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NO EFFECT OF CHROMOSOME 10 FROM A/J MOUSE STRAIN ON MUSCLE PROPERTIES OF C57BL/6J MOUSE. A MOUSE MODEL OF HUMAN METABOLISM AND AGEING

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ABSTRACT

Background. Muscle mass plays a key role in recovery from critical illness or severe trauma. Low skeletal muscle mass is associated with increased mortality risk in patients with coronary heart disease (Nichols et al., 2019). Sarcopenia, low muscle mass, is an increasing problem in our ageing society. The prevalence of sarcopenia varies extremely between elderly cohorts ranging from 7 to over 50% (Bijlsma et al., 2013). Due to their phylogenetic relatedness mice have long served as models of human biology including search for genetic determinants of muscle mass (Morse, 2007). Our aim was to investigate if gene variants that reside in chromosome 10 of A/J mouse strain affect muscle properties of C57BL/6J mouse strain.

Methods. We studied C57BL/6J (B6, n = 11) mouse strain and C57BL/6J-Chr10$^{A/J}$/NaJ (B6.A10, n = 10) strain which also has B6 strain background, but carry chromosome 10 from A/J strain instead of chromosome 10 from B6 strain. Body mass, soleus (SOL) muscle mass and morphometric characteristics as well as tibia length were assessed.

Results. SOL mass did not differ between B6 and B6.A10 strains (9.3 ± 0.7mg vs. 9.8 ± 0.4mg, respectively, $p > 0.05$). There were also no differences in the number of muscle fibres (860 ± 148 vs. 913 ± 136 for B6 and B6.A10 strains, respectively, $p > 0.05$). B6 and B6.A10 strain mice had similar cross-sectional area of type I (1616μm² ± 303 vs. 1752μm² ± 136, B6 and B6.A10 mice, $p > 0.05$) and type II fibres (1689μm² ± 291 vs.1734μm² ± 179, B6 and B6.A10 mice, $p > 0.05$).

Conclusion. Chromosome 10 of B6.A10 mice does not harbour any genes that would affect muscle properties of B6 mice.

Keywords: muscle mass, cross-sectional area, muscle fibres.

INTRODUCTION

Skeletal muscle mass is one of the major components of human body composition (Livshits et al., 2016). Muscle mass plays a key role in recovery from critical illness or severe trauma (Wolfe, 2006). Development of treatments against muscle wasting requires better understanding of genetic, molecular and physiological factors affecting muscle mass.

It appears that differences in muscle mass depend on the number of muscle fibres. Studies of human cadavers show that muscle fibre number of the vastus lateralis varies between 393000 and 903000 fibres in men aged 18–22 years (Lexell, Sjöström, 1988). However, the total number of muscle fibres is mainly determined prenatally when multinucleated myofibres form from myoblasts (Rehfeldt et al., 1999). Studies of skeletal muscle of the number and size of fibres require

sampling muscle tissues. Such procedure is invasive and difficult to carry out on large numbers of volunteers. It appears that mouse models are useful in this regard as muscle size and its underlying indices (number and size of fibres) differ significantly between mouse strains (Lionikas et al., 2013). Consomic mouse strain, when a chromosome from one mouse strain is transferred to another strain provide a possibility to study the function of genes in the chromosome in greater detail. In our study we chose the following model of experiment: C57BL/6J (B6) strain and consomic C57BL/6J-Chr10$^{A/J}$/NaJ (B6.A10) mouse strain. The two strains share genetic background except of chromosome 10, which in case of B6.A10 comes from the A/J strain. A/J strain is more resistant to weight and fat gain when subjected to a high-fat diet than the C57BL/6J strain (Ratkevicius et al., 2010).

It is important to understand relations between muscle characteristics and variations on the genetic level. It helps determine peculiarities of genotype which are associated with smaller muscle and/or muscle function. We hypothesized that combination of genetic variants on mouse chromosome 10 between the A/J and B6 strains could have an effect on muscle morphometric characteristics.

METHODS

The study was carried out at the animal research facility of the Lithuanian Sports University with approval of all the procedures by the Lithuanian Republic Alimentary and Veterinary Public Office (No. G2-45 in 2016). SOL muscles were dissected from 10- to-14–week-old mice. Males from two strains, B6 (n = 11) and consomic B6.A10 (n = 10) were isolated, weighed and frozen in isopentane cooled in liquid nitrogen. The sections of the muscle were cut transversely at 10 μm thickness with a cryotome (Leica CM1520) at −20 °C. Section was mounted on a glass slide and stained following ATPase staining protocol according M. H. Brooke and K. K. Kaiser (1970). The number, cross-sectional area and fibre-type composition of muscle was then determined from the sections using a Nikon Eclipse TS100 microscope.

Quantitative data are presented as mean ± SD unless stated otherwise. For statistical analysis, the data were compiled in IBM SPSS Statistics 25 software or in Excel 2007. Differences between strains were assessed using t-test. Differences between strains were considered statistically significant when $p < 0.05$.

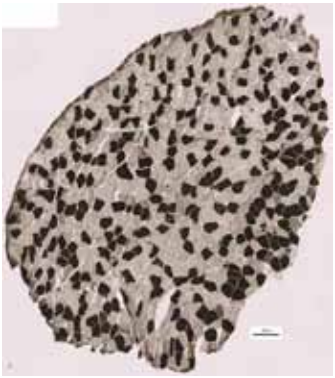

Figure 1. **Typical image of cross section of soleus muscle from C57BL/6J (B6) mouse strain**

REASEARCH RESULTS

Table. **Body weight and tibia length of male mice from B6 and B6.A10 strains**

Strain	**n**	**Body weight (g)**	**Tibia length (mm)**
B6	11	28.28 ± 1.13	18.39 ± 0.13
B6.A10	10	28.64 ± 1.48	18.49 ± 0.19
Statistical difference between strains		$p > 0.05$	$p > 0.05$

Table represents general information of body weight, bone length and number of mice in B6 (C57BL/6J) and B6.A10 (C57Bl/6J-Chr10$^{A/J}$NaJ) mouse strains. Given information shows that there are no statistically significant ($p > 0.05$) differences in body weight, 28.28 ± 1.13 (B6) and 28.64 ± 1.48 (B6.A10), between mice strains. And we also did not find statistically significant ($p > 0.05$) differences in tibia length – 18.39 ± 0.13 (B6) and 18.49 ± 0.19 (B6.A10).

Figure 2 shows SOL mass in B6 and B6.A10 mouse strains. There was no significant difference in the muscle mass between the strains.

Figure 3 shows the total number of fibres in SOL muscle of B6 and B6.A10 mouse strains. There were no differences in the number of muscle fibres between the strains. We found that SOL was made up of 34 ± 5 % of type 1 fibres in B6 strain and 30 ± 3 % of B6.A10 with no significant difference between the strains.

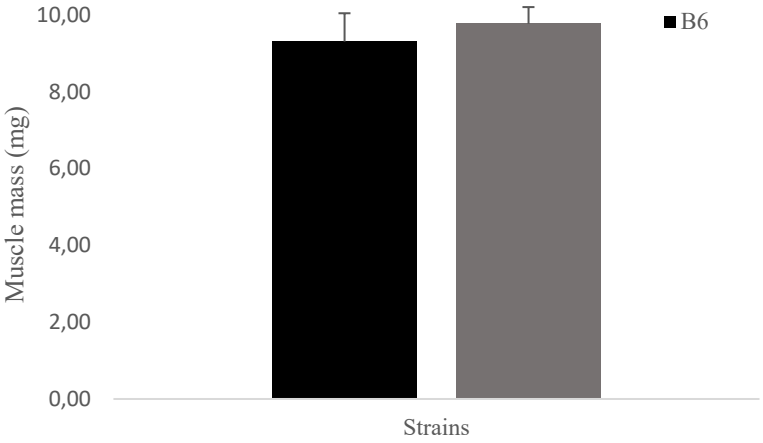

Figure 2. **Soleus mass in B6 and B6.A10 mouse strains (Data are show as means and SD)**

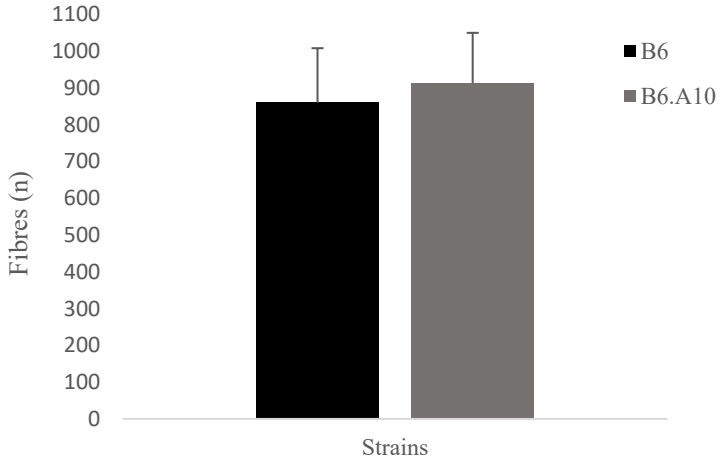

Figure 3. **Total number of muscle fibres (Data are show as means with SD)**

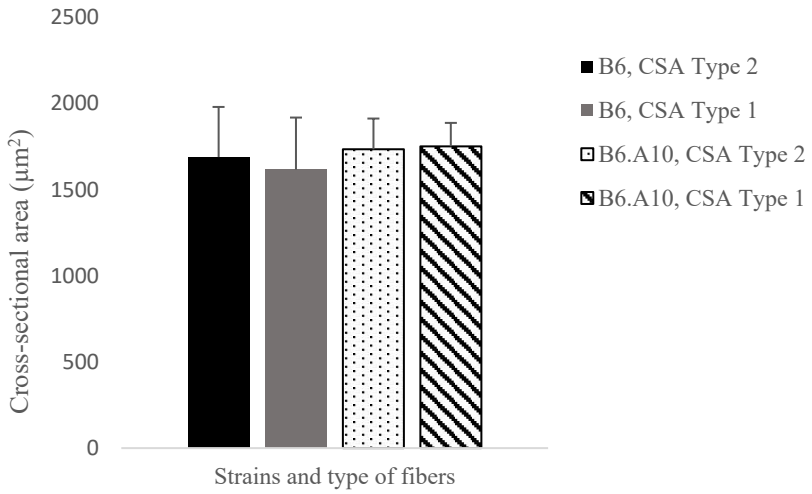

Figure 4. **Cross-sectional area of different types of fibres (Data are show as means with SD)**

Figure 4 represents the mean values of cross-sectional area (CSA) of type 1 and type 2 muscle fibres of SOL muscles. We did not find any statistically differences ($p > 0.05$) between types of fibres in a separate strain and also between strains.

DISCUSSION

Our aim was to investigate if Chromosome 10 of A/J mouse strain contains any genes that would lead to increase in muscle mass if studied on B6 mouse background. However, there was no impact of A/J chromosome 10 on soleus muscle mass, number of muscle fibres and cross-sectional area of muscle fibres in B6 mice. Under certain assumptions of results, this can be construed as evidence that chromosome 10 does not contain any genes which possibly can influence anabolic factors through different pathways. However, it is unclear whether it is suitable for other muscles such as gastrocnemius, extensor digitorum longus and others which contain faster twitch fibres compared to soleus muscle.

Muscle mass in humans and animals reflects a balance between anabolic factors such as IGF-I/growth factor 1/growth hormone, testosterone, nutrition, and exercise, and catabolic factors such as glucocorticoids, thyroid hormones, mediators of systemic inflammatory response, and cytokines (Nemet, Eliakim, 2010). Skeletal muscle mass is also influenced by genetic factors. M. A. Schreger et al. (2004)

found that IGF2 genotype may be associated with fat-free mass and strength at peak age (35 yrs) and across the adult age span. This provides support for the hypothesis that genetic influences may affect muscle mass and function at young age and later in life. TNF-α has been recognized as a potent catabolic factor able to induce muscle wasting through direct and indirect pathways, TNF-α can inhibit protein synthesis over a relatively long period of time, which could ultimately lead to muscle frailty in older people (Liu et al., 2008). W. E. Taylor et al.'s (2001) data suggest that increased expression of myostatin could result in the loss of muscle mass by inhibition of muscle protein synthesis. In addition, myostatin-induced inhibition of DNA synthesis and cell replication could impair the ability of muscle cells to regenerate and restore muscle mass during a catabolic illness or aging. Muscle mass is the result of a dynamic balance between protein synthesis and degradation. This balance is co-ordinately regulated by two major branches of AKT signalling pathways: the AKT (also known as protein kinase B)/mammalian target of rapamycin (mTOR) pathway that controls protein synthesis and the AKT/forkhead box O (FOXO) pathway that controls protein degradation (Rodriguez et al., 2014).

CONCLUSIONS

Our study suggests that a combination of genetic variants on mouse chromosome 10 between the B6 and A/J strains does not influence morphometric characteristics of soleus muscle. This mouse model is inexpedient to wider studies of the possible effect of chromosome 10 on muscle weight, fibre composition and fibre cross-sectional area.

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A/J PELIŲ LINIJOJE ESANTI 10 CHROMOSOMA NEVEIKIA C57BL/J PELIŲ RAUMENŲ SAVYBIŲ. PELIŲ MODELIS ŽMONIŲ METABOLIZMO IR SENĖJIMO TYRIMUOSE

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SANTRAUKA

Tyrimo pagrindimas. Raumenų masė yra vienas iš esminių rodiklių, kurie paveikia atsigavimą po sunkios ligos ar traumos. Maža raumenų masė susijusi su didesne ligonių, sergančių vainikinių arterijų ligomis, mirštamumo rizika (Nichols et al., 2019). Sarkopenijos paplitimas tarp senyvo amžiaus žmonių apima nuo 7 iki 50% populiacijos (Bijlsma et al., 2013). Dėl savo filogenetinio panašumo pelės ilgą laiką tarnavo kaip žmogaus biologijos modeliai, įskaitant genetinių raumenų masę lemiančius veiksnius (Morse, 2007). Mūsų tikslas buvo ištirti, ar A/J pelių linijos 10 chromosomoje esantys genų variantai veikia C57BL/6J pelių linijos raumenų savybes.

Metodai. Ištyrėme C57BL / 6J (B6, n = 11) ir C57BL/6J-Chr10A/J/NaJ (B6. A10, n = 10) pelių linijas. B6.A10 linija turi B6 linijos genetinį foną, tačiau skiriasi 10 chromosoma, kuri yra iš A/J linijos, vietoj 10 chromosomos – iš B6 linijos. Buvo įvertinta kūno masė, plekšninio raumens masė ir morfometrinės savybės, blauzdikaulio ilgis.

Rezultatai. Plekšninio raumens masė nesiskyrė tarp B6 ir B6.A10 linijų (9,3 ± 0,7 mg ir 9,8 ± 0,4 mg atitinkamai, $p > 0,05$). Taip pat nenustatėme skirtumo tarp raumeninių skaidulų kiekio (860 ± 148 ir 913 ± 136, atitinkamai B6 ir B6.A10 linijų, $p > 0,05$). B6 ir B6.A10 pelės turėjo vienodą raumeninių skaidulų skerspjūvio plotą: I tipo skaidulų (1616 μm^2 ± 303 ir 1752μm^2 ± 136, B6 ir B6.A10 linijos atitinkamai, $p > 0,05$) ir II tipo skaidulų (1689 μm^2 ± 291 ir 1734 μm^2 ± 179, B6 ir B6.A10 linijos atitinkamai, $p > 0,05$).

Išvada. 10 chromosoma, esanti B6.A10 pelių linijoje, neturi jokių genų, kurie gali paveikti B6 linijos pelių raumenų savybes.

Raktažodžiai: raumenų masė, skerspjūvio plotas, raumeninės skaidulos.

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