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Growth of the White-Mouse Gastrocnemius Muscle¹

II. In Non-Terrestrial Gravity

CHARLES C. WUNDER AND JOHN W. C. BIRD

Abstract. Exposure of white mice (Swiss Webster, female; NLW, male and female) to 1.5 to 7.0 G's of chronic centrifugation from the age of 5 weeks for durations of 1 to 8 weeks is known to cause some reduction in body growth. However, the retardation of muscular development was not as drastic. When corrections were made for differences between experimental and control body mass by means of *Huxley's Equation for Heterauxic Growth*, the muscles of experimental mice were seen to be larger than those of control animals of the same size. The measurements of muscle size, in order of increasing high-G response were: wet mass, dry mass, and non-collagen nitrogen (NCN) content. These data were examined in terms of the Huxley Heterauxic Equation, as modified from a consideration of *Galileo's Principle of Similitude*:

muscle size \propto (inertial field) (body mass)^{4/3}.

Although all experimental muscle measurements (relative to constant body size) increased with centrifugation, any single detected compensation was much less than the total compensation predicted by this equation. The best empirical relationship found for high-G data was a linear one between the logarithm of effect upon muscles size and logarithm of effect upon body size.

In the accompanying paper (Wunder and Bird, 1969) we noted that concentration of noncollagen nitrogen [NCN] in this muscle increased for mice at normal gravity in the manner expected from meeting the demands of similitude. Previous reports of increasing [NCN] with chronic centrifugation were also reviewed. If the demands of similitude are also met by [NCN] at high gravity, one would expect that for the gastrocnemius muscles from mice grown at high-G, data would satisfy the equation:

$$[\text{NCN}] \propto gB^{1/3}, \quad [1]$$

where g is the resultant inertial field intensity and B is body mass.

METHODS AND MATERIALS

Two strains of mice, NLW and Swiss Webster, were employed in this study. The strains, numbers, sexes, experimental conditions (including resultant inertial field), and relative experimental effects are listed in Table 1. Experimental mice were littermates of the mice described in the accompanying paper (Wunder and Bird, 1969). Those previously described one-G mice served as paired-sample controls for the present high-G mice. Experimental animals

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were exposed to chronic centrifugation for periods of 1, 2, 4, and 8 weeks at resultant fields of from 1.5 to 7 G's in a centrifuge originally described by Walters *et al.* (1960). Control littermates of comparable body mass and sex were placed in identical cages adjacent to the centrifuge but were maintained at normal gravity. The experimental animals were kept under continuous centrifugation with the exception of 10 minutes a day when the animals were removed for replenishing of food, for cage cleaning, and for weighing. Other than for centrifugation the materials and methods were identical to those described in the accompanying paper (Wunder and Bird, 1969).

All data are based upon instances where there was complete survival of all experimental and control members of a litter. As was the case for control animals, there was almost complete survival under these experimental conditions.

TABLE 1

TREATMENT AND CHARACTERISTICS OF HIGH-G MICE
Identification

g (G's)	Exposure time (wk)	Post exposure time (wk)	Series	Sex	Strain	Litter Identification No.	Age at Termination (wk)	Rev./min.	Experimental : Control Ratios	
									Body Mass B _x /B _c	Wet Muscle Mass
									% ± S.E. (Number)	
4-1-0-a	M	NLW	1-11	6	90	73±2	(12)	77±11	(9)	
4-2-0-a	M	NLW	12-14	7	90	85±1	(8)	91±6	(7)	
4-4-0-a	M	NLW	20-26	9	90	72±6	(7)	80±10	(7)	
4-8-0-a	M	NLW	27-34	13	90	80±3	(8)	75±6	(4)	
4-1-0-b	F	NLW	35-42	6	90	78±1	(8)	103±23	(8)	
4-2-0-b	F	NLW	43-48	7	90	80±5	(6)	68±8	(6)	
4-4-0-b	F	NLW	49-57	9	90	78±5	(9)	104±14	(9)	
4-8-0-b	F	NLW	58-66	13	90	71±3	(7)	85±7	(7)	
7-1-0-c	F	SW	101-108	6	96	71±4	(7)	80±5	(7)	
4-1-0-c	F	SW	109-117	6	96	93±6	(8)	81±11	(8)	
1.5-1-0-c	F	SW	118-125	6	96	91±4	(8)	97±10	(8)	
7-4-0-c	F	SW	126-133	9	96	77±5	(7)	87±10	(7)	
7-1-3-c	F	SW	126-133	9	96	94±8	(7)	93±11	(7)	
7-2-2-c	F	SW	126-133	9	96	101±24	(3)	127±52	(2)	
4-4-0-c	F	SW	135-142	9	96	82±2	(7)	81±7	(7)	
4-1-3-c	F	SW	135-142	9	96	87±9	(7)	100±5	(7)	
4-2-2-c	F	SW	135-142	9	96	99±6	(3)	79±7	(3)	
1.5-4-0-c	F	SW	143-150	9	96	86±6	(8)	91±15	(8)	
1.5-1-3-c	F	SW	143-150	9	96	100±5	(7)	111±11	(6)	

TABLE 1 (Cont.)
TREATMENT AND CHARACTERISTICS OF HIGH-G MICE

		Experimental to Control Muscle Ratios (Cont.) % \pm S.E. (Number)					
Dry Mass		% Dry Mass		Total NCN		[NCN] in Dry Muscle	
1.5-2-2-c F	SW	143-150	9	96	106 \pm 3	(3)	87 \pm 9 (3)
3-1-0-d F	SW	201-208	6	55	72 \pm 6	(8)	97 \pm 10 (8)
3-2-0-d F	SW	217-222	7	55	79 \pm 7	(6)	91 \pm 10 (6)
3-4-0-d F	SW	209-216	9	55	103 \pm 2	(7)	90 \pm 13 (7)
3-1-3-d F	SW	223-227	9	55	89 \pm 8	(5)	83 \pm 18 (5)
3-2-2-d F	SW	228-234	9	55	86 \pm 6	(7)	89 \pm 10 (7)
		67 \pm 16	(7)		101 \pm 13	(6)	59 \pm 11 (6)
		122 \pm 30	(7)		118 \pm 19	(7)	110 \pm 18 (7)
		112 \pm 23	(7)		130 \pm 10	(7)	137 \pm 42 (7)
		128 \pm 46	(5)		123 \pm 45	(3)	101 \pm 42 (3)
		104 \pm 18	(7)		110 \pm 24	(7)	128 \pm 37 (7)
		64 \pm 4	(6)		93 \pm 11	(6)	78 \pm 14 (6)
		106 \pm 13	(9)		111 \pm 11	(9)	95 \pm 12 (7)
		128 \pm 13	(7)		99 \pm 4	(5)	130 \pm 22 (6)
		77 \pm 5	(7)		110 \pm 6	(7)	78 \pm 12 (7)
		100 \pm 14	(7)		111 \pm 12	(6)	107 \pm 26 (7)
		99 \pm 17	(8)		113 \pm 8	(7)	106 \pm 21 (8)
		93 \pm 4	(7)		101 \pm 6	(7)	97 \pm 19 (7)
		106 \pm 16	(7)		105 \pm 8	(7)	123 \pm 9 (6)
		136 \pm 49	(2)		107.....	(1)	85 \pm 6 (3)
		78 \pm 7	(7)		87 \pm 6	(7)	128 \pm 29 (6)
		105 \pm 6	(7)		91 \pm 5	(7)	113 \pm 30 (8)
		89 \pm 7	(3)		94 \pm 15	(3)	110 \pm 30 (3)
		104 \pm 16	(8)		140 \pm 40	(8)	130 \pm 29 (8)
		116 \pm 15	(7)		109 \pm 9	(6)	133 \pm 35 (7)
		72 \pm 18	(3)		95 \pm 15	(3)	105 \pm 29 (4)
		98 \pm 15	(8)		118 \pm 14	(8)	118 \pm 13 (8)
		86 \pm 12	(6)		101 \pm 5	(6)	111 \pm 10 (7)
		98 \pm 14	(7)		95 \pm 6	(5)	110 \pm 11 (3)
		98 \pm 35	(4)		166 \pm 71	(4)	
		85 \pm 10	(4)		135 \pm 26	(4)	

RESULTS AND DISCUSSION

The mean ratios of experimental measurement to that for the paired littermate controls are tabulated in Table 1. These mean ratios for various muscle measurements as a function of the mean ratios (B_x/B_e) for body mass are also displayed by the experimental points in the double logarithmic plots shown in Fig. 1. Regression lines for these data are shown by the broken lines. Solid lines indicate ratios to be expected if changes in muscle ratios were only

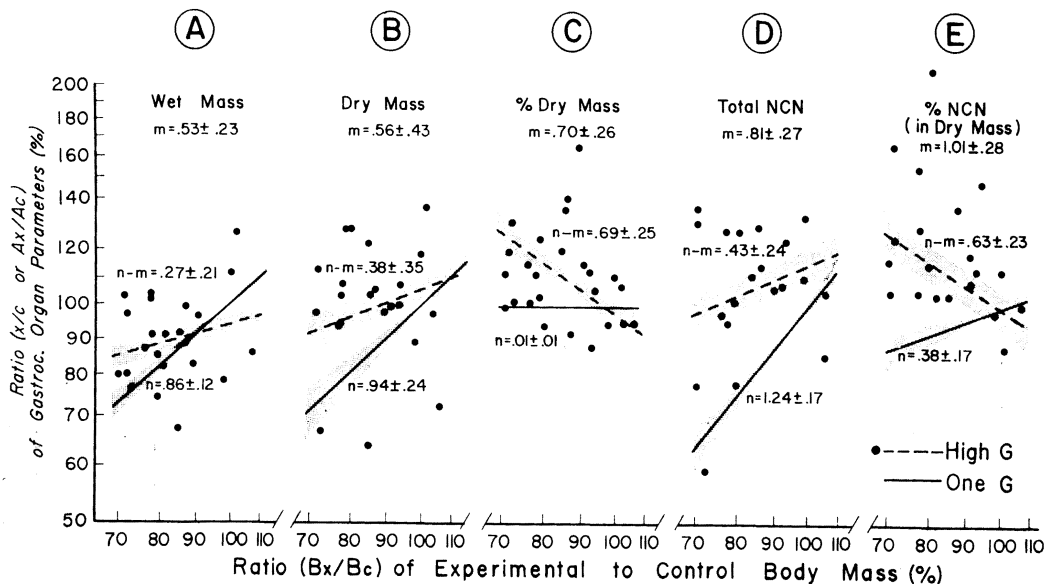


Figure 1. Comparison of measured ratios (points as fit to broken lines) of experimental to control muscle values to that predicted (solid line) for control conditions. Values are displayed on double logarithmic scales. Predicted standard errors of ordinate values are shown. For standard errors of the individual points together with the experimental conditions see Table 1. All normalized data both from continuous exposure and also from recovery experiments in that table are shown here. Slopes $n-m$ or n^1-m computed for parts A, B, C and D from simple least squares linear regression. The experimental line in part E is not computed from the points displayed but from values for individual paired littermates by a multiple regression (first row of Table 2), which yield an n^1-m which is independent of age.

attributable to the way such ratios change as a function of B for control animals. Those solid, control curves are determined from the appropriate values of n or n' shown in Fig. 1-5 of the accompanying manuscript (Wunder and Bird, 1969). For parts A, B, C, and D broken lines were determined from simple regression of the displayed points. For part E the broken line was determined from multiple regression of [NCN] ratios for each pair of mice, in accordance with the first equation in Table 2. Slope of these experimental lines is indicated by $n - m$ or $n' - m$. The magnitude of m should indicate the trend of change in muscle ratios in respect to the relative effect upon body mass.

The effect as indexed by m was significant with respect to wet mass, % dry mass, total NCN and percent NCN of dry mass [NCN] in the muscles. It was most significant in respect to the [NCN]. Significance of difference of individual experimental points from the solid (control) lines can be determined from experimental errors tabulated in Table 1 and the appropriate control error (indicated by with shading about the solid lines).

Although the trends shown for parts A, C, D, and E in Fig. 1 and many individual points are significantly different from the control levels, the differences are not great enough to completely satisfy the demands of similitude indicated by Equation [1]. That would require that the quotient of experimental values as divided by the control levels shown in Figure 1 be equal to the magnitude of the number of G's of inertial field. The effects upon the muscle appear more directly related to effect upon relative body mass than to the actual inertial field intensity itself.

When equation [1] was tested by plotting the appropriate values of $\log [\text{NCN}]_{\text{exp}}/[\text{NCN}]_{\text{cont}}$ as linear functions of $\log g$ and/or $\log (B_x/B_c)$ the dependence upon $\log (B_x/B_c)$ was more apparent than that for $\log g$, suggesting a relationship similar to the empirical equation [2], (see Table 2):

$$\frac{[\text{NCN}]_{\text{exp}}}{[\text{NCN}]_{\text{cont}}} = (B_x/B_c)^{n'-m} \quad [2].$$

This can be visualized in Fig. 1 where all the muscle ratios are plotted on double-logarithmic scales as a function of B_x/B_c . The ratio B_x/B_c , which tends to decrease with g , would serve as an index of gravitational effect relative to the whole animal. Effective experimental intensity would, therefore, be considered greater as one proceeds from right to left (rather than the more conventional left to right) with any curve.

In Fig. 1 the stimulated development of NCN concentration was sufficiently marked to permit separation from the slope $n'-m$ the possible contribution of varying age. As was the case for percent NCN with the one-G curve in Fig. 1 of the accompanying

TABLE 2

Regression Coefficients for (experimental to control [NCN]) log in gastrocnemius muscles performed as a function of log (experimental to control body mass)^{n'-m}, age (in weeks beyond 5 weeks)^{β₁}, log (ratio of experimental to control field)^{β₂}, recovery time (R) in weeks after removal from centrifuge ^gβ₃, sex (M=1 and F=2)^{β₄}, and strain (NLW=1 and SW=2)^{β₅};

$$\log [\text{NCN}]_x / [\text{NCN}]_c = \beta_0 + (n'-m) \log \frac{M}{\bar{M}_1} + \beta_1 \text{Age (wk}^{-1}\text{)} + \beta_2 \log \frac{g}{g_0} + \beta_3 R + \beta_4 \text{Sex} + \beta_5 \text{strain.}$$

β_0	$n'-m$	β_1	β_2	β_3	β_4	β_5
-0.07±0.011	-63±0.23	0.018±0.011	-----	-----	-----	-----*
-0.01±0.03	-0.57±0.23	-----	-----	-----	-----	-----*
-0.10±0.06	-0.70±0.09	0.016±0.010	0.03±0.09	0.013±0.017	-----	-----*
-----	-0.60±0.27	0.008±0.009	-0.06±0.06	0.009±0.017	-----	-----*
-----	-0.64±0.27	0.015±0.010	0.00±0.08	0.010±0.020	-0.04±0.05	0.02±0.05*
-----	-0.49±0.02	-----	-----	-----	-----	-----**
-0.02±0.06	-0.51±0.36	-----	0.01±0.06	-----	-----	-----**
-0.01±0.08	-----	-----	0.08±0.11	-----	-----	-----**

* Computations from all centrifuged animals whether terminated at end of high-g exposure or after reconditioning to 1G.
 ** Computations only with animals terminated at end of exposure.

paper (Wunder and Bird, 1969), this was achieved by multiple regression, which corrected for possible influence of age, W , beyond the 5 weeks of age when the experiment began. Moreover, $[\text{NCN}]$ regression was not based upon the average value for each experiment as represented by the points. Computation was from individual ratios for each experimental animal and its control littermate. This yielded the curve upon which the line in Fig. 1 is based:

$$\log [\text{NCN}]_{\text{exp}}/[\text{NCN}]_{\text{cont}} = -0.07 \pm 0.04 - (0.63 \pm 0.23) \log B_x/B_c + (0.02 \pm 0.01) \text{wk}^{-1}W.$$

Other regressions for this data are listed in Table 2. From the $(n'-m)$ value (-0.57 ± 0.23) in the second row of that table and the n' value (0.43 ± 0.08) in the second row of Table 2 one can obtain an m of 1.00 ± 0.24 . As the computations yield essentially the same values $(1.01$ vs $1.00)$, the present data does not reveal any dependency of m upon age. Any value for the regression of $\log (g/g_0)$ is significantly less than that of unity.

At this time we are not prepared to offer a rigorous explanation as to why equation [2] is more applicable than [1]. Perhaps if the $[\text{NCN}]$ of equation [2], instead of including consideration only for a single muscle change included a consideration for all pertinent adjustments to gravitational adversity as well, such as the relationship between B_x/B_c and g , that equation would prove more applicable. Moreover, equation [1] assumes that muscle functions only against gravity, thus ignoring the other functions for which a muscle is called upon to exert force. Equation 2 might be rationalized by saying that $[\text{NCN}]$ will depend more directly upon "effect" of g than g itself. Decrease in B_x/B_c below unity appears to be a good measure of this effect.

There are obvious limits to the degree of possible compensation. Full agreement with our model is likely only with the most moderate changes in g . Equation [1] would predict that if increasing NCN constituted the only adjustment, then the NCN concentrations in dry tissue would be 16 percent at 4.5 G 's. The equation would therefore predict that at still higher fields the concentration of protein (assuming 6.25 gm per gm of NCN) would exceed 100 percent!

The increasing NCN concentration with mass may be itself be adequate to meet gravitational demands at one- G (Wunder and Bird, 1969). Additional changes, such as those represented by increasing percent of dry mass, must occur for complete adjustment to the added gravitational demands during centrifugation. Implicit in equation [1] is some consideration for the extent to which decreased total body growth may constitute part of the total adjustment. The 20 percent increase in dry muscle mass for the average experiment could constitute a change which is not necessary at

normal gravity. Duling's (1967) recent measurements of vascular resistance with the hindlimbs of centrifuged mass suggest that the relative ability for perfusion of blood increases approximately in proportion to $(B_x/B_e)^{-1}$. Finally, if all other adjustments prove inadequate (as is certainly the case during the first few days of centrifugation) the only recourse would be a decrease in motion and related activity. That phase of adjustment becomes less necessary as other changes condition the animal to high gravity.

The relatively lower fluid composition of this muscle tissue is similar to that previously reported for heart and diaphragm together with a then statistically insignificant trend for this tissue (Bird *et al.*, 1963, as cited by Wunder and Bird, 1969). Because such a change, unlike that for NCN, does not occur merely as a result of greater body mass (Wunder and Bird, 1969, Fig. 3), it could conceivably be a change unrelated to the muscle's requirement for greater weight support at high-G. Perhaps it represents a cardiovascular adjustment whereby an increase in blood volume diminishes the possibility for shock which could result from gravitationally imposed venous pooling of blood with inadequate return to the heart. For these mice, there is a decreased hematocrit suggestive of, among other things, dilution by a shift of tissue fluids into the plasma.

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