Marker-assisted introgression of the Compact mutant myostatin allele \( Mstn^{Cmpt-dl1Abc} \) into a mouse line with extreme growth effects on body composition and muscularity

**Lutz Bünger\(^1\)*, Gerhard Ott\(^2\), László Varga\(^3\), Werner Schlothe\(^4\), Charlotte Rehfeldt\(^5\), Ulla Renne\(^5\), John L. Williams\(^6\) and William G. Hill\(^1\)

\(^1\)Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JT, UK
\(^2\)Fachhochschule Lippe – University of Applied Sciences, Liebigstrasse 87, 32657 Lemgo, Germany
\(^3\)Agricultural Biotechnology Center, PO Box 411, H-2101 Gödöllő, Hungary
\(^4\)Humboldt-Universität zu Berlin, Institut für Nutztierwissenschaften, Landwirtschaftlich-Gärtnerische Fakultät, Unter den Linden 6, D-10099 Berlin, Germany
\(^5\)Research Institute for Biology of Farm Animals, Department of Muscle Biology and Growth, Wilhelm-Stahl-Allee 2, D-18196 Dummerstorf, Germany
\(^6\)Roslin Institute (Edinburgh), Roslin, Midlothian, EH25 9PS, UK

*Current address: Scottish Agricultural College, SLS, GGS, Sir Stephen Watson Building, Bush Estate, Penicuik, Midlothian, EH26 0PH, UK.

Corresponding author: Scottish Agricultural College, SLS, GGS, Sir Stephen Watson Building, Bush Estate, Penicuik, Midlothian, EH26 0PH, UK. Tel: +44 (0)131 535 3227. Fax: +44 (0)131 535 3121. e-mail: L.Bunger@ed.sac.ac.uk

(Received 12 January 2004 and in revised form 17 April 2004 and 30 August 2004)

**Summary**

Myostatin is a negative regulator of muscle growth and mutations in its gene lead to muscular hypertrophy and reduced fat. In cattle, this is seen in ‘double muscled’ breeds. We have used marker-assisted introgression to introduce a murine myostatin mutation, \( Mstn^{Cmpt-dl1Abc} \) [Compact (C)], into an inbred line of mice (DUHi) that had been selected on body weight and had exceptional growth. Compared with homozygous wild-type mice, homozygous \((C/C)\) mice of this line were \(~4–5\%\) lighter, had \(~7–8\%\) shorter tails, substantially increased muscle weights (e.g. quadriceps muscle in males was \(~59\%\) heavier) and an increased ‘dressing percentage’ \((~49\% \text{ vs } 39\%)\), an indicator of overall muscularity. The weights of several organs (e.g. liver, kidney, heart and digestive tract) were significantly reduced, by \(~12–20\%\). Myostatin deficiency also resulted in drastic reductions of total body fat and of various fat depots, total body fat proportion falling from \(~17.5\%\) in wild-type animals of both sexes to \(~9.5\%\) and \(~11.6\%\) in homozygous \((C/C)\) females and males, respectively. Males with a deficiency in myostatin had higher gains in muscle traits than females. Additionally, there was a strong distortion of the segregation ratio on the DUHi background. Of 838 genotyped pups from inter se matings \(~29\%,\ ~63\%\) and \(~8\%\) were homozygous wild type \((+/-)\), heterozygous \((C/+))\) and homozygous \((C/C))\), respectively, showing that \( Mstn^{Cmpt-dl1Abc} \) has lower fitness on this background. This line, when congenic, will be a useful resource in gene expression studies and for finding modifying genes.

1. **Introduction**

Mutations in the gene encoding growth and differentiation factor 8 (GDF8) have been identified in mice as a cause of hypermuscularity. Mice in which production of GDF8 was ablated showed excessive muscularity, with some muscles in homozygotes two to three times the weight of those of their littermates. Because the gene encoding GDF8 affects skeletal muscle development, it was named myostatin (McPherron et al., 1997). In addition to the evidence from the original mouse knockout studies that myostatin regulates muscle development, further studies have implicated myostatin in cell survival (Rios et al., 2002) and in determining the developmental pathway of premusoblasts and adipocytes (Lin et al., 2002; McPherron & Lee, 2002).

Mice, humans and cattle deficient in myostatin have a widespread and dramatic increase in skeletal-muscle
mass (Grobet et al., 1997; Kambadur et al., 1997; McPherron & Lee, 1997; Schuelke et al., 2004). The *myostatin* gene is expressed predominantly in skeletal muscle and, to a lesser extent, fat cells (McPherron & Lee, 2002), and acts as a negative regulator of muscle growth. It is a member of the transforming-growth-factor-β superfamily, which is a large family of secreted growth and differentiation factors that are essential regulators of tissue development and homeostasis. Mutations in the signalling pathway of several family members cause severe diseases.

(i) *Mstn*<sup>Cmpt-dl1Abc</sup> mutation and its modifiers in the Compact mouse

In 1997, the mode of inheritance was reported of a newly identified hypermuscular mouse mutation termed *Compact* (Varga et al., 1997). In a line of mice (here denoted BEH for Berlin high; Bünger, 2001a) selected for high protein amount at the Technical University of Berlin (Germany) (Weniger et al., 1974; Valle Zarate et al., 1994), individuals with a ‘compact’ appearance were noted and a highly muscled line of mice named *Compact* was developed by selection using a muscularity score by visual inspection. Linkage mapping using a Hungarian subpopulation of this line revealed a single locus with a strong association with the *Compact* phenotype on mouse chromosome 1 (denoted *Cmpt*) within an 8.2 cM region (Varga et al., 1997). The *myostatin* gene became a strong candidate gene for *Cmpt*. Sequencing *myostatin* from *Compact* individuals revealed a 12 bp deletion in the propeptide region of the gene, denoted *Mstn<sup>Cmpt-dl1Abc</sup>* (Szabo et al., 1998). The structure of the biologically active growth factor domain is unaffected. The propeptide region, by analogy with other transforming-growth-factors β, might play a role in the proper folding, efficient secretion and regulation of the mature domain of *myostatin* (Szabo et al., 1998). Recent studies have shown that the propeptide is an important inhibitory binding protein for *myostatin* (Lee & McPherron, 2001; Hill et al., 2002). The deleted region in *Compact* mice is highly conserved in all known vertebrate *myostatin* genes (McPherron & Lee, 1997), indicative of its functional importance. It is likely that some activity of mature *myostatin* remains in homozygous *Compact* mice, such that modifier genes might have a significant influence on the phenotypic expression (Szabo et al., 1998). A recent study, using a cross between the Comp 9 inbred line developed from the Hungarian *Compact* subpopulation and CAST/Ei, an inbred line generated from *Mus musculus castaneus*, revealed significant associations with modifiers affecting hypermuscularity of the homozygous *Mstn<sup>Cmpt-dl1Abc</sup>* mutant mice for markers on six chromosomes (Varga et al., 2003).

(ii) Double muscling and the myostatin gene in cattle

Exceptional muscle development, commonly referred to as being ‘double-muscled’ (DM), has been seen in several cattle breeds. Cattle showing the syndrome have greater lean meat yield, particularly in the expensive cuts of meat, and low levels of carcass fat (Hanset, 1982; Arthur, 1995; Casas & Cundiff, 2003). There is also some evidence that these cattle are more feed efficient, the meat is generally much lower in fat and what fat remains is higher in polyunsaturated fatty acids (reviewed by Arnold et al., 2001). Thus the DM phenotype is popular in some parts of continental Europe, but it is less popular in others because of associated welfare problems, particularly calving difficulties. Sequencing of the *myostatin* gene in cattle showed that an 11 bp deletion that truncates the mature protein is associated with the DM phenotype in the Belgian Blue (Grobet et al., 1997; Kambadur et al., 1997; McPherron & Lee, 1997), the Spanish Austrian breeds (Dunner et al., 1997) and four British beef breeds (Smith et al., 2000; J. L. Williams, unpublished). The phenotype associated with this allele is found to be highly variable in, for example, South Devon cattle homozygous for the deletion (Wiener et al., 2002). A different point mutation affecting a single amino acid is associated with the DM phenotype in Piedmontese cattle (Kambadur et al., 1997; McPherron & Lee, 1997). Subsequent studies have shown a *myostatin* allelic series, with loss-of-function mutations associated with DM in different breeds (Grobet et al., 1998).

There are reports of negative side effects of the DM phenotype on several traits in cattle, including reproductive traits (e.g. dystocia), physical fitness (e.g. cardiovascular disadvantages), conformational abnormalities and undesired shifts in muscle-fibre-type distribution (Holmes & Ashmore, 1972; Arthur, 1995; Wegner et al., 2000; Arnold et al., 2001; Coopman et al., 2003). There is also evidence that the degree to which some of these phenotypes are displayed also depends on the genetic background (Wiener et al., 2002). As all the evidence for negative side effects was obtained from cattle the question arises if there are similar effects in a multiparous species.

(iii) Potential use of myostatin in other species

To date, mutations in the *myostatin* gene of farm animals have been described only in cattle, but breeding companies in other meat species are believed to be screening for mutations in this gene with the hope of finding variants associated with increased muscularity and/or leanness. Research in *myostatin*-blocking agents is also of interest for the control of, for example, obesity and muscular dystrophy in humans (McPherron & Lee, 2002; Whittemore et al., 2003),
for the reduction of muscle wastage in HIV patients (Gonzalez-Cadavid et al., 1998), the maintenance of muscle strength in older age (Seibert et al., 2001) and the reaction to strength training (Ivey et al., 2000). Given the unpredictability of the phenotype and potential negative side effects, it is important that the impact of variation in this gene on a wide range of welfare, fitness and production traits is fully explored before it becomes a focus of selection for breeders of meat-producing livestock.

(iv) This work

Here, we describe the marker-assisted introgression of $\textit{Mstn}^{\text{Cmpt-dl1Abc}}$ into a line of mice [Dummerstorf high, inbred (DUHi): Bünger et al., 2001a] that had been selected for growth and so serves as a model for highly selected breeds of meat-producing livestock. This introgression line is being developed into a congenic line and, during its establishment, inter se matings were made to examine the effects of the myostatin mutation on the DUHi background. A phenotypic screen, including detailed carcass dissection, was developed and carried out to quantify the muscular phenotype to extend earlier studies that based the measurement of muscularity on a classification of the phenotype on a visual inspection using a five-grade scale (Varga et al., 2003).

The objectives were to evaluate the marker-assisted introgression, to estimate the effects of the $\textit{Mstn}^{\text{Cmpt-dl1Abc}}$ mutation in both heterozygotes and homozygotes on the muscularity and body composition of an extreme high growth line, and to facilitate future investigations of the side effects of the mutation on reproduction and welfare traits in this model multiparous species.

2. Materials and methods

(i) Origins and derivation of the two growth selected lines

Two of the long-term high-body-weight selected lines, DUHi and BEHi (Berlin high, inbred), imported into our lab and subsequently inbred (Bünger et al., 2001a) were used in this marker-assisted introgression study. The DUHi strain (from which DUHi was derived by inbreeding in Edinburgh) was originally developed in Dummerstorf, Germany, by selection for high body weight at 42 d (days) (Bünger et al., 1983, 2001a). It is the heaviest known inbred mouse line, with male body weights of over 80 g at 70 d, about twice those of its unselected control and five times that of the smallest available inbred line (Bünger et al., 2001b). BEHi was derived from a line (BEHi) founded from mice bought from various pet shops. It was initially selected on protein mass (Weniger et al., 1974; Barkemeyer et al., 1989; Barkemeyer & Horst, 1990), then for high weight combined with low fat (Valle Zarate et al., 1994) and finally on body weight. With male weights of ~60 g at 70 d, the BEHi line is also much heavier than its corresponding low line BELi (21 g), but is lighter and substantially leaner than DUHi. The ‘Compact’ line in Berlin was derived from the first mice seen in early generations of the BEH line that showed this phenotype. It has been shown to be homozygous for $\textit{Cmpt}$, presumably through the selection for body weight, because its control line (in Berlin) was still segregating for this mutation. Animals of the Berlin Compact line were used as the founders for the Hungarian Compact line, and there was no further exchange of genetic material between the laboratories in Berlin and Hungary. Animals imported to our laboratory in Edinburgh came from generation 64 of the BEH line from Germany. Because both the Hungarian (Varga et al., 1997; Szabo et al., 1998) and the Edinburgh subpopulations were derived from the Compact and the high-growth line, respectively, with the same origin in Berlin, these subpopulations might have been homozygous for the $\textit{Mstn}^{\text{Cmpt-dl1Abc}}$ mutation from their foundation.

(ii) Introgression of the $\textit{Mstn}^{\text{Cmpt-dl1Abc}}$ mutation

Animals were derived from a single litter of an F1 cross between a female of BEHi (generation 13 in our lab) homozygous for the $\textit{Mstn}^{\text{Cmpt-dl1Abc}}$ mutation and a wild-type DUHi male (generation 8 in our lab). This was followed by five generations of recurrent marker-assisted backcrossing to the DUHi line, using both sexes as the recurrent parent, to produce a backcross line (denoted DUHi$^c$) segregating for the $\textit{Mstn}^{\text{Cmpt-dl1Abc}}$ mutation. Heterozygous animals of this line were mated inter se in generations 5 (i.e. the fourth backcross) and 6 (denoted gen5 and gen6, with expected proportions of 93·8% and 96·9%, respectively, of the DUHi genotype) to give wild-type, heterozygous and homozygous animals, which were used for the detailed dissection. The DUHi$^c$ line was subsequently maintained by inter se matings using heterozygous parents until gen10. At each generation, a few matings were also made between homozygous C/C mice but, because of fertility problems, no stable homozygous line has yet been developed.

(a) Genotyping for $\textit{Mstn}^{\text{Cmpt-dl1Abc}}$

All genotyping for $\textit{Mstn}^{\text{Cmpt-dl1Abc}}$ for the marker-assisted introgression (up to gen10) were carried out in the Hungarian lab, as described earlier (Szabo et al., 1998). In view of the apparent distortion in viability of the different genotypes, in contrast to data obtained on
Table 1. Body, organ and tissue weights, and anatomical dimensions in mice homozygous (C/C), heterozygous (C/+), and wild type (+/-) for MstnCompact-d1AAbs

|                         | Females |                         | Males |                         | All |
|-------------------------|---------|-------------------------|-------|-------------------------|-----|
|                         | C/C     | C/+                    | +/+   | C/C                    | +/+ | All |
|                         | n = 8   | 67                     | 35    | 12                     | 74  | 56  |
| Body weight dimensions  |         |                        |       |                        |     |     |
| Body weight (g)         | 59.7ab  | 65.4d                  | 62.0e | 76.8c                  | 85.2a | 81.0b | 7.28 | S-G |
| Tail length (cm)        | 11.6cd  | 12.0b                  | 11.9b | 11.7bd                  | 11.8bc | 11.6d | 0.313 | YG |
| Max width lumbar region (cm) | 10.5e  | 11.0b                  | 11.3c | 10.36d                 | 10.95b | 11.3e | 0.592 | G |
| Width shoulder (cm)     | 3.71ab  | 3.39cd                 | 3.3d  | 3.91a                  | 3.57bc | 3.43d | 0.388 | G |
| Width neck (cm)         | 1.35b   | 1.29b                  | 1.26b | 1.55a                  | 1.39b | 1.33b | 0.304 | G |
| Height neck (cm)        | 1.16bc  | 1.04d                  | 1.02d | 1.32a                  | 1.25ab | 1.18c | 0.176 | G |
| Width upper rear leg (cm) | 1.90ab | 1.76cd                | 1.69e | 2.01a                  | 1.80bc | 1.74de | 0.168 | G |
| Width lower rear leg (cm) | 0.9a   | 0.98bc                 | 0.91d | 1.15a                  | 1.01b | 0.95c | 0.086 | G |
| Width upper foreleg (cm) | 1.07bc  | 0.99d                  | 0.95e | 1.24a                  | 1.09bc | 1.04c | 0.106 | G |
| Width lower foreleg (cm) | 0.654bc | 0.637c                 | 0.610d | 0.735a                 | 0.672bc | 0.636cd | 0.075 | G |
| Organ and tissue weights |         |                        |       |                        |     |     |
| Heart (g)               | 0.265c  | 0.319b                 | 0.311b | 0.318b               | 0.362a | 0.373a | 0.073 | S-G |
| Kidney (g)              | 0.740d  | 0.795c,d               | 0.841bc | 0.902b            | 1.070a | 1.091a | 0.144 | G |
| Liver (g)               | 3.94c   | 4.444b                 | 4.56a  | 3.55d                 | 4.04c | 4.33b | 0.500 | G |
| Lung (g)                | 1.03a   | 1.04a                  | 1.02a  | 0.915a                | 0.981a | 0.975a | 0.281 | -   |
| Spleen (g)              | 0.279b  | 0.263b                 | 0.287a | 0.163a               | 0.198a | 0.203a | 0.067 | S |
| Stomach, intestines (g) | 10.21b  | 11.73a                 | 12.16a | 9.25a               | 10.90b | 11.60a | 1.43  | S-G |
| Head, feet, tail (g)    | 7.34f   | 7.61bc                 | 7.60hc | 8.17a               | 8.03a | 7.81b | 0.588 | SX |
| Fur (g)                 | 6.74de  | 6.53a                  | 6.82d  | 7.42e               | 8.57b | 9.02a | 0.815 | SXG |

Traits marked with superscript symbols were measured only in gen5; corresponding sample sizes (n) were 3, 12, 18, 15, 18, 26.

Body weight was fitted as a covariate for all traits except body weight.

Means sharing a common character in their superscript are not significantly different (P > 0.05).
six: standard deviation pooled over all groups.
SXG: traits that are significant (P < 0.05) are sex, sex-by-genotype interaction and genotype effects; –, effect not significant.

other genetic backgrounds, the genotypes of animals in gen10 (80 animals: 28+/-, 45 C/+ and seven C/C) were confirmed by genotyping at the Roslin Institute using a different primer set designed from the mouse mstn sequence (Genbank accession U840055) flanking the site of the 12 bp deletion in the Compact gene (forward, 5'-GTATTGATGGTGAAGACAGTGTTCG-3'; reverse, 5'-GGAAGGTTACAGCAAGATCATCG-3', with predicted fragment sizes of 112 bp for + and 100 bp for C).

(iii) Management of animals

(a) General management

Mice were fed a standard expanded breeding diet [Rat and Mouse No. 3, Special Diet Services, Witham, UK; digestible crude oil, 3.9% ; digestible crude protein, 20.9%; starches, 27.3%; sugars, 11.2%; digestible energy, 12.1 MJ kg⁻¹] from weaning onwards and maintained with controlled lighting (12 h light) at 21 ± 1 °C. Animals were usually housed after weaning at 21 d in full-sib groups (between three and eight individuals) except when litters were small, when age-matched offspring of the same sex of two dams were weaned into one cage. After weaning, mice were caged in plastic cages (MB1, North Kent Plastics, Rochester, UK). Litters of more than 12 pups were usually reduced to 12 in the first 3 days after birth, except when animals were in short supply.

(b) Body weights and dissection

Body weights were taken routinely at 42 d and 70 d, and litter sizes (number born alive) at birth and at 21 d (weaning and tissue harvest for genotyping) were recorded in all generations. Nearly all animals in gen5 and gen6, except for some heterozygotes required for breeding, were subjected to detailed dissection at 70 d after continued exposure to CO₂ and the traits listed in Tables 1 and 2 were measured. A digital image of each mouse was taken (Fig. 1) before the dissection continued. Initially (gen5), body dimension traits were measured using a calliper, which turned out to be too time consuming during the dissection. Therefore, some actual measurements [e.g. height of neck region (Table 1)] were discontinued after gen5 and values (gen5 and gen6) were predicted from the images.
This approach had high correlation coefficients (0.896–0.999) with the calliper measurements and high repeatability coefficients (0.935–0.999 or higher) between three repeats of values derived from image analysis (G. Ott, unpublished).

### Table 2. Muscling and fatness traits in mice homozygous (C/C), heterozygous (C/+), and wild type (+/+) for Mstn<sup>Comp-dl1Abc</sup>

|                  | Females |                      | Males |                      | All  |
|------------------|---------|-----------------------|-------|-----------------------|------|
|                  | C/C  n=8 | C/+ n=67 | +/+ n=35 | C/C  n=12 | C/+ n=74 | +/+ n=56 |
| **Muscling traits** |         |          |          |             |         |          |
| Carcass weight (g) | 33.2<sup>b</sup> | 29.7<sup>d</sup> | 28.1<sup>e</sup> | 35.9<sup>a</sup> | 30.7<sup>c</sup> | 28.4<sup>f</sup> | 1.87 | SXG   |
| Carcass (%)       | 49.1<sup>a</sup> | 41.9<sup>b</sup> | 39.8<sup>d</sup> | 48.7<sup>a</sup> | 41.1<sup>c</sup> | 38.7<sup>e</sup> | 2.18 | S—G  |
| Left leg (g)      | 4.92<sup>b</sup> | 4.21<sup>d</sup> | 3.89<sup>e</sup> | 5.55<sup>a</sup> | 4.58<sup>c</sup> | 4.10<sup>d</sup> | 0.458 | SXG   |
| M. quadriceps (right) (g) | 0.629<sup>b</sup> | 0.493<sup>d</sup> | 0.434<sup>e</sup> | 0.744<sup>a</sup> | 0.554<sup>c</sup> | 0.468<sup>d</sup> | 0.071 | SXG   |
| **Fatness traits** |         |          |          |             |         |          |
| Total body fat (g) | 10.3<sup>c</sup>,d | 12.9<sup>b</sup> | 14.2<sup>a</sup> | 8.06<sup>d</sup> | 9.75<sup>d</sup> | 11.6<sup>b,c</sup> | 3.69 | S—G  |
| Total body fat (%) | 9.47<sup>b</sup> | 16.2<sup>a</sup> | 17.5<sup>a</sup> | 11.6<sup>b</sup> | 15.6<sup>a</sup> | 17.4<sup>a</sup> | 4.76 | S—G  |
| Posterior subcutaneous fat (g) | 1.13<sup>b,c</sup> | 1.67<sup>a</sup> | 1.79<sup>a</sup> | 0.79<sup>d</sup> | 1.08<sup>d</sup> | 1.27<sup>b</sup> | 0.449 | S—G  |
| Epididymal fat (g) | 1.62<sup>c</sup>,d | 2.59<sup>b</sup> | 2.88<sup>a</sup> | 1.01<sup>e</sup> | 1.45<sup>d</sup> | 1.81<sup>c</sup> | 0.753 | S—G  |
| Perirenal and retroperitoneal fat (g) | 1.17<sup>b,c</sup> | 1.81<sup>a</sup> | 1.82<sup>a</sup> | 0.54<sup>d</sup> | 0.98<sup>c</sup> | 1.23<sup>b</sup> | 0.345 | SXG   |
| Interscapular brown fat<sup>a</sup> (g) | 0.241<sup>b,d</sup> | 0.291<sup>a,b</sup> | 0.312<sup>a</sup> | 0.238<sup>c,d</sup> | 0.261<sup>b,c</sup> | 0.312<sup>a</sup> | 0.085 | ——G  |
| Interscapular white fat<sup>a</sup> (g) | 0.538<sup>a,b</sup> | 0.680<sup>a</sup> | 0.714<sup>a</sup> | 0.383<sup>b</sup> | 0.487<sup>b</sup> | 0.620<sup>a</sup> | 0.277 | S—G  |

Traits marked <sup>a</sup> were measured only in gen6; corresponding sample sizes (n) were 5, 55, 17, 9, 59, 30.

Body weight was fitted as a covariate for all traits except Carcass % and Total body fat%.

Means sharing a common character in their superscript are not significantly different (P>0.05).

**SD**: standard deviation pooled over all groups.

**SXG**: traits that are significant (P<0.05) are sex, sex-by-genotype interaction and genotype effects; ——, effect not significant.

Fig. 1. Landmarks (P1 to P17) used to quantify body dimensions (Table 1).
After removing and weighing all organs and tissues given in Tables 1 and 2, the empty carcass was weighed. After harvesting three muscles [left and right M. quadriceps femoris (a muscle group consisting of four muscles: Mm. rectus femoris, vastus lateralis, vastus intermedius and vastus medialis)] and the Pars lumbalis segment of the M. longissimus dorsi] for subsequent histological assays (C. Rehfeldt et al., unpublished), dry-matter weight (DM) of the whole body (carcass, organs and tissues, but without the three muscles) was determined by freeze drying the prepared carcass and the fat percentage predicted from DM percentage by linear regression (Hastings & Hill, 1989).

(iv) Statistical methods

Least-square means (Y) were calculated using the following model,

\[ Y = M + G + R + S + F(R) + (all\ interactions\ between\ G,\ R,\ S) + e \]

where M is the overall mean, G is a genotype effect, R is a generation (= replicate) effect, S is a sex effect, F(R) is a family effect nested within generation and e is the residual error. All effects were fitted as fixed except F and e, which were fitted as random. For analysis of organ and tissue weights except for fat percentage and carcass percentage, body weight was fitted as a covariate. ANOVA was undertaken with GLM using the SAS System for Windows Release 6.08 (SAS Institute, Cary, NC, USA). The additive genetic effect (a) was estimated as one-half of the contrast between the two homozygous groups C/C and +/+ . To estimate the dominance effect (d), a model was fitted in which G was replaced by two effects – H, with two classes, homozygotes and heterozygotes, and G(H), a nested effect distinguishing the two homozygous classes. Then, d was estimated as the contrast between the two H classes, which has the consequence that the unweighted means of the two homozygous groups were used, although the sample sizes were very different (Tables 1, 2). An additional parameter, \( h = 0.5(d + a)/a \) was also computed directly from the estimates of d and a; for example, h takes values of 0, 0·5 and 1·0 if C/C is, respectively, fully recessive, additive or completely dominant, with intermediate values reflecting partial dominance. Values of h provide helpful information but must be looked at with caution when a is very small or nonsignificant. Least-squares means were estimated for each sex for all traits, but additive and dominance effects were estimated for both sexes only when the generation \( \times \) sex interaction was significant.

3. Results

(i) Genotype frequencies

(a) Parental lines

The founder BEHi female was not genotyped but all 11 offspring turned out to be C/+ and, additionally, a random samples of 52 animals from the DUHi line (inbred generation 12; founder male for DUHi was from inbred generation 5) were genotyped and all had the expected +/+ genotype. Finally, 41 animals from inbred generation 13 from line BEHi were also genotyped and all were homozygous C/C.

(b) Backcross matings

In gen3 to gen6, a total of 294 pups resulted from backcross matings, of which 257 were genotyped. These two figures differed because of postnatal losses (including those culled to reduce litter size to 12) and because late litters were not genotyped. Of these backcross animals, 45·5% (117) were heterozygote (C/+ ) and 54·5% (140) homozygous wild type (+/+ ) (Table 3). Although this suggests a heterozygote disadvantage, the deviation from the expected 1:1 ratio was not significant \( (P > 0.05) \) in any generation or over all four generations [calculated \( \chi^2 (\chi^2) = 2.06, \chi^2 (\chi^2; 1 df, 0.05) = 3.84 \) , and there was homogeneity across generations [contingency table, \( \chi^2 = 0.242, \chi^2 (3 df, 0.05) = 7.81 \) ].

(c) Inter se matings

From inter se matings from gen4 to gen10 a total of 838 pups were genotyped (Table 3), of which 244 (29-1%) were homozygous wild type (+/+ ), 528 (63·0%) heterozygotes (C/+ ) and 66 (7·9%) homozygous for Compact (C/C). This is a highly significant distortion of the expected segregation ratio (Table 3). Although numbers in some individual generations were small, the deviations from the expected 1:2:1 ratio were significant \( (P < 0.05) \) in all generations except gen5, which was just below the significance threshold \( (\chi^2 = 5.8) \); also, there was (just) homogeneity across generations [contingency table, \( \chi^2 = 20.1 \), \( \chi^2 (12 df, 0.05) = 21.0 \) , allowing summary over these generations. With C/C excluded, the frequencies of the two remaining genotypes \( (C/+ , +/+ , C/C) \) did not depart significantly from the expected 2:1 ratio \( [\chi^2 = 1.04, \chi^2 (1, 0.05) = 3.84] \). Indeed the apparent small excess of +/+ over C/+ in backcross matings was reversed here (Table 3).

(ii) Body weight and dimensions

Sex effects were significant for most of the traits in this category apart from body and tail length, and could not be accounted for by differences in body weight.
because it was fitted in the model as a covariate (Table 1). Genotype had a significant effect on all of the traits, but for only one (body length) was there a significant $S \times G$ interaction in which males and females reacted differently to the MstnCmpt-dl1Abc mutation.

(a) Comparison of homozygotes

Homozygous Compact C/C animals of both sexes were 4–5% lighter than their +/+ litter mates (Table 1). Compact females were significantly shorter than +/+ females, but the males were not. C/C animals of both sexes had significantly shorter tails (7–8%). Taking body length and tail length together, Compact animals were about 4% shorter than their wild-type controls. The maximal width in the lumbar region (‘belly width’) of Compact animals was less than that of the wild type (by over 8%), but their shoulder region was about 12% wider, both differences being significant. The increased muscularity in Compact animals of both sexes is also reflected in increased neck measurements (height and width), which were both increased by about 10%. Similarly, leg measurements were also increased in Compact by 7–12% in females and by 15–21% in males. The estimates of additive effects, $a$ (Table 4), are based on half the homoygous differences (C/C less +/+ ) and are, of course, significant when the contrast is significant. They varied in direction, with measurements of muscularity being positive and tail length and ‘belly width’ negative (Table 1).

(b) Dominance effects

Dominance was investigated by comparing heterozygotes (C+) and the unweighted means of the two homozygotes. If $a$ and $d$ have the same sign, this implies that the MstnCmpt-dl1Abc allele is dominant over the wild-type allele, completely dominant if $d = a$. Conversely, if they take opposite sign, the Compact allele is (partially) recessive. For body weight, C/+ animals of both sexes are significantly heavier than both homozygotes, reflected in high, significant $d$ values and $h < -1$ (the sign of $h$ being that of $a$) (Table 4). This is also the case for body length, with heterozygotes again superior, but there was a significant $S \times G$ interaction. For most of the other dimensional traits, $h$ varied between 0.23 and 0.40, indicating that MstnCmpt-dl1Abc is partially recessive (C/+ animals closer in performance to +/+ than C/C).

(iii) Organ and tissue weights

Although the effect of body weight was removed as a covariate, the effects of sex were significant for the weights of all of the organ and tissue traits except the lung, and the effects of genotype were significant for all but lung, spleen and ‘head, feet, tail’ weights (Table 1). There were significant genotype $\times$ sex interactions only for the weight of fur and of the combined ‘feet/head/tail’, with larger effects seen in males.

(a) Comparison of homozygotes

Heart, kidney and liver were reduced in homozygous C/C animals by 12–18% (Table 1). The spleen and the combined stomach and intestine weight were reduced by around 18% in Compact animals, with a low, non-significant reduction of the spleen in females. The combined weight of the head, feet and tail was slightly lower in C/C females and larger in C/C males than in +/+ . Whereas the weight of the fur was little changed in C/C females, it was significantly reduced in C/C males by 18%.

**Table 3. Offspring numbers and tests of segregation ratios from backcross matings and inter se matings**

| Generation | Backcross matings | Inter se matings |
|------------|-------------------|-----------------|
|            | C/+ | +/+ | All | $\chi^2$ | df | C/+ | C/C | All | $\chi^2$ | df |
| 3          | 19  | 23  | 42  | 0.38  | 1  | 21  | 6   | 67  | 9.2  | 2  |
| 4          | 31  | 34  | 65  | 0.13  | 1  | 12  | 6   | 51  | 5.8  | 2  |
| 5          | 45  | 54  | 99  | 0.82  | 1  | 56  | 17  | 223 | 40.2 | 2  |
| 6          | 22  | 29  | 51  | 0.96  | 1  | 66  | 6   | 184 | 47.8 | 2  |
| 7          |     |     |     |       |    | 36  | 19  | 159 | 18.7 | 2  |
| 8          |     |     |     |       |    | 25  | 5   | 75  | 13.7 | 2  |
| 9          |     |     |     |       |    | 28  | 7   | 79  | 12.2 | 2  |
| 10         |     |     |     |       |    | 244 | 66  | 838 | 132.3 | 2 |
| Total      | 117 | 140 | 257 | 2.06  | 1  | 291 | 63  | 100 | 20.1 | 12 |
| Percentage | 45.5| 54.5| 100 |       |    | 29.1| 63  | 100 |       |    |

$\chi^2$, calculated $\chi^2$ values. Tabular $\chi^2$: the values at $P < 0.05$ (df, $\chi^2$) are (1, 3.84), (2, 5.99), (3, 7.81) and (12, 21.0).
Traits marked recessive, partially recessive, additive, partially dominant, completely dominant or overdominant, respectively).

Body weight was fitted as a covariate for all traits except body weight.

Width neck

Height neck

Width upper rear leg (cm)

Width lower rear leg (cm)

Width upper foreleg (cm)

Width lower foreleg (cm)

Heart (g)

Kidney (g)

Liver (g)

Lung (g)

Spleen (g)

Stomach, intestines (g)

Head, feet, tail (g)

Fur (g)

| Traits | Both sexes | Females | Males |
|--------|------------|---------|-------|
| | $a$ | $se$ | $d$ | $se$ | $h$ | $a$ | $d$ | $a$ | $d$ |
| Body weight (g) | -1.62 | 0.88 | 5.76 | 0.98 | -1.28 | -0.15 | 0.19 | 0.04 | 0.15 |
| Body length (cm) | -0.055 | 0.031 | 0.167 | 0.037 | 1.03 | 0.07 | 0.19 | -0.04 | 0.15 |
| Tail length (cm) | 0.45 | 0.059 | 0.089 | 0.069 | 0.40 | 0.03 | 0.15 | 0.04 | 0.15 |
| Max width lumbar region (cm) | -0.21 | 0.034 | 0.11 | 0.040 | 0.23 | 0.01 | 0.15 | 0.03 | 0.15 |
| Width shoulder (cm) | 0.21 | 0.034 | -0.112 | 0.039 | 0.23 | 0.01 | 0.15 | 0.03 | 0.15 |
| Width neck (cm) | 0.076 | 0.027 | -0.038 | 0.031 | 0.25 | 0.01 | 0.15 | 0.03 | 0.15 |
| Height neck (cm) | 0.071 | 0.016 | -0.026 | 0.017 | 0.32 | 0.01 | 0.15 | 0.03 | 0.15 |
| Width upper rear leg (cm) | 0.122 | 0.017 | -0.051 | 0.020 | 0.29 | 0.01 | 0.15 | 0.03 | 0.15 |
| Width lower rear leg (cm) | 0.094 | 0.009 | -0.034 | 0.010 | 0.32 | 0.01 | 0.15 | 0.03 | 0.15 |
| Width upper foreleg (cm) | 0.083 | 0.011 | -0.038 | 0.012 | 0.27 | 0.01 | 0.15 | 0.03 | 0.15 |
| Width lower foreleg (cm) | 0.036 | 0.007 | -0.005 | 0.009 | 0.44 | 0.01 | 0.15 | 0.03 | 0.15 |

| Organ and tissue weights |
|--------------------------|
| Heart (g) | -0.025 | 0.007 | 0.024 | 0.008 | 0.03 |
| Kidney (g) | -0.073 | 0.014 | 0.040 | 0.017 | 0.23 |
| Liver (g) | -0.351 | 0.050 | 0.143 | 0.059 | 0.30 |
| Lung (g) | -0.013 | 0.028 | 0.026 | 0.033 | -0.47 |
| Spleen (g) | -0.012 | 0.007 | -0.001 | 0.008 | 0.55 |
| Stomach, intestines (g) | -1.07 | 0.14 | 0.52 | 0.17 | 0.26 |
| Head, feet, tail (g) | 0.026 | 0.059 | 0.084 | 0.070 | 2.14 |
| Fur (g) | -0.421 | 0.082 | 0.063 | 0.101 | 0.43 |

$ae$, standard error pooled over all groups.

Significant dominance effects were found for a few organs: heart, liver and ‘stomach, intestines’ weights, with the Compact allele tending to be partially recessive ($h=0.03–0.30$) (Table 4).

Muscling traits

The effects of sex were significant for all muscling traits (Table 2). Males had higher ‘muscling weights’ than females, even though the effect of body weight was accounted for. The proportion of the carcass to the total body weight was higher in females than in males. The genotype effects were significant for all traits in this category. There were also significant genotype ¥ sex interactions for the weight of the carcass, the leg and for M. quadriceps, with higher Compact effects in males.

Comparison of homozygotes

Homozygous C/C animals had much heavier carcasses than +/+ individuals, the differences amounting to 18% (in females) and 26% (in males). There were also substantial effects on the ratio of the carcass to the total body weight: the relative carcass weight increased from around 40% to 49% in both sexes. The weight of the whole leg (left) was 27% (in females) and 35% (in males) higher in C/C than in wild-type animals, and that of the M. quadriceps (right) increased by 45% in females and by 59% in males (Table 2).

Dominance effects

For all traits, there were significant negative dominance effects ($d$) for the C allele, with slightly higher values in females. Because $a$ and $d$ differ in their sign (Table 4) and the degrees of dominance ($h$) for the muscling traits were between 0.24 and 0.31, the C allele is partially recessive (Table 5).

Fatness traits

Sex effects were significant for all fat traits except the proportion of fat and the interscapular brown fat depot, with higher fat amounts in females (Table 2).
Table 5. Genotypic values and degrees of dominance for muscling and fatness traits

| Muscling traits | Both sexes | Females | Males |
|----------------|------------|---------|-------|
|                | a | se | d | se | h | a | d | a | d |
| Carcass weight (g) | 3.16 | 0.187 | −1.22 | 0.228 | 0.31 | 2.57 | −1.40 | 3.75 | −1.05 |
| Carcass percentage (%) | 4.84 | 0.265 | −2.53 | 0.288 | 0.24 | 0.514 | −0.276 | 0.73 | −0.168 |
| Left leg (g) | 0.62 | 0.046 | −0.222 | 0.055 | 0.32 | 0.097 | −0.053 | 0.138 | −0.038 |
| M. quadriiceps (right) (g) | 0.118 | 0.007 | −0.045 | 0.009 | 0.31 | 0.007 | −0.045 | 0.009 | 0.31 |

| Fatness traits | Both sexes | Females | Males |
|----------------|------------|---------|-------|
| Total body fat (g) | −1.864 | 0.369 | 0.30 | 0.43 | 0.50 | −0.329 | 0.315 | −0.344 | 0.084 |
| Total body fat (%) | −3.469 | 0.578 | 1.93 | 0.63 | 0.46 | −0.516 | 0.075 | 0.177 | 0.089 | 0.38 |
| Posterior subcutaneous fat (g) | −0.283 | 0.045 | 0.127 | 0.053 | 2.91 | −0.337 | 0.044 | 0.200 | 0.051 | 0.24 |
| Epididymal fat (g) | −0.516 | 0.075 | 0.177 | 0.089 | 0.38 | −0.337 | 0.044 | 0.200 | 0.051 | 0.24 |
| Perirenal and retroperitoneal fat (g) | −0.036 | 0.010 | 0.001 | 0.012 | −2.4 | −0.103 | 0.032 | 0.022 | 0.039 | 0.50 |
| Interscapular brown fat (g) | −0.036 | 0.010 | 0.001 | 0.012 | −2.4 | −0.103 | 0.032 | 0.022 | 0.039 | 0.50 |
| Interscapular white fat (g) | −0.103 | 0.032 | 0.022 | 0.039 | 0.50 | −0.337 | 0.044 | 0.200 | 0.051 | 0.24 |

a, additive effect of \( C = 0.5[(C/C) - (+/+)] \); d, dominance effect in \( C/+ = (C/+ - 0.5[(C/C) + (+/+)]) \); if \( a \) and \( d \) have the same sign, \( Mstn^{cmp-dl1abc} \) is partially dominant and if it has the opposite sign then it is partially recessive; \( h \), degree of dominance \( = (d + a)/2a \) (for \( h < 0, h = 0, h < 0.5, h > 0.5, h = 1, h > 1, Mstn^{cmp-dl1abc} \) is underdominant, completely recessive, partially recessive, additive, partially dominant, completely dominant or overdominant, respectively).

Values in bold are significant \((P<0.05)\).

The genotype effects were significant for all fatness traits, but the sex \( \times \) genotype interaction was significant for only one fat depot (Table 2).

(a) Comparison of homozygotes

Homozygous \( C/C \) animals of both sexes had significantly less total body fat: 27% less in females and 31% less in males, corresponding to a \( C/C \ vs \ (+/+ \) difference in proportion fat of 8.0% (in females) and 5.8% (in males). Similarly all individual fat depots measured were reduced, by 23–44% in females, with the highest reductions in epididymal fat, and by 24–56% in males, with the highest reduction in perirenal and retroperitoneal fat (Table 2).

(b) Dominance effects

There were significant dominance effects \( (d) \) for four of the seven fat traits. The degrees of dominance \( (h) \) were mostly between 0.24 and 0.50, apart from two unrealistic values for the posterior subcutaneous fat and the interscapular brown fat. This indicates again that the \( C \) allele is partially recessive (Table 5).

4. Discussion

(i) Genotype frequencies

The analysis of the genotyped pups available from \( inter \ se \) matings of the backcross line showed 29% \(+/+\), 63% \( C/+ \) and only 8% \( C/C \). As the ratios of \( C/+ \) to \(+/+\) in both backcross and \( inter \ se \) matings were as expected, only homozygote individuals seem to be affected. This very strong distortion of the segregation ratio shows that \( C/C \) individuals have either a lower fertilization rate or a lower subsequent survival rate. Although there is a recent report of an apparent loss of homozygous embryos in superovulated cows carrying the 11-bp deletion associated with double muscling in cattle that were bred with heterozygous bulls (Potts et al., 2003) this distortion was unexpected, because earlier mouse crosses involving the \( Mstn^{cmp-dl1abc} \) and other backgrounds (undertaken by L. Varga, unpublished) did not show any significant deviation from the 1:2:1 ratio. For example, in a large F2 population from crosses of \( Mstn^{cmp-dl1abc}/Mstn^{cmp-dl1abc} \) animals of the Comp9 strain and inbred CAST/Ei founders, genotype frequencies agreed well with the expected ratio (Varga et al., 2003). There was, however, indication of a distorted segregation in the experiment of McPherron et al. (1997) in which the GDF-8-encoding gene (i.e. myostatin) was disrupted by homologous gene targeting. Of 678 offspring from crosses of F1 heterozygotes, 25% were \(+/+\), 56% \(+/-\) and 19% \(-/-\), showing a significant but less strong departure from the expected ratio than in our experiment. Our results suggest that the deleterious effect of \( C/C \) results from an interaction with other genes in the DUHi background. Because the line DUHi was not fully
Table 6. Litter size at birth (LS0) and at weaning (LS21) resulting from inter se matings in lines DUHi* and DUHi

| Generation | 6  | 7  | 8  | 9  | 10 | 6–10 | Diff | se  |
|------------|----|----|----|----|----|------|------|-----|
| DUHi*      | n  | n  | n  | n  | n  |      |      |     |
| LS0        | 27 | 25 | 17 | 9  |     | 9    | 9-4  | 0.1 |
| LS21       | 8.5| 7.4| 9.5| 8.4| 8.9| 8.8  | 0.63 |
| DUHi       | n  | n  | n  | n  | n  |      |      |     |
| LS0        | 16 | 12 | 10 | 13 | 13 | 10-0 | 3.9  |     |
| LS21       | 7.7| 10.7| 10.7| 10.2| 11.5| 10.4| 3.0  |     |

Diff, difference between DUHi* and DUHi for LS0 and LS21 with its standard error (se).

congenric at the time of this study, the possibility can not be ruled out that remaining alleles from the BEHi background other than at the Compact locus might have caused this distortion, but the probability of this seems low. This would imply an interaction between such linked introgressed genes and the DUHi background, for this distortion of the segregation ratio is not seen in BEH and, in view of the large phenotypic ground, for this distortion of the segregation ratio is such linked introgressed genes and the DUHi back-

This experiment was not designed to analyse the deficiency of the C/C animals and therefore the time at which the losses occurred was not recorded. Because tissues for genotyping were not collected before weaning, losses could have occurred at any point from fertilization until weaning. In order to estimate whether losses were pre-, peri- or postnatal and to check whether a reduction in litter size from inter se matings was associated with the C/C shortage, we compared the average size of litters at birth and at weaning from generations 6–10 (excluding previous generations when litter size in DUHi* is affected by heterozygosity in the first generations after the F1) in the recombinant line DUHi* (from inter se matings) with that of the contemporaneous generations of the parent line DUHi (Table 6). Because there were, on average, only 8% C/C animals in line DUHi*, a reduction in litter size of ~17% would be expected, corresponding at this litter size to a reduction of about 1.5–2 pups. This seems not to be the case (Table 6). Litter size at birth in line DUHi* is similar to that of the recurrent backcross partner line DUHi, which would be expected if there was no loss of C/C embryos. Because genotyping was undertaken on weaned animals, there could, however, still be a difference in postnatal or preweaning losses of homozygous pups. The litter size at weaning is influenced by management procedures (reduction of larger litters to 12), but this would affect both lines (DUHi and DUHi*) in a similar way. However, both lines had similar litter sizes at weaning as well (Table 6), suggesting that differences might have occurred at fertili-

gametes carrying the C allele, with no substantial effects on the total number of fertilized ova. We hope to investigate this hypothesis further in future studies.

(ii) Body weight and dimensions

Compact had a significant effect on most traits measured. Animals carrying two copies of the C allele were slightly lighter and shorter (especially in the tail), with decreased maximal width in the lumbar region and increased measurements reflecting muscularity, like shoulder width, neck width and height, and upper leg and lower leg widths.

At first sight, our results regarding the effects of Compact on body weight seem to be in contradiction to observations of transgenic mice with a disrupted myostatin gene. Over an age range of 2–5 months, animals of both sexes with the myostatin gene knocked out were ~20–30% heavier than wild-type litter mates (McPherron et al., 1997), whereas our Compact animals on the DUHi background were slightly lighter. There are two major differences between these studies, however: the mutation and the genetic background. The Compact mutation is in the propeptide and does not affect the mature protein, whereas the knockout construct of McPherron et al. replaced the myostatin coding region with a reporter gene. It is known that the propeptide interacts with the mature protein, so it is possible that, in mice with the C allele, there might be an aberrant myostatin function, whereas, in the knockout, there is no myostatin protein produced. The genetic background for the knockout was C57BL/6, a line with body weights of about 30 g at 2–3 months of age in males and 23 g in females. By contrast, DUHi* animals are two or three times heavier at a similar age, males weighing about 80 g and females over 60 g. On this genetically high-growth DUHi* background, the myostatin deficiency caused by the murine mutation Mstn<sup>Cre<sup>dl</sup>Abc</sup> does not result in additional body growth, whereas it does on the less extreme BEHi background. One explanation might be that

https://doi.org/10.1017/S0016672304007165 Published online by Cambridge University Press
growth is already near a maximum and the C/C animals prioritize muscle protein over other tissues like fat, some other organs and possibly bone. The latter seems to be supported by the finding of decreased body and tail lengths.

The reduced belly width seems mainly to reflect a reduced fatness and a hypertrophied skeletal musculature, leading to a change in the abdominal shape. The effect on traits mainly determined by the amount of muscle tissue (shoulder, neck and leg measures) is not as extreme (~7–21%) (Table 1) as observed in mice with myostatin deficiencies created by transgenic methods, in which the weight of individual muscles was found to be increased by a factor of two or three (McPherron et al., 1997; Hamrick et al., 2000). Our measures in Table 1 are linear dimensions, however, such that a 10% change in the linear dimensions corresponds to an increase of over 30% in volume and, assuming a similar tissue density, in weight. However, the weight of M. quadriceps, which was increased by 45% in females and 59% in males, also showed that the Compact effects are less extreme in this high-growth background.

The body weight of heterozygous animals in this study is significantly higher than that of both homozygotes in both sexes. Such superiority of heterozygotes over either corresponding homozygote is not unique [e.g. Lipsitch et al. (2003) termed it ‘allele-specific overdominance’, observing a higher disease resistance in heterozygous individuals] but needs further investigation and seems to contrast with results obtained in transgenic mice with a disrupted myostatin function on a ‘normal growth’ background (C57BL/6), in which the weights of the heterozygotes were between those of the two homozygotes (McPherron et al., 1997). However the C/+ vs +/+ difference (~5%) is similar in our study, and the low body weight of the C/C animals in our study seems to be the reason for this C/+ superiority.

(iii) Organ and tissue weights

The Compact mutation is associated with 12–20% reduced weights of essential organs such as heart, kidney, liver and gut in both sexes (Table 1). Reductions of internal organs have also been seen in double-muscled cattle when compared with those of wild-type controls (Ansay & Hanset, 1979). These reductions in organ size might be a partial explanation for the reduced stress resistance found in double muscled cattle (e.g. Arthur, 1995; Arnold et al., 2001). The reduced heart size seems especially of note: although it consists mainly of (cardiac) muscle it is not hypertrophied like the skeletal muscles, indicating that effects of myostatin on the regulation of these muscles differs. The cause of the substantially reduced fur weights in males but not in females is unclear.

(iv) Muscling traits

Because the carcass defined here consists mainly of muscle and bone (see also Grobet et al., 2003), the significant increases in carcass weights seen in Compact animals of 18% (in females) and 26% (in males) are not surprising (Table 2). It is of note that the absolute carcass weights of males increased significantly more than those of females, but the proportion of the carcass to total body weight increased similarly in both sexes, from ~40% to ~49%. Carcass weight might be superior to scoring a single muscle, which would not take account of changes of the ratios between different muscles owing to selection. Measuring the carcass would provide a more objective measure but can not yet be undertaken in vivo in mice; however, computer-aided tomography (CT) scanning might provide a possible route for live mice as for larger animals (e.g. Jones et al., 2002).

(v) Fatness traits

The DUH line is very heavy but not extremely obese, although males have 17–22% body fat compared with ~10% in an unselected control (Bünger et al., 1998). Because myostatin deficiency has substantial anti-obesity effects in mice (Lin et al., 2002; McPherron & Lee, 2002) it was important to estimate the effects of the Compact mutation on fatness in the DUHi2 line. Homozygous (C/C) animals of both sexes have significantly less total body fat and corresponding reductions in fat percentage from 17.5% to 9.5% (in females) and from 17.4% to 11.6% (in males). Similar fat proportion ranges were reported for males (6–10%) and females (9–10.5%) for the Hungarian Compact line, which weighs up to 36 g (in males) and 31 g (in females) at ~70 d (Fekete et al., 1996). The reduction in total body fat in our study was accompanied by a reduction in all measured individual fat depots by 23–56% (Table 2). All fat traits were significantly affected by genotype and sex, with the exception of total body fat proportion and the brown portion of the interscapular fat depots, in which no sex difference were found. Generally, there were significant genotype x sex interactions.

(vi) Myostatin effects in heterozygotes and dominance effects

Homozygotes for one of the double-muscling alleles in cattle and the MstnCompact-dl1Abc allele in mice seem to have several negative side effects, so a breeding scheme based on a homozygous terminal sire line and wild-type female line might be a viable alternative breeding strategy in farm animals. Its utility depends inter alia on the performance of heterozygotes. In this
study, heterozygotes were substantially heavier than both homozygotes in both sexes. Overall, for the muscle and fat traits, about one-quarter to one-third of the total difference between the two homozygotes is present in the C/+ vs +/+ difference (i.e. C was partially recessive for these traits) (see h values, Tables 4, 5). Similar effects, increased muscling and decreased fat, to those reported here for heterozygous animals have been seen in heterozygous South Devon cattle, while increased calving problems occurred only in homozygotes (Wiener et al., 2002).

This experiment has clearly shown that MstnCmpt-dl1Abc has substantial negative effects on fat deposition and positive effects on muscle mass, even though the mouse line into which it was introgressed might be close to an upper body weight limit (Bünger et al., 2001 b). MstnCmpt-dl1Abc is associated with a similar, possibly less extreme, phenotype to that of mice with ablated myostatin expression created using transgenic technology (McPherron et al., 1997). Thus, MstnCmpt-dl1Abc can be regarded as another allele affecting myostatin activity (Szabo et al., 1998) and, like deletions, knockouts and substitutions, results in hypermuscularity. Although the precise molecular mechanisms might differ between these different mutations, the impact on the phenotype of perturbing myostatin expression is similar.

MstnCmpt-dl1Abc is therefore a valuable model for other mammalian species and will help to inform on the consequences of the widespread use of such natural or induced mutations if they become available in animal breeding. Variation in the magnitude of the mstn gene suggests that myostatin interacts with other genes in the control of normal muscular and adipose development. Myostatin also appears to affect other production and welfare traits negatively, and it is likely that these will also vary in magnitude with dependence on the genetic background (Nadeau, 2001). It would therefore be important to introgress Compact onto different genetic backgrounds and to investigate its effects on a wide spectrum of traits.

We are grateful to the BBSRC for financial support of the whole project and to SEERAD for funding to LB to complete the final analysis. JWL acknowledges support from Defra through LK0655. We thank Li-fang Liang and Geoff Simm for valuable comments on the manuscript, and Mike Robb, Charlotte Bruley, Adrian White, Barbara Urquhart and Krisztina Sóvári for technical assistance.

References

Ansay, M. & Hanset, R. (1979). Anatomical, physiological and biochemical differences between conventional and double-muscled cattle in the Belgian blue and white breed. Livestock Production Science 6, 5–13.

Arnold, H., Della-Fera, M. A. & Baile, C. A. (2001). Review of myostatin history, physiology and applications. International Archives of Bio science 2001, 1014–1022.

Arthur, P. F. (1995). Double muscling in cattle: a review. Australian Journal of Agricultural Research 46, 1493–1515.

Barkemeyer, H. & Horst, P. (1990). Consequences of long-term selection for protein deposition on growth of mice. Journal of Animal Breeding and Genetics – Zeitschrift für Tierzüchtung und Züchtungsbiologie 107, 52–60.

Barkemeyer, H., Horst, P. & Schlote, W. (1989). Antagonism between growth and fitness of mice as a consequence of long term selection for protein deposition. Journal of Animal Breeding and Genetics – Zeitschrift für Tierzüchtung und Züchtungsbiologie 106, 433–442.

Bünger, L., Schüler, L., Kupat, B. & Renne, U. (1983). Selection for growth in model animals (laboratory mice) 2. Direct selection response. Archiv für Tierzucht – Archives of Animal Breeding 26, 281–293.

Bünger, L., Laidlaw, A. H., Bullfield, G., Eisen, E. J., Medrano, J. F., Bradford, G. E., Pirchner, F., Renne, U., Schlote, W. & Hill, W. G. (2001 a). Inbred lines of mice derived from long-term on growth selected lines: unique resources for mapping growth genes. Mammalian Genome 12, 678–686.

Bünger, L., Renne, U. & Buis, R. C. (2001 b). Body weight limits in mice – long-term selection and single genes. Encyclopedia of Genetics (ed. E. C. R. Reeve), pp. 337–360. Fitzroy Dearborn.

Bünger, L., Renne, U., Dietl, G. & Kuhla, S. (1998). Long-term selection for protein amount over 70 generations in mice. Genetical Research 72, 93–109.

Casas, E. & Cundiff, L. V. (2003). Maternal grand sire, grand dam and sire breed effects on growth and carcass traits of crossbred cattle. Journal of Animal Science 81, 904–911.

Coopman, F., De Smet, F., Gengler, N., Haegeman, A., Jacobs, K., Van Poucke, M., Laevens, H., Van Zeveren, A. & Groen, A. F. (2003). Estimating internal pelvic sizes using external body measurements in the double-muscled Belgian Blue beef breed. Animal Science 76, 229–235.

Dunner, S., Charlier, C., Farnir, F., Brouwers, B., Canon, J. & Georges, M. (1997). Towards interbreed lbd fine mapping of the mb locus: double-muscling in the asturiana de los valles breed involves the same locus as in the Belgian Blue cattle breed. Mammalian Genome 8, 430–435.

Fekete, S., Szakáll, I., Andrássoszky, E., Kösa, E. & Hullár, I. (1996). Body composition of mice of different body condition score and sex. Acta Veterinaria Hungarica 44, 399–410.

Gonzalez-Cadavid, N. F., Taylor, W. E., Yarasheski, K., Sinha-Hikim, I., Ma, K., Ezzat, S., Shen, R., Lalani, R., Asa, S., Mamita, M., Nair, G., Arver, S. & Bhasin, S. (1998). Organization of the human myostatin gene and expression in healthy men and HIV-infected men with muscle wasting. Proceedings of the National Academy of Sciences of the USA 95, 14938–14943.

Grobet, L., Martin, L. R., Poncelet, D., Pirottin, D., Brouwers, B., Riquet, J., Schoeberlein, A., Dunner, S., Menissier, F., Massabanda, J., Fries, R., Hansen, R. & Georges, M. (1997). A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle. Nature Genetics 17, 71–74.

Grobet, L., Poncelet, D., Royo, L. J., Brouwers, B., Pirottin, D., Michaux, C., Menissier, F., Zanotti, M., Dunner, S. & Georges, M. (1998). Molecular definition of an allelic series of mutations disrupting the myostatin

https://doi.org/10.1017/S0016672304007165 Published online by Cambridge University Press
function and causing double-muscling in cattle. Mammalian Genome 9, 210–213.

Grobet, L., Piriotin, D., Farnir, F., Poncelet, D., Royo, L. J., Brouwers, B., Christians, E., Desmecht, D., Coignoul, F., Kahn, R. & Georges, M. (2003). Modulating skeletal muscle mass by postnatal, muscle-specific inactivation of the myostatin gene. Genesis 35, 227–238.

Hamrick, M. W., McPherron, A. C., Lovejoy, C. O. & Hudson, J. (2000). Femoral morphology and cross-sectional geometry of adult myostatin-deficient mice. Bone 27, 343–349.

Hanset, R. (1982). Major genes in animal production, examples and perspectives: cattle and pigs. Proceedings of the 2nd World Congress on Genetics applied to Livestock Production, Madrid 5, 439–452.

Hastings, I. M. & Hill, W. G. (1989). A note on the effect of different selection criteria on carcass composition in mice. Animal Production 48, 229–233.

Hill, J. J., Davies, M. V., Pearson, A. A., Wang, J. H., Hewick, R. M., Wolfman, N. M. & Qiu, Y. (2002). The myostatin propeptide and the follistatin-related gene are inhibitory binding proteins of myostatin in normal serum. Journal of Biological Chemistry 277, 40735–40741.

Holmes, J. H. & Ashmore, C. R. (1972). A histochemical study of development of muscle fiber type and size in normal and ‘double muscled’ cattle. Growth 36, 351–372.

Ivey, F. M., Roth, S. M., Ferrell, R. E., Tracy, B. L., Lemmer, J. T., Hurlbut, D. E., Martel, G. F., Siegel, E. L., Fozard, J. L., Metter, J., Fleg, J. L. & Hurley, B. F. (2000). Effects of age, gender, and myostatin genotype on the hypertrophic response to heavy resistance strength training. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 55, M641–M648.

Jones, H. E., Lewis, R. M., Young, M. J. & Wolf, B. T. (2002). The use of X-ray computer tomography for measuring the muscularity of live sheep. Animal Science 75, 387–399.

Kambadur, R., Sharma, M., Smith, T. P. & Bass, J. J. (1997). Mutations in myostatin (GDF8) in double-muscled Belgian Blue and Piedmontese cattle. Genome Research 7, 910–916.

Lee, S. I. & McPherron, A. C. (2001). Regulation of myostatin activity and muscle growth. Proceedings of the National Academy of the Sciences of the USA 98, 9306–9311.

Lin, J., Arnold, H. B., Della-Fera, M. A., Azain, M. J., Hartzell, D. L. & Baile, C. A. (2002). Myostatin knock-out in mice increases myogenesis and decreases adipogenesis. Biochemical and Biophysical Research Communications 291, 701–706.

Lipsitch, M., Bergstrom, C. T. & Rustom, A. (2003). Effect of human leukocyte antigen heterozygosity on infectious disease outcome: the need for allele-specific measures. BioMed Central, Medical Genetics 4, 2.

McPherron, A. C., Lawler, A. M. & Lee, S. J. (1997). Regulation of skeletal muscle mass in mice by a new TGF-β superfamily member. Nature 387, 83–90.

McPherron, A. C. & Lee, S. J. (1997). Double muscling in cattle due to mutations in the myostatin gene. Proceedings of the National Academy of Sciences of the USA 94, 12457–12461.

McPherron, A. C. & Lee, S. J. (2002). Suppression of body fat accumulation in myostatin-deficient mice. The Journal of Clinical Investigation 109, 595–601.

Nadeau, J. H. (2001). Modifier genes in mice and humans. Nature Reviews Genetics 2, 165–174.

Potts, J. K., Echterkamp, S. E., Smith, T. P. & Reecy, J. M. (2003). Characterization of gene expression in double-muscled and normal-muscled bovine embryos. Animal Genetics 34, 438–444.

Rios, R., Carneiro, I., Arce, V. M. & Devesa, J. (2002). Myostatin is an inhibitor of myogenic differentiation. American Journal of Physiology – Cell Physiology 282, C993–C999.

Schuelke, M., Wagner, K. R., Stolz, L. E., Hübner, C., Riebel, T., Kömen, W., Braun, T., Tobin, J. F. & Lee, S. J. (2004). Myostatin mutation associated with gross muscle hypertrophy in a child. The New England Journal of Medicine 350, 2682–2688.

Seibert, M. J., Xue, Q. L., Fried, L. P. & Walston, J. D. (2001). Polymorphic variation in the human myostatin (GDF-8) gene and association with strength measures in the Women’s Health and Aging Study II cohort. Journal Of The American Geriatrics Society 49, 1093–1096.

Smith, J. A., Lewis, A. M., Wiener, P. & Williams, J. L. (2000). Genetic variation in the bovine myostatin gene in UK beef cattle: allele frequencies and haplotype analysis in the South Devon. Animal Genetics 31, 306–309.

Szabo, G., Dallmann, G., Müller, G., Patthy, L., Soller, M. & Varga, L. (1998). A deletion in the myostatin gene causes the compact (Cmpt) hypomuscular mutation in mice. Mammalian Genome 9, 671–672.

Valle Zarate, A., Horst, P. & Weniger, H. J. (1994). Antagonism between growth and productive adaptability in mice. Archiv für Tierzucht – Archives of Animal Breeding 37, 185–198.

Varga, L., Szabo, G., Darvasi, A., Müller, G., Sass, M. & Soller, M. (1997). Inheritance and mapping of compact (cmap), a new mutation causing hypermuscularity in mice. Genetics 147, 755–764.

Varga, L., Müller, G., Szabo, G., Pinke, O., Korom, E., Kovaecs, B., Patthy, L. & Soller, M. (2003). Mapping modifiers affecting muscularity of the myostatin mutant (Mstn<sup>cmap-cmpt</sup>) Compact mouse. Genetics 165, 257–267.

Wagner, J., Albrecth, E., McDlerd, I., Teuscher, F., Papstein, H. J. & Ender, K. (2000). Growth- and breed-related changes of muscle fiber characteristics in cattle. Journal of Animal Science 78, 1485–1496.

Weniger, H. J., Horst, P., Steinhauf, D., Major, F., Wolf, M. & Tawfik, E. S. (1974). Model experiments on selection for endurance and its relation to growth. Part I. Introduction, methods and preliminary investigations on the basic population. Journal of Animal Breeding and Genetics – Zeitschrift für Tierzüchtung und Züchtungsbiologie 91, 265–270.

Whitemore, L. A., Song, K., Li, X., Aghajanian, J., Davies, M., Girgenrath, S., Hill, J. J., Jalenak, M., Kelley, P. & Knight, A. (2003). Inhibition of myostatin in adult mice increases skeletal muscle mass and strength. Biochemical and Biophysical Research Communications 300, 965–971.

Wiener, P., Smith, J. A., Lewis, A. M., Woolliams, J. A. & Williams, J. L. (2002). Muscle-related traits in cattle: the role of the myostatin gene in the South Devon breed. Genetics Selection Evolution 34, 221–232.

https://doi.org/10.1017/S0016672304007165 Published online by Cambridge University Press