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Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Differential growth of skeletal muscle in mice selected divergently over 108 generations for low and high body weight*

Elżbieta Wirth-Dzięciołowska[1,3], Małgorzata Zimowska[2], Marta Gajewska[3], Monika Żmuda[1], Stanisław J. Rosochacki[4,]**

[1] Warsaw Agricultural University, Department of Genetics and Animal Breeding, Ciszewskiego 8, 02-786 Warszawa, Poland

[2] Warsaw University, Faculty of Biology, Institute of Zoology, Department of Cytology, Miecznikowa 1, 02-096 Warszawa, Poland

[3] The Maria Skłodowska-Curie Memorial Cancer Centre and Institute of Oncology in Warsaw Roentgena 5, 02-781 Warszawa, Poland

[4] Polish Academy of Sciences, Institute of Genetics and Animal Breeding, Jastrzębiec, 05-552 Wólka Kosowska, Poland and Białystok Technical University, Chair of Sanitary Biology and Biotechnology, Wiejska 45e, 15-351 Białystok, Poland

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Experiments were performed on 180 mice from two lines dubbed light (L) and heavy (C), selected divergently for body weight over 108 generations. The main hypothesis was that the changes occurring in body weight and muscle weight as a result of directed divergent selection could be associated with changes in the transcription of some miogenic genes and/or with the level of proteins regulating myogenesis and with the composition of muscle fibres.

Hind limb muscle masses from females and males of the two lines were weighed at 1 and 3 weeks and 3 month of age. Morphological analysis for histological cross-sections of the *gastroenemius* muscle was carried out in 3-week and 3-month-old mice. The percentage comparison of nuclei in muscle fibres were analysed, too. Levels of MYOD1, MYF-5 and myogenin at the same time points were determined using Western blotting. Microsatellite markers for *MyoD1, Myf-5* and *MYOG* were used to compare allele frequencies of analysed genes in both lines. There were differences in muscle

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**Corresponding author: email: s.rosochacki@ighz.pl

weight between the sexes at age of 3 months. Muscles of the hind limbs were heavier in males than in females by 23.7% in line C, and by 14% in line L. Significant differences in muscle mass were accompanied by changes in muscle fibre size. The number of large-diameter muscle fibres increased with animals' age, and in females fibres of diameters of 60-80 µm accounted for 38% of the total in line C, as compared with 94% preponderance of smaller (20-60 µm) fibres in the muscle of line L females. The numbers of nuclei were clearly greater in line C than in line L individuals, as well as in 3-month-old animals as compared with those at 3 weeks of age.
Selection have brought about change, not only in myogenesis, but also in the frequency of alleles of microsatellite markers *MyoD1, Myf-5* and at the myogenin locus, thus suggesting that molecular differences between the lines have arisen. Differences in the levels of MYOD1, MYF5 and MYOG are evident between both sexes and the selected lines of mice.

KEY WORDS: mice / body weight / divergent selection / myogenesis / fibre size / MyoD

Skeletal muscle is a functionally unique tissue, exhibiting great plasticity and ability to regenerate in response to injury. During embryonic development, myoblasts emerge from mesenchyme-borne precursor cells. Specific signals then cause them to exit the cell cycle and initiate differentiation [Rehfeld *et al*. 2000]. They begin to synthesize muscle-specific proteins (MRF – myogenic regulatory factors) and start to form polynucleated myotubes. During its early stages, myogenesis is under the control of specific regulators from the MyoD family [Berkes and Tapscott 2005, Borycki and Emerson 1997].

The functional units of skeletal muscle are muscle fibres, differing with regard to their morphology, biochemistry and physiology, as well as their oxidative and glycolytic activity [Berchtold *et al*. 2000, Smith *et al*. 1999]. Due to a high degree of specialization, muscle fibers have lost their ability to proliferate and differentiate, as well as to form new cells capable of repairing or enlarging the tissue. These processes are thus dependent on populations of mononuclear satellite cells, which retain their ability to replicate for their entire lifespan. These provide new nuclei and participate in skeletal muscle regeneration [Anderson 2006, Ferrari *et al.* 1998, Zammit *et al*. 2002]. As during myogenesis, these processes occur under the control of myogenic regulatory factors, capable of inducing the expression of muscle-specific genes. The *MYOD1* gene plays a significant role in the activation and/or differentiation of satellite cells, this perhaps relating to its ability to induce the expression of the p21 protein inhibiting the transition of cells from stage G1 to S. The role of the *MYF-5* gene may be related to the control of myoblast determination, while the *MYOG* and *MYF-6* genes are active during final myoblast differentiation [Sakuma *et al.* 1999]. The continued expression of *MYF-6* in adult animals suggests a regulatory function in later stages of life [Hughes *et al*. 1997]. MYF-6 transcripts account for more than 30% of proteins (the others – MYF-5, MYF-3, MYOG – for less than 10%). The activity of the *MyoD* gene family may be regulated during myoblast differentiation by the way of phosphorylation or compartmentalisation. These also regulate the expression of the acetylcholine receptor and acetylcholinesterase [Melo *et al.* 1996].

The main goal of directed selection towards increased body weight in animals has been to obtain increased muscle weight, which in turn is connected with increased

protein and fat content. Earlier experiments studied differences in body weight between mice selected for high (C) or low (L) body weight after 21 days of life [Wirth-Dzięciołowska *et al*. 1996, 2000]. Body lipid levels were found to be higher in females than males reflecting a faster deposition of fat tissue, as evidenced by more numerous and larger adipocytes in line L mice [Wirth-Dzięciołowska *et al.* 1997]. Faster protein turnover in muscles of line L mice than line C mice was also observed [Rosochacki *et al.* 2005].

On the basis of those earlier results, we undertook further research:

– to determine whether changes in body weight concerning light or heavy skeletal muscle arise out of directed mice selection;
– to assess the composition of muscle fibre, and the percentage of nuclear content in mouse gastrocnemius muscle during development;
– to determine the degree to which arising differences reflect changes in the transcription and/or levels of proteins regulating myogenesis processes.

Material and methods

Experimental animals

The experiments were performed on mice of lines L (light) and C (heavy) selected for body weight over 108 generations. These lines were derived from outbred population obtained through crossing of four inbred lines of mice: A/St, BALB/c, BN/a and C57BL/6Jn. Selection criteria and breeding conditions of mice were described previously [Wirth-Dzięciołowska *et al*. 1996].

Experimental procedure

The research was performed on 180 mice of both sexes from the L and C lines, aged: 1 week (age at which juveniles are still immobile), 3 weeks (when the selection is performed) and 3 months (when both sexes achieve sexual maturity). At each point, 15 males and 15 females of both lines were sacrificed using a lethal dose of Narcotan, following a 14-hour period of fasting. All experimental procedures involving animals were approved by a Local Ethics Commission (permission No 4/2002).

To determine the percentage contribution of muscles to total body weight, hind limbs and then muscles were removed. Body, leg and muscle weights were measured on Sartorius scale, to an accuracy of 0.001 mg.

Determination of fibre diameter

Histological studies and checks on MRF levels in hind-limb muscles from 3 randomly selected males and 3 females of both lines were performed on 3-week and 3-month-old animals. Isolated tissues were frozen in isopentane chilled with liquid nitrogen (Merck) and stored at -80°C. The muscles were cut into 10 μm sections using a cryotome at -25°C. After hydration, the sections were stained with Harris acid haematoxilin and 1% aqueous eosin (BDH). The stain was washed out and the slices

of tissue dehydrated with alcohol and kept in ethanol (Merck). The sections were photographed at random in 5 replicates for each muscle using a Nicon microscope with a digital camera and ACT-1 software. The number and diameters of fibers were determined, and the percentage composition of nuclei calculated using Lucia G software.

Electrophoresis and Western blotting

Muscles were homogenized in 20 mM Tris-HCl, 5 mM EGTA, 5 mM EDTA, 0.01% leupeptin, 0.5 mM PMSF, and 10 mM β-mercaptoethanol (pH 7.5) on ice. The extracts were centrifuged (10000 g, 10 min), and the supernatant was suspended in loading buffer (150 mM Tris-HCl, 20% glycerol, 2 mM β-mercaptoethanol, 0.004% bromophenol blue, pH 7.4). Protein content was determined using the Bradford method [Bradford 1976]. Twenty µg of protein was placed in a well and electrophoresis performed (SDS PAGE, 10% gel, 100V). Following the transfer [Towbin *et al*. 1979], the PVDF membranes (Roche) were incubated with the antibody I against MyoD, MYF-5 or myogenin (Santa Cruz Biotech) diluted to 1:200 (overnight at 4°C), and then with antibody II (Santa Cruz Biotech) conjugated with HRP (1.5 h, 25°C) and then with a chemiluminescence reagent (Roche). Exposed films were archived using the GelDoc2000 system, the bands being analysed using the Quantity One package (BIORAD).

Isolation of DNA

DNA was isolated from 1-1.5 cm tail sections using the phenol method. DNA was then suspended in 500 µl deionised H_2O. It was amplified using PCR and appropriate primers, as previously described in Gajewska *et al.* [2002]. The amplified products were separated electrophoretically in a 4% high-resolution agarose gel, MetaPhore 3:1 (FMC Bioproducts) with Agarose for Routine Use (Sigma) containing ethidium bromide in TBE buffer. Different molecular mass markers were used, i.e. LowLadder – 20 bp (Sigma) and pUCMix – 100 bp (Fermentas). Allele lengths were measured using the GelExpert kit (Nucleotech, San Mateo CA, USA).

To determine the frequencies of microsatellite sequence alleles near the *loci* of the genes coding for the proteins of interest, we selected for two microsatellites:

– D1Mit200 and D1Mit218 from chromosome 1 flanking the *MYOG locus*,
– D7Mit267 and D7Nds1 from chromosome 7 near the MYOD1 gene,
– D10Mit134 and D10Mit135 from chromosome 10 near the *MYF-5 locus*.

Statistical analyses

Two two-factor analyses of variance were used to calculated means and standard deviations, as well as percentage compositions of muscle mass in relation to overall masses of leg and body:

– to determine the differences between experimental lines of mice, using model:

$$x_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

where:

x_{ijk} – body weight of mice;
μ – intercept;
α_i – sex effect;
β_j – line effect;
$(\alpha\beta)_{ij}$ – interaction between line and sex;
e_{ijk} – error term.

– to determine the differences occurring in the traits studied between mice of the two lines (ANOVA with regression for body mass), using model:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma(x_{ijk} - \overline{x}) + e_{ijk}$$

where:

y_{ijk} – muscle weight;
μ – intercept;
α_i – sex effect;
β_j – line effect;
$(\alpha\beta)_{ij}$ – interaction between line and sex;
$\gamma(x_{ijk} - \overline{x})$ – regression of analysed trait (muscle weight) on body weight;
e_{ijk} – error.

Results and discussion

Body weight

Statistically significant differences in body weight between line C and line L mice were observed during the analyzed period (P<0.0001 on day 7 and at 3 months of life – Tab. 1). Significant differences between males and females were only observed at 3

Table 1. Differences in body weight between mice of lines L and C on their 7th, 21st and 98th day of life, and between sexes at the age of 3 months

Age (days)	Number of animals	L line				C line			
		male		female		male		female	
		x	SD	x	SD	x	SD	x	SD
7	54	3.25^{a}	0.54	3.32^{b}	0.55	4.71^{a}	0.61	4.39^{b}	0.54
21	66	6.73^{a}	1.19	6.69^{b}	1.30	12.47^{a}	1.91	12.04^{b}	1.72
98	60	22.20^{A}	4.04	19.08^{A}	2.58	40.36^{B}	2.80	32.54^{B}	2.39

^{ab}Differences between lines significant at P<0.0001.
^{AB}Differences between sexes significant at P<0.001.
x – body weight (g), SD – standard deviation.

months (P<0.0001). Percentage body mass differences between mice of the two lines and in each age group were higher in the males and peaked at 3 weeks of age.

Hind-limb weight

Animals of the two lines differ significantly in the absolute weight of limbs at all studied points of life (Fig. 1). Differences in hind-limb weight between the sexes were only observed on day 98 of life, the average weight of males' hind legs being higher by 0.2 g in line L, and ca. 0.4 g in line C. Figures for the percentage contribution of hind-limb weight made to total body weight increased with age in both lines, being 7.6%, 10.25% and 13.6% in line L and 8.0%, 11% and 13.7% in line C, for 1-week, 3-week and 3-month-old animals, respectively. Only on day 7 of life were the differences obtained confirmed as significant using ANOVA with regression on body weight (P<0.01). Comparisons of weights of right and left limbs in individual mice indicated no asymmetry Fig1A.

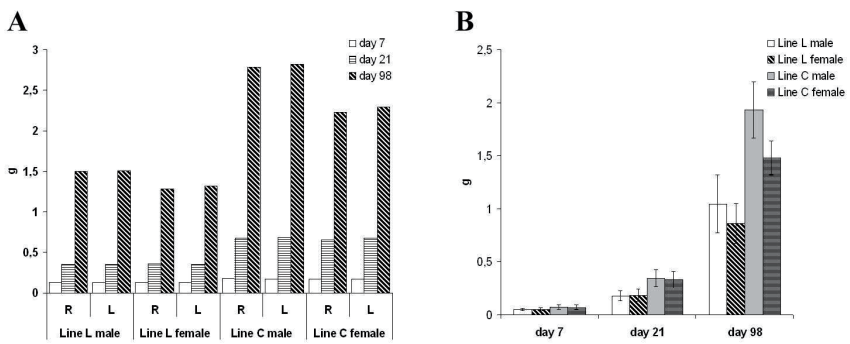

Fig. 1. (A) Right (R) and left (L) hind limb muscle weights (g) for mice selected for high and low body weight over 108 generations. (B) Right-limb muscle weight (g±SE) across selection and sex groups on 7th, 21st and 98th days of life.

Hind-limb muscle weight

Right hind limb masses differed significantly between mice of the two lines at all points of the study. The aforesaid significant difference between males and females also applied at day 98 (P<0.0001). The mean muscle weights were: day 7– 0.053 g line L and 0.073 g line C; day 21– line L 0.184g and line C 0.336 g. On the 98th day of life, the mean muscle weights in line L were 1.045 and 0.863 g for males and females, respectively. In line C, the mean muscle weight was 1.931 g in males and 1.477 g in females Fig1B. The greatest observed percentage difference in muscle mass characterised day 21 data, reaching 46% (as compared with 26.5% and 43% differences on days 7 and 98 respectively). The differences between relative muscle masses in lines L and C on day 21 were confirmed using ANOVA with regression for body mass (P< 0.001). In this age group, muscles in line L account for a greater proportion of body weight than those in the heavy line.

Histological analysis and fibre diameter

Morphological observations revealed differences in the histological cross-sections of muscles (Fig. 2). The percentage contribution the *gastrocnemius* muscle made to body mass in 21-day-old line L males was 0.6%, this being 0.1 percentage points less than in line C males. This muscle was proportionately larger in females, and constituted 0.75% and 0.9% of body weight in lines L and C, respectively.

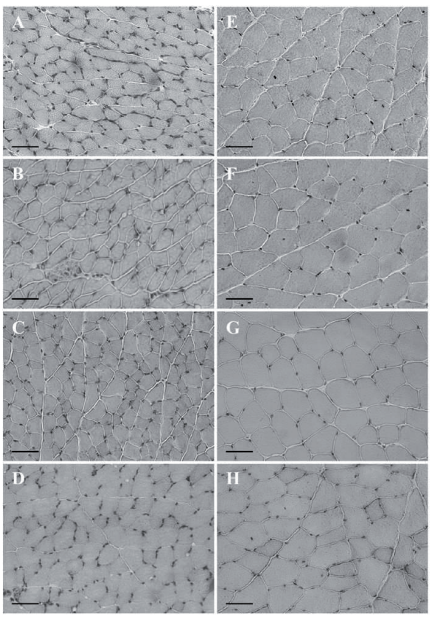

Fig. 2. Histological staining of a *gastrocnemius* muscle cross-section. Light line A – 3-week-old female, B – 3-week-old male, E – 3-month-old female, F – 3-month-old male. Heavy line H – 3-week-old female, D – 3-week-old male, G – 3-month-old female, H – 3-month-old male (scale 50 μm).

To determine the composition of fibres of various diameters in the structure of muscles accurately, we studied the percentage composition of individual fibres belonging to 5 classes at 20 μm intervals, encompassing diameters below 19.99 μm and up to 80 μm. The diameter of *gastrocnemius* fibres differed in relation to age group in both lines of mice (Fig. 3 A, B).

Heavy females had a larger number of thin fibres (19.99-39.99 μm) as compared with females of the same age group in line L (78% *vs*. 71%). The number of fibres in the 40.00-59.99 μm range was similar in females of both groups and fibres over 60.00 μm began to appear. In light 21-day-old males, the muscle fibre diameter was between 19.99 and 49.99 μm, 90% being composed of fine fibres of diameter 19.99-39.99 μm. In males of line C there was a greater variability of fibres, with a normal distribution (from 19.99 to 59.99 μm). Fibres of 19.99-39,99 μm diameter accounted for the greatest share (73%). Both light males and females, and heavy females had

fibres of less than 19.99 μm, while these were absent from heavy males of this age group. (Fig. 3A, B).

Muscle weight gain observed in three-month-old animals was connected with increased participation of the *gastrocnemius* muscle in overall body weight (from 0.85% in L males to 0.95% in L females, and at 1.0% in both sexes of line C). The relative number of large-diameter muscle fibres also increased. Females of both lines displayed greater variability of muscle fibre size than males (from 20 to above 80 μm). Both lines were characterised by a decreased percentage composition of fibres ranging from 29.99 to 39.99 μm in diameter (27% in line L and 13% in line C), as well as an increased role for those of diameters 39.99-49.99 μm (34% in L females as against 28% in C females). Furthermore, the share of fibres sized 59.99-69.99 μm grew significantly (to 21% in line C, cf. 7% in line L), particularly in line C females, in which large fibres of more than 80 μm diameter appeared (Fig. 3A).

Males of the two lines showed similar distributions of fibre sizes, fibres of 29.99-39.99 μm being dominant (36%). In L males, 39.99-49.99 μm fibres appeared after 3 weeks of life, and constituted 25% of the total, with fibres of up to 70.00 μm appearing as well. Heavy males and light males had a similar variability distribution, except that those in larger diameter classes occurred more frequently. Neither light nor heavy males had fibres of diameters below 19.99 μm (Fig. 3B).

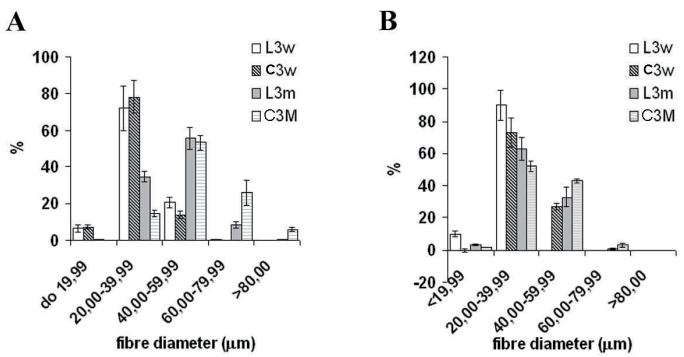

Fig.3. Diameter of *gastrocnemius* muscle fibres in females (A) and males (B) of mice from L and C lines. Results are expressed with regard to the percentage contributions of 5 diameter classes of fibre (±SE).

Percentage composition of nuclei in muscle fibres

In the 3rd week of life of mice, the differences in numbers of nuclei were clearly greater between line L and C males (on average 1.8 for L males and 3.0 for C males) than between females (2.1 and 2.5 for L and C respectively – Fig. 4A, B). Line C also featured a greater difference between the sexes than line L. The average number of nuclei was significantly greater in 3-month-old animals than in those aged 3 weeks, though the differences for this trait between the lines and sexes were not significant.

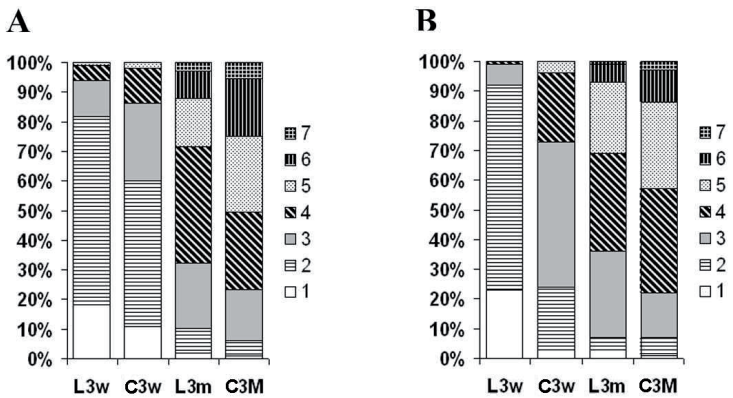

Fig. 4. Percentage content of nuclei in the *gastrocnemius* muscle of females (A) and males (B) of the L and C lines, at 3 weeks and 3 months – 1-7 number of nuclei/cell.

Level of myogenic transcription factors

The levels of MYOD1, MYF-5 and myogenin in males and females from the two selected lines were determined at 3 weeks and 3 months using Western blotting. The level of MYOD1 in 3-month-old individuals was lower in the females of both selected lines and in C males than those measured at 3 weeks. In line L males, MYOD1 remained at similar levels throughout the study. The differences in MyoD1 levels between the 3rd week and 3rd month of life are most visible in line C mice, wherein the highest MyoD1protein levels occur in heavy females at 3 months (Fig. 5A).

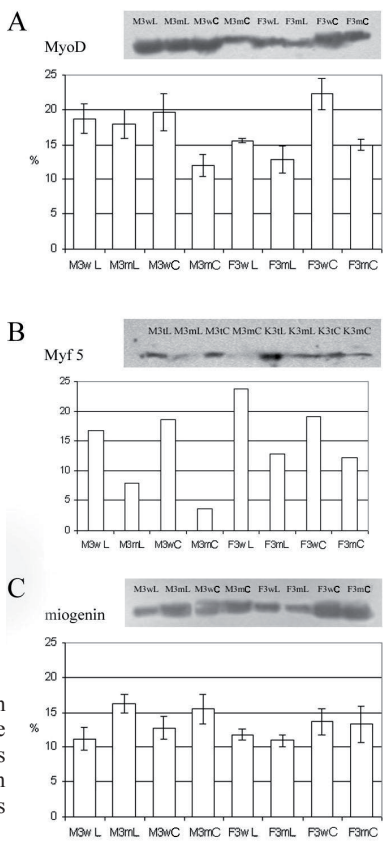

Fig. 5. Representative Western blot analysis on extracts from the *gastrocnemius* muscles of male and female mice from the selected lines, at 3 weeks (3w) and 3 months (3m). Results are mean levels of MYOD1 (A), MYF-5 (B), and myogenin (C) from 3 animals, as replicated 3 times and expressed as AU±SE.

The level of MYF-5 in 3-month-old individuals was lower in both females and males of the selected lines than that observed in the 3rd week. However, in both lines, this difference was more pronounced in males than in females (Fig. 5B).

The level of myogenine is somewhat higher in 3-month-old males than in 3-week-old males, particularly in line L, whereas in the females, the protein level remained at a similar level in both the 3rd week and the 3rd month (Fig. 5C).

Allele frequency at the studied gene *loci*

Significant differences in the frequency of microsatellite markers at individual *loci* were observed between the mice of the two selected lines (Figs. 6-8).

In the case of the D7Mit267marker for *MYOD-1*(Fig. 6A), the two lines exhibited decidedly different genotypes. Line C is characterized by allele *a*, and line L exhibits a combination of alleles *b* and *c*. Both lines exhibit the same D7Nds1 microsatellites with *a, b* and *c* alleles, though at varying frequencies (Fig. 6B).

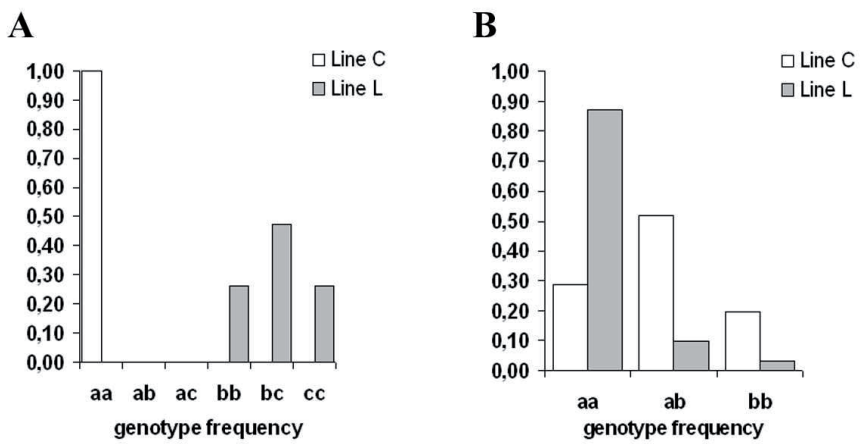

Fig. 6. Frequency of genotypes at the *MYOD1 locus* on chromosome 7. Examination of microsatellites D7Mit267 (A) and D7Ndsl (B).

Analogous differences were observed between the lines in terms of microsatellite D10Mit135 marking *MYF-5*. In line C, only *aa* genotype could be observed, but in line L genotype *bb* accounted for about 80% (Fig.7A). In the case of D10Mit134, alleles *a* and *b* were present in L mice, alleles *b* and *c* in C mice. (Fig.7B)

In line L, one type of marker of microsatellite D1Mit218 allele *b* that characterizes *MYOG* occurs, whereas in line C this allele is present both homo- and heterozygously (Fig. 8A). Moreover, clear differences were present in terms of the second microsatellite, D1Mit200. Line C exhibits only one allele *a,* whereas allele *b* is predominant in line L (Fig. 8B).

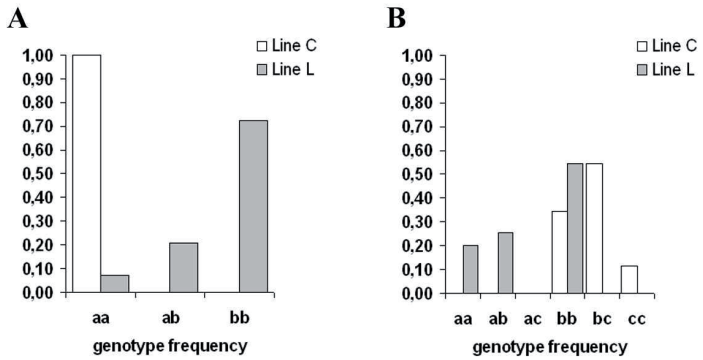

Fig. 7. Frequency of genotypes at the *MYF-5 locus* on chromosome 10. Examination of microsatellites D10Mit135 (A) and D10Mit134 (B).

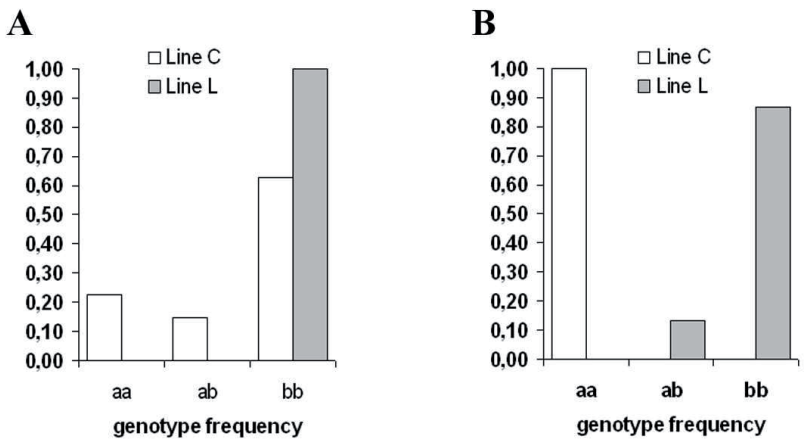

Fig. 8. Frequency of genotypes in the *MYOG locus* on chromosome 1. Examination of microsatellites D1Mit200 (A) and D1Mit218 (B).

The main goal of long-term selection programs to increase body weight was to achieve an increase in muscle weight and to study the changes occurring in muscles as a result of the selection program. Hooper [1976] analyzed the effect of selection for low and high body weight in mice, and concluded that differences in murine biceps weight on day 70 of life were caused in part by the length of muscle fibres, but were mostly due to different numbers of sarcomeres per fibre. Postnatal skeletal muscle growth is mostly due to an increase in length and thickening of fibres, but not an increase in their number [Rehfeld *et al.* 2000]. Hypertrophy pertained to fibres formed during embryonic development and was related to the fusion of mononuclear satellite cells with polynuclear muscle fibres and an increased production of muscle proteins.

Slightly different results were obtained by Aberle and Doolittle [1976] who selected mice for increased body weight. The selected line was heavier by 70-75% than that of control mice. The greater muscle weight on day 60 in the selected line as compared with the control was a function of the number of fibres and not their size. However, clear differences in muscle mass between the sexes were due to the greater diameter of muscle fibres in males than females. Morphometric analyses of skeletal muscle in mice of large body weight growth starting in neonates and in 21-, 42- and 63-day-olds were performed by Summers and Medrano[1994]. They concluded that adults of this line achieved body weight gain and muscle weight gain some 40% greater than in the control. The cross-section surface areas of the GAS, SOL and TA muscles were also significantly greater, a phenomenon that was related to an increase in numbers of fibres in the studied muscles by 30% in relation to the control.

Our selection program, carried on for 108 generations, has resulted in differences in body weight immediately following birth. These differences increased as the growth advanced. On day 7, mice of line C were heavier by 38% than light line mice, and on day 21 by 83% . Sexual dimorphism appeared at 3 months of age, particularly markedly in the heavy line. The males from line C were 19.4% heavier than the females, whereas this difference was only of 14% in line L (Tab. 1). Body weight differences in the two lines were accompanied by differences in the masses of hind limbs, of 27% and 46% on days 7 and 21, respectively. At 3 months, there were differences in muscle mass between the sexes. In line C the males muscles of the hind limb were heavier than those in females by 23.7%, and in line L by 14% (Fig. 1).

Comparing lines selected for heavy and light body weight Aberle and Doolittle [1976] showed that the larger mass of muscles in males was the result of a larger cross-sectional area of muscle fibres, rather than a greater number.

Our research confirms these results and shows that the differences in muscle weight are accompanied by differences in the sizes of muscle fibres (Fig. 2). The average diameter of the fibres making up the mouse *gastrocnemius* muscle in 3-week-old female mice of the L and C lines was influenced by a greater percentage of thinner fibres in relation to the same muscle in males. Furthermore, in line C males, we showed a 60% greater number of nuclei than in L males, and a 30% greater number than in females of the two lines. In 3-month-old animals, the percentage composition of larger fibres had grown, and these differences were mostly marked in heavy mice (Fig. 3). We also found that DNA content per g of tissue was lower in L than in C animals [Rosochacki *et al*. 2005]. This is also related to the number of nuclei in the muscles of older animals, as compared to younger ones, being (on average, in both males and females) about twice as high in 3-month-old mice as compared to 3-week-old ones (Fig. 4).

Martin *et al.* [1979] pursued a dual selection program for increases and decreases in body weight in the post-weaning period. They obtained significant differences in body weight and muscle weight between the selected lines and the control, which were due to a higher level of growth hormone synthesis. Hooper *et al*.[1986] compared

day 21 body, muscle and organ weights of control males, males selected for body weight gain and castrated males of both groups. In the castrated males, the measured parameters were significantly smaller than in the line selected for body weight gain, this indicating that testosterone levels are responsible for body weight growth and an increase in muscle cell size.

Our results may support these findings indirectly. Heavy male mice 3 months old had already been sexually mature for at least 4 to 5 weeks, whereas in the light line sexual maturations were barely beginning in both males and females. The males of the C line also had a greater contribution to total weight due to lower limb muscle weight, and a greater percentage composition of thicker fibres in the *gastrocnemius* muscle, in comparison with females in the same group or light males. As previous research revealed, at that time heavy males exhibited higher peripheral blood testosterone levels than light males [Wirth-Dzięciołowska *et al*. 1996].

During embryonic development, cellular signalling caused myoblasts to exit from the cell cycle, stops division and initiates the process of differentiation. The cells begin to synthesize muscle-specific proteins and finally connect into polynuclear myotubes, which form the muscle during the maturation process . The other population of myoblasts remaining in close proximity to the myofibrils does not form the myotubes. These cells, called satellite cells, are capable of division, supply new nuclei during postnatal muscle growth, and participate in muscle regeneration [Rehfeld *et al.* 2000].

Myogenic determination, differentiation and muscle growth remain under the control of specific gene products belonging to the MyoD family. The changes observed in muscle fibre size are thus related to the activity of myogenic factors of the MyoD family, which are responsible for the proliferation and differentiation of satellite cells [Sacuma *et al*. 2000].

Molecular examination of microsatellite markers on material from the C and L lines indicates significant differences in the segregation of marker alleles conjugated with MyoD genes and confirms that mice selected for body weight gain are characterized by variable genotypic frequencies for microsatellites marking the *loci* of the *MYOD-1*, *MYOG* and *MYF-5* genes, between the selected lines (Figs. 6, 7 and 8). Allelic frequency differences observed in the *loci* of the genes in question are characterized by the absence of certain alleles in some *loci,* which suggests molecular-level differences between the lines. This allows for the supposition that muscle development may occur differently in each line of mice, and that the muscles of adult individuals from these groups may differ in terms of the structure of the fibres, their size and activity (Figs. 2-4). The mass differences observed in the muscles of line L and C mice might be the result of both the number and/or size of muscle fibres, as well as changes in the levels of proteins necessary for development of structural proteins. Our previous work showed that the capacity for protein synthesis was greater in the C than L lines and we also showed that protein turnover was faster in the L than in the C group of mice, a contention supported by the higher measured activities of proteolytic enzymes in the muscles of L mice [Rosochacki *et al.* 2005].

Each of the genes from the *MyoD* family has a different expression pattern. *MYF-5* is activated first, and then *MYOG*. *MYOD1* (*MYF3*) is activated as the third one, after myoblasts have begun to differentiate. Though the roles of many transcription factors during myogenesis are clear, much less is known about the role during the postnatal growth of skeletal muscle. It is known that, following activation, satellite cells express MyoD / Myf-5, and that both factors are detectable in these cells during proliferation in the postnatal period. MyoD1 is also important in the termination of the cell cycle in myoblasts, and in the control of proliferation, thanks to its ability to induce p21 expression, which inhibits the exit from the G1 phase and the onset of S. On the other hand, myogenin and Myf-6(MRF4) are expressed in cells undergoing the final phase of differentiation [Berkes and Tapscott 2005, Sabourin and Rudnicki 2000, Sakuma *et al.* 1999]. Myf-6 (MRF4) is the only gene-yielded expression in prenatal and postnatal stages at a high level. The level of MYF-5 transcripts resembles that of other genes from the *MyoD* family in accounting for less than 10% of MYF-6 transcripts in mature muscle.

Levels of the proteins MYF-5, myogenin and MyoD1 were observed in the studied animals on days 21 and 98. MYF-5 peaked in 3-week-old animals as opposed to 3-month-old ones. The highest MYF-5 level was noted in 3-week-old light and heavy females, whereas the lowest observed level characterised 3-month-old males, irrespective of line. In 3-month-old individuals (irrespective of their gender), the level of MYOD1 was lower than it had been observed in the 3rd week, both in males and in females of the two lines, just as in the case of MYF-5 level. Significant differences were only observed for the heavy line, thus indicating more intensive proliferation during earlier stages of development. In light-line males, MYOD1 remained at a similar level at both examined stages. In line C animals, we observed significantly lowered MYOD1 levels in 3-month-old animals as compared with those 3 weeks old. In light mice the same tendency is also visible, yet at much lower intensity. On average, the difference between the MYF-5 protein levels in 21-day-old and 3-month-old females was of about 1.7 fold, while that between 21-day-old males and 3-month-old males was almost threefold (Fig. 5B). Such much lower level in muscles of 3-month-old males is in agreement with the low postnatal level of this protein only being involved in the formation, activation and regeneration of satellite cells, as opposed to miotube formation.

The myogenin levels were very similar in the individuals studied, though on average they were slightly higher in 3-month-old males than in others. In females, this protein was at a similar level at 3 weeks and at 3 months in both lines. Only in males did the myogenin expression level change somewhat, being higher in 3-month-old animals. In females it stayed virtually unchanged. The myogenin level is likely related to the number of muscle fibres forming during myogenesis, and the number of nuclei in the myofibrils, as well as the percentage composition of nuclei in the fibre (highest in 3-month-old individuals). It may well be that this corresponds with changes in fibre diameter observed in males. In young males, we observed small differences in

muscle fibre size, and the occurrence of mainly small- and medium-diameter fibres. In 3-month-old animals, a whole spectrum of fibre sizes is visible, from very fine up to more than 80μm in diameter.

Histological analyses confirm the differentiated layout of muscle fibres in younger and older mice (Fig. 2 and 3). The muscle of 3-week-old mice of both lines was seen to be dominated (to the tune of 70-90%) by fibres of 20-40 μm in diameter, with only a small percentage of coarser and/or finer fibres being present. In 3-month-old animals we noted no fibres with diameters below 20μm, while 30-60 μm fibres accounted for 70-80% of the total, with some of those observed having diameters as great as 80 μm or more. Fiedler *et al*. [1998] showed that the intensive growth of muscles in pigs was also caused by increased sizes and numbers of myofibrils. Similar data were obtained by Rehfeld and Bünger [1990], who showed significant differences in the thickness and number of myofibrils in mice selected for higher muscle protein content.

Pilot research performed to estimate proteolytic processes in 3-month-old skeletal muscles [Rosochacki *et al*. 2005], allows us to hypothesize that our lines of mice have differentiated in respect to this trait. We have noted faster protein turnover in light mice, as compared with their heavy counterparts. Cathepsin D was the main enzyme participating in myofibril remodelling, in which changes in other proteolytic markers were observed (PSCatD, PIA, LIA).

This is the first report treating together the connection between the proteolysis occurring in muscles and tissue remodelling established with the aid of histology which, most likely, reflects changes occurring earlier in the expression of some genes from the *MyoD* family participating in myogenesis. It seems that the selection of mice for increased or decreased body weight gain over 108 generations may be a good animal model for the study of comprehensive tissue remodelling.

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