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Muscle-bone relationships in mice selected for different body conformations

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Abstract

Some muscle-bone relationships were studied in terms of gastrocnemius muscle weight, femur and tibia length and femur and tibia weight in four lines of mice (CBi-, CBi+, CBi/L and CBi/C) artificially selected for different body conformations and in the unselected control line (CBi). CBi- (low body weight - short tail) and CBi+ (high body weight - long tail) lines were divergently selected following the positive genetic correlation between body weight and tail (skeleton) length (agonistic selection). In contrast, CBi/L (low body weight - long tail) and CBi/C (high body weight - short tail) were also divergently selected but against the aforementioned correlation (antagonistic selection). The relationship between bone length and muscle weight was interpreted based on the assumption that the increased tension generated by the longitudinal growth of a bone, brings about an increase in the mass of the muscles attached to it. All CBi+, CBi/C and CBi/L mice showed enlarged femurs and tibias, but only those genotypes simultaneously selected for high body weight (CBi+ and CBi/C) showed heavier muscles than controls. The CBi+ and CBi- genotypes with agonistic selection differ in bone length and muscle weight, as it would be expected of the allometric modification of their body conformation, showing the associated longitudinal bone growth-muscle growth. CBi/C and CBi/L mice, with a non-allometric modification of body conformation, exhibited the same bone length but different muscle weight. Consequently, the antagonistic criterion allowed to confirm that the genetic influence on of the proposed muscle-bone relationships could be modified, thus making it possible to lengthen the bone through selection of a long skeleton and to avoid the correlated effect on muscle mass, by selecting for a low body weight, bringing forth presumptive evidence that both processes were genetically independent.

Keywords: Muscle Weight, Femur Length, Femur Weight, Body Conformation, Mice

Introduction

According to the cellular nature of vertebrates growth, the organs of the body can be sorted into one of three broad groups: (1) those which proliferate throughout the life span of the individual, (2) those that essentially cease proliferation as they approach maturity, but retain the capability to re-establish proliferation under the appropriate stimulus and (3) those in which once proliferative capacity ceases, it is never regained[1]. Muscles and bones, two types of organs biomechanically linked during development, are included in different groups: the second and the third, respectively. In the living animal, muscles are essential for maintaining the shape of the body in a particular position, while their coordinate movements result in locomotion, being both, posture and locomotion, basic for animal survival. On the other hand, whereas muscles are actively involved in posture and movement, bones play a passive role in the transmission and storage of these various forms of energy[2].

Schiessl et al.[3] and Turner[4], on discussing bone-muscle relationship, retrieved two paragraphs from D'Arcy Thompson's classical book *On growth and form* which closely connect muscle and bone changes. Hooper[5] observed a broad similarity between the quantitative genetic control of muscle and bone growth, when lines of mice divergently selected for body weight were compared on a body weight basis and proposed the hypothesis that the epiphyseal plate may act as a "pacemaker" for skeletal muscle growth. In his own words "the increased tension generated by the longitudinal growth of a bone brings about an increase in the mass of the muscles attached to it,

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Trait	Genotype				
	CBi-	CBi/L	CBi	CBi/C	CBi+
	low body weight – short tail	low body weight – long tail	unselected control line	high body weight – short tail	high body weight – long tail
Body weight (g)	30.3^{a} ± 0.47	32.0^{a} ± 0.39	40.5^{b} ± 0.56	51.7^{c} ± 0.82	55.6^{d} ± 1.38
Body fat (%)	6.40^{a} ± 0.485	7.09^{a} ± 0.343	$16.57^{b,c}$ ± 1.004	14.93^{b} ± 0.751	19.21^{c} ± 1.021
Fat free body weight (g)	28.3^{a} ± 0.40	29.7^{a} ± 0.32	33.7^{b} ± 0.40	43.9^{c} ± 0.70	44.8^{c} ± 0.66
Tail length (cm)	8.14^{a} ± 0.056	12.20^{b} ± 0.078	10.11^{c} ± 0.059	8.60^{d} ± 0.070	11.69^{e} ± 0.055
Body length (cm)	9.81^{a} ± 0.050	10.14^{b} ± 0.056	10.48^{c} ± 0.048	10.96^{d} ± 0.089	11.65^{e} ± 0.087
Total length (cm)	17.9^{a} ± 0.08	22.3^{b} ± 0.10	20.6^{c} ± 0.08	19.6^{d} ± 0.14	23.3^{e} ± 0.11
Femur weight (mg)	69.1^{a} ± 0.58	85.2^{b} ± 0.39	83.1^{b} ± 1.01	109.5^{c} ± 1.18	127.1^{d} ± 1.63
Femur length (mm)	14.56^{a} ± 0.070	16.84^{b} ± 0.082	15.88^{c} ± 0.075	16.73^{b} ± 0.062	16.72^{b} ± 0.087
Tibia weight (mg)	57.5^{a} ± 0.86	64.7^{b} ± 0.57	60.0^{a} ± 0.91	72.0^{c} ± 0.68	92.0^{d} ± 1.34
Tibia length (mm)	16.23^{a} ± 0.048	18.44^{b} ± 0.079	17.39^{c} ± 0.067	18.30^{b} ± 0.061	18.58^{b} ± 0.091
Gastrocnemius muscle weight (mg)	165.9^{a} ± 3.42	183.9^{b} ± 3.13	199.8^{c} ± 2.71	263.1^{d} ± 3.84	239.1^{e} ± 4.15

^{a,b,c,d,e} Values with different superscript differ at least at $p < 0.05$
Sample size: n = 14 animals per genotype

Table 1. Muscle-bone related traits (mean ± standard error) in male mice selected for body conformation and in the unselected control line.

while the greater force exerted by these large muscles increases the diameter and mass of the bone". After five generations of divergent selection for a tibia length/radius length ratio he observed not only the expected direct response, as the ratio responded to selection in both directions, but also a correlated response in the weight of three related muscles: *tibialis anterior, biceps brachii* and *sternomastoid,* which were interpreted as evidence of the assumption that the weight of a muscle may be influenced by the length of the bone to which it is attached.

Turner[4] analyzed two cases that seem to be exceptions to this general rule: the myostatin-null mouse with individual muscles that are up to three times larger than normal but with unaffected femurs in either size or shape[6] and two unrelated strains of mice with a considerable difference in femoral bone mass[7] but with the same muscle mass estimated from lean body mass figures. The derived interpretation of these exceptions refers to the feasibility that genetic influences can build large muscles or dense bones beyond the functional demand of physical exercise, overwhelming the biomechanical linkage between bone and muscle.

Lines of mice from the CBi stock have been artificially selected for different body conformations for more than 90 generations. As a consequence of combining body weight and skeletal length, either following (agonistic selection) or opposing (antagonistic selection) the positive genetic correlation between them, these lines differ in several skeletal characteristics, femoral histomorphometry, patterns of bone mineral apposition and biomass supported per unit of skeletal mass[8-12]. Derived from the same base population, and with differences stemming from a controlled selective procedure, these lines represent a model suitable for testing the importance of genetic factors on the previously discussed muscle-bone relationships.

Material and methods

Animals. Four lines of mice selected for different body conformations (CBi+, CBi-, CBi/C and CBi/L), and their unselected control line (CBi) were used. Selected lines were the outcome of two selection experiments in which the selective criterion was a biological quantitative index combining body weight and tail length at 49 days of age. A detailed description of the selective procedure is given elsewhere[13]. Briefly, two lines were selected for either high or low values of the index (divergent selection) according to the positive genetic correlation between both traits (agonistic selection: CBi+ high body weight - long tail; CBi-: low body weight - short tail) and the other two were also obtained by divergent (two-way) selection but against the aforementioned correlation (antagonistic selection: CBi/C high body weight - short tail; CBi/L: low body weight - long tail).

All mice were reared in groups of six gender-matched companions, in polypropylene cages (32 x 24 x 10 cm) with wood shavings for bedding and kept under the same breeding conditions (23 ± 1 °C, with alternate 12 h light-dark periods). Food (Cargill Lab Chow) and water were provided *ad libitum*.

Muscle-bone traits. Fourteen males and 14 females from each line, randomly chosen from litters of eight to ten animals in generation 75 of selection were used. Mice were killed by etherization at 150 days of age. Immediately afterwards, each animal was weighed to the nearest tenth of a gram. Tail length and total length were measured to the nearest millimeter; body length was estimated by subtracting tail length from total length. Both gastrocnemius muscles were excised and weighed to the nearest 0.1 mg. After that, both femurs and both tibias were removed by dissection, carefully cleaned by hand to remove all the adhering soft tissues, weighed to the nearest 0.1 mg and their length (from the highest point of the greater trochanter to the medial condyle for the femur, and from the center of the condyles to the tip of the medial malleolus for the tibia) were measured with a micrometric calliper.

Each whole mouse was then chopped roughly into little pieces and homogenized in a blender. Approximately 10 g of the homogeneous mixer was used for fat analysis using Folch's technique[14]. Body weight was also expressed on a fat-free basis based on body composition values.

Statistical analysis. Univariate analyses of variance[15] were calculated for all traits. Male and female animals were treated separately throughout the analysis, so comparisons were made among genotypes within gender.

Results

Average values for weight and length traits are given in Tables 1 and 2, for male and female mice, respectively. The expected direct responses to selection for body weight and tail length were observed in both genders. Consequently, CBi+ and CBi/C mice, positively selected for body weight were heavier than CBi- and CBi/L animals selected for low body weight. The unselected control line CBi showed intermediate mean values. The same applied for tail length as CBi+ and CBi/L lines, selected for long tail, showed longer tails than controls while CBi- and CBi/C mice, negatively selected for this trait, exhibited shorter tails than CBi.

Differences in body weight between CBi+ and CBi/C mice can be ascribed to differences in body fat content as long as they became non significant when body weight was expressed on a fat free basis.

Data confirm previous reports about other morphometric skeletal traits measured in the same genotypes[8,11]. CBi- and CBi+ mice showed shorter or longer bodies, femurs and tibias than controls depending on direction of the selective pressure on tail (skeletal) length. Conversely, CBi/L and CBi/C mice only modified their total length as expected and were longer and shorter than controls, respectively. This response was fully associated to the tail length modification as long as body length depicted a different pattern. This latter trait, as well as femur length and tibia length, enlarged in both genotypes regardless of the direction of the selection for skeleton length. This particular response is undoubtedly ascribed to the selective pressure applied on body weight in relation to the skeletal function as a scaffold for the soft tissue. In CBi/C animals, the selective criterion played against the aforementioned skeletal function as it intended to increase body weight in spite of a reduction of the skeletal growth in length. Conversely, the same criterion did not affect the expected response in the downward selection line as CBi/L mice lengthened their bones in response to selection pressure on tail length despite of the absence of any body weight demand.

Femur weight was higher in CBi/L than in CBi- mice because CBi/L mice were longer. For the same reason, no differences were evident between CBi/L and CBi animals, particularly in males. The same relationships held for the tibia.

Gastrocnemius muscle weight varied in parallel with fat free body weight. Thus, CBi+ and CBi/C animals showed the heaviest muscles and CBi- and CBi/L, the lightest.

Discussion

Dynamic factors such as skeletal elongation and functional demands represent definite stimuli for muscle growth. Growth in length of a muscle correlates with the lengthening of the bone to which it is attached and biomechanically linked, while both muscle and bone mass growth depend on the functional demands within the restrains imposed by the organism genotype. Bone growth in length, a consequence of the endochondral ossification that takes place in the presence of an active epiphyseal plate, seems to be genetically determined[16], while growth in width is a concomitant mechanism sensitive to weight loading[17], determined by the increasing biomass and the dynamic functional demands which adjust bone structure to muscle strength. Although

Trait	Genotype				
	CBi-	CBi/L	CBi	CBi/C	CBi+
	low body weight – short tail	low body weight – long tail	unselected control line	high body weight – short tail	high body weight – long tail
Body weight (g)	27.0^{a} ± 0.35	25.6^{a} ± 0.21	32.0^{b} ± 0.59	47.6^{c} ± 0.98	57.8^{d} ± 1.12
Body fat (%)	14.31^{a} ± 0.424	9.18^{b} 0.293	15.36^{a} ± 1.132	23.76^{c} ± 1.332	33.69^{d} ± 1.434
Fat free body weight (g)	22.3^{a} ± 0.29	23.3^{a} ± 0.20	27.0^{b} ± 0.28	36.2^{c} ± 0.48	38.1^{c} ± 0.86
Tail length (cm)	8.39^{a} ± 0.050	11.90^{b} ± 0.051	9.86^{c} ± 0.046	8.69^{d} ± 0.056	11.89^{b} ± 0.053
Body length (cm)	9.46^{a} ± 0.063	9.56^{a} ± 0.040	10.04^{b} ± 0.054	10.81^{c} ± 0.047	11.69^{d} ± 0.040
Total length (cm)	17.9^{a} ± 0.07	21.5^{b} ± 0.06	19.9^{c} ± 0.08	19.5^{d} ± 0.09	23.6^{e} ± 0.07
Femur weight (mg)	62.5^{a} ± 0.59	74.8^{b} ± 0.97	78.5^{b} ± 0.94	109.7^{c} ± 0.92	114.2^{d} ± 1.80
Femur length (mm)	15.34^{a} ± 0.075	16.98^{b} ± 0.065	16.23^{c} ± 0.051	17.18^{b} ± 0.061	17.63^{d} ± 0.093
Tibia weight (mg)	50.2^{a} ± 0.34	55.7^{b} ± 0.59	56.2^{b} ± 0.57	69.0^{c} ± 0.96	78.6^{d} ± 1.81
Tibia length (mm)	17.12^{a} ± 0.067	17.81^{b} ± 0.053	17.66^{b} ± 0.038	18.55^{c} ± 0.044	19.34^{d} ± 0.076
Gastrocnemius muscle weight (mg)	140.9^{a} ± 2.48	143.3^{a} ± 2.37	161.6^{b} ± 1.64	223.8^{c} ± 3.27	223.5^{c} ± 3.25

^{a,b,c,d,e} Values with different superscript differ at least at $p < 0.05$
Sample size: n = 14 animals per genotype

Table 2. Muscle-bone related traits (mean ± standard error) in female mice selected for body conformation and in the unselected control line.

muscle mass or weight, the trait discussed in this study, does not accurately reflect muscle strength, which depends strongly on the peak cross section area of the muscle, a trait not measured, it was used in the same sense Hooper did it: a correlated response to bone lengthening.

Experiments using artificial selection to alter mice body growth are very common and widely found in scientific literature[18-20]. Conversely, information regarding the developmental mechanisms underlying such responses is scarce[21]. To this regard, this study could bring some insight on the interplay of genetic and nongenetic factors on some muscle-bone relationships.

According to the first part of Hooper's assumption, if "the increased tension generated by the longitudinal growth of a bone brings about an increase in the mass of the muscles attached to it", then several situations involving CBi genotypes could be predicted:

1. CBi+, CBi/C and CBi/L mice, with the same femur and tibia length should show heavier muscles than controls without differing among them. Concerning this first prediction only two of the three genotypes that enlarged their femurs showed heavier muscles than controls. The expected response was only observed in CBi+ and CBi/C genotypes also selected for high body weight. The antagonistic criterion showed that the broad similarity between the quantitative genetic control of muscle and bone growth observed by Hooper does not mean that increased bone length directly causes increased muscle mass. This criterion enabled the selection of a long skeleton avoiding the correlated effect on muscle weight by simultaneously selecting for a low body

weight, like in CBi/L animals. The same results suggest the impossibility of reducing the bone growth in length if, at the same time, the selective pressure intends to increase body weight and, consequently, muscle weight, like in CBi/C mice. This latter response would be at least partially related to the second assumption in Hooper's hypothesis "the greater force exerted by these larger muscles increases the mass of the bone" which was not under consideration in this study.

2. CBi- mice with shorter femurs than CBi ones should also exhibit lighter muscles. In relation to this prediction, results confirm that CBi- mice with shorter bones than controls also exhibit lighter muscles, a response that fits Hooper's proposal but does not add any key data on the subject because CBi- animals were selected not only for a short skeleton but also for a low body weight intending to maintain the natural physiological association between body weight and tail length.

3. CBi/C and CBi+ mice with similar bone length should show similar muscle weight. The response to this prediction provides further insight as CBi/C males showed heavier muscles than their CBi+ counterparts with equally long bones, while CBi/C females, with shorter bones than CBi+, exhibited the same muscle weight. These relationships reflect a response of different magnitude to the selective pressure on both traits. CBi/C mice, despite having been selected for a short skeleton, would have enlarged their bones due to the mechanical influence of their heavier muscles resulting from the positive selection for body weight. It seems logical that some steady state should be determined by these two opposite forces, one tending to enlarge the bones as required by their weight-supporting function of the soft tissue and the other intending to preclude that enlargement in response to the selective pressure for a short skeleton. This steady state seems to have been achieved in CBi/C but not in CBi+ mice. This latter genotype would have lengthened their bones in response to the selective pressure for a long skeleton. This lengthening should have been accompanied by a compatible increase in muscle weight in response to selection for high body weight. Furthermore, just like CBi/L animals which enlarged their femurs without a concomitant modification in muscle weight, CBi+ mice were able to build longer bones than required for biomass support.

4.CBi- and CBi/L mice, with quite different femur length because of selection for different skeletal length, should exhibit different muscle weight. This prediction involves two genotypes selected for low body weight but with divergent selection for bone length. CBi-, the downward line of the agonistic selection, showed shorter femurs and tibias than CBi/L, the downward genotype of the antagonistic selection. This response in CBi- mice was associated to lighter muscles, despite their similarities in body weight and fat free body weight. These results are compatible with Hooper's idea and indicate the possibility of exploiting a source of genetic variance for the proposed muscle-bone relationship, independent from body weight, that directly relates bone lengthening and muscle mass increase. CBi- and CBi/L mice responded to divergent selection for skeleton length, showing either longer or shorter bones than controls and these genetically-determined differences in longitudinal growth do resulted in different muscle weights. It should be noticed that, though CBi/L mice showed heavier muscles and longer femurs than CBi-, they did not exhibit heavier muscles than controls in spite of their longer femurs because the first case relates to two genotypes selected both for low body weight and either short or long tail (skeleton) length, whereas the second compares a genotype selected for low body weight and long tail with its unselected control line.

In summary, both agonistically selected genotypes (CBi+ and CBi-) differed in femur length and muscle weight as expected in terms of the allometric, and therefore harmonious, modification in body conformation, showing the natural association between longitudinal bone growth and muscle growth observed by Hooper and probably ascribed to concomitant differences in body weight. These lines also differ (CBi+ > CBi-) in the biomass sustained per unit of skeletal mass and in femoral cortical thickness[8]. On the other hand, the two antagonistically selected genotypes (CBi/L and CBi/C) showed similar differences concerning weight supported per unit of skeletal mass and femoral cortical thickness (CBi/C > CBi/L) despite showing a non-allometric, and therefore non-harmonious, modification of their body conformations[11]. The fact that these lines showed the same bone length but different muscle weight suggests that both processes are genetically independent, demonstrating the feasibility of surpassing the "pacemaker" role suggested by Hooper for the epiphyseal plate and observed after CBi- vs. CBi/L comparisons.

Artificial selection has proved to be a very important genetic tool for generating animal models. When selection is performed by means of an antagonistic index, physiological and anatomical incompatibilities between traits can be introduced after few generations[22]. In the present case, the antagonistic approach allowed a deeper and more thorough view of muscle-bone relationships which are rather complex. It has been suggested[23] that antagonistic selection altered the negative feedback relationship between the architectural design of the femur diaphyses (cross-sectional moment of inertia) and the mechanical quality of the cortical tissue (modulus of elasticity) inducing the development of inadequately strong bones. Preliminary reports[24] indicate that agonistic selection also affected the modeling-dependent compensation for differences in bone material quality leading to inadequately less or more robust bones than controls.

Assuming that these features are normally controlled by the bone mechanostat as proposed by Frost[25], these results would indicate that some components of that cybernetic system are inherited separately and, as a consequence, they could be distinctly transmitted to the progeny. This could be also the case concerning to bone length and muscle weight.

As a cumulative conclusion the results herein described would seem to suggest that a greater longitudinal bone growth not necessarily implies more muscle mass, a statement related with the first part of Hooper's proposal.

Although this study does not intend to discuss the relationship between femur strength and voluntary muscle forces, a topic related to the second part of Hooper's hypothesis, it could be argued that during growth the rules that govern mechanical influences on longitudinal bone growth could and probably do differ from the rules that govern mechanical influences on whole-bone strength. In that regard, body and tail lengths would be determined by factors that control endochondral growth in growth plates which could be and probably are independent from those that control muscle strength and whole-bone strength relationships.

Regarding the exceptions mentioned by Turner[4] it can be argued that in the myostatin-deficient mice a point mutation in a single gene acting as a negative regulator of skeletal muscle is responsible for the alteration, without any further concomitant change in the genome. It must be realized that the impressive musculature of the so called "mighty mouse" is the outcome of the lack of expression shown by one gene and, in this sense, this fact represents an abnormal event. According to Sellier[26], growth can be defined as a composite quantitative character regulated by a large number of physiological pathways, partly differing according to the stage of life. Efstratiadis[27] pointed out that "genes are not lonely players". Therefore, drawing conclusions about normal functions from the results of their perturbation defines the limitations of the attempt, a concept that can be also applied to the effects of transgenesis, the process by which a gene (foreign gene or transgene) is carried into the genome of another species, incorporated into its germline and transmitted to its progeny. The different femoral bone mass and the similar muscle mass showed by C3H/HeJ and C57Bl/6j strains[4,6] resemble the situation observed with CBi- and CBi/L mice in terms of genetic variation that directly relates bone lengthening and muscle mass increase. However, in the former comparison nothing can be said about the genetic reasons underlying such particularity, while in the latter one such difference could be ascribed to a particular selective process.

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