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Genetic Obesity and the Muscle Satellite Cell[1] (41854)

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Abstract. The nuclear content of skeletal muscles from lean and obese (ob/ob) male mice was investigated. Four lean and four ob/ob mice were examined at 2, 3, 5, 8, and 16 weeks of age. Differences in body weight between the two phenotypes were significant after 5 weeks of age. For all ages in the soleus and after 3 weeks of age in the gastrocnemius, muscle wet weight and length were less in ob/ob mice than in lean mice. Age did not influence the incidence of myonuclei in fiber cross sections for either muscle. Compared to lean mice the incidence of myonuclei per fiber cross section was significantly lower for ob/ob mice in the soleus muscle but not in the gastrocnemius muscle. The incidence of satellite cells in fiber cross sections and the percentage of nuclei within the basal lamina that were satellite cell nuclei decreased as age increased. Muscle fibers of ob/ob mice contained fewer myonuclei either because of shorter muscle length (gastrocnemius), or because of the combined effect of shorter muscle length and fewer myonuclei per unit length (soleus). The lower apparent number of myonuclei in the muscles of ob/ob mice was associated with a lower apparent number of satellite cells.

In postnatal skeletal muscle total muscle mass is directly related to total muscle DNA content (1). Since the amount of DNA per nucleus is constant within an animal, DNA content must be a direct reflection of nuclear content. The rate of myonuclear proliferation, then, will influence the rate of accretion of muscle mass. Addition of new myonuclei postnatally to growing myofibers is the result of mitosis in satellite cells and the subsequent incorporation of daughter nuclei into the myofiber (2). Therefore, the quantity and/or rate of mitotic activity of satellite cells are presumably important variables that orchestrate postnatal muscle growth.

In certain models of obesity, such as obese (ob/ob) mice and obese Zucker rats, the growth potential of the lean body mass is diminished (3, 4). This characteristic is expressed in the early postweaning period in the ob/ob mouse and has been suggested as a predisposing factor for obesity (3, 5). The role of satellite cells in the reduction of growth potential of lean body mass has not been previously described in this model or any other model of obesity. The relation between muscle nuclear content and other linear measurements of muscle growth were examined in this study in lean and ob/ob mice at various postnatal ages.

Materials and Methods. Four pairs of male lean (ob/+ or +/+) and obese (ob/ob) mice were obtained from the Michigan State University breeding colony of C57BL/6J-ob/+ mice at 2, 3, 5, 8, and 16 weeks of age. Mice were weaned at 3 weeks of age and fed a stock diet (Wayne Lab-Blox, Allied Mills, Chicago, Ill.) *ad libitum.* Mice were housed at 23–25°C in solid-bottomed cages with lights on from 0700 to 1900 hr each day.

At 2 weeks of age, preobese and nonobese mice were tentatively identified by oxygen consumption measurements (6). Preobese ob/ob mice consume less oxygen than nonobese (ob/+ or +/+) mice. Identification of preobese and nonobese mice was confirmed by measurement of body fat after removal of the gastrocnemius and soleus muscles and stomach contents. Carcasses with 10–15% fat were designated lean while those with 17–22% were designated ob/ob (6). Immediately after sacrifice with diethyl ether, hind limbs were fixed *in situ* by infusion of buffered (0.1 *N* phosphate, pH 7.2) 2% glutaraldehyde solution into the left ventricle after first flushing the system with a solution of 0.5% sodium citrate and 0.9% NaCl. The posterior vena cava was

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cut prior to the infusion. After 30 min, blocks of tissue approximately 1 mm square and several millimeters in length, were taken from the white portion of the medial head of the gastrocnemius muscle and from the soleus muscle. The blocks were fixed for an additional hour with 2% glutaraldehyde in phosphate buffer. Then the blocks were washed with buffer containing 4.5% sucrose and stored at 0–3°C in sucrose buffer for up to 4 weeks before being shipped on ice to the Richard B. Russell Agricultural Research Center where the blocks were postfixed in osmium and finally embedded in Epon.[2] Sections, approximately 70 nm thick (silver interference) were put onto 250-μm-mesh copper grids and stained with lead citrate and uranyl acetate. Cross sections of 100 muscle fibers from each animal were examined for fiber diameter (minimum diameter) and for the presence of myonuclei and of satellite cell nuclei. From these data, the percentage of fibers in cross section that exhibited myonuclei and satellite cell nuclei and the percentage of nuclei within the basal lamina that were satellite cell nuclei were calculated (7). At 2, 5, and 16 weeks of age, nuclear lengths were determined on longitudinal muscle sections (1 μm thick) that were stained with methylene blue–azure II.

Analysis of variance was conducted according to the Statistical Analysis System of Barr *et al.* (8). An arcsine transformation was conducted on the percentage data prior to analysis.

Results. Morphologically, satellite cells of lean and obese mice were indistinguishable in their ultrastructural characteristics. At 2 weeks of age, relatively more of the satellite cells in both phenotypes exhibited an ultrastructure characteristic of an activated cell (9).

Obese mice were significantly heavier (Table I) than lean mice after 5 weeks of age. The gastrocnemius muscle of obese mice, when compared to lean mice, was lighter in weight and shorter in length after 3 weeks of age. After 5 weeks of age, fibers in the gastrocnemius muscle of obese mice had a smaller diameter than did the fibers of lean mice.

Wet weight and length of the soleus muscle of obese mice were significantly lower over all ages when compared to lean mice. Fiber diameter was initially smaller then larger at 3 and 5 weeks of age, before again becoming smaller at 8 and 16 weeks of age in soleus muscle of obese mice when compared to lean mice.

Results from measurement of muscle nuclear content are summarized in Table II. In the gastrocnemius muscle, the percentage fibers with myonuclei present did not vary significantly with phenotype or age. But, in the soleus muscle, the percentage fibers with myonuclei present was significantly lower across all ages in ob/ob mice than in lean mice. The percentage fibers with satellite cell nuclei present decreased with age in both muscles, as did the percentage nuclei within the basal lamina that were satellite cell nuclei. Nuclear length, determined in 5-week-old mice was similar between phenotypes. The overall mean nuclear length was 12.1 ± 0.1 μm for gastrocnemius muscle and 12.0 ± 0.1 μm for soleus muscle.

Discussion. Phenotypic expression of the obese condition in ob/ob mice normally occurs about 5 weeks postnatally (10). Bergen *et al.* (3) previously reported that by 5 to 6 weeks of age total fat-free residue and total protein were less in hind limbs of ob/ob mice than in lean mice. Our data, as well as that of others (11–14), also showed that the skeletal muscle mass was less in ob/ob mice than in lean mice. Furthermore, the temporal sequence that led to the expression of phenotypic differences in muscle weight, length and fiber diameter varied for the two muscles. Phenotypic differences in these traits were already evident at 2 weeks of age in the soleus muscle; no differences existed in the gastrocnemius muscle until 5 to 8 weeks of age. In many species, e.g., fowl (15) and pig (16), the incidence of myonuclei in fiber cross sections increases with age. On the other hand, mice of the C57BL/6J strain, as well as those of several other strains of mice (17, 18), exhibit little change after 2 weeks of age in the percentage fibers with myonuclei present. The reason for this species variation is not known.

The percentage fibers with satellite cell nu-

[2] Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products which may be suitable.

TABLE I. EFFECT OF PHENOTYPE AND AGE ON BODY WEIGHT AND MUSCLE DIMENSIONS[a]

Trait	Phenotype	Age (weeks) 2	3	5	8	16	Standard error
Body weight[b] (g)	Lean	7.5	10.0	19.6	27.5 **	32.4 **	0.9
	Obese	7.1	11.0	18.8	40.6	55.6	0.9
Gastrocnemius							
Wet weight[b] (mg)	Lean	8.0	17.2	37.9 **	64.6 **	67.0 **	2.1
	Obese	6.4	17.3	28.5	48.3	46.4	2.1
Length[b] (mm)	Lean	51	66	81 **	94 *d*	96 **	2
	Obese	51	65	71	90	85	2
Fiber diameter[b] (μm)	Lean	18	24	25	43 **	46 d	1
	Obese	17	24	24	35	43	1
Soleus							
Wet weight[c] (mg)	Lean	1.6	2.4	5.2	8.4	9.4	0.4
	Obese	1.5	2.2	4.3	6.7	8.6	0.4
Length[c] (mm)	Lean	51	62	73	81	81	2
	Obese	49	62	65	80	73	2
Fiber diameter[b] (μm)	Lean	18 *	19 **	27 *	38 **	36 **	8
	Obese	15	23	30	31	33	8

[a] Each value is the least-squares mean from four animals.
[b] Phenotype × age interaction was significant, $P < 0.01$.
[c] Main effects of phenotype and of age were significant, $P < 0.01$. Interactions were not significant ($P > 0.05$).
[d] Within trait and age, effect of phenotype tended to be significant ($P < 0.10$).
* $P < 0.05$ for phenotypic comparison within trait and age.
** $P < 0.01$ for phenotypic comparison within trait and age.

clei present and the percentage nuclei within the basal lamina that are satellite cell nuclei normally decreases with age in the mouse (17–19). However, due to small sample size and very low satellite cell nuclear frequencies, statistical analyses were not conducted on satellite cell nuclear traits. Discussion of these limitations is presented in detail elsewhere (20–22). To obtain an estimate of phenotypic differences for nuclear traits, data for muscle length and nuclear traits were pooled within phenotype across ages (Table III). The apparent number of nuclei was calculated by the formula PL/d, where P equaled the percentage fibers in cross section with nuclei present, L equaled muscle length, and d equaled nuclear length. Considering only phenotypic differences, then, the gastrocnemius and soleus muscles were both about 8% shorter in ob/ob mice than in lean mice. Apparent myonuclear number was 14 and 23% less in the gastrocnemius and soleus muscles, respectively, of ob/ob mice when compared to the muscles of lean mice. The lower apparent number of myonuclei in the gastrocnemius muscle of ob/ob mice was primarily due to differences in muscle length (Table I) while the lower number in the soleus muscle was due to differences in muscle length and in the percentage fibers with myonuclei present (Table II).

Apparent satellite cell number was also lower in muscles of ob/ob mice than in muscles of lean mice. No appreciable difference was observed in the percentage of nuclei within the basal lamina that were satellite cell nuclei. Similarity in this latter trait between phenotypes, both across ages (Table III) and within the various ages (Table II), suggests that incorporation of satellite cell nuclei per se into myofibers was not influenced by phenotype. The fact that there are fewer myonuclei contained within the two muscles of the ob/ob

TABLE II. EFFECT OF PHENOTYPE AND AGE ON MUSCLE NUCLEAR CONTENT[a]

Trait	Phenotype	Age (weeks) 2	3	5	8	16
				%		
Gastrocnemius						
Fibers with myonuclei[b]	Lean	60.2 ± 3.4	57.5 ± 3.7	53.2 ± 4.3	61.5 ± 2.5	61.0 ± 11.2
	Obese	63.5 ± 1.4	61.0 ± 2.1	54.2 ± 2.2	54.2 ± 1.9	56.5 ± 2.6
Fibers with satellite cell nuclei	Lean	11.0 ± 1.1	3.5 ± 0.6	1.5 ± 0.5	1.8 ± 0.2	0.5 ± 0.3
	Obese	8.5 ± 1.8	3.8 ± 0.8	1.2 ± 0.2	1.5 ± 0.3	0.8 ± 0.5
Nuclei within basal lamina that were satellite cell nuclei	Lean	15.5 ± 1.5	5.7 ± 0.9	2.9 ± 1.0	2.4 ± 0.4	0.7 ± 0.4
	Obese	11.6 ± 2.1	5.8 ± 1.2	2.2 ± 0.4	2.7 ± 0.6	1.3 ± 0.9
Soleus						
Fibers with myonuclei[b,c]	Lean	61.5 ± 2.5	61.0 ± 2.1	58.5 ± 4.9	56.5 ± 4.4	68.8 ± 6.7
	Obese	58.0 ± 1.4	59.2 ± 4.9	54.0 ± 1.8	45.5 ± 6.1	51.5 ± 9.9
Fibers with satellite cell nuclei	Lean	11.8 ± 0.8	5.5 ± 2.0	2.2 ± 0.5	2.2 ± 0.5	0.5 ± 0.5
	Obese	9.2 ± 2.3	5.5 ± 1.0	3.0 ± 0.6	3.5 ± 0.3	0.5 ± 0.3
Nuclei within basal lamina that were satellite	Lean	16.2 ± 1.2	8.0 ± 2.6	3.6 ± 0.5	3.8 ± 0.8	1.0 ± 0.9
	Obese	13.5 ± 2.8	8.9 ± 2.3	5.3 ± 1.0	7.7 ± 1.6	1.3 ± 0.9

[a] Values are means ± SEM of four animals; observations were made on cross sections of fibers.
[b] For both muscles the percentage fibers with myonuclei present did not change significantly ($P > 0.05$) with age, and the phenotype × age interaction was also not significant ($P > 0.05$).
[c] The percentage fibers with myonuclei present was significantly lower ($P < 0.05$) in the soleus muscle of obese mice when compared to lean mice.

mice is associated with the finding that there are fewer satellite cells in ob/ob mouse muscle when compared to the muscle of lean mice. Purchas *et al.* (23) recently reported that the proportion of satellite cell nuclei in the gastrocnemius muscle of ob/ob mice which showed proliferative activity was lower than in the same muscle of lean mice at 2 and 3 weeks of age. Thus, the total myonuclear content of ob/ob mice is probably restricted by mitotic activity of the satellite cell as well as by the number of satellite cells.

TABLE III. PHENOTYPIC MEANS FOR SELECTED TRAITS[a]

Trait	Phenotype	Muscle Gastrocnemius	Soleus
Muscle length (mm)	Lean	78	70
	Obese	72	65
Fibers with myonuclei (%)	Lean	60.7	61.2
	Obese	57.9	53.6
Apparent myonuclear number[b]	Lean	3910	3570
	Obese	3440	2900
Fibers with satellite cell nuclei (%)	Lean	3.6	4.5
	Obese	3.1	4.3
Apparent satellite cell number[b]	Lean	232	262
	Obese	184	233
Nuclei within basal lamina that were satellite cell nuclei (%)	Lean	5.6	6.5
	Obese	5.1	7.3

[a] Values were pooled across ages; $N = 24$.
[b] Calculated by formula $n = PL/d$, where P = percentage fibers in cross section with nuclei present, L = muscle length, and d = nuclear length.

1. Allen RE, Merkel RA, Young RB. Cellular aspects of muscle growth: Myogenic cell proliferation. J Anim Sci **49**:115–127, 1979.
2. Moss FP, Leblond CP. Satellite cells as the source of nuclei in muscles of growing rats. Anat Rec **170**:421–436, 1971.
3. Bergen WG, Kaplan ML, Merkel RA, Leveille GA. Growth of adipose and lean tissue mass in hind limbs of genetically obese mice during preobese and obese phases of development. Amer J Clin Nutr **28**:157, 1975.
4. Shapira JF, Kircher I, Martin RJ. Indices of skeletal muscle growth in lean and obese Zucker rats. J Nutr **110**:1313–1318, 1980.
5. Carnie JA, Smith DG. Diabetes, obesity and heat production—possible links. Fed Eur Biochem Soc Lett **110**:8–10, 1980.
6. Thurlby PL, Trayhurn P. The development of obesity in preweanling ob/ob mice. Brit J Nutr **39**:397–402, 1978.
7. Campion DR, Marks HL, Richardson RL. An analysis of satellite cell content in the semimembranosus muscle of Japanese quail (*Coturnix coturnix japonica*) selected for rapid growth. Acta Anat **112**:9–13, 1982.
8. Barr AJ, Goodnight JH, Sall JP, Helwig JT. A User's Guide to SAS 76 (SAS Institute, Inc., Raleigh, NC, 1976).
9. Campion DR. The muscle satellite cell. A review. Int Rev Cytol **87**:225–247, 1984.
10. Ingalls AM, Dickie MM, Snell GD. Obese, new mutation in the house mouse. J Hered **41**:317, 1950.
11. Thurlby PL, Trayhurn P. Regional blood flow in genetically obese (ob/ob) mice. Pflueger Arch **385**:193–201, 1980.
12. Cuendet GS, Loten EG, Jeanrenaud B, Renold AG. Decreased basal, noninsulin stimulated glucose uptake and metabolism by skeletal muscle isolated from obese-hyperglycemic (ob/ob) mice. J Clin Invest **58**:1078–1088, 1976.
13. Trostler N, Romsos DR, Bergen WG, Leveille GA. Skeletal muscle accretion and turnover in lean and obese (ob/ob) mice. Metabolism **28**:928–933, 1979.
14. Trostler N, Amin R, Shafrir E. Increased protease activity in muscles of obese (ob/ob) mice. Int J Obesity **6**:557–566, 1982.
15. Moss FP. The relationship between the dimensions of the fibers and the number of nuclei during normal growth of skeletal muscle in the domestic fowl. Amer J Anat **122**:555–564, 1968.
16. Campion DR, Richardson RL, Reagan JO, Kraeling RR. Changes in the satellite cell population during postnatal growth of pig skeletal muscle. J Anim Sci **52**:1014–1018, 1981.
17. Schultz E. A quantitative study of the satellite cell population in postnatal mouse lumbrical muscle. Anat Rec **180**:580–596, 1974.
18. Cardasis CA, Cooper GW. An analysis of nuclear numbers in individual muscle fibers during differentiation and growth: A satellite cell–muscle fiber growth unit. J Exp Zool **191**:347–358, 1975.
19. Allbrook DB, Han MF, Hellmuth AE. Population of muscle satellite cells in relation to age and mitotic activity. Pathology **3**:233–243, 1971.
20. Abercrombie M. Estimation of nuclear population from microtome sections. Anat Rec **94**:239–247, 1946.
21. Maruenda E, de, Franzini-Armstrong C. Satellite and invasive cells in frog sartorius muscle. Tissue Cell **10**:749–772, 1978.
22. Atherton GW, James NT. Stereological analysis of the number of nuclei in skeletal muscle fibers. Acta Anat **107**:236–240, 1980.
23. Purchas RW, Romsos DR, Allen RE, Merkel RA. Muscle growth and satellite cell proliferative activity in obese (ob/ob) mice. J Anim Sci, submitted, 1984.

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