the 50% pups with the highest protein weight of the two males tested. Population size was maintained at 80 pairs during selection. After 80 generations, the selection process reached an asymptote towards the selection plateau. The population size in both lines was 80 pairs per generation. The litter size was standardized to nine. Offspring were weaned at 21 d. Animals were fed ad libitum with a breeding diet containing 12.5 MJ/kg metabolisable energy, with an average content of 22.5% crude protein, 5.0% crude fat, 4.5% crude fibre, 6.5% crude ash, 13.5% water, 48.0% N-free extract, vitamins, trace elements, amino acids and minerals (Altromin diet 1314, Lage, Germany). Live body weight and the weight of the epididymal fat pads were recorded for each animal.

Counting of adipocytes

To normalize the transcription rates in adipose tissue, the number of adipocytes in the epididyma1 fat pads was compared between the lines. Epididymal fat pads of males were frozen in liquid nitrogen and stored at -80°C. The tissue was cut into 20 µm slices with a microtome cryostat at -30°C (FRIGOCUT 2800, Leica, Nussloch, Germany). Fat cells were microscopically analyzed for diameter using an automated image analyzer (AMBA, IBSB Berlin, Germany). Adipocyte volume was calculated assuming the cell was spherical. The number of adipocytes was calculated from the weight of the tissue, the average cell volume and the density of lipids at 0.927 g/cm^3 .

Measurements of serum proteins

The concentrations of GH, IGF-1, insulin and leptin were measured in circulating blood. Blood was co1- lected by decapitation of either 20 males (for analyses of individual samples) or 80 males (for analyses of pooled samples) of every line at the ages of three weeks and six weeks in generations 80, 81 and 82. Serum samples were prepared by a 10 min centrifugation step at 3000 rpm and stored at -20°C, either separately for every individual (IGF-1, leptin) or combined from 4-8 individuals of the same group (insulin, GH) and analyzed individua11y or as pools. GH concentrations were determined by radioimmunoassay (RIA) in pooled samples according to Sinha et al,[21] in the laboratory of F. Talamantes (University of California, Santa Cruz, CA). The IGF-1 quantification was based on a RIA, in which the interference of IGF binding proteins is blocked by incubation in the presence of excess IGF-2.[22] The antiserum used (no. 878/4) has a 1ow cross reactivity with IGF-2 (< 0.05%). The assay was validated according to the recommendations of Blum and Breier[22] and Bang et al.[23] and was performed as described by Wolf et al.[24] Insulin concentrations were assayed radioimmunologically for 20 animals per line, using the Human RIA-Sax-Insulin™ Kit (Laboratorium Saxoniae GmbH, Sebnitz, Germany). Due to the reduced specificity of the a-human insulin antibody to mouse insulin, the insulin concentrations measured in DU6 and DU6P are given as relative values to the control line (DUKs). Mouse leptin was measured in individual serum samples by the Quantikine™ ELISA (RkD Systems, Wiesbaden, Germany).

RNA isolation and Northern blotting

For each line, the test groups of male mice descending from different litters were subjected to gene expression investigation at the ages of three weeks (31- 35 animals in a group, generations 80 and 81) and six weeks (15 -16 animals in a group, generation 82). Mice were killed by decapitation between 09.00-10.00 h; epididymal fat pads, pituitary glands and liver portions were collected and rapidly frozen in liquid nitrogen. Total RNA was isolated from the entire pituitary glands or from up to 50 mg of tissue sample using RNeasy™ Kit (QIAGEN, Hilden, Germany) by a method

including tissue homogenization, and selective RNA adsorption on silica-gel membranes in the presence of a high-salt buffer system. RNA was quantified spectrophotometrica11y at 260 nm. Estimated amounts of 10 pg (liver) or 5 pg (adipose tissue and pituitary) of total RNA were precipitated with ethanol, separated by electrophoresis on 1% agarose-0.66 M formaldehyde gels and transferred to GeneScreen^{TM} nylon membranes (DuPont NEN, Bad Homburg, Germany) with 10x SSPE buffer for 20h. The RNA sample prepara- tions of the respective tissues from 38-40 mice of the same age representing the three lines DU6, DU6P and DUKs with 12-15 animals each were analyzed simultaneously from one membrane.

Northern blot hybridization

Labelled hybridization probes were generated via standard PCR replacing 95% of unlabelled dCTP by [α-^{32}P]-dCTP (3000 Ci/mmol, Amersham International, Amersham, UK). Primers and annealing conditions for generating the probes are presented in Table 1.

For the β-actin probe, a *Pst I* fragment of the cloned cDNA was labelled by random nonamer priming with [α-^{32}P]-dCTP using a Megaprime^{TM} Kit (Amersham). Probes were purified by gel-filtration on a Sephadex G-50 column (Pharmacia, Frieburg, Germany), denatured and diluted to 0.7-2.0x10^6 cpm/ml with hybridization solution (6xSSC, 0.5 % SDS, 5xDenhardt's solution, 100 µg/m1 herring sperm DNA (Sigma, Deisenhofen, Germany)). Hybridization was performed at 65°C for about 24 h after 1-2 h prehybridization. Filters were washed at 65°C with up to 0.1 x SSC, 0.1% SDS stringency and were then exposed to X-ray films RetinaXBD90 (RETINA, Wolfen, Germany) for autoradio-graphy. Following exposure, the filters were stripped and re-used for subsequent hybridization.

Specific mRNA abundance in individual probes was quantified densitometrically with the ONE-Dscan^{TM} image analysis software (CSPI, Scanalytics, Billerica, MA) as the ratio of integrated optical densities from the specific probe signal to β-actin signal and designated as Relative Densitometric Value (RDV).

Table 1 Hybridization probes

Probe	Primers	Annealing conditions (temperature, time, cycles)
Gdc1; exon 6, 230 bp	AGCCTGTGTCTCTACTCCTCAAA ACATCTCCCTG CCTACCTTTCC	61°C,60s, 5/58°C, 40s, 30
Lpl exon 2, 209 bp	TAAACCAAATAATCCCTCCAA ATCACTCTTACCGTCCATCCAT	57°C,60s, 5/55°C, 40s, 30
Ap2; exon 4, 155 bp	ATGTGTTATGAAAGGCGTGACT ATTGCTTGCTTATTAGTGGAAAA	57°C,60s, 5/55°C, 40s, 30
Gh; exon 3, 416 bp	TAGGGGAATCTTAGCCAATGC AGCCATGACTGGATGAGCAG	60°C, 60 s, 30
Igf-1; fragment of bovine cDNA, 328bp	AGACAGGGGCTTTTATTTCAACAA GTAACTCGTG CAGAGCGAAGGAT	64°C,60s,5/62°C,40s,5/60°C,40s,25

Gdc1 = glycerolphosphate dehydrogenase 1; *Lpl* = lipoprotein lipase; Ap2 = adipocyte protein 2; *Gh* = growth hormone; *IGF-1* = insulin-like growth factor 1.

Statistical analysis
Data were subjected to the Welch-test (two sided) using the SigmaStat software package (Jandel, San Rafael, CA). To identify the group or groups that differed from the others, all pair-wise multiple com- ' parison procedures were carried out. Significant dif- ference between the test groups was defined at P ~ 0.05. Data from different membranes were ana- lyzed separately. For convenience of presentation, data for each experiment were centered to the mean value of the control line.

Results

Selection effect on exterior traits
Data on the body composition characteristics of the animals from the three investigated lines are presented in Table 2. Mice of both selected lines, DU6 and DU6P, demonstrated accelerated growth in comparison to controls. Line DU6 showed the highest growth rate, with mean body weight elevated by 89% at the age of three weeks and by 118% at the age of six weeks, compared to control animals. The corresponding values for line DU6P were 62% and 94% at the age of three weeks and six weeks, respectively. All three lines differed significantly from one another in body weight at both ages studied. The higher body weights in the selected lines were accompanied by line specific increases in protein and fat accumulation.
The protein and the total fat amount were highest in DU6P at three weeks. At the age of six weeks, the protein amount was elevated by about 70% in both selected lines compared to the control; however, the total fat amount in line DU6 was 3.2-fold higher than in the DUKs and increased 2.2-fold compared to DU6P at six weeks. There was no evidence of sig- nificant differences in the number of adipocytes in epididymal fat pads, between the lines, at the age of three weeks and six weeks. However, from 3-6 weeks the fat pad and the adipocyte volume in mice of DU6 increased 6-fold, and in DU6P and DUKs about 3-fold (Table 3).

Blood plasma concentrations of selected factors
The GH, IGF-1, insulin, and leptin plasma concentrations at three weeks and six weeks of age, are summarized in Table 4. At three weeks, the concentrations of GH and IGF-1 were significantly elevated in both selected lines when compared to the control line. Simultaneously, the concentration of leptin was signinicantly decreased in DU6P compared to DU6 and the control line. At the age of six weeks, maximal GH and IGF-1 concentrations were observed in line DU6P: GH was significantly higher (+ 244%) than in DU6 mice; the IGF-1 concentrations were 2.5-fold higher than in the controls. Mice of the high body weight selected line DU6 were characterized by extremely high concentrations of insulin and leptin. Insulin was more than 2-fold higher in line DU6 than in the other lines. The concentrations of leptin were 3.9-fold and 3.5-fold elevated in DU6 in comparison with DU6P and DUKs. The coefficients of correlation between the individual concentrations of leptin in circulating blood and the weights of the epididyma1 fat pads was 0.7 for the DU6 and the DU6P mice and 0.3 for the control animals.

Growth factor transcripts
The data from the analysis by Northern blot hybridization with *Gh* and *Igf-1* probes are summarized in Figure 1. At the age of three weeks, the animals of both growth-selected lines had 2.5-fold higher levels of pituitary GH mRNA than the controls. To measure the total IGF-1 mRNA concentration, the 0.9-1.2 kb and the 7.0-7.5 kb mRNA species were added. The 1.5-1.9 kb transcript species amounted to maximal 5% of total IGF-1 mRNA in individual samples. In general, their concentrations were too low for a reliable quantification. IGF-1 transcripts were elevated 2.3-fold and 1.8-fold in liver of DU6 and DU6P, respectively. A representative blot is shown in Figure 2. At the age of six weeks, all three lines were characterized by nearly the same levels of GH

mRNA, whereas Igfl mRNA abundance remained elevated in fat DU6 mice, in contrast to both DU6P and DUKs. The ratio between the different Igfl mRNA species was observed to change with age in a line-specific manner (Figure 2). The percentage of the 7-7.5 kb mRNA declined in the lines DU6 and DUKs ($P< 0.01$) with increasing age, D the longer transcript. In this line the percentage of the 7- 7.5 kb mRNA species was thereby 1.7-fold and 2.2-fold higher at six weeks of age than in the DU6 and the DUKs animals, respectively.

Table 2 Body composition characteristics of the selected and control lines

	3 weeks			*6 weeks*		
Traits	*DU6*	*DU6P*	*DUKs*	*DU6*	*DU6P*	*DUKs*
BW (g)	20.0 ± 3.9^a	17.2 ± 2.6^b	10.6 ± 2.4^c	61.5 ± 3.2^a	54.8 ± 4.3^b	28.2 ± 2.1^c
Protein (g)	2.48 ± 0.15^a	3.16 ± 0.28^b	1.89 ± 0.14^c	8.28 ± 0.93^a	8.24 ± 0.97^a	4.85 ± 0.41^b
Fat (g)	0.81 ± 0.10^a	1.16 ± 0.22^b	0.70 ± 0.11^a	6.11 ± 1.38^a	2.76 ± 0.82^b	1.92 ± 0.36^c
EFW (mg)	45 ± 33^a	46 ± 28^a	17 ± 12^b	1017 ± 507^a	471 ± 219^b	278 ± 89^c

DU6 = line selected for high body weight; DU6P = line selected for high carcass protein content; DUKs = unselected controls. BW = body weight; Protein = total protein mass in the body; Fat = total fat mass in the body; EFW = epididymal fat weight. Data are means ± s.d; values designated with different letters within an age group are significantly different between the lines, $P < 0.05$ (Welch-test, two sided).

Table 3 Adipocyte characteristics of epididymal fat pads

	3 weeks			*6 weeks*		
	DU6	*DU6P*	*DUKs*	*DU6*	*DU6P*	*DUKs*
Average adipocyte diameter (μm)	27.9 ± 5.4	28.6 ± 5.1	23.2 ± 4.1	48.1 ± 7.5	39.9 ± 7.0	32.6 ± 6.4
Estimated adipocyte volume ($\mu m^3 \times 10^2$)	127.7 ± 92.5	133.1 ± 72.9	71.4 ± 35.5	625.1 ± 271.8	364.9 ± 197.5	204.4 ± 139.9
Estimated adipocyte number ($\times 10^6$)	5.87 ± 1.49	5.10 ± 1.64	3.80 ± 1.33	13.25 ± 8.08	9.26 ± 4.14	11.48 ± 5.8

DU6 = line selected for high body weight; DU6P = line selected for high carcass protein content; DUKs = unselected controls

Table 4 Blood plasma concentrations of factors known for their effect either on growth and/or obesity or on defects of the endocrine system

	3 weeks			*6 weeks*		
	DU6	*DU6P*	*DUKs*	*DU6*	*DU6P*	*DUKs*
GH (ng/ml)†	5.08 (4.96; 5.40)[a]	5.6 (2.74; 11.3)[a]	0.56[b]	0.96 (0.58; 1.78)[a]	3.3 (1.0; 6.1)[b]	1.52 (0.84; 3.88)
IGF-1 (ng/ml)*	315 ± 82^a	369 ± 81^a	258 ± 48^b	775 ± 209^a	849 ± 239^a	346 ± 38^b
Insulin‡	0.76 ± 0.17	0.75 ± 0.14	1 ± 0.33	2.78 ± 2.11^a	1.15 ± 0.39^b	1 ± 0.32^b
Leptin (ng/ml)*	2.81 ± 1.62^a	1.99 ± 0.62^b	2.73 ± 1.49^a	11.61 ± 9.41^a	3.00 ± 2.06^b	3.29 ± 2.28^b

DU6 = line selected for high body weight; DU6P = line selected for high carcass protein content; DUKs = unselected controls
GH = growth hormone; IGF-1 = insulin-like growth factor 1.
* Data are means ± sd.
†Because GH concentrations were not normally distributed data are given as medians and quartile range.
‡ The insulin concentrations are presented as relative values to the control DUKs; values designated with different letters within an age group of a trait are significantly different between the lines at $P < 0.05$; no letter indicates that there is no significant difference to any other line.

Adipocyte-specific transcripts

As shown in Figure 3, the mRNA abundance for glycerolphosphate dehydrogenase 1 (GDC-1) was significantly higher in epididyma1 adipose tissue probes of the fat line than of the protein-selected and control lines at both ages studied (2-2.8-fold). Lipoprotein lipase (LPL) mRNA amounts were significantly increased in mice aged three weeks for both DU6 and DU6P by factors of 2.7 ($P< 0.001$) and 1.8 ($P< 0.05$), respectively, compared to the mean con- centration in the DUKs line; the sma11 difference between DU6 and DU6P was significant at $P< 0.05$. However, no difference was observed between the groups at the age of six weeks. Measurements of the Ap2 transcript amounts revealed no line-specific differences in any age group, instead individua1 variability within each line was rather high.

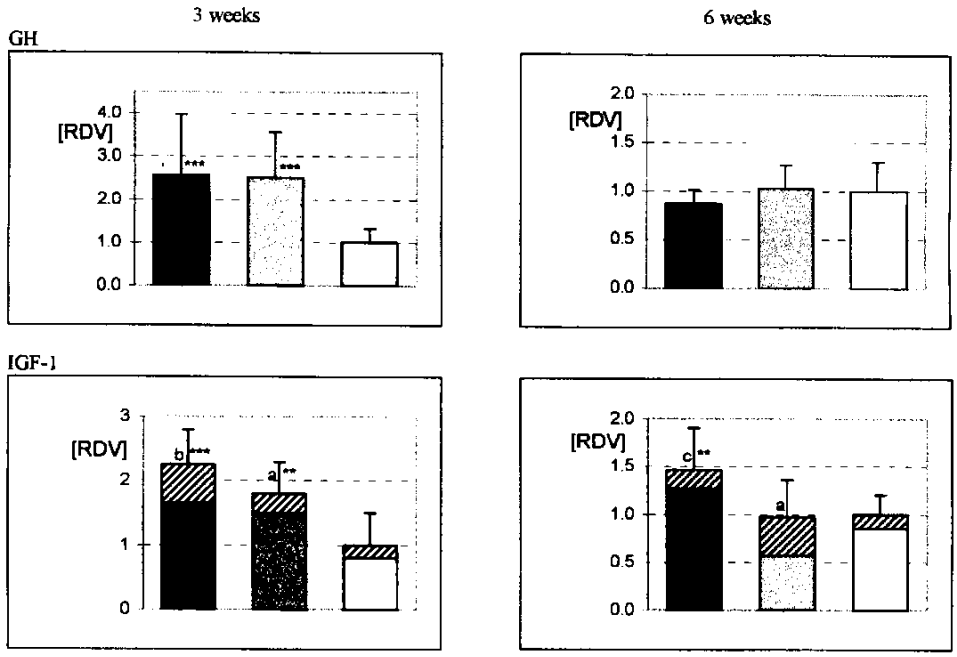

Figure 1 Relative densitometric values (RDV) for Northern blot hybridization panels representing growth hormone (GH) and insulin-like growth factor 1 (IGF-1) mRNA abundance in pituitary gland and liver, respectively, in the animals of the lines DU6 (selected for high body weight, dark bars), DU6P (selected for high carcass protein, grey bars) and DUKs (unselected controls, open bars) at the ages of three weeks and six weeks. Hatched bars represent 7.0–7.5 kb IGF-1 mRNA subspecies. Data are normalized to the mean values in the control line; means and s.d. are shown. *, a-b $P < 0.05$; **, a–c $P < 0.01$; ***, a–d $P < 0.001$ indicate the groups significantly different from the control; letter indices stay for the difference between both selected lines.

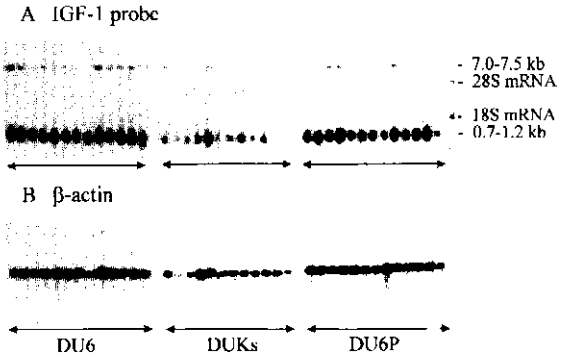

Figure 2 Representative Northern blot of total RNA from liver tissue of the animals of the three lines at the age of three weeks subsequently hybridised to (A) insulin-like growth factor 1 (IGF-1) and (B) β-actin probes.

Discussion

Here we report differences in the serum concentrations of regulatory proteins and in the expression of genes, which might be candidates for the genetic variation between selected mouse lines differing in selection response of body weight and fat content. The observed quantitative differences in IGF-1, GH, insu- lin and leptin plasma concentrations, as well as in the differences of GH, IGF-1, GDC-1 and LPL mRNA expression, between the lines, suggest that the expres- sion of these genes is either a direct genetic response to selection or a secondary effect resulting from the correlated response to genetic variants of regulatory factors like, for examples, hormones which have been fixed during selection.

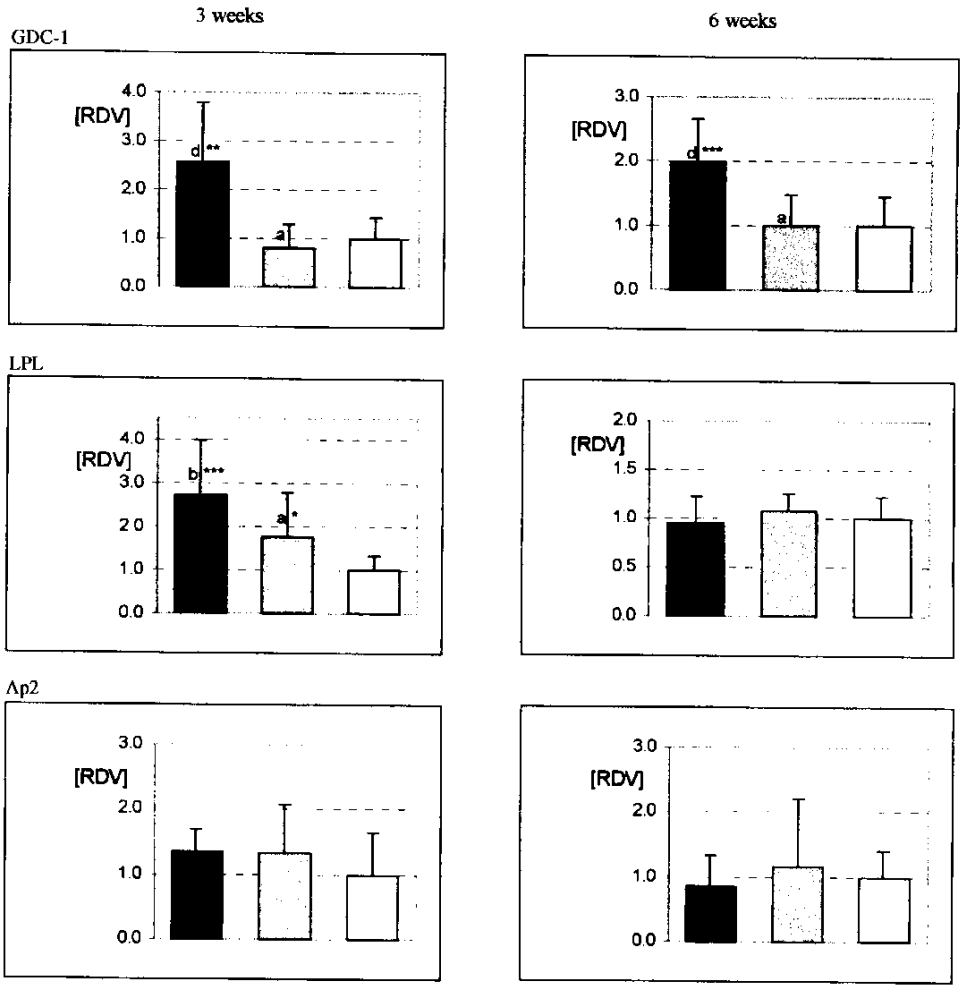

Figure 3 Relative densitometric values (RDV: mean values and s.d., normalized to the mean values in the control line) for Northern blot hybridization panels representing mRNA abundance in the epididymal fat pads in the animals of the lines DU6 (dark bars), DU6P grey bars) and DUKs (open bars) at the ages of three weeks and six weeks. *, a–b $P < 0.05$; **, a–c $P < 0.01$; ***, a–d $P < 0.001$ indicate the groups significantly different from the control; letter indices stay for the difference between both selected lines. Gdc1 = glycerolphosphate dehydrogenase 1; LPL = lipoprotein lipase; Ap2 = adipocyte protein 2.

The significant increase in the transcription rates for GH and IGF-1 in both selected lines compared to the control implies a relationship with the enhanced growth rates exhibited by the animals around weaning, when the period of intensive growth begins. In particular, elevated GH transcription rates might account for the accelerated growth with high protein accretion in the selected lines[26]. For transgenic mice overexpressing GH, it has been demonstrated that accelerated growth is not induced until the age of three weeks[27].

Increased GH levels being the main modulator of IGF-1 transcription[28] is probably the reason for the observed elevation of the IGF-1 serum concentrations in both selected lines, compared to the contro1 group, at the age of three weeks. IGF-1 expression is only independent of GH during the early postnatal period29.

At the end of the juvenile stage, when growth becomes much slower, only the mice selected for high protein content still showed higher GH transcription rates, whereas for IGF-1 mRNA, the DU6 mice also had higher hepatic mRNA concentrations than the controls. The higher mRNA concentration of the IGF-1 gene variant at day 42, characteristic for DU6, may be attributed to the interaction with GH independent cis- and trans- acting transcription factors. In this case, a GH-dependent regulatory mechanism for the IGF-1 gene seems unlikely because decreased GH secretion together with reduced pituitary GH mRNA levels is a common feature of obesity in rodents and humans[30]. Insulin may be a candidate for a positive regulation of IGF-1 gene transcription; in cultured rat hepatocytes IGF-1 mRNA is increased by insulin stimulation.[31,32] The

elevated IGF-1 mRNA and serum concentrations of DU6 mice observed in our study, thus might be attributable to the moderate hyperinsulinaemia in this group compared to DU6P mice and controls.

Our investigation leave open the reason why the significantly increased IGF-1 levels of the DU6P subjects, compared to the controls, are not reAected by higher amounts of IGF-1 mRNA. However, we observed line-specific developmental changes in the ratio of the different IGF-1 mRNA species. The relative amounts of the 1ong 7-7.5 kb mRNA decreased in older animals of lines DU6 and DUKs, but not in DU6P. This group of transcripts is likely to be subjected to regulatory mechanisms affecting mRNA stability[33]. There is some evidence for a factor interacting with specific structures in the 3'- UT region, dependent on the metabolic condition of the organism. This factor seems to be determined by the selection process and might be associated with the posttranscriptional upregulation of the hepatic IGF-1 secretion in DU6P mice.

The significantly increased transcription niveaus for LPL at three weeks, and for GDC-1 at both ages, observed in the high body weight selected line DU6
compared to the other lines, are apparently consistent with the increased fat accumulation in this line. Up to weaning, the animals of the selected lines were con- suming a high-fat diet with the materna1 milk (Brockmann, unpublished data) and thus their body fat depot comes, to a great part, from nutritional resources. The sma11 difference between DU6 and DU6P at this age may be the result of similar intake of nutrients in milk, which may be insufficient to allow the expression of the genetic potential. In contrast to juvenile animals, fat accumulation in adult mice is dependent on the utilization of nutritional glucose and on the lipo- genesis rate; differences in adiposity are mostly due to differences in fat cell size and filling.[5] The results of the histological analyses of epididyma1 fat pads of the mouse lines confirmed hypertrophy of adipocytes as the reason for the heavier fat pads observed in the selected line DU6.

Hyperleptinaemia was observed only in the DU6 after weaning, concomitant with increased serum insulin concentrations and elevated fat mass. This is consistent with the concept that leptin mRNA and serum leptin concentrations of both human patients and animals are increased in parallel with body fat[35,36] and increased adipocyte cell size.[37] Interestingly, epididymal fat mass was not significantly associated with leptin concentrations in the control group, suggesting a different regulation of leptin secretion, .dependent on the selection process. Above that, the leptin resistance proven for the mice of line DU6 corresponds with recently published data;[38] DU6 have higher daily intakes of metabolisable energy and higher energy gains during the growth phase compared to DU6P and DUKs. In contrast, no difference was proven in resting metabolic rates between the lines.[38] Hyperinsulinaemia over long periods is an additional factor leading to increased leptin concen- trations *in vivo*.[39]

It is noteworthy that in spite of their approximately doubled body weight compared to the controls, the DU6P mice showed no difference in leptin concentrations at six weeks of age. We therefore conclude that body weight is an inadequate indicator for the leptin secretion in subjects with an elevated portion of lean body mass.

Insulin is considered to be responsible for high rates of lipogenesis by controlling glucose utilization and the expression of main lipogenic enzymes.[40] Therefore, the above mentioned enhanced fat accumulation in weaned DU6 mice is likely to be primarily due to an insulin-modulated increase of lipogenic activity in adipocytes; this notion is supported by the observed elevation of the transcript concentrations for GDC-1, the gene associated with fat synthesis. Both enzymes, LPL and GDC-1 are subjected to transcriptiona1 up- regulation by insulin.[16,17] In accordance with these reports, increased mRNA concentrations of GDC-1 were found in DU6 at both ages analyzed, however, differences in mRNA levels of LPL were limited to the age of three weeks only, and were not observed at six weeks. The increased mRNA amount of GDC-1 in DU6 mice might be caused by variants in the encod- ing gene or its regulatory elements itself. We did not identify any line specific activity of the *Ap2* gene, which was extremely different between individuals within every line. In contrast to GDC-1 and LPL, for which the idea of a coordinate

induction or suppres- sion of the expression during differentiation and by hormonal stimuli *in vitro*[4,15,41] is in line with our observations, no such relation was applicable to Ap2.

PPARγ (peroxisome proliferator activated receptor gamma) is an additional factor which has been identified to regulate leptin secretion and to promote the adipocyte differentiation process. Response elements of PPARγ have been identified in different adipocyte genes including lipoprotein lipase[42] and adipocyte binding protein Ap2[43]. Recently, increased amounts of PPARγ have been demonstrated in adipose tissue of DU6 mice compared with DUKs animals.[44] This might explain the higher gene expression levels of GDC-1 at both ages and of LPL at three weeks in line DU6 compared with the lean lines DU6P and DUKs, whereas Ap2 was not different between the lines.

Conclusions

For the first interpretation of our data, we assumed that changes in transcript amounts are at least qualitatively corresponding to changes in protein amount as reported for other *in vivo* and *in vitro* models.[45-47] However, further studies on possible translational and post-translational regulatory events would be necessary for a complete understanding of the fina1 effect of the altered expression assessed herein.

The present results suggest a genetic response of the expression levels of GH and IGF-1 in the selected lines characterized by high body weight. In addition, differences in transcription amounts of *IGF-1, GDC-1* and *Lpl* either direct genetic effects on the system or secondary changes resulting from hormonal effects of, for example, insulin or leptin. The expression at the *Gh* and *Ap2* loci appears to be secondary for differentiation in fat content.

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