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Maximum Likelihood Factor Analysis of the Effects of Chronic Centrifugation on the Structural Development of the Musculoskeletal System of the Rat*

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Summary. At the age of 30 days female Sprague-Dawley rats were placed on a 3.66 m radius centrifuge and subsequently exposed almost continuously for 810 days to either 2.76 or 4.15 G. An age-matched control group of rats was raised near the centrifuge facility at earth gravity. Three further control groups of rats were obtained from the animal colony and sacrificed at the age of 34, 72 and 102 days. A total of 16 variables were simultaneously factor analyzed by a maximum-likelihood extraction routine and the factor loadings presented after rotation to simple structure by a varimax rotation routine. The variables include G-load, age, body mass, femoral length and cross-sectional area, inner and outer radii, density and strength at the mid-length of the femur, dry weight of gluteus medius, semimenbranosus and triceps surae muscles.

Factor analyses on A) all controls, B) all controls and the 2.76 G group, and C) all controls and centrifuged animals, produced highly similar loading structures of three common factors which accounted for 74%, 68% and 68%, respectively, of the total variance. The 3 factors were interpreted as:

1. An age and size factor which stimulates the growth in length and diameter and increases the density and strength of the femur. This factor is positively correlated with G-load but is also active in the control animals living at earth gravity.

2. A growth inhibition factor which acts on body size, femoral length and on both the outer and inner radius at mid-length of the femur. This factor is intensified by centrifugation.

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3. A muscle growth inhibition factor which is probably correlated with age and G-load but is also active at earth gravity.

A tentative biomechanical interpretation of these 3 factors has been ventured.

Key words: Hypergravity Growth factors Femur Muscle

Introduction

Subjecting a growing mammal to an increased gravitational field via a centrifuge may evoke rather complex and different growth reactions in the various epiphyseal plates and the periosteal and endosteal surfaces along the shaft of a long bone (Amtmann and Oyama, 1973, 1976; Smith, 1977). While an ambient accelerative field consistently inhibits longitudinal growth of mammalian long bones (Smith and Burton, 1971; Wunder, 1971; Amtmann and Oyama, 1976), the alterations in their cross-sectional shape due to a hypergravic environment are poorly understood (Amtmann, 1974; Amtmann and Oyama, 1973, 1976).

During postnatal human growth the mode of stressing of the femoral mid-shaft probably will not change much, at least after the start of bipedal walking. The magnitude of loading, however, will on average increase continuously, because the total body mass increases much faster than the cross-sectional area of the bone. For example, if man could keep child-sized legs while his trunk grew normally, the effect on the legs would be as if gravity increased gradually from 1 G to 20–30 G. A theoretical and statistical analysis of transverse sections of human femora at different ages revealed that the cross-sectional dimensions are not primarily related to the longitudinal growth of the bone but most likely to the partial body mass (trunk, two arms and one swinging leg) superimposed in upright gait upon the femur and bending it medially (Amtmann, 1974).

In the growing rat, the relationship between body mass and cross-sectional area at mid-length of the femur seems subject to similar principles (Amtmann, 1974). If, indeed, a growing rat continuously adapts its femoral mid-shaft to an increasing level of stress, this may be brought about by a periosteal and endosteal apposition or resorption of bone and/or an increase of bone density and strength. A similar, but more pronounced pattern of reaction of the bones to increased mechanical stresses should be found in rats subjected for almost their whole lives to an increased gravitational field, if, that is, the mechanical effects are not obscured by other effects of centrifugation (see Amtmann and Oyama, 1976). This implies that the mode of locomotion and the level of activity are not significantly altered by centrifugation, as compared to animals living at earth gravity. A change in the motor pattern should change the time averaged principal stress directions in the femoral mid-shaft and consequently evoke internal bone remodeling to align the structural tissue components with the new average principal stress directions.

Although our earlier bivariate analyses of the present experiment revealed significant correlations between G-load and femoral length, cross-sectional size and shape, dry-weights of three selected hind leg muscles (Amtmann and Oyama, 1973, 1976), bone density (Jaekel et al., 1977), and ultimate compressive strength of bone (Kimura et al., in press), a simultaneous analysis of all variables has not been performed.

The aim of the present research is to factor analyze the collected data of the previous studies in order to seperate the effects of centrifugation from those of size and age.

Materials and Methods

The female Sprague-Dawley rats used in this analysis were those used in previous studies (Amtmann and Oyama, 1973, 1976; Jaekel et al., 1977; Kimura et al., in press). Control animals were treated in the same manner as the experimental group, but were not centrifuged. The animals were put on a 3.66 m radius centrifuge at the age of 30 days and subsequently exposed continuously for 810 days to a resultant of centrifugal and gravitational forces of 2.76 G or 4.15 G, except for twice weekly service stoppages of approximately 20 min. Since the body masses of the centrifuged group were markedly smaller than those of the age-matched controls, other groups of rats were included for comparison. These animals were 34, 72 and 102 days old. After sacrifice the right and left femora of each animal were removed and carefully cleared of adhering tissue. The length of the femur was measured. The bones were cut perpendicular to the shaft axis at mid-length and 1 mm thick cross sections removed, photographed and then enlarged to facilitate measurement of the cross-sectional dimensions. The "inner" and "outer" radii were calculated by the formula $(\text{area}/\pi)^{1/2}$ inserting the total cross-sectional area (bone substance and marrow cavity) and the area of the marrow cavity, respectively (see Amtmann, 1974) Along with the femur, three hind leg muscles were dissected and dried to a constant weight at 105° C. Two different kinds of bone density measurements were performed by photon absorptiometry using a ^{125}I Profile Scanner: (1) Measurement parallel to the shaft axis. The 1 mm thick dry bone section from the mid-length was scanned by the photon beam parallel to the shaft axis. The maximum absorptions on the anterior, posterior, medial and lateral positions were measured. (2) Measurement perpendicular to the shaft axis. The photons scanned the dry bone about 2 mm proximal from the mid-length, perpendicular to the shaft axis. The absorption of the beam from the anterior to the posterior positions was used for comparison. The bone density was expressed by a γ-ray linear absorption $\ln(I_0/I) = \mu \cdot d$, where I_0 is the intensity of the unattenuated radiation and I the attenuated intensity of the beam after passage through the bone of diameter d. In the parallel measurements, the linear absorption is equal to the absorption coefficient μ (mm^{-1}) since the bone thickness, $d = 1$ mm. The absorption in the 4.15 G centrifuged group was not measured. A compression test was performed by a universal testing machine with a cross-head speed of 1 mm/min. The 1 mm thick dry bone section from the mid-length was compressed parallel to the shaft axis. The breaking force was divided by the cross-sectional area to yield the ultimate strength. For the factor-analytic technique the reader may consult Überla (1968), Weber (1974), Timm (1975) and Schiller (1976).

Results

As bivariate statistical analyses of the variables have already been presented in detail in our earlier research, only the means of the control animals and those centrifuged at 2.76 G and 4.15 G, respectively, are shown in Table 1.

Analysis A. The average age of the control rats is only 439.3 days because other groups of younger rats with body masses comparable to the 840 day old centrifuged groups were included. The control animals were 34, 72, 102 and 840 days old. Since these rats lived at earth gravity the G-load was set equal to unity and excluded from Analysis A. The 40 observations of the 15 variables were intercorrelated (upper triangular correlation matrix in Table 2)

Table 1. The effect of chronic centrifugation on various bone and muscle parameters of female rats, as assessed by Maximum-likelihood factor analysis

X	Variable	$N=40$	$N=36$	$N=10$	Analysis A All controls, $N=40$			Analysis B All controls and 2.76 G group. $N=76$			Analysis C All controls, 2.76 G and 4.15 G groups. $N=86$		
					Factor			Factor			Factor		
					1	2	3	1	2	3	1	2	3
1	Age, days	439.3[a]	840	840	88[b]	−04	−23	−07	−87	−28	87	33	−33
2	Gravity	1.00	2.76	4.15	–	–	–	−55	−47	−33	23	52	−54
3	(Body mass)$^{1/3}$, g$^{1/3}$	7.02	6.97	6.64	86	−13	06	36	−62	26	68	−15	40
4	Femur length, mm	35.87	35.23	33.99	79	−16	29	40	−57	31	62	−27	43
5	(Cross-sectional area/π)$^{1/2}$, mm	1.39	1.43	1.38	93	−23	−22	23	−69	−26	71	−13	−15
6	Inner radius at mid-shaft, mm	1.12	1.01	1.05	10	94	−24	86	16	−17	08	−80	−12
7	Outer radius at mid-shaft, mm	1.79	1.75	1.75	79	−56	−24	77	−63	−09	77	−59	00
8	Parallel absorption, anterior	0.34	0.38	–	81	−11	10	−01	−83	00	–	–	–
9	Parallel absorption, posterior	0.35	0.38	–	83	01	13	−04	−80	08	–	–	–
10	Parallel absorption, medial	0.32	0.36	–	76	06	15	−17	−76	08	–	–	–
11	Parallel absorption, lateral	0.34	0.39	–	84	00	14	−11	−85	04	–	–	–
12	Perpendicular absorption	0.47	0.53	–	92	−27	−10	24	−93	−03	–	–	–
13	Compressive strength, N/mm^2	172.4	189.6	177.3	40	−23	04	−04	−41	−02	35	07	00
14	(Muscle mass)$^{1/3}$, g$^{1/3}$, triceps surae	0.74	0.69	0.63	−25	12	82	−05	28	88	−25	00	91
15	(Muscle mass)$^{1/3}$, g$^{1/3}$, semimembranosus	0.59	0.58	0.52	25	06	79	−05	−15	71	−15	12	81
16	(Muscle mass)$^{1/3}$, g$^{1/3}$, gluteus medius	0.77	0.76	0.73	11	12	78	04	−03	85	06	−02	84
					6.79[c]	2.75	1.63	4.67	3.74	2.50	2.90	2.75	1.82

[a] Mean value; [b] read +0.88; [c] eigenvalue

Table 2. Correlation matrices

		All controls, $N=44$, upper triangular matrix															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
All controls and 2.76 G group, $N=76$	1	1.00	—	81[a]	67	86	18	77	72	67	68	65	85	39	−46	[illegible]	−09
	2	59	1.00	—	—	—		—	—	—	—	—	—	—	—	—	—
	3	52	−06	1.00	70	79	20	74	71	69	70	75	84	36	−26	30	19
	4	40	−14	61	1.00	70	17	65	69	67	64	74	75	40	[illegible]	[illegible]	24
	5	69	22	44	43	1.00	35	92	71	73	61	76	93	38	−42	[illegible]	−08
	6	−16	−45	17	22	11	1.00	66	15	05	01	06	37	25	−35	−22	−30
	7	51	−11	64	64	63	58	1.00	68	62	53	63	90	44	−47	[illegible]	−16
	8	72	40	50	51	56	−13	51	1.00	77	69	70	74	39	−11	[illegible]	08
	9	65	34	43	48	53	−19	47	73	1.00	69	78	77	43	−06	[illegible]	17
	10	62	36	40	41	46	−26	34	67	56	1.00	70	73	27	−15	[illegible]	12
	11	72	50	50	47	55	−20	44	71	73	71	1.00	78	33	−08	[illegible]	14
	12	78	31	63	61	68	03	77	75	74	69	75	1.00	46	−37	[illegible]	−01
	13	35	22	15	17	23	−07	23	41	37	32	32	40	1.00	[illegible]	[illegible]	15
	14	−51	−39	−01	08	−41	−14	−29	−20	−12	−15	−18	−29	−11	[illegible]	[illegible]	70
	15	00	−07	37	34	−06	−15	−01	08	08	18	19	09	−06	[illegible]	1.00	65
	16	−20	−26	32	28	−24	−14	−03	−03	06	09	04	01	02	[illegible]	[illegible]	1.00

[a] Read +0.81

and factor analyzed using a principal axis extraction routine for an initial estimate of the factor stucture. This initial solution was used for the maximum-likelihood-extraction routine which converged after 31 iterations to factor loadings which were rotated to simple structure by a varimax rotation routine [Table 1 (Analysis A)]. From this analysis it appears that 74% of the total variance can be attributed to the three factors extracted which account for 45, 18, and 11% respectively of the total variance.

Factor 1 has high loadings on age, all body dimensions reflecting size, and on bone density measurements. In contrast to the high loading on the outer radius of femoral mid-shaft the loading on the inner radius is negligible. The loadings on the cube roots of the dry weights of the muscles are also very low. That is, if the dimension reflected by Factor 1 increases, age, body mass, femur length, cross-sectional area, outer radius, bone density and bone strength also increase.

Factor 2 has its highest loadings on the inner and outer radius at mid-length of the femur while the loadings on age, body mass, femoral length, parallel absorption and muscle masses are minimal. However, cross-sectional area, perpendicular photon absorption and compressive strength at mid-shaft exhibit a moderate parallel covariation with this dimension.

Factor 3 is clearly a muscle component and is essentially unrelated to body mass and compressive strength of the femoral mid-shaft. This factor exhibits moderate, but negative loadings on age and all three cross-sectional dimensions of the femur. Femoral lengths show a parallel covariation with the dimension reflected by Factor 3.

Analysis B. In Analysis B all the observations of Analysis A and those of the animals centrifuged at 2.76 G for 840 days are simultaneously factor analyzed using the original of the lower triangular 16 × 16 correlation matrix of Table 2, whose coefficients are rounded up to two decimal places. As can be deduced from the eigenvalues of the table, 68% of the total variance can be attributed to the three factors whose loadings were obtained after 59 iterations using the maximum-likelihood-extraction and then the varimax rotation routines. These factors account for 29, 23 and 16%, respectively, of the total variance. The similarity between the factor structures of Analyses A und B is expressed by the factor congruence coefficients presented in Table 3. As the table shows, the factor loadings of Factor 1 in Analysis B (BF_1) correspond very closely with Factor 2 in Analysis A (AF_2), since $q_{21} = -0.91$. The negative sign indicates that the signs of the loadings are mirror-inverted, which, however, may not apply to the very low loadings due to error variation.

Since the similarity between BF_2 and AF_1, and BF_3 and AF_3, respectively, is even higher, the total congruence coefficient is $q = 0.97$, if BF_2, BF_1, BF_3 are assigned to AF_1, AF_2, AF_3 and the signs of BF_1 and BF_2 are mirror-inverted. As follows from Schiller (1976), one can consider $q \geq 0.9$ to be significant; that is, the factor structures of the Analyses A and B are in very high congruency.

Factor 1 exhibits a relatively high, but negative loading on the G-load and a negligible loading on age. As follows from the moderate to very high loadings on body mass and on the femoral dimensions, the effect of centrifuga-

Table 3. The similarity of the factor structures of Table 1

		Analysis B			Analysis C		
		F_1	F_2	F_3	F_1	F_2	F_3
Analysis A	F_1	0.40	−0.98	0.02	0.97	0.30	0.10
	F_2	−0.91	0.32	0.25	−0.50	0.88	0.16
	F_3	−0.20	0.00	0.97	−0.24	0.17	0.96
Total			q = 0.97			q = 0.94	

tion reflected by Factor 1 is inhibitory on body and bone size. Bone density, strength and muscle masses are unaffected by Factor 1.

Factor 2 also has a relatively high loading on the G-load and a very high one on age. Body mass, the femoral dimensions with the exception of the inner radius of the mid-shaft, bone density and strength increase with increasing force.

Factor 3 shows very high loadings on muscle masses and a moderate and negative loading on G-load.

Analysis C. Analysis C comprises the observations of all control and centrifuged animals except the density values which were not available for the 4.15 G animals. The factor loadings showed convergence after 110 iterations. As in Analysis B, 68% of the total variance can be attributed to the three factors which account for 26, 25 and 17%, respectively, of the total variance.

The factor congruence coefficient in Table 3 reveals that the factor loadings of AF_1, AF_2 and AF_3 are congruous with CF_1, CF_2 and CF_3, respectively, and the total similarity of the factor structures of Analyses A and C is significant ($q = 0.94 > 0.9$). Factor 1 has a relatively low and Factor 3 a relatively high loading on the G-force, as compared to the loadings of BF_2 and BF_3, respectively. Besides the very high loadings on the muscle masses, Factor 3 exhibits moderate loadings on age, body mass and femoral length. The factor loadings of AF_3, BF_3 and CF_3 which are very similar suggest that with increasing age and G-load body mass, femoral length and the dry weights of the three hind leg muscles decrease under the influence of Factor 3.

Discussion

The factor-analytic technique used in the present research is scale free; that is, the technique does not depend on the unit of measurement used for the observations. Therefore, when an observation is multiplied by a constant, the factor loadings for the observation are multiplied by the same constant and the estimated unique variance is multiplied by the square of the constant (Timm, 1975). The solutions of other techniques, e.g. common factor analysis, or principal component analysis, depend on the scale of the variables, i.e. they are different if standardized or unstandardized variables are factor analyzed (Weber, 1974; Timm, 1975). To accelerate the convergence of the iterative procedure,

Table 4. Maximum-likelihood factor analysis of normally distributed random numbers after varimax rotation. N = sample size

Variable	Loadings of factor I				
	$N=40$	$N=40$	$N=40$	$N=60$	$N=80$
1	−96[a]	−36	−11	−04	09
2	04	95	26	−32	14
3	20	−09	−50	12	84
4	14	−12	−15	−07	09
5	41	09	−02	09	−13
6	00	13	10	−29	54
7	20	−18	−35	32	−11
8	17	09	98	26	−16
9	18	−01	06	03	07
10	24	−37	−14	−09	−04
11	37	10	−24	25	09
12	−32	−06	24	13	−11
13	11	13	−24	−96	−01
14	−13	−12	05	−01	−03
15	26	−05	09	21	−11
χ^2	127[b]	69	135	141	135
DF	105	105	105	105	105
P	>0.05	>0.05	>0.01	>0.01	>0.01

[a] Read −0.96; [b] sphericity test

the maximum-likelihood-extraction routine was applied to the solution of a common factor analysis, as suggested by Weber (1974). The interpretation of the factor structures is based on a varimax rotation solution, whose individual factor loadings cannot be judged by a significance test so far. Weber (1974) suggested as a rule of thumb that loadings greater than 0.30 or 0.40 should be considered as significant, depending on the size of the sample. This valuation is obviously incorrect, since we performed some maximum-likelihood factor analyses on normally distributed random numbers, which resulted in factor loadings as large as 0.98 (Table 4).

However, the loadings arranged according to the size of their absolute values showed a consistent distribution depending on the sample size. The higher values, moreover, were randomly distributed across the variables, i.e. the probability of a specific loading was the same for all variables. From our factor analyses on random numbers we infer that the factor structure of a single factor analysis on actual biological data cannot be distinguished from a random variable factor structure even if the loadings are as high as 0.99 in absolute value. Only if the same structure appears in several factor analyses on independently collected data may a biological interpretation be ventured. In the present research we conducted three analyses, namely on the data of all controls; of all controls plus the 2.76 G group of animals; and of all controls plus the 2.76 and 4.15 group of animals. The similarities of the three factor structures, as quantified by the congruence coefficients of Schiller (1976), are very high ($q=0.97$ and 0.94, Table 3) and significant. Moreover, a very similar factor structure (Table 5) was revealed by Amtmann (1978) in the corresponding data

Table 5. Maximum-likelihood factor analysis of the effect of running exercise on growing rats. Original data from Tsomplektsis (1976). Factor loadings after varimax rotation from Amtmann (1978)

	Variable	Factor		
		1	2	3
1	Exercise hours per day	−44[a]	10	−02
2	Duration of exercise in days	06	−06	−96
3	Age, days	−16	−50	05
4	Body mass$^{1/3}$ ($g^{1/3}$)	11	−33	−44
5	Femur length (mm)	04	−27	−46
6	Cross-sectional area$^{1/2}$ (mm)	39	−79	−35
7	Outer radius at mid-shaft (mm)	86	−27	−24
8	Inner radius at mid-shaft (mm)	92	33	−02

[a] Read −0.44

collected from rats, which were daily exercised for 0, 1, 2 or 3 hours on a tread-mill and sacrificed after 9, 18, 27, 36 or 45 days (Tsomplektsis, 1978). The following interpretation of the factor loadings, therefore, seems legitimate to us although it may be distorted by some random variation.

In Analysis A only control rats living at earth gravity were factor analyzed. Since these rats were 34, 72, 102 and 840 days old at the day of their sacrifice it seems obvious that the first factor which accounts for the greatest amount of the total variance extracted by the maximum-likelihood-extraction routine is an age and size factor. As age, and therefore total body mass, increases, femoral length and cross-sectional area at mid-shaft are increased by this factor at work. The enlargement of the cross section is produced exclusively by increase in the outer radius. The inner radius, however, exhibits only a negligible correlation with the first factor. This result is congruous with our earlier research (Amtmann and Oyama, 1976). In Fig. 1b and 2b of that report we displayed the scatter of the square-root of cross-sectional area/π and of the outer and inner radii against the length of the femur. From these figures and Table 2 of the same paper it follows that the bivariate correlation between femoral length and the inner radius is insignificantly low ($r = -0.12$ in the weight controls and $r = 0.30$ in the age controls). Very much in line with this, the partial correlations between square-root of cross-sectional area/π and inner and outer radii are respectively $r = 0.30$ and $r = 0.68$, if the effect of femoral length is eliminated from the total correlations. The same loading structures as in the first factor of Analysis A were found in the second factor of Analysis B and first factor of Analysis C (Table 1 and 3). The signs of the loadings of Factor 2 in Analysis

B are mirror-inverted as compared to the signs of Factor 1 in Analyses A and C, which is in line with the model of factor analysis. Since this age and size factor exhibits a moderate loading (−0.47) on the G-load in Analysis B and a negligible loading (0.23) in Analysis C, the relation between G-load and that factor may be due to chance. However, this age and size factor also shows high loadings on bone density and moderate ones on compressive strength; that is, both density and strength in the femoral mid-shaft are increased by this factor at work. Although the results of Analyses A and B suggest that a high percentage of the observed alterations in density may be due to the effects of age, there is good reason to assume that the effects are intensified also by centrifugation which would imply that the loading −0.47 on gravity in the second factor of Analysis B is significant. In our earlier research on the same rats (Jaekel et al., 1977), we found the density of the femora at mid-shaft to be significantly higher in the centrifuged rats than in the animals subjected to earth gravity, if the photon absorptiometric measurements were made by applying the photon beam perpendicularly to the shaft axis and if cross sections of equal size were compared. In line with this, Kimura et al. (in press) showed that the ultimate compressive strength at mid-length of the femora is 10% greater in the rats centrifuged at 2.76 G, as compared to all control animals, if the mean values are adjusted with respect to body mass and outer radius, respectively. The loadings on gravity and strength of bone in Analysis C are congruous with these assumptions but they are less pronounced. If our line of reasoning is correct, centrifugation as expressed by this age and size factor has a slightly stimulating effect on the longitudinal growth, the density and the strength of the femoral bone. This effect is enhanced with increasing age and body mass. The principal effect of an increased accelerative force, as produced by centrifugation, is an increase in the weight-to-mass ratio, which calls for greater muscle forces to equilibrate joint moments. This necessitates greater bone strength in the bone shaft to compensate bending moments. The stimulating effect should therefore also be augmented with increasing age and increasing body mass, as was pointed out in the Introduction and by Amtmann (1974). The very high loading on body mass in the first factor of Analysis A, which was performed on growing rats living at earth gravity, seems to substantiate this interpretation of the stimulating effect due to scale. In a recent centrifugation experiment on growing rats, Smith (1977) also noticed a stimulating effect on the longitudinal growth of the femur and on the formation of secondary ossification centres at a very low G-level (G = 1.05). He assumed this effect to be due to pure rotation rather than to hypergravity, because the rotational control animals were kept in cages mounted in the centre of the centrifuge. However, the cages of the rotational control animals were not suspended freely swinging over the centre of the centrifuge but kept rigid with a horizontal floor position, so that by the rotation of the centrifuge horizontal forces were produced which the animals additionally had to equilibrate in their joint systems during locomotion. It seems to us that the observed effects on femoral bone growth are more likely due to an increased mechanical stressing than to non-physical factors evoked by pure rotation. Rotational effects are obvious in the behavioural response of rats during the first several weeks of hyper-G exposure. However, the rats habituate to the rotational motion after several weeks and thereafter show no signs of being adversely affected. The separation of rotational effects from G-effects is a complex problem which may be solved by subjecting different animals to the same G-load on centrifuges of varying radii, as Smith (1977) proposed himself.

The loading structure of the second factor of Analysis A is congruous with the loading structures of the first factor in Analysis B, whose signs are mirror-inverted, and the second factor in Analysis C, as was revealed by the similarity coefficients in Table 3. In all three analyses this factor exhibits very high loadings on the inner and outer radii at mid-length of the femur while the loadings on age, parallel absorption and the muscle masses are negligible. The outer and inner radii of the femoral mid-shaft are decreased under the positive influence of this factor. In the control animals this factor also exhibits negligible loadings on body mass and femoral length. A simultaneous analysis of all control animals and those of the 2.76 G group, however, reveals a high loading on G-load and moderate ones on body mass and femoral length. The signs of the loadings indicate that body mass, femoral length and both the cross-sectional radii are decreased with increasing G-load. Thus, the factor loadings suggest that this factor reflects the established effect of centrifugation on growing rats, namely growth inhibition (Amtmann and Oyama, 1973, 1976; Smith, 1977). Since the same loading pattern, although not marked with respect to body mass and femoral length, has been found in the control rats, this factor is very likely not related to rotational but to hypergravic influences.

It seems obvious from the analyses that two factors operate upon the growing femur. The size and age factor stimulates the growth in length and diameter and increases bone density and strength, while the growth inhibition factor acts on body size, bone length and the cross-sectional radii. Both factors are most likely correlated with gravitational effects, which is surprising, since the two factors operate in opposite directions.

However, a similar factor structure was revealed by Amtmann (1978) in rats, which were exercised for 0, 1, 2 or 3 hours daily on a tread-mill and sacrificed after 9, 18, 27, 36 or 45 days (Tsomplektsis, 1976). A maximum-likelihood factor analysis showed (Table 5) that the outer and inner radii of the femoral cross section at mid-length were decreased by the first factor, which is highly correlated with the training load per day, while femoral length, cross-sectional area and outer radius were increased by the third factor, which is highly correlated with the duration of exercise in days, but not with the age of the animal. Density and strength of the bones have not been evaluated so far. The second factor showed high loadings on age and cross-sectional area. Although it seems rather premature to identify the factor structures of these two experiments, a similarity between the first factor of the tread-mill experiment and the first and second factors of Analyses B and C, respectively, of the present research is likely. This would mean that the alterations of the cross-sectional dimensions of the femur in the centrifuged rats is related to daily running activity of the animal on the centrifuge; that is, with increasing running activity in the hypergravic environment the cross-sectional radii would be decreased. The level of activity of the animals on the centrifuge, however, has not yet been analyzed quantitatively. In a preliminary study, the rats subjected to 4.15 G showed a significantly lower rate of running activity than the controls. However, as the results of both experiments suggest, an increased level of mechanical stress in repetitive leg movements during one day may evoke a different and opposed adaptational reaction in the bones to that produced by a prolonged exercise load subjecting the bones to the same level of stresses for several months.

Stronger muscle forces, necessary in a hypergravic accelerative field to equilibrate joint moments, should also subject the bones to increased bending moments, which one would expect to be compensated by an increased outer and inner bone diameter. In the reverse case, the outer and inner radii should be reduced along with a decreased muscle activity. In contrast to what one would expect from a superficial consideration, bone substance is deposited at the endosteal surface and removed at the periosteal surface in the animals which are exposed to hypergravity or subjected to an increased exercise load per day. The results of the present research suggest that the adaptational reactions of a long bone diaphysis to an increased level of bending stress are constituted by a decrease of both the outer and inner radii. If this tentative interpretation of the loading structure of Factor 1 of Analysis B is correct, the earlier bone remodeling models (Frost, 1964; Pauwels, 1968; Kummer, 1973; Gjelsvik, 1973 a and b) must be modified with respect to the reaction of bone tissue to increased bending stresses.

As shown by the similarity coefficients of Table 3, the loading structures of the third factors in all three analyses display a definite parallelism. This factor has the highest positive loadings on muscle masses and a moderate, but negative loading on G-load in Analysis B and C. The association of this factor with G-load, however, may be due only to the experimental design, because the animals exposed to higher G-loads by centrifugation were on average almost twice as old as the control animals. Since the mass of hind leg muscles should be correlated with leg length, the positive loading on femoral length seems plausible. This result is in line with our earlier bivariate analyses on the weight control rats (Amtmann and Oyama, 1976; Fig. 4b). In all three analyses the loading on the age of the animal is negative, which indicates that with increasing age the relative masses of the three muscles evaluated are decreased by this factor at work. As a tentative interpretation, one may draw an analogy between the effect of Factor 3 and the influence of the age and size factor on the density and strength of the femoral bone. If, with increasing age, the strength per unit of area is increased in the muscles, the total muscle mass necessary to equilibrate joint moments during locomotion may be less in an older animal, as compared to a younger one which is of the same total body size. Since the histology of the muscles has not been analyzed in the present research, no further conclusions can be reached. In our earlier partial correlation analysis (Amtmann and Oyama, 1976) of the present data we found rather controversial relationships between muscle masses and the cross-sectional parameters if the effects of femoral length were eliminated from the corresponding correlations. The present multivariate analysis suggests that with increasing muscle weights the cross-sectional area and both the outer and inner radii are decreased by the third factor. However, the loadings on the cross-sectional parameters are rather low and may be due to chance. Therefore, our earlier conclusion that centrifugation has a significant effect on the relationship between cross-sectional dimensions at mid-length of the femoral shaft and the dry weights of muscles may be due to spurious correlations.

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