Protein turnover, body composition, muscle characteristics, and blood hormones in response to different direction of growth selection in mice

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ABSTRACT

Whole body protein turnover and tissue traits were studied in growing male mice long-term selected (78 generations) for carcass protein mass (DU-6P, protein line), for body weight (DU-6, growth line) or for an index combining body weight and treadmill performance (DU-6+LB, growth+fitness line) and in the unselected randomly bred control (DU-Ks). For the estimation of protein turnover data six mice of each line were housed individually in metabolic cages and were fed *ad libitum* a commercial stock diet (crude protein 268 g, gross energy 19 MJ/kg DM). At the end of the experiment body weight of all selected mice was about twice that of the controls. Fractional rates of protein synthesis (scaled to the corresponding body protein pools) were significantly higher in selected mice than in controls, but were not significantly different between the protein and growth line in contrast to the protein deposition rates. Chemical body composition was changed in dependence on selection traits. In the growth line body fat content was highest at the expense of protein content; in the protein line the proportion of lean body mass (muscle protein) was highest. This is supported by results of a previous experiment (generation 68) on 60-days old mice showing an enlargement of cross sectional area of the EDL muscle. The number of capillaries per fibre as indicator for nutrient and oxygen supply of the muscle was adequately increased with the enlargement of muscle cross section in all selection lines, but it was even doubled in the growth+fitness line. No significant alterations in blood thyroid hormone levels (T_3, T_4) or in free glutamine content of muscles extracts were observed in response to selection. Plasma IGF-I levels were higher in selected lines than in controls.

KEY WORDS: body composition, protein synthesis, ^{15}N, end-product method, tracer technique, IGF-I, thyroid hormones, mice

INTRODUCTION

Protein accretion in animals depends on genotype, age, activity and nutritional status. These determinants exert at least some of their effects via hormonal mechanisms. Relationships between blood hormone levels (thyroid hormons, insulin and IGF-I) and muscle protein turnover rates were observed in rats fed on different protein levels (Jepson et al., 1988a; Millward, 1989). To explore whether selection-linked relationships exist between blood hormone levels and parameters of protein metabolism long-term selected lines of mice were used as a model. These mouse lines exhibit significantly different phenotypes with regard to growth and chemical body composition due to long-term selection for high body weight or high carcass protein content (Bünger et al., 1985, 1987). In the present studies these lines were characterized concerning growth parameters, protein turnover and some blood hormones. Additionally, histological/histochemical data of muscles from mice of generation 68 of selection were collected and illustrated in dependence on the used selection traits, since marked genetic relations were observed previously between muscle structure and growth characteristics (Rehfeldt et al., 1989).

MATERIAL AND METHODS

Animals and diets

The experimental mouse lines were established from an outbred base population (Fzt:DU) bred from 4 outbred (NMRI, Han:NMR, CFW, CF1) and 4 inbred strains (CBA, AB, C57Bl, XVII), (Bünger et al., 1985; Schüler, 1985). These lines selected on day 42 of age for 78 generations were:

DU-Ks, randomly bred control, unselected (control line),
DU-6P, selected for high carcass protein content (protein line),
DU-6, selected for high body weight (growth line),
DU-6+LB, selected for an index combining body weight and physical fitness (growth+fitness line).

The experiment was conducted with growing male mice from each line. Throughout the trials, the mice were housed at a constant temperature (22°C) and 50 % humidity with a 12 h light/dark cycle (light from 6.00 to 18.00). Twelve mice per line (Generation 78) were weaned at 21 days of age and divided randomly into 2 subgroups. Six mice were adapted to single housing in screen-bottom metabolic cages (diameter 21 cm, height 12 cm, 3-floor battery, E. Becker and Co GmbH, Castrop-Rauxel, Germany) immediately after weaning (subgroup 1, used for N balance studies and estimation of protein turnover data). The remaining 6 mice

(subgroup 2, used for body analyses) were housed singly in polystyrene breeding cages (13 cm wide x 15 cm height) throughout the experiment. All mice were fed *ad libitum* with a commercial pelleted standard diet (Altromin 1310, Altromin international, Lage/Lippe, Germany; see Table 1) with free access to water.

TABLE 1

Nutrient composition of the Altromin stock diet, g/kg DM

Crude protein	268	Lysine	14.2
Crude fibre	42	Methionine + Cysteine	10.5
Crude fat	61	Threonine	9.9
Ash	69	Arginine	16.3
Gross energy (MJ)	19.1		
Protein energy as percentage of gross energy	35		

The metabolism experiment with subgroup 1 consisted of three subperiods: a 5-days preperiod for adaption of mice to experimental conditions, a 5-days main period for estimation of nitrogen (N) balance (26 days of age at start) and a 1-day tracer period for measurement of whole body protein synthesis. During the N balance period urine and faeces were collected immediately in sulphuric acid (5 %) and homogenized before N analysis of aliquots.

A ^{15}N-labelled amino acid (AA) mixture with an similar AA composition as the stock diet was used (all AA with the exception of proline, cysteine and tyrosine were ^{15}N-labelled, average enrichment of the mixture 84 atom% excess) for a 1-day tracer experiment. This mixture was dissolved in water before application of 150-200 µl of the solution by stomach tube to each animal (35-41 mg ^{15}N/kg body weight). At the end of the experiment (33 d of age) mice of both subgroups were anesthesized with urethane and killed by terminal bleeding (cardiac puncture). Blood was stored on ice before centrifugation at 4°C at 1450 x g. For the estimation of insulin-like growth factor-I (IGF-I) concentrations EDTA plasma was used. The thyroid hormone (T_3, T_4) levels were measured in blood serum. Bodies of subgroup 1 were dissected into 3 fractions (eviscerated carcass, organs and skin+head+paws) for the estimation of nitrogen distribution. *Plantaris* and *tibialis* muscles of the right hindlimb were analyzed for free glutamine (GLN) content. Bodies of subgroup 2 were used for the estimation of the chemical body composition after the removal of gut contents. All samples were preserved at -20° C until analysis. Tissue fractions and carcasses were autoclaved, homogenized and freeze-dryed before analysis of the chemical composition (protein, fat, ash).

Analytical methods

Tissue water content was measured by freeze-drying the homogenates until weight constance and by terminal drying at 105°C. Nitrogen analysis of all sam-

ples was performed by the semimicro Kjeldahl method; ^{15}N excess was estimated by emission spectrometry, using the ^{15}N-analyzer NOI-6c (FAN Fischer Analysen Instrumente GmbH, Leipzig, Germany). Ash and fat content of food and carcass samples were determined with conventional methods (ash: 7 h ashing at 600°C; fat: Soxhlet method without hydrolysis using petrol ether as solvent, Naumann and Bassler, 1993). The gross energy content of the diets was determined with an adiabatic bomb calorimeter (C 400, Janke and Kunkel, Staufen, Germany).

GLN concentration in the muscles was analyzed according to a modified method of Jepson et al. (1988b) on aliquots of muscle sulphursalicylic acid (SSA) extracts by ion-exchange chromatography using the physiological run and shortened program of the AA analyzer Biochrom 20 (Pharmacia-Biotech Europe GmbH, Freiburg, Germany). Plasma IGF-I concentration was estimated after acid-alcohol extraction using a ^{125}J-RIA (Nichols Institute, Diagnostica GmbH, Bad Nauheim, Germany). Thyroxine (T_4) and 3,5,3'-triiodothyronine (T_3) levels were measured by a ^{125}J-RIA (RIA mat®, Byk-Sangtec Diagnostica GmbH and Co, Dietzenbach, Germany).

Muscle fibre characteristics

To support the results on protein metabolism and body composition the findings on muscle structure characteristics of these mouse lines estimated in a previous experiment (generation 68) were included. These mice were killed by cervical dislocation at 60 days of age and the lower leg muscle group was prepared from the bone. Serial transverse sections (10μm) from the midbelly of the EDL (*M. extensor digitorum longus*) were cut in a cryostat (-20°C) mounted on slides and stained by NADH-tetrazolium-reductase (Novikoff et al., 1961) for fibre types, alkaline phosphatase for capillaries (Gomori, 1952, described by Spannhof, 1967) or by haemalum-eosin for nuclei (Romeis, 1989), respectively.

Projected microscopic images of muscle cross section were used to determine EDL cross sectional area by planimetry and to count total muscle fibre number. Muscle fibre cross sectional area was calculated by dividing the EDL cross sectional area by the total muscle fibre number. Muscle fibre type frequencies of red, intermediate and white fibres were measured on the basis of about 350 fibres in the middle of the cross section by the semiautomatic muscle fibre analyzer "MBA2" (Beyersdorfer et al., 1985). The numbers of nuclei or capillaries were counted at a 400 x magnification by an ocular grid (8 times) corresponding to an area of 0.5 mm, containing about 400 fibres.

Calculations

The protein content of tissue was calculated by multiplying the nitrogen (N) content by 6.25. Estimation of the whole body protein synthesis rate was based on

the measurement of the cumulative renal excretion of the label (end product method) over a 24-h period after a single ^{15}N dose. The protein breakdown rate was subsequently calculated as the difference between the synthesis rate and protein accretion rate measured as nitrogen balance. The kinetic parameters of the protein metabolism were calculated using a three-compartment model, and the CADEMO computer program (Rasch et al., 1987, as described by Krawielitzki et al., 1989). Relations between traits were determined by simple linear correlation coefficients.

Data of the metabolism experiment are presented as means (± SD) for each group. Effects were evaluated by one-way ANOVA, and Tukey HSD test for unequal n using the Statgraphics 5.0 (1995) Package. Data of muscle structure characteristics were evaluated by the GLM procedure of the SAS System (SAS 6.12 Package).

RESULTS

Growth performance

Feed intake of selected mice was about 1.5 times higher, daily gain (with the exception of line DU-6+LB) and body weight were about 2 times higher than in controls at the same chronological age (Table 2). Feed intake of both subgroups during the balance period as well as body weights at the end of the experiment

TABLE 2

N balance data of mice selected for different growth traits over 78 generations and of the control line from day 26 to 31 of age

Parameter/line	Control DU-Ks	Protein line DU-6P	Growth line DU-6	Growth+fitness line DU-6+LB
No. of animals	6	6	6	6
Mean body weight, g	22.2 ± 1.1[a]	37.5 ± 3.4[b]	36.9 ± 2.1[b]	35.5 ± 1.3[b]
Feed intake, g DM/d	4.3 ± 0.3[a]	6.9 ± 0.2[b]	5.8 ± 0.2[c]	6.7 ± 0.3[b]
Daily gain, g/d	1.2 ± 0.2[a]	1.9 ± 0.2[b]	2.2 ± 0.4[b]	1.4 ± 0.4[a]
N intake, mg/d	175 ±14[a]	284 ± 9[b]	275 ± 10[b]	249 ± 7[c]
N excretion, mg/d				
urine	84 ±11[a]	127 ± 9[b]	147 ± 6[c]	130 ± 6[b]
faeces	39 ± 3[a]	70 ± 3[b]	61 ± 5[c]	53 ± 4[c]
N accretion, mg/d	52 ± 3[a]	87 ± 8[b]	67 ± 9[c]	66 ± 5[c]
N accretion/N intake, %	29.2± 1.9[a]	30.6 ± 2.5[a]	24.3 ± 2.7[b]	26.6 ± 1.5[ab]

values are means and SD
a ,b – significant differences between the groups are indicated by different letters (P< 0.05)

were nearly similar within the limits of variation (data not shown). At almost equal N intakes per day mice of the protein line accreted significantly more N per day and utilized significantly more N for accretion than those of the growth line as evidenced by a lower urinary N excretion (46 and 54% of intake in DU-6P and DU-6, respectively). N intake and daily gain of index selected mice was lower than in the other selection lines, and N accretion was intermediate. Apparent N digestibility was not different between the lines and varied between 76 and 78%.

Body composition

At the end of the experiment (33 days of age) body weights of selected mice were nearly doubled in comparison to controls. In animals selected for high body weight a decreased protein, ash and water content and an increased fat percentage in the body was observed in comparison to the other lines (Table 3). Body composition was almost unchanged in mice selected for protein mass or for an index combining growth and treadmill endurance. Highest proportion of lean body mass (LBM, skinned eviscerated body without head, legs and tail) was determined in the protein line. N proportions of LBM (as a percentage of total body N) were lower in all lines, but were in the same range as LBM (36.0, 41.5, 38.1 and 38.4 % in DU-Ks, DU-6P, DU-6 and DU-6+LB, respectively, data not shown).

In response to selection changes in relative organ weights (expressed as percentage of body weight) were observed. The deviations from unselected controls illustrate Figure 1.

TABLE 3

Body composition of mice selected for different growth traits over 78 generations and of the control line at 33 days of age

Parameter/line	Control DU-Ks	Protein line DU-6P	Growth line DU-6	Growth+fitness line DU-6+LB
No. of animals	6	6	6	6
Final body weight, g	26.1 ± 1.6^a	42.9 ± 2.1^b	45.9 ± 3.2^b	39.2 ± 3.0^b
Dry matter, DM, %	29.8 ± 0.3^a	29.9 ± 0.4^a	30.6 ± 0.6^a	28.9 ± 0.5^a
Protein, % DM	61.3 ± 1.3^a	63.2 ± 0.9^a	53.8 ± 0.9^b	61.1 ± 2.2^a
Fat, % DM	29.0 ± 1.6^a	27.3 ± 1.1^a	37.9 ± 0.8^b	29.6 ± 2.3^a
Ash, % DM	9.7 ± 0.3^a	9.5 ± 0.6^a	8.3 ± 0.4^b	9.3 ± 0.2^a
Lean body mass, LBM, % of BW	40.5 ± 1.2^a	43.5 ± 0.6^b	40.8 ± 1.2^a	41.0 ± 0.6^a

values are means and SD

a, b – significant differences between the groups are indicated by different letters (P< 0.05)
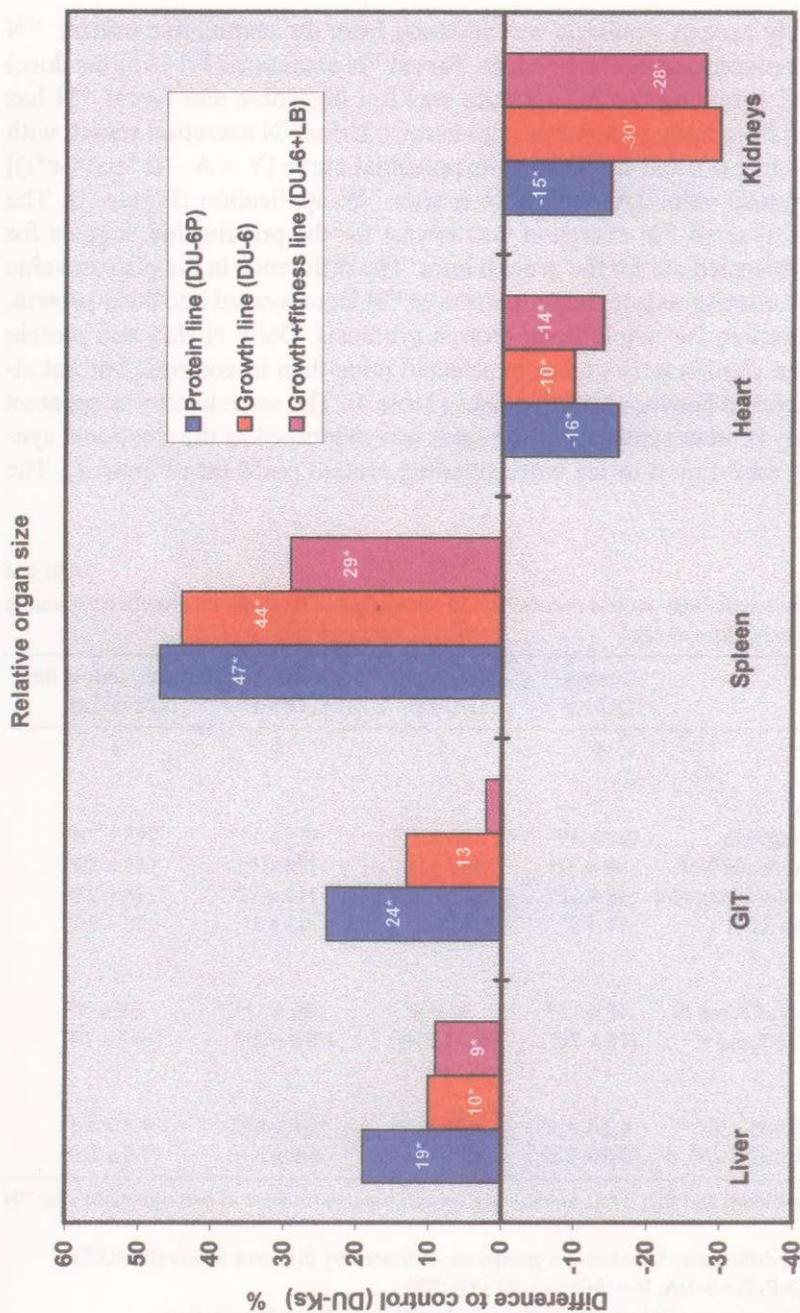
Figure 1. Selection-linked changes of the relative organ weights in selected mice in comparison to unselected control mice at 33 days of age
Legend to figure 1
Means of 6 animals per line
* significantly different to control at P <0.05

Protein turnover data

Whole-body protein synthesis was assessed from the cumulative urinary ^{15}N excretion as a percentage of the ^{15}N dose. Faecal ^{15}N excretion (3-6 % of the dose) was neglected assuming the AA mixture was full digestible and faecal ^{15}N has rather derived from body proteins or gut microbs. Urine ^{15}N excretion started with a delay of 0.4 to 0.6 h and followed an exponential curve $[Y = A - B*exp(-c*t)]$ reaching a plateau value (A) within 24 h after ^{15}N application (Figure 2). The plateau value of renal ^{15}N excretion was lowest for the protein line, highest for controls and intermediate for the growth lines. The difference in the plateau value against 100 % corresponds to the proportion of ^{15}N incorporated into body protein, which is a measure for whole body protein synthesis. Daily N flux and protein synthesis were significantly higher in selected mice than in controls, but not significantly different between selection lines (Table 4). The same trend was apparent when the daily protein synthesis of the lines was expressed as the fractional synthesis rate by adjusting it to the corresponding protein pools (see Figure 3). The

TABLE 4

Parameters of the whole body protein metabolism in mice selected for different growth traits and in the control line at 31 days of age

Parameter/line	Control DU-Ks	Protein line DU-6P	Growth line DU-6	Growth+fitness line DU-6+LB
No of animals*	5	5	5	5
Metabolic rates				
flux rate, Q, mg N/d	182 ± 19^a	326 ± 27^b	325 ± 17^b	295 ± 20^b
synthesis rate, S, mg N/d	98 ± 11^a	199 ± 18^b	178 ± 9^b	165 ± 20^b
break down rate, B, mg N/d	46 ± 12^a	112 ± 22^b	111 ± 10^b	96 ± 23^b
synthesis/Flux R, %	54 ± 3^a	61 ± 1^b	55 ± 1^a	55 ± 3^a
Pool sizes				
metabolic pool, P1, mg N	46 ± 12^{ab}	36 ± 6^a	36 ± 11^a	59 ± 8^b
protein pool , P2, mg N	718 ± 28^a	1077 ± 47^b	1009 ± 29^b	1055 ± 63^b
Half lives				
T1/2 for P1 0.693/c, h	4.2 ± 0.9^a	3.0 ± 0.7^a	3.4 ± 0.6^a	3.4 ± 0.7^a
T1/2 for P2 0.693*P2/B, d	10.8 ± 2.5^a	6.7 ± 0.6^b	6.3 ± 0.6^b	7.6 ± 0.9^b

values given are means and SD; * one animal/line was eliminated because of extreme values for ^{15}N excretion

a, b – significant differences between the groups are indicated by different letters (P< 0.05)

Q =E*d/e, S =Q-E, B =S-NA, R =100*S/Q, P1 =Q/c*24

E =N excretion in urine, e =^{15}N excretion in urine, d =^{15}N dose, NA = N accretion
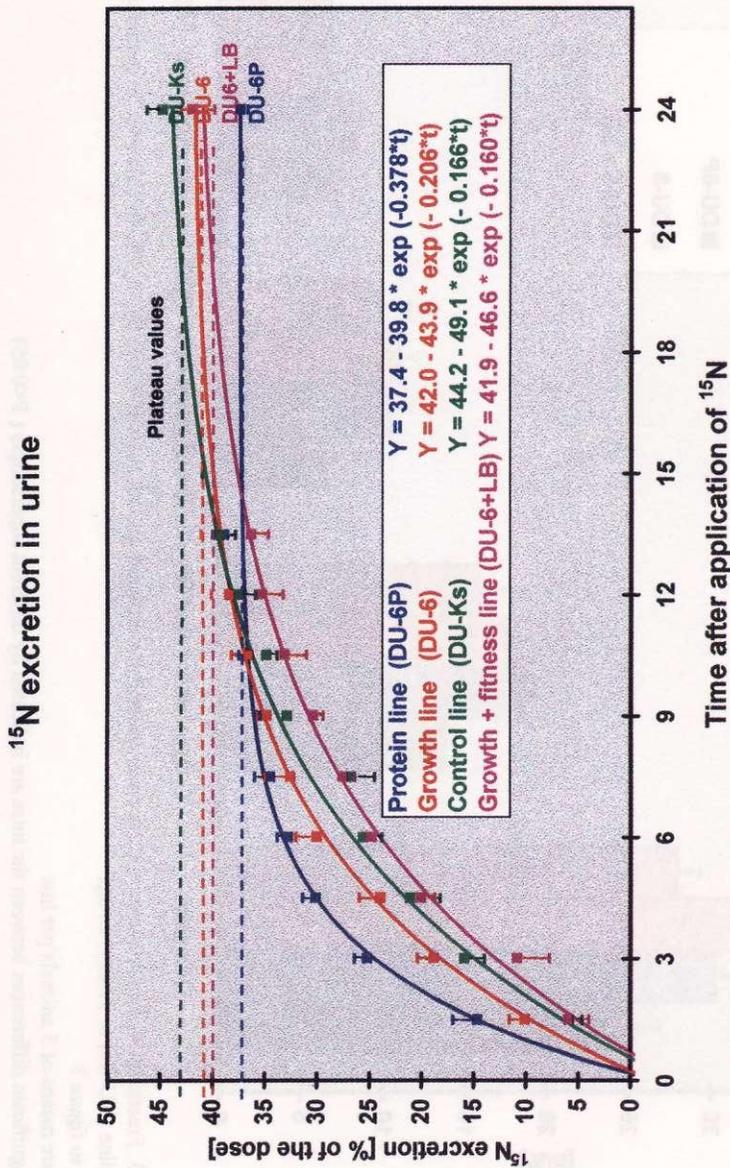
Figure 2. Urinary ^{15}N excretion after a single dose of ^{15}N amino acids in mice selected for different growth traits (DU-6P, DU-6, DU-6+LB) over 78 generations and of the control line (DU-Ks) at 32 days of age

Legend to figure 2

Each measuring point of the curves $Y = A - B*exp(-c*t)$ is a mean of 5 animals

A = Plateau value (% of dose)   B = Distance for t=0 and t=A (plateau value) on the ordinate   c = Slope of the curve (exponential coefficient)

t = Time after ^{15}N application (hours)
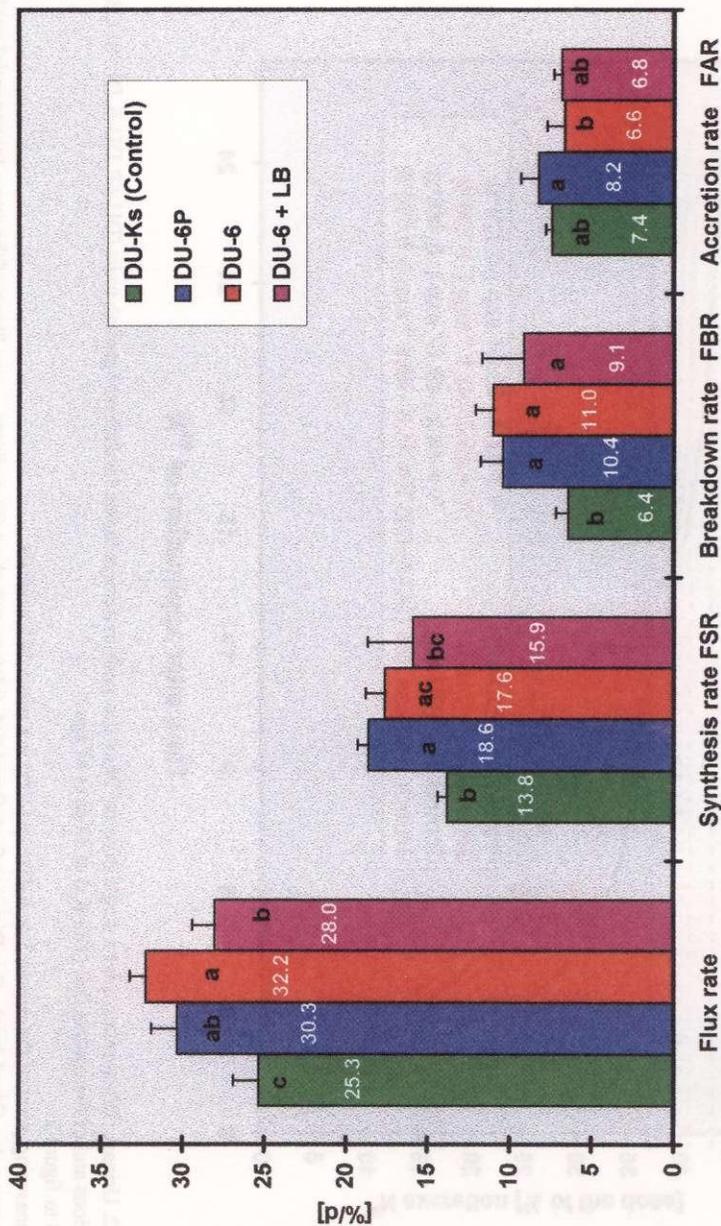
Figure 3. Fractional metabolic rates (%/d) in mice selected for different growth traits (DU-6P, DU-6, DU-6+LB) over 78 generations and in the control line (DU-Ks) at 32 days of age

Legend to figure 3

Values are means of 5 animals per line

a, b –significant differences between the lines are indicated by different superscripts (P<0.05)

difference in the fractional rate of protein accretion between the protein line
(8.2 ± 0.7 %/d) and the growth lines (6.6 ± 0.7 and 6.8 ± 0.4 %/d in DU-6 and
DU-6+LB, respectively) was still significant.

Protein turnover rates of the growth+fitness line showed a tendency towards
lower values than in the other both selection lines. N reutilization for protein syn-
thesis indicated by the ratio of protein-synthesis/flux was significantly higher in
the protein line than in the other selection lines. However, efficiency of protein
deposition [defined as (accretion/synthesis) x 100] was lower in all selection lines
than in controls (0.44, 0.38, 0.40 and 0.53 in DU-6P, DU-6, DU-6+LB and
DU-Ks, respectively). Remarcable is the significant greater metabolic pool (P1) of
the growth+fitness line (DU-6+LB) in comparison to the other selection lines.
Because of identical half lives (T1/2) for the metabolic pool in all lines this sug-
gests that the turnover of this line is more rapid than of the other lines. But this
finding should be verified in further experiments.

Muscle characteristics

Changes in body composition and protein accretion were also reflected by re-
sults on muscle growth. Thereby it has to be considered that these mice were of a
later age of postnatal life (60 days) and derived from a former generation (68). As
shown in Table 5, cross sectional area of the EDL muscle was increased in all
selection lines. Selection response tended to be higher in the protein line (50%)
and in the growth+fitness line (54%) than in the growth line (30%).

The increases in muscle girth were mainly realised by fibre hypertrophy in
DU-6P and DU-6 mice. In DU-6+LB mice total fibre number and mean fibre size

TABLE 5

Body weight and histological muscle fibre characteristics of *M. extensor digitorum longus* (EDL) of
mice selected for different growth traits over 68 generations and of the control line at 60 days of age
(LS means and SE)

	Control DU-Ks	Protein line DU-6P	Growth line DU-6	Growth+fitness line DU-6+LB
No. of animals	10	12	10	7
Body weight, g	33.4 ± 1.3^a	56.7 ± 1.2^b	64.1 ± 1.3^c	52.1 ± 1.4^d
Cross sectional area, mm^2	1.31 ± 0.11^a	1.97 ± 0.11^b	1.70 ± 0.11^b	$2.02 + 0.13^b$
Mean muscle fibre area, μm^2	1270 ± 87^a	1621 ± 76^b	1537 ± 87^b	1585 ± 99^b
Total muscle fibre number	1058 ± 61^a	1238 ± 56^b	1124 ± 61^{ab}	1284 ± 73^b
Nuclei/mm^2 fibre area	967 ± 64^a	727 ± 61^b	840 ± 61^a	847 ± 79^a
Nuclei/ muscle cross section	1155 ± 104^a	1348 ± 90^{ab}	1507 ± 99^b	1599 ± 128^b
Capillaries/fibre	0.34 ± 0.03^a	0.54 ± 0.03^b	0.47 ± 0.04^b	0.71 ± 0.04^c
Capillaries/mm^2	334.9 ± 31.7^a	390.2 ± 28.9^a	$342.9 + 33.4^a$	$569.1 + 37.8^b$

a,b – significant differences between the groups are indicated by different letters ($P < 0.05$)

Muscle fibre type frequencies

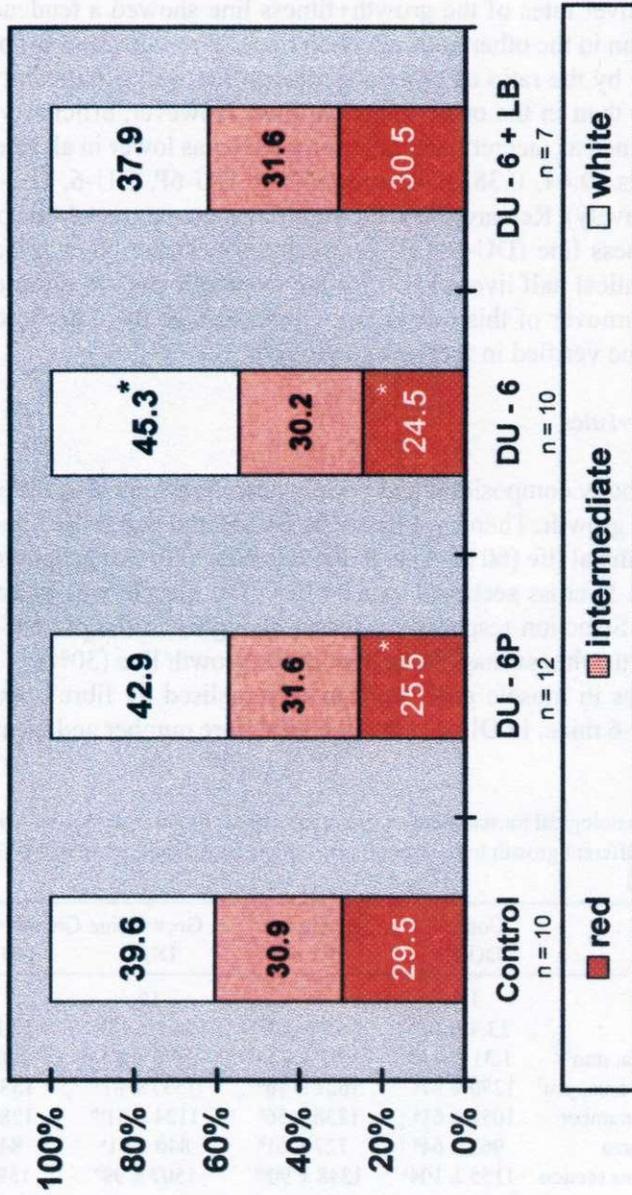|	red	intermediate	white
Control (n = 10)	29.5	30.9	39.6
DU - 6P (n = 12)	25.5*	31.6	42.9
DU - 6 (n = 10)	24.5*	30.2	45.3*
DU - 6 + LB (n = 7)	30.5	31.6	37.9

■ red ▨ intermediate □ white

Figure 4. Frequencies of muscle fibre types in EDL muscle of mice selected for different growth traits (DU-6P, DU-6, DU-6+LB) over 68 generations and of a control line (DU-Ks) at 60 days of age

Legend to figure 4

* significantly different to control at P<0.05

were increased by selection at almost the same extent. Increases in muscle size were accompanied by changes in myonuclear distribution. The total nuclear number per muscle cross section was more enhanced in the growth+fitness line (38%) and in the growth line (30%) as compared to the protein line (17%). Because of the non-proportional increases in fibre size and nuclear number, the nuclear/cytoplasm ratio, measured as nuclear number/mm² fibre area, decreased significantly by selection (by 25%) for protein mass. The data on blood capillary distribution as indicator for nutrient and oxygen supply revealed, that the number of capillaries was adequately increased with the enlargement of muscle cross section in the protein and growth line. However, in the growth+fitness line the number of capillaries per fibre was even doubled, and capillary density was enhanced by 70% in comparison to control line.

As shown in Figure 4 the distribution of metabolic muscle fibre types which indicates the main pathways of energy production was also influenced by long-term selection. The proportion of white (glycolytic) and intermediate fibres was significantly increased in the protein and growth line at the expense of red (oxidative) muscle fibres. Muscles of the growth+fitness line showed the highest red and the lowest white fibre proportions compared to all lines without significant differences to control.

Blood hormones and GLN content

Although there were significantly different growth pattern in selected and unselected mice as well as between the selection lines plasma hormone concentrations were not clearly different (Table 6). At least this is in part caused by the immense variation between individuals (especially within the protein line) and by the small data base. Because of small blood sample volumes, it was not possible to

TABLE 6

Blood and tissue traits of mice selected for different growth parameters and of the control line at 33 days of age

Parameters/ Line	Control DU-Ks	Protein line DU-6P	Growth line DU-6	Growth+fitness line DU-6+LB
T_3, nmol/L	0.86 ± 0.11ᵃ (7)	0.88 ± 0.15ᵃ (7)	0.84 ± 0.12ᵃ (9)	0.84 ± 0.24ᵃ (9)
T_4, nmol/L	48.8 ± 9.2ᵃ (7)	38.8 ± 10.8ᵃ (7)	43.0 ± 7.8ᵃ (9)	42.7 ± 10.7ᵃ (7)
IGF-I, ng/ml	446 ± 51ᵃ (10)	591 ± 118ᵇ (9)	610 ± 96ᵇ (9)	598 ± 71ᵇ (12)
GLN content in muscle extracts, µmol/g muscle	2.64 ± 0.50ᵃ (12)	2.82 ±0.63ᵃ (12)	2.92 ±0.56ᵃ (11)	2.06±0.89ᵃ (11)

values are means and SD. Number of animals in parantheses
a, b – significant differences between the groups are indicated by different letters (P< 0.05)
T_3= triiodothyronine; T_4= thyroxine; IGF-I= insulin-like growth factor-I; GLN= glutamine

include all animals into the analyses. Neither the total T_3 nor the total T_4 levels in the blood were significantly different between the lines, but the IGF-I levels of selected mice were significantly higher than in controls. The free GLN content in muscle extracts was not significantly different between the lines because of high variation.

DISCUSSION

In this study we have investigated the influence of long-term selection for different growth traits on protein metabolism using a mouse model. The estimated fractional rates of the whole-body protein synthesis are comparable with those reported for 20 g mice by Garlick and Marshall (1972) and for 40 g mice by Waterlow et al. (1978) but are lower than values for normal (31 %/d) and fast growing (33 %/d) mice of the same age published by Bernier et al. (1987). Generally, all measurements on protein turnover in these lines were confirmed with a higher animal number (Schadereit et al., 1998). However, it should be noted, that the compartmental method used in the present studies includes some assumptions which are not completely valid leading to under- or overestimations of the absolute synthesis rates. Therefore the values can only be considered as an approximation allowing a relative comparison between the lines. The higher N accretion in the protein line (DU-6P) in comparison to the growth (DU-6) line seems to be due to a combination of enhanced protein synthesis and decreased protein breakdown. It can not be excluded that the approach was not sufficiently sensitive to elicit differences in protein synthesis between DU-6P and DU-6. The lower ratio of protein synthesis/protein accretion in the protein line in comparison to the growth line (2.1 and 2.5, respectively) can be interpreted to indicate a lower protein breakdown rate in DU-6P mice. This assumption was evidenced by a significantly lower rate of N^t-methylhistidine excretion (index for the rate of myofibrillar protein degradation) observed in this line (Schadereit et al., 1998). The higher protein turnover level of selected mice at least in part may be caused by the increase of some visceral organs in response to enhanced food intakes of selected animals (see Figure 1), as the visceral organs contribute highly to whole body protein-synthesis (in mice 35%; Bernier et al., 1987) which is not combined with protein deposition (Bergner, 1989; Simon, 1989). At the end of the metabolism experiment (33 d of age) the whole bodies of the selection lines had the same protein content, however, the body fat content in DU-6 was enhanced by more than 60 % and in DU-6+LB by 11% compared to the protein line. The lean body of mice selected for an index combining body weight and endurance fitness (active type) compared to mice selected for body weight (inactive type) is consistent with results obtained in humans (Millward et al.,1994).

Differences in protein accretion between the selection lines were also evident by muscle structure characteristics studied in a previous experiment and in older animals. As shown by histological data the enlargement of *EDL* muscle cross sectional area was slightly lower after selection for body weight compared to selection for protein mass or for growth-fitness. Differences were also apparent at the cellular level. In the protein line muscle nuclear/cytoplasm ratio declined markedly by selection (by 25%) showing that protein accretion per nucleus was enhanced considerably. This effect was also found previously in *EDL* and *rectus femoris* muscles of mice in generation 43 of selection and was also evident by lower DNA/protein ratios (Rehfeldt and Bünger, 1990). The proliferative capacity of satellite cell indicated by myonuclear numbers was more enhanced by selection for body weight or for growth-fitness compared to selection for carcass protein content. Assuming that total fibre number is mainly a result of prenatal proliferation the overall proliferative capacity of muscle was mostly enhanced in the growth+fitness line. Moreover, the muscles of this line were characterised by enormous improvements of blood capillary supply and by fibre type frequencies indicating the oxidative pathway as major way for energy production. All these changes as well as the tendency towards lower glutamine levels of muscle extracts seem to meet the demands of high physical activity included into the selection index.

Taken together with previously obtained results (Rehfeldt and Bünger, 1990) selection for body weight or for protein mass changes the kinetics of postnatal fibre type conversion to a more glycolytic phenotype as also reported by Swatland and Cassens (1972) on rats and by Nøstvold et al. (1979) on pigs. Extreme lean breeds of pigs (e.g. Pietrain), cattle and chickens exhibit relatively high white fibre percentages which correlate to the PSE (pale, soft, exudative) meat condition (Linke, 1972; Fiedler and Ender, 1984) and to stress-susceptibility in pigs (Fiedler et al., 1993).

The status of some growth-related hormones was characterized to examine whether there are selection-linked changes in relation to growth parameters. No significant differences in thyroid hormone levels (T_3, T_4) were observed between the lines. This confirms results on chickens divergently selected for body weight (Mc Nabb et al., 1989) where the circulating T_3 was not consistently higher in the growth-selected line or results of Lauterio et al. (1986), where circulating T_3 of growth-selected broiler strains was mostly higher than in controls while thyroxine levels did not differ at most posthatch stages. The failure in finding simple relations between the patterns of plasma T_3 or T_4 levels and the patterns of growth is explainable in the view of the complexity of thyroid hormone actions.

In our studies higher plasma IGF-I levels were found in all selected mice. There is considerable evidence of positive correlations between plasma IGF-I levels and improved nutrient supply in rats (Nam et al., 1990; Jahreis, 1993; Tomas and Pym, 1995; Tarim et al., 1997) and broiler chickens (Tomas et al., 1991), whereas no

clear growth-related IGF-I pattern was observed in pigs (Seve and Ponter, 1997) or broiler chickens (Mitchell and Burke, 1995) in which this relationship was lost at 28 days of age. Despite the very small data base per selection line relationships between IGF-I and growth parameters could be observed. Correlation coefficients calculated within each line did not reach statistical significance mainly due to the limited number of data points (7-12) and the high variation within the parameters. In all lines IGF-I levels were related to the daily N intake or N balance values ($r = 0.62$ to $r = 0.64$, respectively) and with the exception of index selected mice to the body weight ($r = 0.61$ to 0.77). Including all lines into this calculation the coherence was stronger. These findings are consistent with literature (Blair et al., 1988; Bates and Pell, 1991; Powell-Braxton et al., 1993) and the magnitude of the relation between body weight and IGF-I level equals to values given by Blair et al. (1987) who estimated correlation coefficients of $r = 0.79$ in 35 day-mice and of $r = 0.53$ in 110-day mice. This shift to a stronger relation in young mice was supported by large scale studies by Souffrant and Kratzsch (personal communication). In all four mouse lines a further increase of the body weight was observed from day 42 of age onwards, but the plasma IGF-I levels dropped at this age. That means, moderate to strong phenotypic correlations can only be expected in young animals.

No clear relationships could be seen between plasma IGF-I values and protein synthesis in our mouse lines. This confirms results of Bates and Pell (1991) who did not find consistent positive correlations between the fractional rate of whole body or muscle protein synthesis and plasma IGF-I concentration in growth hormone (GH) and/or clenbuterol treated dwarf mice. Interestingly, IGF-I levels paralled the increase in body weight only in GH or GH+clenbuterol treated animals, but not after clenbuterol treatment alone. Also in pigs (Seve and Ponter, 1997), the mean absolute values of plasma IGF-I concentration could not explain between-genotype differences in protein synthesis rates of different tissues. For example, Pietrain pigs had the lowest plasma IGF-I concentration and the highest muscle protein synthesis rate. From their studies with mice Sonntag et al. (1992) postulated that in normal aging animals the decrease in IGF-I is associated with the decline in protein synthesis, but that other regulatory mechanisms appear to have an important role in this process. In which way physiological variations in IGF-I level influence the protein turnover is not clear.

The muscle glutamine (GLN) content was not significantly different in the mouse lines and a moderate correlation to the protein synthesis of the whole body could only be seen in the index-selected growth line and in the protein line ($r = 0.68$ and $r = 0.56$, respectively). Highly correlated changes of both the protein synthesis and the GLN level in muscles were reported for rats (MacLennan et al., 1987; Jepson et al., 1988b) and for humans (Rennie et al.,1994). The nature of the mechanism and the physiological significance of the muscle GLN stores are not fully understood.

The muscle GLN pool is thought to act as a labile store of nitrogen mobilized in times of stress (fasting, surgery, scpsis, cxogcnous glucocorticoids and exercise) and the fall of intramuscular GLN content in the index-selected growth line may be caused by an enhanced demand for GLN of this line. The provision of exoge-nous GLN and the preservation of a high GLN pool may be related to the conser-vation of muscle protcin as cvidenced by studies in rats (Hickson et al., 1995). Current results (Rennie et al., 1996) suggest the possibility that GLN pool size is a part of an osmotic signalling mechanism to regulate whole body protein metabo-lism.

CONCLUSIONS

The experimental results on selection lines of mice show that nutrient partition and body composition were altered depending on the used selection traits. In mice selected for high body protein content, the higher protein deposition in the carcass is due to an improved efficiency of nitrogen utilization, a somewhat higher whole body protein synthesis combined with a slightly lower protein breakdown rate. Selection for body mass results in an increase of body fat, but not if combined with endurace fitness. Cellular structure of skeletal muscle is markedly changed by selection. The correlated selection response of skeletal muscle structure clearly differs in rclation to sclection trait and is consistent with differences in body com-position, protein metabolism and endurance fitness. IGF-I seems to be a signifi-cant determinant in realization of growth selection response.

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ŚTRESZCZENIE

Wpływ kierunku selekcji na obrót białka (protein turnover), skład ciała, charakterystykę mięśni i hormony krwi u myszy

Oznaczano obrót białka w ciele oraz cechy tkanek u rosnących samców myszy z linii selekcjonowanych przez długi okres (78 pokoleń) na: zawartość białka w tuszy (DU-6P, linia „białkowa"), masę ciała (DU-6, linia „wzrostowa") lub wskaźnik łączący masę ciała i wydajność ruchu na bieżni (DU-6 + LB, linia „wzrostowa" + „sprawnościowa") oraz u kontrolnej grupy nieselekcjonowanej i kojarzonej losowo (DU-Ks). Dla oznaczenia obrotu białka sześć myszy z każdej linii utrzymywano indywidualnie w klatkach przemianowych i żywiono do woli przemysłową standardową paszą (białko ogólne 268 g, energia brutto 19MJ/kg s.m.). Przy końcu doświadczenia masa ciała wszystkich wybranych myszy była około 2 razy większa niż kontrolnych. Cząstkowe tempo syntezy białka (wyznaczonej w stosunku do „puli" białka ciała) było istotnie większe u myszy selekcjonowanych niż kontrolnych, lecz różniło się nieistotnie między liniami „białkową" i „wzrostową" w przeciwieństwie do tempa odkładania białka. Skład chemiczny ciała zmieniał się w zależności od przyjętego kierunku selekcji. W ciele myszy linii „wzrostowej" zawartość tłuszczu była większa kosztem zawartości białka; w linii „białkowej" udział beztłuszczowej masy ciała (białko mięśni) był największy. Znajduje to potwierdzenie w wynikach wcześniejszego doświadczenia (68 pokoleń), przeprowadzonego na 60-cio dniowych myszach, u których stwierdzono zwiększenie powierzchni przekroju mięśnia *extensor digitorum longus*. Liczba naczyń włosowatych we włóknie mięśniowym, przyjęta jako wskaźnik zaopatrzenia mięśnia w składniki odżywcze i tlen, zwiększała się odpowiednio wraz ze zwiększaniem się powierzchni przekroju mięśnia u wszystkich selekcjonowanych linii, i była niemal dwukrotnie większa u myszy linii „wzrostowej" + „sprawnościowej". Nie stwierdzono istotnych zmian w poziomie hormonów tarczycy (T_3, T_4) w krwi ani w zawartości wolnej glutaminy w mięśniach w zależności od kierunku selekcji. Poziom IGF-I w osoczu krwi był wyższy u myszy z linii selekcjonowanych niż kontrolnych.