(NASA-CR-149804) EFFECTS ON BODY SIZE AND COMPOSITION OF CHRONIC EXPOSURE TO ALTERED GRAVITY Final Report, 1 Aug. 1973 - 31 Mar. 1977 (Virginia Univ.) 103 p HC A06/MF A01 CSCL 06P G3/52
N77-19749
Unclas
21616

NASA RESEARCH GRANT

NGR47-005-213

EFFECT ON BODY SIZE AND COMPOSITION OF CHRONIC EXPOSURE TO ALTERED GRAVITY

BY

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FINAL REPORT

MARCH, 1977

FINAL REPORT

NGR47-005-213

A Research Grant from NASA

to

The University of Virginia

For The Period August 1, 1973 To March 31, 1977

Titled

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March, 1977

UNCLASSIFIED
No Restriction on Distribution

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I. INTRODUCTORY

At the time when this grant was proposed, it was evident that American investigators would have no opportunity for at least several years to study the physiological response of small mammals to weightlessness (0 G). However, as a result of the efforts of a few investigators, substantial progress had been made in studying the physiological effects of accelerations greater than terrestrial gravity (1 G) simulated by chronic centrifugation. Thus, it appeared that the best way to prepare for eventual opportunities to study weightlessness and at the same time to add to the sum of knowledge of gravitational physiology was to build on the progress achieved with centrifugation.

This research grant was intended to support research on the effects of chronic centrifugation upon body size and composition in the rat. Because of the similar stresses placed upon the body by acceleration and physical exercise and because space voyagers had successfully employed exercise as a conditioner at 0 G, it was proposed to study the effects on body size and composition of exercise with and without concomitant centrifugation. (Exploration of the differences between exercise and acceleration which were revealed was also profitable.)

Because it was known that gross body mass could be altered by dietary regimens, it was proposed to study the interaction with centrifugation of high protein and high fat diets.

Soon it became evident that some body composition parameters were vigorously defended against efforts to perturb them with ΔG, exercise, diet and other factors. It was then realized that one would be handicapped in interpreting the effects of acceleration on body composition without knowledge of the possible involvement of physiological regulation. This led us into a profitable reexamination of data collected for other purposed which yielded evidence that some body components are physiologically regulated and others are not. Because of evidence that some systems of physiological regulation do not begin to function effectively in the rat until several weeks after birth, it became desirable to study the effects of our various experimental variables on the young growing rat as well as on the adult.

At the very end of the study period it became evident that hypodynamia in its various forms is a special case of our experimental variable activity. It may be regarded as the ultimately mild exercise regimen, below ad libitum activity which is usually the control condition. Hence, as the period of support closed we had just completed a preliminary study of cage restraint.

The briefest outline of our studies by category is provided by the TABLE OF CONTENTS. However, the succession of topics in the TABLE is a logical one and, in many respects, does not agree with the chronological development of our ideas presented above.

NOTE

Most of the investigations listed in the TABLE OF CONTENTS are presented here in their final form. However, statistical and graphical analyses of II C., IV.A., and V.A. are not yet completed Consequently, the presentations of them are tentative and incomplete.

II. ACCELERATION STUDIES

A. Body Size and Acceleration.

The following is a manuscript accepted for publication in the next number of Life Sciences And Space Research. Its principal contribution is that it provides a first approximation to a general theory for the body composition response of mammals (including man to ΔG.)

(Life Sciences And Space Research. in press.)

BODY SIZE AND CHRONIC ACCELERATION

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Body composition studied as a function of acceleration (1-4.7 G) in mice and rats showed fat-free body mass (FFBM) to be a predictable function of G-force while corroborating the known lability of body fat. Of 9 studied components of FFBM only skeletal muscle, liver and heart contributed to the observed changes induced by Δ G. (Body water / FFBM) was independent of Δ G When FFBM (as a percentage of 1 G controls) was plotted versus G for mice, rats and monkeys (1-4 7 G) and men (0-1 G), the mass of the fat-free compartment passed through a maximun at 1 G The data distribution in the figure suggested possible effects of body size and of age

COSPAR Identification No V 7.5

Numerous reports in the literature have demonstrated a change in body size of several species of mammals following exposure to a change in chronic acceleration (Δ G). For laboratory mammals Δ G has been achieved by chronic centrifugation and for man, by exposure to weightlessness. The body-size parameter usually monitored is live weight. The usefulness of this datum is impaired by its heterogeneous nature, comprising fat and numerous non-fatty tissues and organs in addition to gut content and fur. Interpretation of the results obtained is facilitated if one fractionates the body into fat and the fat-free body mass (FFBM) as a first approximation and further fractionation of the FFBM is desireable. This approach requires body composition analyses which are encountered infrequently. We have attempted here to collate and interpret the few useful data which we have been able to find

Data are available on four species: male Swiss Webster white mice [1] , female Sprague-Dawley rats [2,3], male monkeys (Macaca nemestrina) [4] and men [5,6,7]. The body composition data are more detailed for the mice and rats, while in the case of the monkey and man, the only available data useful to us are live weight and body water values or values of FFBM calculated from water In each case the individuals studied were adults. Data on mice exposed during the period of rapid growth [1] were not used.

Mouse and rat data are summarized in Table 1. The coefficients of variation demonstrate the stability of the FFBM and the lability of fat in either absolute or relative

units. These findings corroborate several earlier reports, for example, in adult rats at terrestrial gravity fatness changed in response to: treadmill exercise [8], combinations of exercise and/or force-feeding [9], surgical stress [10], changes in the daily light cycle [10] and changes in diet [11]. None of these conditions in the adult altered the FFBM. Consequently, the evidence in Table 1 that FFBM changes with $\Delta' G$ stands in clear contrast to its marked stability at terrestrial gravity. Among nine components constituting the FFBM which have been studied [2] only skeletal muscle, liver and heart contributed to the changes noted in FFBM. In the rat these changes have been shown to be prompt, reversible and rectilinearly related to ΔG [2].

In our experience the water fraction of the fat-free compartment is so stable under steady-state conditions that it is frequently more useful as an index of technical precision than of state of hydration. In Table 1 the mouse values are somewhat low, possibly because, unlike the rat data, fur and gut content were included with FFBM. The water fraction of the rats on high-protein diet are seen to be lower than for those on chow, a statistically significant ($P<.0001$) difference pointed out earlier [3] The data of others [12] show a similar effect of high-protein diet.

The data in Table 1 can be combined with those on monkeys and men in a single plot of body composition parameters versus

acceleration if the parameters are normalized in terms of percentage of the control value at 1 G. When this is done for fat (either in grams or percentage), no meaningful relationships are apparent. Such a plot of FFBM is presented in Fig. 1. Obviously, there is agreement among the investigators that acceleration levels above 1 G reduced FFBM. Also the data suggest a body size effect. As size increases from mouse to monkey, the lines show an increasing negative slope. On the other hand the two sets of rat data may reflect an age difference since the FFBM at 1 G was 300 g in one case [2] and 242 g in the other [3], reflecting an age difference of approximately 75 days. Probably such uncontrolled variables would result in an envelope of values for each species comparable in breadth to that required to include both sets of rat data In the monkey study [4] total body water was measured and the FFBM estimated by assuming it contained 73.2% water.

Values for man were obtained by multiplying the reported mean daily loss (percent/day) in "lean body mass" of Skylab crews as tabulated in [7], by the mission duration in days. The human point plotted in Fig. 1 showing a loss of 3.9% is the crew mean for Skylab mission number 2. This mission with a bicycle ergometer available was judged most nearly comparable with the other plotted species where ad libitum activity was allowed within the living cages On later Skylab missions the crews had access to additional exercisers which simulated

load bearing in a gravitational field [7] As a result the mean crew loss in "lean body mass" for the whole mission was 1.4% in Skylab 3 and 1.1% in Skylab 4. In studies of Apollo missions the mean reduction in total body water was 2.4% [6]. Assuming the FFBM had 73% water [13] this corresponds to a loss of ∿ 3.3% in FFBM, as plotted in Fig. 1. Limb circumference measurements corrected for variations in thickness of subcutaneous fat were made on cosmonauts on the Salyut mission [5] and lend semi-quantitative corroboration to the reports on Apollo and Skylab.

Considering the general implications of Fig. 1, we might predict from extrapolations that at 0 G the FFBM will be 104% in the mouse, 104.5 and 106.5% in the rat, and 110 5% in the monkey. By contrast, a homology with the human data suggests that these species would all be below 100% when weightless. But it appears reasonable to assume that if FFBM were measured in each species from 0 to 4 7 G, it would pass through a maximum at 1 G. However, until data on weightless laboratory mammals or data on men at hyper-gravity are in hand, we cannot confidently choose between extrapolated values and homologous values and we must consider four alternative explanations·

1. The steady-state values of mouse, rat and monkey at 0 G will be species-related and may fall either above or below 100% of control.
2. The steady-state values at 0 G will be body size-related and may fall either above or below 100%.

(8)

3. Adaptation to weightlessness was incomplete in the men. After completion, FFBM in men would be in excess of 100%.

4. Factors primarily responsible are as yet unidentified.

For the present, we will accept the hypothesis of a continuous function passing through a maximum at 1 G with secondary displacements due to combinations of the factors body size, age, species and perhaps others.

It is probably helpful to consider involvement of regulatory mechanisms in the functions described by Fig. 1. The relative constancy of the FFBM at terrestrial gravity in spite of numerous perturbing influences (see above) probably reflects physiological regulation of at least its major components. However, the same may not be true of the prompt and predictable changes in FFBM evoked by Δ G [3]. If we accept the propositions that regulatory mechanisms are generally the result of evolutionary processes and that terrestrial gravity has not shown a physiologically significant change during the evolution of terrestrial life, then living organisms could not possess regulatory defenses against chronic changes in acceleration *per se*. However, Δ G does change weight-load and mammals possess physiological mechanisms (e.g., hypertrophy - atrophy of muscle and bone) for responding to changes in load encountered at terrestrial gravity. Thus, the general pattern of changes in FFBM of men on orbital missions with and without exercisers which stimulate weight-bearing [7] was in accord with *a priori*

expectations that weightlessness would cause atrophy of skeletal muscle which might be prevented by an appropriate exercise regimen. Atrophy in skeletal muscle, the largest component of the FFBM, could alone account for the reduction in FFBM reported for the weightless men. The reductions in FFBM seen with accelerations greater than 1 G are more difficult to explain. Chronic acceleration must exert effects beyond the increased weight-load which we expect to evoke hypertrophy and an increase in FFBM. We can suggest no explanations but reaffirm our conviction that the observed response is physiologic rather than pathologic for two reasons. First, rats exposed to hypergravity approach their new lower level of FFBM from either above or below [3] which suggests a steady state rather than a progressive decline. Secondly, weanling rats at 4.15 G show normal growth curves running approximately parallel to those for controls at 1 G during exposures as long as one year [unpublished].

If the major components of the FFBM are regulated, the pertinent masses must be monitored as a function of time. It is difficult to conceive of "ponderoceptors," i.e., receptors capable of monitoring FFBM as weight but their existence must be considered. Since alteration of chronic acceleration can change weight independently of mass, it constitutes a possible test for the existence of ponderoceptors. The appropriate response of a regulatory system based upon ponderostasis would be a decrease in FFBM during

centrifugation and an increase during weightlessness Fig. 1 shows that the former occurs but the latter does not Thus ponderostatic regulation is not supported by these results.

The reduction of ∿ 4% in the FFBM of weightless men appears to be of doubtful practical importance. But since it can possibly be explained primarily as a result of skeletal muscle atrophy and since skeletal muscle constitutes approximately one-half the FFBM, then the reduction in that component could be ∿ 8%. If the mass reduction should be greatest in the antigravity muscles, the percentage reduction would be even greater.

ACKNOWLEDGMENTS

This study was supported by
NASA research grant number NGR 47-005-213.

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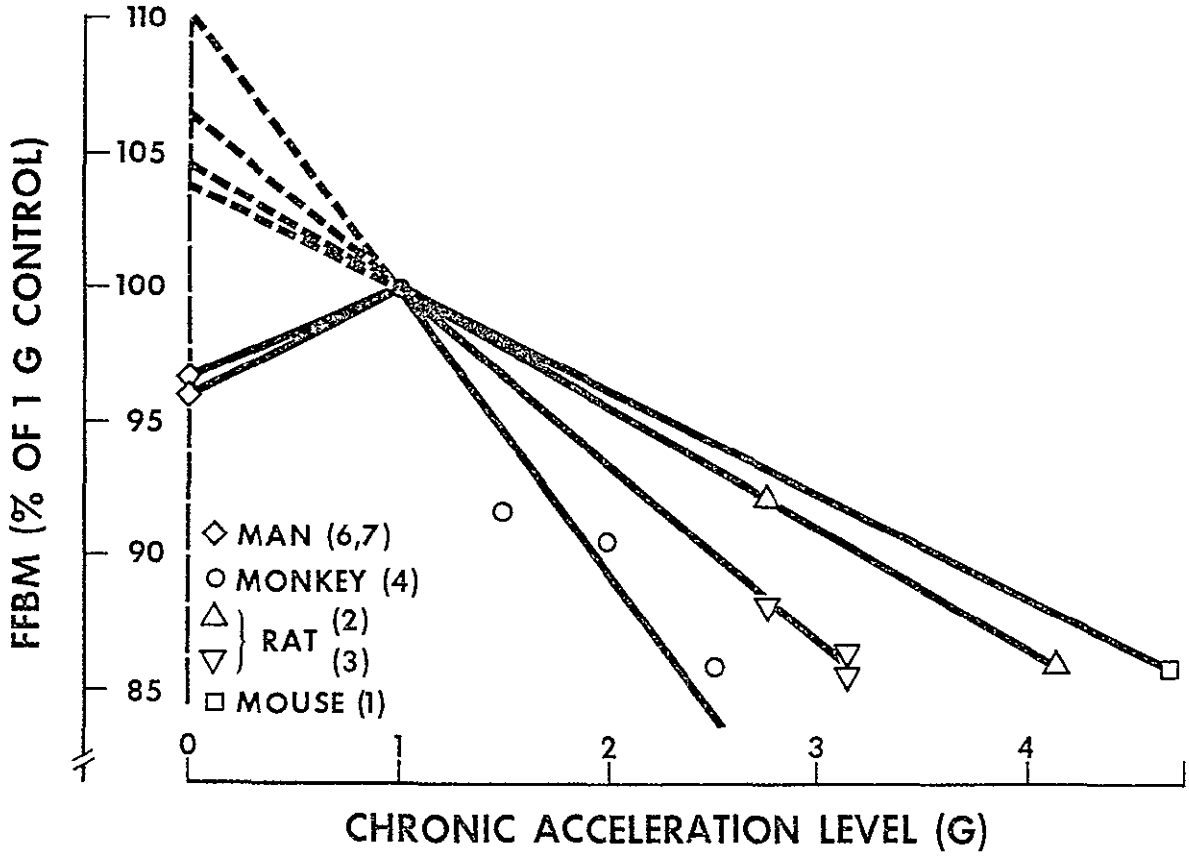

Fig. 1. Effect of chronic acceleration on fat-free body mass (FFBM) in four species. Sources of the data indicated by references

Table 1. Effect of chronic acceleration on body composition (group means ±SEM).

Species, Reference	Acceleration-G, Exposure Period		
White mouse [1]	1.0, 1 mo.		4.7, 1 mo.
N per group, Diet	9, chow		9, chow
fat (g)	3.08±.55[b] (53)[e]		0.86±.17[b] (59)
FFBM[a] (g)	33.14±.93[c] (8)		28.41±.77[c] (8)
fat/FFBM (%)	9.28±1.67[c] (53)		3.01±.59[c] (58)
H_2O/FFBM (%)	70.53±.34 (1)		70.44±.24 (1)
White rat [2]	1.00, 2 mo.	2.76, 2 mo.	4.15, 2 mo.
N per group, Diet	7, chow	8, chow	8, chow
fat (g)	45±4[d] (23)	25±2[d] (22)	15±2[d] (37)
FFBM (g)	300±8[d] (7)	277±8[d] (8)	258±4[d] (4)
fat/FFBM (%)	15.5±1.5[c] (25)	9.0±.7[c] (22)	5.8±.7[c] (34)
H_2O/FFBM (%)	73.4±.3 (1)	74.2±.3 (1)	73.8±.2 (1)
White rat [3]	1.00, 28 da.	3.18, 28 da.	3.18, 28 da.
N per group, Diet	6, chow	6, chow	5, high fat
fat (g)	46±6[d] (32)	25±2[d] (19)	51±7 (31)
FFBM (g)	263±8[d] (7)	228±5[d] (5)	225±17[d] (17)
fat/FFBM (%)	18±3 (41)	12±1[c] (20)	22±2[c] (20)
H_2O/FFBM (%)	72.8±.5 (2)	73.9±.3 (1)	73.2±.3 (1)
White rat [3]	1.00, 24 da.	2.76, 24 da.	
N per group, Diet	8, high prot.	7, high prot.	
fat (g)	48±4[d] (24)	33±5[d] (40)	
FFBM (g)	233±5[d] (6)	203±5[d] (6)	
fat/FFBM (%)	21±2 (27)	17±3 (46)	
H_2O/FFBM (%)	71.4±.4 (2)	71.4±.7 (2)	

[a] FFBM = fat-free body mass. [b] P<.02 by t test. [c] P<.01 by t test

[d] P<.01 by analysis of variance. [e] The values in parentheses are coefficients of variation (SD as % of mean).

B. Regulation of Body Mass in Rats Exposed to Chronic Acceleration.

This paper published in 1975 made the following general contributions which are useful in the context of NASA's program. Body fatness showed the same lability during centrifugation that characterizes its response to a variety of other variables. However, the fat-free body mass (FFBM) yielded several lines of evidence suggesting that it is regulated physiologically Of the many factors studied, ΔG is the only one capable of altering FFBM in the adult. Probably this reflects the fact that ΔG is the only one of these factors which living organisms have never had an opportunity to adapt to during the whole course of evolution.

AMERICAN JOURNAL OF PHYSIOLOGY
Vol 228 No 3, March 1975 *Printed in U S A*

Regulation of body mass in rats exposed to chronic acceleration

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PITTS, G C, L S BULL, AND J OYAMA *Regulation of body mass in rats exposed to chronic acceleration* Am J Physiol 228(3) 714–717 1975 —Female rats approximately 6 mo old were chronically centrifuged for up to 30 days at 2 76 *G* or 3 18 *G* and sacrificed at intervals for body-composition study Both fat and the fat-free body mass (FFBM) were reduced during the 1st wk of centrifugation, with the fat showing considerably more variation both within and between groups The FFBM was reduced below control level to the same extent in rats fed commercial chow, a high-fat diet, or a high-protein diet or in rats prefasted to produce a body-mass deficit at the start of centrifugation There were no centrifugation-associated changes in body water content It was concluded that body fat showed no evidence of regulation, FFBM is regulated at any constant level of acceleration between 1 and 4 15 *G*, and the change in FFBM induced by a change in acceleration is probably not regulated

gravity, centrifugation, body composition, regulation, body fat, fat-free compartment

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THE AMOUNT OF BODY FAT IS relatively labile in studies carried out at earth gravity For example, in adult rats it changed in response to treadmill exercise (7), combinations of exercise and/or force-feeding (9), surgical stress (10), and changes in the fraction of darkness in the 24-h cycle (10) However, none of these conditions altered the fat-free body mass (FFBM) Thus, the finding that hypergravity conditions simulated by chronic centrifugation altered FFBM and that the observed changes bore a simple mathematical relationship to *G* force (8) is notable by contrast and assumes considerable importance with respect to the possible regulation of body mass

Because previous studies of the body-composition effects of chronic centrifugation in rats (8) and mice (2) have been based on analyses of the carcasses of animals sacrificed terminally, one cannot determine whether the reported changes represent steady-state conditions or merely one time point in transient effects The present study, incorporating body-composition analyses performed at intervals during the centrifugation regimen, was designed to establish the time course of changes in body fat and FFBM We have also investigated the effects on body composition of dietary alterations in energy and protein balances both at 1 *G* and during centrifugation since resistance of body composition parameters to such changes in balance would be presumptive evidence of physiological regulation

METHODS

Female Sprague-Dawley-derived rats (Simonsen Laboratories, Inc, Gilroy, Calif) approximately 6 mo of age were received on *day −10* (centrifugation initiated on *day 0*) On *day −3* they were segregated into three mass-matched groups one eating a chow diet ad libitum, one a high-fat diet ad libitum, and one fasting All groups had water available ad libitum at all times On *day 0* seven randomly chosen rats of each group were sacrificed for body-composition studies The remainder were randomly segregated into four groups—*group I* 1 00 *G*, chow ad libitum, $n = 20$, *group II* 3 18 *G*, chow ad libitum, $n = 20$, *group III* 3 18 *G*, high-fat diet ad libitum, $n = 17$, *group IV* 3 18 *G*, having a mass deficit due to previous fasting but returned to chow ad libitum on *day 0*, $n = 20$ The mean body mass per group may be seen in Fig 1 Body-composition data during the centrifugation regimen were obtained on randomly designated rats sacrificed at *days +7*, *+24*, and *+30*

Two additional experiments were carried out later In one, female Sprague-Dawley-derived rats from the same source were segregated into two mass-matched groups—*group I-HP* 1 00 *G*, high-protein diet, $n = 24$, *group II-HP* 2 76 *G*, high-protein diet, $n = 16$ Animals were sacrificed on *days 0*, *+7*, and *+24*

In the last experiment Sprague-Dawley-derived female rats (Flow Laboratories, Dublin, Va) ~300 g in body mass were segregated into two mass-matched groups *Group I-PF* 1 00 *G*, chow diet, $n = 14$, on each experimental day each rat was limited to the mean weight of food consumed by *group I* on that day in the earlier study *Group II-PF* 1 00 *G*, chow diet, $n = 17$, on each experimental day each rat was limited to the mean weight of food consumed by *group II* on that day in the earlier study The animals ate all the food provided on each day This feeding arrangement is referred to as pair-feeding although groups were paired and not individuals On *days 0*, *+7*, and *+24* rats were sacrificed for body-composition studies

Centrifugation was continuous, 7 days/wk for 24 or 30 days except for one service stop per day of 35 9 ± 5 7 (SE) min The animal compartments holding 16 rats, 2 to a cage, were suspended in pivotal-yoke assemblies so that the resultant of gravitational and centrifugal forces was perpendicular to the cage floor The cage floor was approximately 8 3 feet from the axis of rotation Each compartment was

illuminated with fluorescent lights providing 12 h of light and 12 h of darkness per day

The ground chow diet (Simonsen maintenance diet) contained 4 2 kcal/g of gross energy (determined by oxygen bomb calorimetry) It was composed primarily of natural products supplemented with vitamins and minerals and contained 24% crude protein and 6% fat by weight

The high-fat diet was that of Schemmel et al (12) containing 7 3 kcal/g gross energy It was composed primarily of purified components including 60% fat and approximately 23% protein by mass Rats on this high-fat diet ad libitum at earth gravity ingest more calories per day and absorb a higher fraction of digestive tract contents than do those on the chow (1) In the present case *group III* was absorbing ~50% more dietary energy per day than *group I* prior to the start of centrifugation It was hoped that this higher level of absorbed energy in *group III* rats might partly compensate for the transient reduction in food intake commonly reported in rats for the first few days of centrifugation (3)

The high-protein diet had the following percentage composition by mass protein (hydrolyzed casein) 71, hydrogenated fat (Crisco) 15, corn oil 3, vitamin diet fortification mixture (Nutritional Biochemicals Corp, Cleveland) 3, Hegsted salt mix 5, and residue 3 Its gross energy content was 5 8 kcal/g During a 20-day metabolic trial eight weanling female rats on this diet grew at a rate very similar to that of eight rats on Purina laboratory chow It was hoped that this diet would raise daily protein intake toward maintenance values during the first few days of centrifugation when feeding activity is reduced, thus removing protein as a nutritional limitation

The rats were killed by decapitation, bled out, sheared as closely as possible with animal clippers and the content of the gastrointestinal tract was removed and weighed Body mass after sacrifice, plus mass of shed blood, minus mass of fur and gut content, minus the mass of extracted lipid yielded FFBM The carcass was ground and duplicate aliquots were taken for determination of water content by freeze-drying and fat content by Soxhlet extraction with petroleum ether (BP 30–60°C)

For the most part, analyses of variance for factorial designs (15) were utilized in statistical analyses of body-composition data with two groups times two, three, or four periods of centrifugation

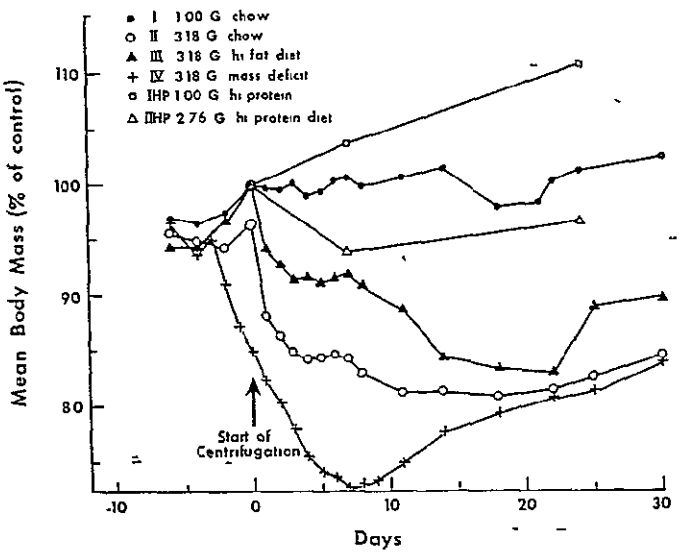

FIG 1 Effects of centrifugation on live body mass *Groups I, II,* and *IV* on chow diet are expressed as a percentage of *I* on *day 0 Group III* on high-fat diet is expressed as a percentage of *III* on *day 0 Groups I-HP* and *II-HP* on high-protein diet are expressed as a percentage of *I-HP* on *day 0*

TABLE 1 *Body fat by group and days of exposure to chronic centrifugation*

Group	Body Fat, g				
	Period of exposure, days				
	0	7	24	30	60*
I 1 00 *G* chow	64 ± 9 (7)	54 ± 6 (6)	46 ± 6 (6)	56 ± 4 (8)	45 ± 4 (8)
II 3 18 *G*, chow	64 ± 9 (7)	48 ± 4 (6)	26 ± 2 (6)	21 ± 2 (8)	23
III 3 18 *G* high fat diet	74 ± 5 (7)	76 ± 4 (6)	51 ± 7 (5)	48 ± 7 (6)	
IV 3 18 *G*, chow mass deficit	61 ± 6 (7)	35 ± 6 (6)	14 ± 2 (6)	18 ± 2 (8)	
I-HP 1 00 *G*, high-protein diet	53 ± 3 (8)	53 ± 4 (8)	48 ± 4 (8)		
II-HP 2 76 *G*, high-protein diet	53 ± 3 (8)	42 ± 3 (8)	33 ± 5 (7)		
I-PF 1 00 *G*, pair-fed to *I*	33 ± 2 (6)		38 ± 3 (8)		
II-PF 1 0 *G* pair-fed to *II*	33 ± 2 (6)	24 ± 2 (8)	12 ± 2 (9)		

Values are means ± SE numbers in parentheses are numbers in group
* Sixty day values are from a previous study (8) in which 3 levels of *G* were used Our value for 3 18 was calculated from the equation $y = 53\ 3 - 9\ 5x$

RESULTS

Mean live weights by groups before and during centrifugation are presented in Fig 1 All centrifuged groups showed the decrease in body mass during the 1st wk of exposure, which has been previously reported (2, 3, 8, 16) The relative order of magnitude of these initial weight losses was *IV* > *II* > *III* > *II-HP* Although body weights of the pair-fed groups (*I-PF* and *II-PF*) are not represented in Fig 1, it should be noted that they followed closely the weight curves of the groups to which they were pair-fed On *day +24 group II-PF* was 67 g below *I-PF* and *group II* was 63 g below *I*

Table 1 presents data on the quantity of body fat by groups and by sampling days, Table 2 summarizes the results of analyses of variance on both fat and the FFBM The following responses of body fat should be noted Comparing *groups I* and *II*, differing only in level of acceleration, the difference in fat level was significant ($P < 0\ 01$ for groups), there was a progressive fall in fat content during centrifugation ($P < 0\ 01$ for days), and the progressive change in body fat during the period of observation was different in the two groups ($P < 0\ 01$ for interaction) Comparing *groups III* and *I* to test whether the high-fat diet ameliorated the effects of centrifugation, the level of body fat between groups did not differ significantly However, fat decreased progressively during centrifugation in both groups with the

TABLE 2 *Results of analyses of variance on body-composition effects of centrifugation*

Statistic Evaluated	Groups Compared	Main Effects: Groups	Main Effects: Days*	Interaction
Body fat, g	*I* and *I-HP*	†		
	I and *II*	0 01	0 01	0 01
	I and *III*		0 05	0 05
	I and *IV*	0 01	0 05	
	I-HP and *II-HP*	0 01		
Fat-free body mass, g	*I* and *I-HP*	0 01	0 01	
	I and *II*	0 01	0 05	
	I and *III*	0 01		
	I and *IV*	0 01	0 01	0 05
	II and *III*		0 05	
	II and *IV*		0 01	0 05
	III and *IV*		0 01	
	I-HP and *II-HP*	0 01	0 05	

* Calculations of days effect were based on all days in which data were collected (see Table 1) except *day 0*, which represents precentrifugation condition † Blank spaces indicate no statistical significance ($P > 0\ 05$) In all other spaces, probability of null hypothesis is less than value indicated

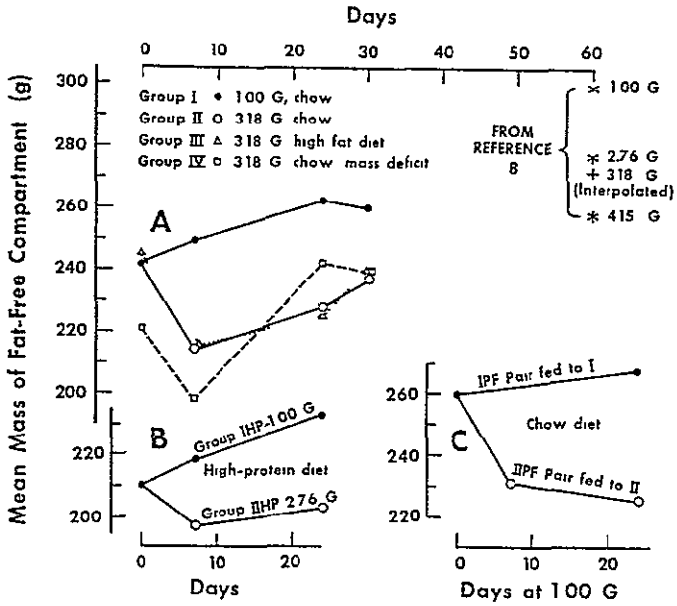

FIG 2 Effects of centrifugation on fat-free body mass

steeper, continued drop in *III* being significantly different from that in *I* ($P < 0\ 05$ for interaction) *Group IV*, starting centrifugation with a weight deficit (Fig 1), surprisingly showed no fat deficit, its body fat content being comparable with that of *group I* on *day 0* However, its body fat level was significantly lower than that of *group I* after centrifugation began During the first 7 days of centrifugation it dropped lower than in any other group The level of body fat in *II-HP* was lower than in *I-HP* However, the effect of days did not attain statistical significance

The values representing 60 days of exposure were not included in the statistical analysis (Table 2) because they were collected in an earlier study (8) and interpolation was required to obtain the value at 3 18 *G*

The effects of the experimental variables on the FFBM are presented in Table 2 and Fig 2 and can be summarized briefly In all possible comparisons centrifuged groups had lower FFBMs than noncentrifuged groups In nearly all groups the effect of days was significant, indicating the presence of continued growth There were two significant interactions involving *group IV* that merely verify statistically that the time course of change in FFBM in *group IV* is different from that of any other group (Fig 2) In Fig 2*A* on *day 30* all groups appear to be converging That this is a statistical accident is indicated by the 60-day values showing that the centrifugation-induced reduction of FFBM persists for at least 2 mo

Table 3, presenting mean values for energy and protein intake, established our success in altering balance of those factors Energy absorption in centrifuged groups ranged from 6 5 to 75 8 kcal/day per two rats, this latter value being 60 and 78 % of values for the noncentrifuged groups (*I-HP* and *I*, respectively) With respect to dietary protein the ingestion in centrifuged groups ranged from 0 48 to 9 96 g/day per two rats, the latter value (*group II-HP*) being roughly comparable with the noncentrifuged groups

Because body water expressed as a percentage of FFBM shows small interindividual variability (4) and is quite independent of various changes in regimen (5, 7–10), we have employed it as an index of changes in gross state of hydration The only statistically significant change in this value was that demonstrated by comparing either group on high-protein diet with any other group, *I-HP* and *II-HP* being lower in each case The mean ± SE for the combined water data from *groups I, II, III,* and *IV* ($n = 98$) was 73 4 ± 0 8 %, for *I-HP* and *II-HP* ($n = 39$) it was 71 4 ± 2 % By the Student *t* test the difference between these values is statistically significant ($P < 0\ 001$)

DISCUSSION

Chronic centrifugation has proved to be a valuable tool in the study of regulation of body mass Centrifugation of approximately half-grown chickens until they were 75+ % grown (300+ days of exposure) revealed that mature body size bore an inverse rectilinear relationship to accleration field strength in the range of 1–2 *G* and that the smaller body size during continued centrifugation was "closely

TABLE 3 *Daily energy and protein intakes for 2 rats in a single cage during first 7 days of centrifugation*

	Groups*					
	I	*II*	*III*	*IV*	*I-HP*	*II-HP*
Numbers of values	64	67	64	66	56	49
Energy absorbed, kcal/day†	96 7 ±2 2	20 4 ±1 3	32 2 ±2 1	6 5 ±1 0	127 6 ±2 2	75 8 ±10 9
Protein ingested, g/day†	7 18 ±0 17	1 51 ±0 10	1 22 ±0 08	0 48 ± 07	16 6 ± 3	9 96 ±1 42

Values are means ± SE As tested by Student *t* with $P < 0\ 01$ as criterion for rejecting the null hypothesis, each value for energy absorption is different from every other With respect to protein ingestion, while all other centrifuged groups are significantly below controls at earth gravity, *II-HP* is not significantly different from *I* However, *II-HP* is significantly below its simultaneous control, *I-HP* * See Table 1 for regimen of each group † Energy values represent number of kilocalories absorbed from gut lumen Since fraction absorbed had not been determined for protein, protein values represent grams ingested

regulated" (13) Smith and Burton (13) concluded from calculations of centrifugation results reported for mice and rats that a similar relationship obtained in mammals Acting on our conviction that one must separately evaluate fat and the fat-free compartment in studying body-mass regulation (6) and expanding the acceleration range studied (1–4 15 *G*), we found that, as has been reported in mice (2), both fat and the FFBM participated in the reduction of mass after 60 days of centrifugation (8) In both cases the relationship of mass to acceleration was inverse and in the case of FFBM it was clearly rectilinear (8)

The present study provides no evidence that quantity of body fat is regulated either at earth gravity or in the centrifuged animal The mean value for each group appears to be determined by the details of the existing regimen (diet, *G* force, duration of exposure) and within each group the interindividual variability is high, the coefficient of variation (SD × 100/mean) for each group ranging from 14 to 35 %

By contrast with the results on body fat, we have obtained the following four lines of evidence suggesting that FFBM is physiologically regulated in rats subsisting continuously at any one level of acceleration

a) Low interindividual variation within groups The coefficients of variation were 4–12 % in Table 1 and 4–8 % in the earlier study (8)

b) The failure of increased intake of energy or protein (Table 3) to raise the steady-state level of FFBM during centrifugation

c) The ability of centrifuged rats to approach the new lower level of FFBM from below as well as from above (Fig 2*A*)

d) The maintenance of late growth paralleling that of a control group at earth gravity for 30 (Fig 2) or 60 days (8)

However, the adjustment to a new level of FFBM associated with a change in level of chronic acceleration does not suggest regulation Indeed, the rectilinear relationship between FFBM and *G* force (8) strongly resembles Prosser's idealized diagram for an unregulated parameter (11), and it suggests that there may exist a continuous spectrum of FFBM's depending solely on acceleration Also, once FFBM is perturbed to a new value by a change in acceleration there is no evidence that it moves back toward the previous value (Fig 2) unless acceleration level is returned toward the previous value

Our data permit us to infer some of the properties of the centrifugation-induced change in FFBM For example, it appears to be quite independent of changes in quantity of body fat In Table 1 we see that starting with the 7th day of centrifugation every centrifuged group showed a sustained downward trend in body fat while FFBM's were showing a sustained upward trend During the 1st wk of centrifugation (*days 0* and *7*) *group III* showed no change in body fat (t = 0 30, $P > 0\ 05$) but a decrease in FFBM (t = 4 40, $P < 0\ 01$) During this same period all other centrifuged groups showed decreases in both fat and FFBM

Some of our data suggest the mechanisms that the body employs to reduce FFBM when chronic acceleration is increased The pair-feeding experiment (Fig 2*C*) showed that a group at earth gravity (*II-PF*) that was pair-fed to a centrifuged group (*II*) underwent a comparable reduction in FFBM This corroborated a similar finding on mice (16) and demonstrated that of the two effector mechanisms, change in rate of energy intake and change in rate of energy expenditure, the former played the primary role in the change in FFBM that *group II* underwent during the 1st wk of centrifugation Finally, the data on *group II-HP* in Fig 2*B* and Table 3 suggest that, even with ample energy available as fat and with excess protein being ingested, the animals nevertheless made selective use of metabolic pathways so that body protein content (FFBM) was reduced

We acknowledge the technical assistance of Mr John H Key, Jr and Mr George T Tillman, Jr

This study was supported in part by National Aeronautics and Space Administration Research Contract NAS2-1554 and Research Grant NGR47-005-213

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Received for publication 17 June 1974

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C. Growth during Centrifugation.

This is an unpublished study on which the body composition analyses have only recently been completed. As a result of a centrifuge failure before centrifugation of the animals was completed, we were unable to employ the analytical methods which were used in the growth experiments listed under III.A. Consequently, the information yield was seriously reduced.

The Materials and Methods are presented in the protocol which follows.

RAT GROWTH DURING CHRONIC CENTRIFUGATION

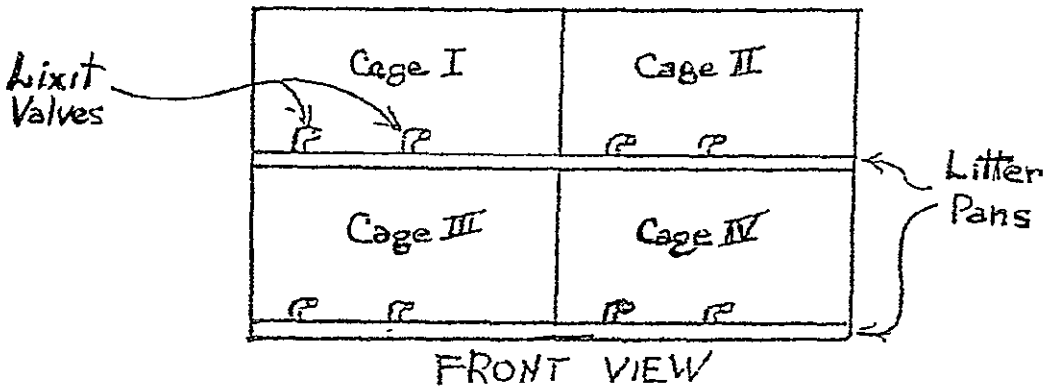
A. Objectives

1. Using weanling female rats, to describe growth to maturity at 4.15G (compared with controls at 1G) in terms of 10 individual body components and 4 chemical components.

2. To determine whether the effects of centrifugation are reversible after retirement from the centrifuge.

B. Equipment

Two cage assemblies modified as diagrammed below:



One such assembly to go on the centrifuge at 4.15G. This houses the "experimental" group and will be known as the E assembly. One to stay in the room above the centrifuge at 1G. This houses the "control" group and will be known as the C assembly. As can be seen in the diagram above, the individual cages are designated E-I, E-II, E-III and E-IV or C-I, C-II, etc.

C. Animals

Weanling female Simonsen rats to be used. ~115 to be ordered. The animals will be placed 14 per cage in the cage assemblies for the Experimental Group (E) and 12 per cage in the cage assembly for the Control Group (C).

D. Schedule of centrifuge stops for weighing and servicing.

Time from start of centrifugation	Service required
-1 day	Weigh all rats. Eliminate unacceptables. Segregate into groups E & C.
0 day (Sept. 14)	Weigh all rats. Check Lixit valves and food. Sacrifice 8 pups for body composition study.
0 thru 30	Weigh all rats once a week, half on Monday or Tuesday and half on Thursday or Friday. Check all Lixits and food containers at each stop.
28 (Oct. 12)	Sacrifice 2 rats from each cage (I, II, III, and IV). N will equal 8E and 8C.
28 thru 63	Weigh all rats once a week, half on Monday or Tuesday and half on Thursday or Friday. Check all Lixits and food containers at each stop.
63 (Nov. 16)	Sacrifice 6 rats from the E group and 6 from the C group. Also retire 8 rats from the E group to 1G in the room above the centrifuge. This will be designated the R group.
63 thru 105	Weigh all rats (E, C and R) once a week, group E on Tuesdays and group C on Fridays or vice versa. Check all Lixits and food containers at each stop.
105 (Dec. 28)	Sacrifice 6 rats from the E group and 6 from the C group.
105 thru 180	Weigh all rats (E,C and R) once a week. After Dec. 28 Lonnie should find it necessary to make only one visit a week. On the day that Lonnie is not present for the centrifuge stop someone else should make sure to check Lixits and food supply.

(cont'd)

180 (Mar. 12, '74)	Sacrifice 8 rats from each group (E,C and R).
180 thru 365	Weigh all rats once a week. Check Lixits and food at each stop.
365 (Sept 14, '74)	Sacrifice 8 rats from each group (E and C). All surviving rats to be weighed and retired from the centrifuge. (Consult Dr. Pitts for continuation of schedule.)

E. Schedule for sacrifices.

Date	Duration of Centrifugation (weeks)	Rat Age (weeks)	Sacrifices (number & group)
9/14/73	0	4	8C
10/12/73	4	8	8C & 8E
11/16/73	9	17	6C & 6E, also 8E retired (R)
12/28/73	15	23	6C & 6E
3/12/74	26	30	8C, 8E & 8R
9/14/74	52	56	8C & 8E

Remaining rats retired to 1G.

3/14/75 Remainder in C & E.

F. IN CASE OF DIFFICULTIES:

Consult Dr. Oyama
Call Collect - Dr. Grover C. Pitts
Dept. of Physiology
School of Medicine
Univ. of Virginia
Charlottesville, Va. 22901
Area Code - 804-924-2585

Growth in live mass by groups is presented in Fig. 1. An examination of the growth curves for the two major groups suggests that they had reached a steady state with little to be learned by continuing the study. However, our body composition studies (Figs. 2,3 and 4) corroborate reports in the literature that the major body compartments do not reach plateaus until well after one year of age. This largely explains our feeling that the centrifuge failure which occurred at 305 days was an unfortunate setback to the study. The group that was retired on the 63rd day of centrifugation from 4.15 G to 1 G rejoined the 1 G growth curve by the 150th day. However, we cannot determine whether the group retired on the 290th day would have rejoined the 1 G group.

The detailed body composition results are presented in Table 1. At the head of the columns in the Table the roman numerals represent sacrifice groups as follows:

Number	Duration of exposure (days)
I	0
II	28
III	63
IV	105
V	179
VI	305 (centrif. failure)

Statistical analyses have not yet been carried our on these data. However, the tabulated results are more easily interpreted when plotted (Figs. 2,3,and 4). Fig 2 corroborates our earlier finding that the fat-free compartment is reduced in size by centrifugation. That reduction is contributed to by water, muscle (Fig. 2), bone, liver (Fig. 3), kidneys and heart (Fig. 4). Skin (Fig. 2), the pulmonary system (Fig. 3), gut and CNS (Fig. 4) appear unaffected by centrifugation.

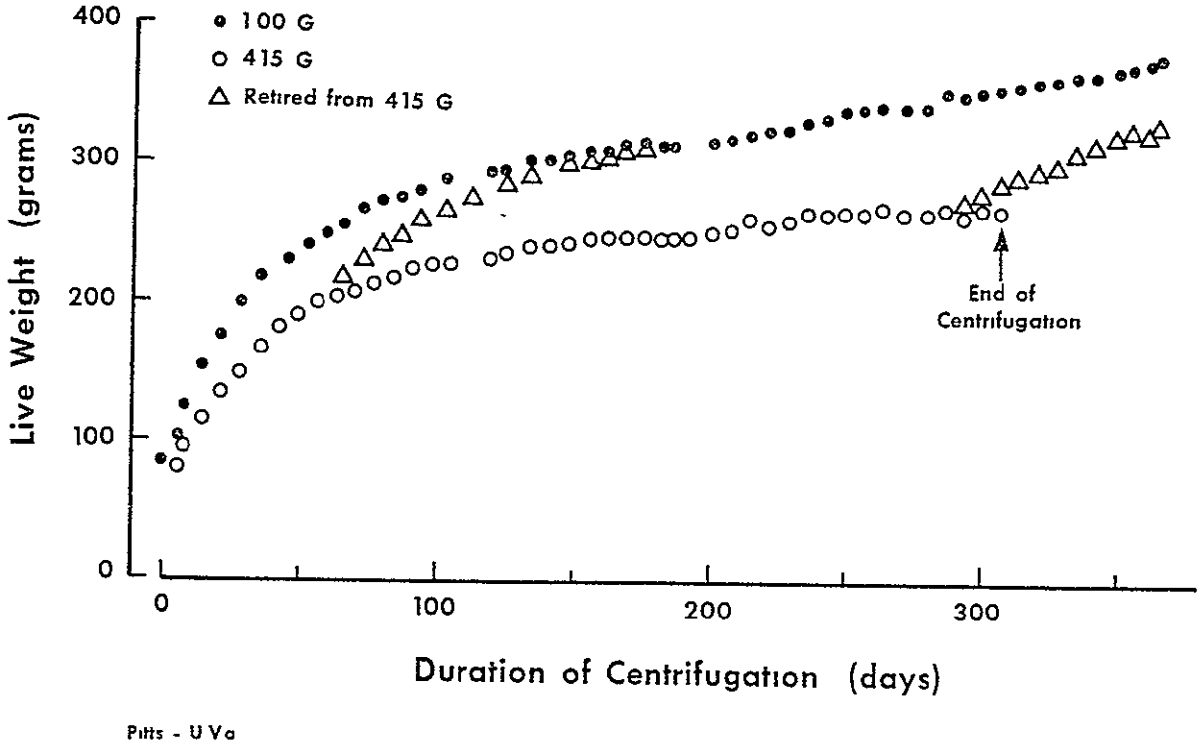


Fig. 1. Growth in live mass by groups.

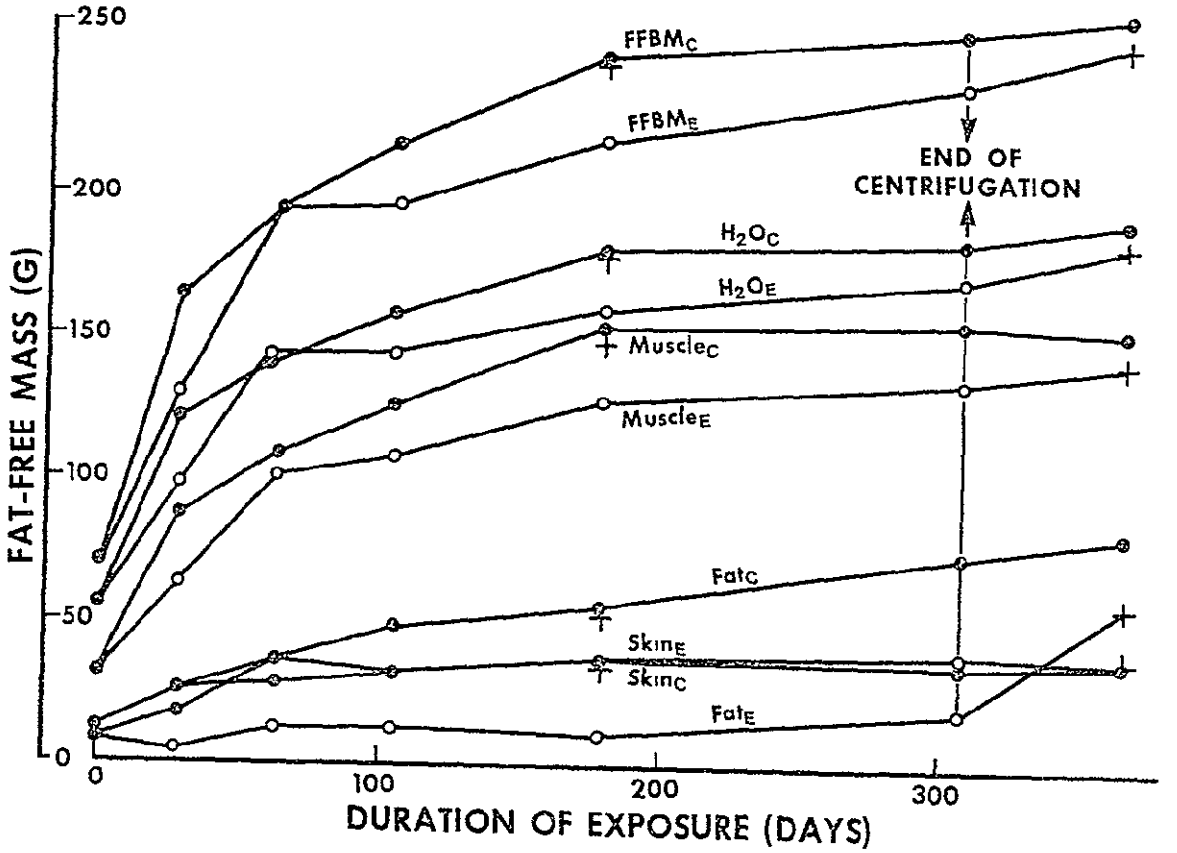


Fig. 2. Mean fat-free mass of individual components. Values on groups retired from 4.15 G to 1 G are indicated by +. Subscripts C = control group (1 G) and E = experimental group (4.15 G). FFBM = fat-free body mass.

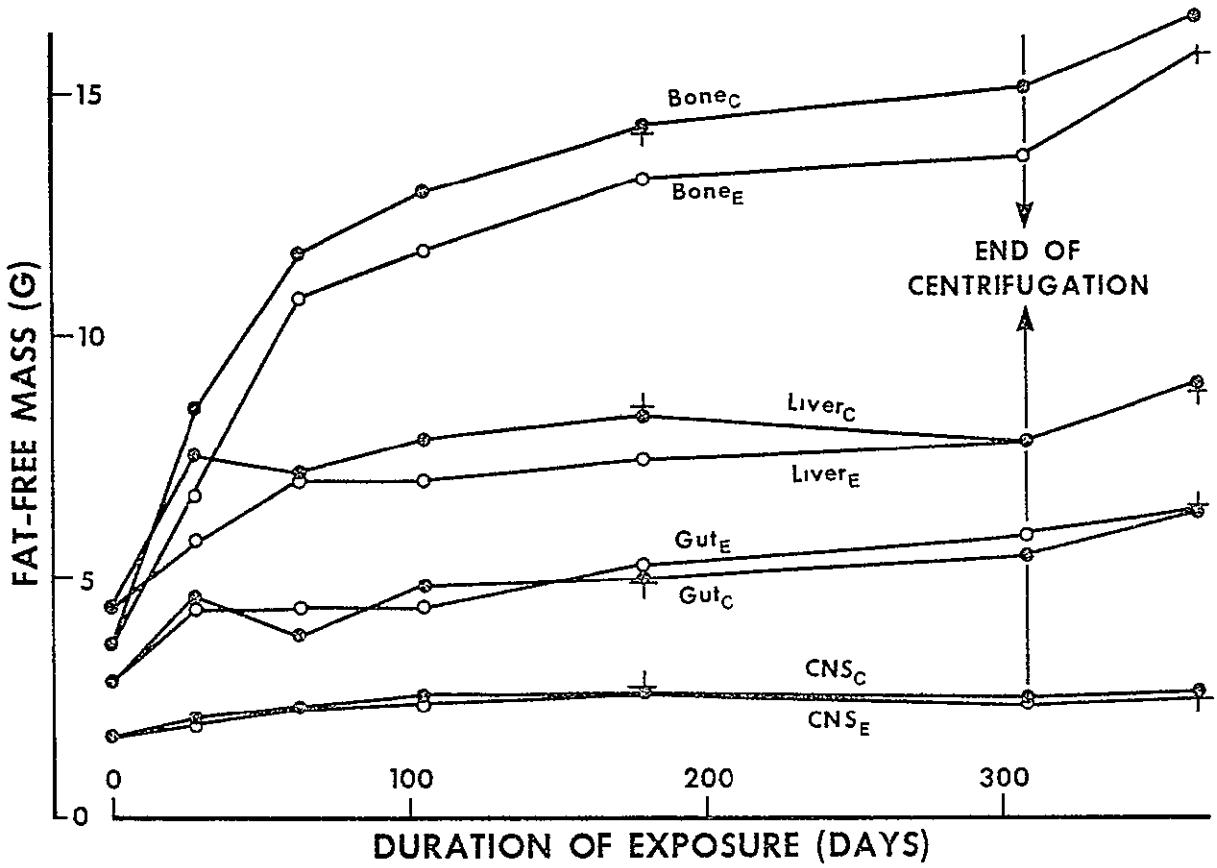


Fig. 3. Mean fat-free mass of individual components. Values on groups retired from 4.15 G to 1 G are indicated by +. Subscripts C = control group (1 G) and E = experimental group (4.15 G).

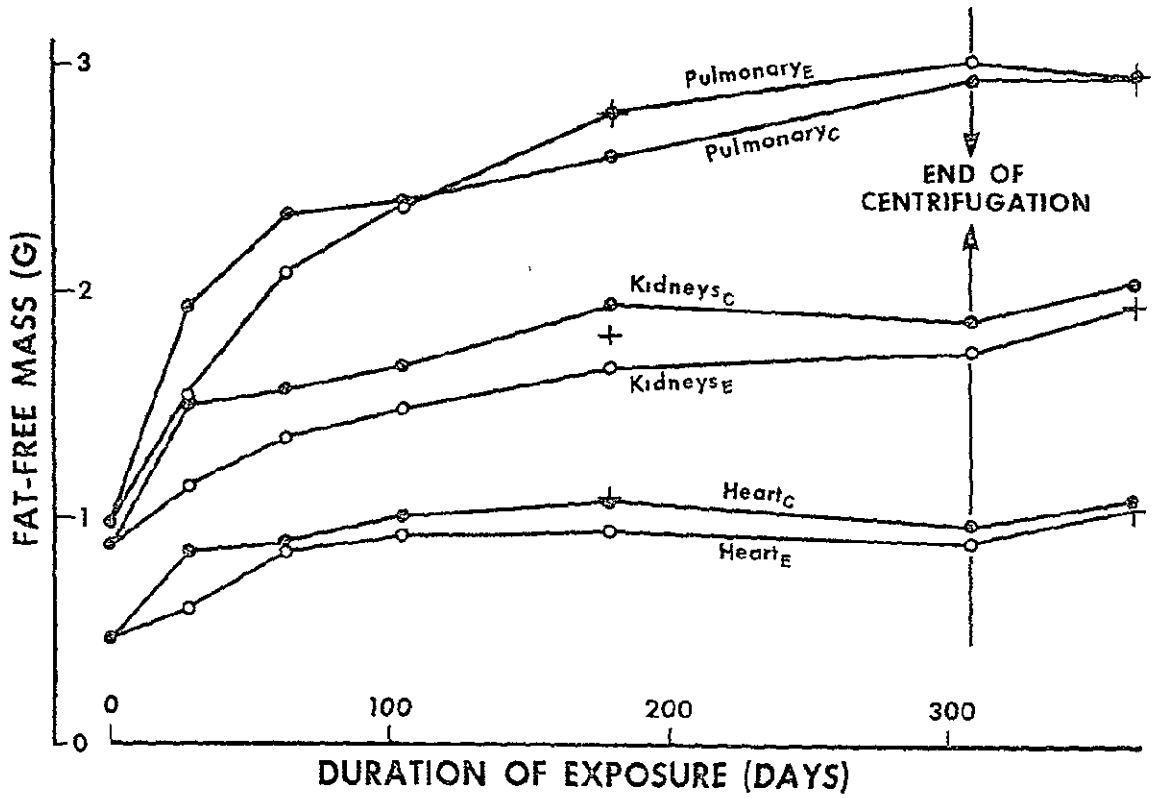


Fig. 4. Mean fat-free mass of indiviaual components. Values on groups retired from 4.15 G to 1 G are indicated by +. Subscripts C = control group (1 G) and E = experimental group (4.15 G).

Table 1. Rat growth during chronic centrifugation. Body composition (mean ± SEM) by sacrifice groups.

Component	I-Basal N=8	II-1G N=8	II-4.15G N=8
CLW (g)	78.9 ± 1	181.5 ± 6.1	134.5 ± 3.7
Total Body Fat (g)	8.41 ± 0.57	17.49 ± 1.14	4.6 ± 1.79
TBF/CLW (%)	10.6 ± 0.6	9.7 ± 0.6	3.4 ± 0.4
FFBW (g)	70.5 ± 0.6	163.9 ± 5.6	129.9 ± 3.2
Total H_2O (g)	54.9 ± 0.4	122 ± 4	98.4 ± 2.4
H_2O/FFBW (%)	77.6 ± 0.4	73.9 ± 0.5	75.5 ± 0 4
FF Muscle (g)	31.8 ± 0.4	87 ± 4	63.3 ± 1.9
FF Skin (g)	10.8 ± 0.31	24.42 ± 0.76	22.73 ± 0.63
FF Dry Bone (g)	3.59 ± 0.04	8.46 ± 0.27	6.66 ± 0.20
FF Liver (g)	4.25 ± 0.1	7.64 ± 0.39	5.69 ± 0.24
FF Gut (g)	2.84 ± 0.14	4.64 ± 0.16	4 37 ± 0.25
Fresh CNS (g)	1.69 ± 0.02	2 1 ± 0.03	1.93 ± 0.04
FF Heart (g)	0.46 ± 0.02	0.85 ± 0.04	0.59 ± 0.03
FF Kidneys (g)	0.89 ± 0 03	1.5 ± 0.05	1.13 ± 0.04
FF Lungs and Trachea (g)	0.98 ± 0.02	1.93 ± 0.1	1.53 ± 0.09
Fresh Adrenals (mg)	20 ± 1	46 ± 3	36 ± 2
FF Musc/FFBW (%)	45.2 ± 0.5	52 9 ± 1 1	48 5 ± 0.6
FF Skin/FFBW (%)	15.2 ± 0.4	15 ± 0.3	17.4 ± 0.3
FF Dry Bone/FFBW (%)	5.1 ± 0.1	5.2 ± 0.2	5.1 ± 0 1
FF Liver/FFBW (%)	6 ± 0.1	4 7 ± 0.2	4.4 ± 0.1
FF Gut/FFBW (%)	4 ± 0.2	2.8 ± 0.1	3.4 ± 0.2
FF Kidneys/FFBW (%)	1.25 ± 0.04	0.91 ± 0.02	0.86 ± 0.02
Fresh CNS/FFBW (%)	2.41 ± 0.03	1.29 ± 0.05	1.48 ± 0.04
FF Heart/FFBW (%)	0.65 ± 0.02	0.51 ± 0.02	0.45 ± 0.02
FF Lungs & Trach/FFBW (%)	1.37 ± 0.03	1.18 ± 0.05	1.17 ± 0.05

Table 1. continued

Component	III-1 G N=6	III-4.15 G N=6	IV-1 G N=6
CLW (g)	231.3 ± 8.2	209.8 ± 7.2	267 ± 4.5
Total Body Fat (g)	36.47 ± 2.27	13 22 ± 1.05	48.64 ± 6.36
TBF/CLW (%)	15.7 ± 0.6	6.3 ± 0.5	18.1 ± 2.1
FFBW (g)	194.9 ± 7.6	196.6 ± 7.2	218.3 ± 3.3
Total H_2O (g)	139.9 ± 4.1	145.6 ± 5.5	157 ± 2.6
H_2O/FFBW (%)	71.8 ± 0.4	74.1 ± 0 3	71.9 ± 0.3
FF Muscle (g)	108.9 ± 5.5	100.5 ± 4	126 6 ± 3.6
FF Skin (g)	27.12 ± 0.9	34.62 ± 1.58	30.94 ± 0.5
FF Dry Bone (g)	11.66 ± 0.48	10.79 ± 0 37	12.99 ± 0.22
FF Liver (g)	7.1 ± 0.74	7.11 ± 0.25	7 93 ± 0.2
FF Gut (g)	3.72 ± 0 36	4.32 ± 0.07	4.92 ± 0.17
Fresh CNS (g)	2.34 ± 0.03	2.21 ± 0.04	2 58 ± 0.04
FF Heart (g)	0.9 ± 0.03	0.85 ± 0 05	1 ± 0.06
FF Kidneys (g)	1.57 ± 0.11	1.36 ± 0.05	1.67 ± 0.04
FF Lungs & Trachea (g)	2.35 ± 0.11	2.08 ± 0.14	2.41 ± 0.15
Fresh Adrenals (mg)	55 ± 2	50 ± 4	53 ± 2
FF Musc/FFBW (%)	55.8 ± 1.4	51.1 ± 0.4	57.9 ± 0.9
FF Skin/FFBW (%)	13.9 ± 0.1	17 6 ± 0.4	14.2 ± 0 2
FF Dry Bone/FFBW (%)	6 ± 0.1	5.5 ± 0.1	6 ± 0.1
FF Liver/FFBW (%)	3.9 ± 0.1	3.6 ± 0.1	3.6 ± 0.1
FF Gut/FFBW (%)	2.1 ± 0.1	2.2 ± 0.1	2.3 ± 0.1
FF Kidneys/FFBW (%)	0.8 ± 0.04	0.69 ± 0.02	0.77 ± 0.02
Fresh CNS/FFBW (%)	1.2 ± 0	1.1 ± 0	1.2 ± 0
FF Heart/FFBW (%)	0.45 ± 0.01	0.41 ± 0.03	0.45 ± 0.03
FF Lungs & Trach/FFBW (%)	1.2 ± 0.05	1.05 ± 0.05	1.10 ± 0.08

Table 1 continued

Component	IV-4.15 G N=6	V-1 G N=8	V-4.15 G N=8
CLW (g)	208.8 ± 8.1	305.9 ± 9.5	209 ± 4.2
Total Body Fat (g)	12.78 ± 1.64	56.1 ± 7.18	11.62 ± 1.14
TBF/CLW (%)	6.1 ± 0.6	18 ± 1.8	5 ± 0.5
FFBW (g)	196.1 ± 7.2	249.8 ± 3.9	220 ± 3
Total H_2O (g)	142.9 ± 5	180.7 ± 3.1	159.5 ± 3.1
H_2O/FFBW (%)	72.9 ± 0.2	72.4 ± 0.2	72.5 ± 0.2
FF Muscle (g)	108.3 ± 3 1	154.4 ± 2.6	128 ± 2.7
FF Skin (g)	32.77 ± 1.14	36.6 ± 1.43	36.54 ± 1.09
FF Dry Bone (g)	11.82 ± 0.46	14.41 ± 0.3	13.33 ± 0.34
FF Liver (g)	7.01 ± 0.55	8.37 ± 0.51	7.43 ± 0.22
FF Gut (g)	4.37 ± 0.24	5.01 ± 0.27	5.26 ± 0.17
Fresh CNS (g)	2.34 ± 0.06	2.64 ± 0.03	2.55 ± 0.06
FF Heart (g)	0.92 ± 0.06	1.07 ± 0.04	0.94 ± 0.08
FF Kidneys (g)	1.48 ± 0.03	1.94 ± 0.07	1.66 ± 0.05
FF Lungs & Trachea (g)	2.37 ± 0.12	2.6 ± 0.1	2.8 ± 0.18
Fresh Adrenals (mg)	55 ± 2	56 ± 2	56 ± 3
FF Musc/FFBW (%)	55.2 ± 0.9	61.8 ± 0.6	58.2 ± 0.7
FF Skin/FFBW (%)	16 7 ± 0.4	14.7 ± 0.5	16.6 ± 0.4
FF Dry Bone/FFBW (%)	6 ± 0.1	5 ± 0.1	6.1 ± 0.1
FF Liver/FFBW (%)	3.6 ± 0.2	3.4 ± 0.2	3.4 ± 0.1
FF Gut/FFBW (g)	2.2 ± 0.1	2 ± 0	2.4 ± 0.1
FF Kidneys/FFBW (g)	0.75 + 0.02	0 ± 0.03	0 ± 0.02
Fresh CNS/FFBW (%)	2.0 ± 0	1.1 ± 0	1.1 ± 0
FF Heart/FFBW (%)	0.47 ± 0.02	0.43 ± 0.02	0.43 ± 0 02
FF Lungs & Trach/FFBW (%)	1 08 ± 0.12	1.04 ± 0.04	1.27 ± 0.09

Table 1. continued

Component	V-retired to 1 G N=8	VI-1 G N=10	VI-4.15 G N=9
CLW (g)	299.1 ± 6.8	331.3 ± 14.2	253.3 ± 6.9
Total Body Fat (g)	52.88 ± 4.07	74.99 ± 10.16	18 86 ± 1.75
TBF/CLW (%)	17.6 ± 1.1	22 ± 2.2	7.4 ± 0.6
FFBW (g)	246.2 ± 4.7	258.9 ± 6.2	240.5 ± 6
Total H_2O (g)	178.1 ± 3 4	184.8 ± 5	170.5 ± 4.3
H_2O/FFBW (%)	72.4 ± 0.3	72.1 ± 0.4	72.7 ± 0 2
FF Muscle (g)	147.8 ± 3.5	156 ± 4.5	135.4 ± 4.2
FF Skin (g)	35.84 ± 0.89	35.16 ± 0.67	38.93 ± 1.16
FF Dry Bone (g)	14.22 ± 0.22	15.21 ± 0.33	13.79 ± 0 4
FF Liver (g)	8.5 ± 0.22	7.9 ± 0.36	7.82 ± 0.39
FF Gut (g)	4.94 ± 0.18	5.47 ± 0.18	5 96 ± 0.24
Fresh CNS (g)	2.67 ± 0.05	2.58 ± 0.03	2.42 ± 0.07
FF Heart (g)	1.09 ± 0.06	0.96 ± 0.04	0.88 ± 0.04
FF Kidneys (g)	1.8 ± 0.02	1.88 ± 0.06	1.71 ± 0.04
FF Lungs & Trachea (g)	2.78 ± 0.12	2.93 ± 0.1	3.01 ± 0.09
Fresh Adrenals	59 ± 2	54 ± 3	65 ± 3
FF Musc/FFBW (%)	60 ± 0.4	60.8 ± 0.5	57.7 ± 0.8
FF Skin/FFBW (%)	14.6 ± 0.2	13.7 ± 0.2	16.6 ± 0.3
FF Dry Bone/FFBW (%)	5.8 ± 0.1	6 ± 0.1	5.9 ± 0.2
FF Liver/FFBW (%)	3.5 ± 0.1	3.1 ± 0.2	3.3 ± 0 1
FF Gut/FFBW (%)	2 ± 0.1	2.1 ± 0.1	2.6 ± 0.1
FF Kidneys/FFBW (%)	0.7 ± 0.01	0.73 ± 0.02	0.73 ± 0.02
Fresh CNS/FFBW (%)	1.1 ± 0	1.0 ± 0	1.1 ± 0
FF Heart/FFBW (%)	0.44 ± 0 02	0.37 ± 0.01	0.37 ± 0.01
FF Lungs & Trach/FFBW (%)	1.1 ± 0 06	1.14 ± 0.03	1.27 + 0.03

III. EXERCISE STUDIES

A. Exercise, Dietary Obesity and Growth

This paper was published in 1977. Besides making several contributions to the basic physiology of rat growth and its regulation, it makes the following general contributions which are useful in the context of NASA's program. Growth in mass is one of the most fundamental and interesting properties of life in general and if investigators are given an opportunity to study weightless small mammals, they will almost certainly choose growth as one of the properties to be studied. This paper provides a previously lacking background for interpreting rat growth as a function of a wide range of accelerations. It characterizes early parabolic growth, late hyperbolic growth and late rectilinear growth. It also suggests which individual body tissues and organs are likely to respond to environmental stimuli such as acceleration and which are not.

Exercise, dietary obesity, and growth in the rat

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PITTS, G C, AND L S BULL *Exercise, dietary obesity, and growth in the rat* Am J Physiol 232(1) R38–R44, 1977 or Am J Physiol Regulatory Integrative Comp Physiol 1(1) R38–R44, 1977—Four regimens high-fat diet, exercised (*I*), chow, exercised (*II*), high-fat, sedentary (*III*), and chow, sedentary (*IV*) were initiated in 35-day-old male rats Growth was exponential in *I* and *II* and exponential progressing to rectilinear in *III* and *IV* The exponential model predicted the decreasing rank order in asymptotic weight to be *III, IV, I, II* Body composition data (9 components) showed rank order in masses of fat and the fat-free body mass compartment (FFBM) to be the same as for asymptotic live weight The rectilinear growth mode probably reflected fat accretion High-fat diet increased and treadmill exercise decreased FFBM, the latter being reversible These effects depended on regimen initiation by the 5–7th wk of age During growth, masses of H_2O, muscle, and skin increased as functions of body size, bone as a function of age, and heart, liver, gut, testes, and CNS were influenced by combinations of size, age, activity, and diet Growth in body size was expressed more precisely with FFBM, instead of live weight, as the index of size

exercise, obesity, growth, body composition, body size, diet, body fat, fat-free tissues

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THE OBSERVATIONS THAT EXERCISE may increase or reduce body size, depending upon relative effects on body fat and skeletal muscle (1, 12, 27), and that volitional hyperphagia of certain diets may increase body size (20, 28) suggest that the body mass and composition of adult mammals may, in part, reflect their exercise and dietary regimens during growth The present study is addressed to the question how do endurance-type exercise and a high-fat diet administered during growth influence body mass and composition of rats?

Ideally, the question should be considered on several levels simultaneously On a very basic level it involves energy balance with changes in energy stored in bodily tissues Our two experimental variables, exercise and diet, probably exert their influence, at least in part, by altering the energy balance

On another level, the question must be considered from the standpoint of physiological regulation The establishment of some regulatory mechanisms occurs at a relatively late age in the rat pup For example, it has been shown that control of energy ingestion is established after weaning and then only progressively (9) Thus, changes in regimen which might have no effect on the adult, being compensated by regulatory mechanisms, could possibly exert an influence when initiated in the growing pup

Finally, our approach through body composition analysis is critical Total body weight is a datum of limited value to us because it encompasses a group of heterogeneous tissues As a first approximation the gross body mass may be compartmented into fat, which is primarily stored energy, and the fat-free body mass (FFBM) with a variety of functions We have further subdivided the fat-free compartment and the overall approach has enabled us to discern relationships which are otherwise obscured

METHODS

Weanling male CFE rats from Carworth, 35 days old and weighing ~100 g, were used They were individually caged in a room at 22 ± 1°C with 12 h of light (9 P M to 9 A M) and 12 h of darkness per day All experimental activities occurred during their normal waking (dark) period by the light of a 15-W red bulb On *day 1* (35 days of age) six randomly chosen individuals were killed for body composition studies and the others were divided into four weight-matched groups of 25 animals each *group I*—high-fat diet, exercised, *group II*—chow, exercised, *group III*—high-fat diet, sedentary, and *group IV*—chow, sedentary

At 141 days of age the exercise regimen was terminated but the respective diets were continued Ten animals from each group were killed at this time for body composition studies At 293 days of age, the experiment was terminated and all remaining animals were killed for body composition studies The period before termination of the exercise regimen is called the exercise period and that after termination, the recovery period

Exercise was on a motor-driven treadmill (Warren E Collins, Braintree, Mass) twice a day for 7 days a week Grade, speed, and duration of each session were increased progressively with the capabilities of the growing animals By the 33rd day (68 days of age) the subsequently constant conditions of 18 m/min up a 14% grade for 30 min a session were attained A treadmill session raised rectal temperature (3-cm depth) an average of 2 7°C with no observable symptoms of fatigue Because in our experience rats will not voluntarily push themselves beyond mild physical exertion, it was necessary to use an electrical shocking device located at the rear of each compartment Animals that ran poorly due to sore feet or other reasons were eliminated The "sedentary" groups remained in small living cages (18 x 18 x 24 cm)

The chow diet was ground laboratory chow (Ralston Purina Co) containing a gross protein minimum of 23% by weight and of fat, 4 5% The high-fat diet makes rats

obese or subobese (28) Its percentage composition by weight was lard 60 00, casein 25 00, Osborn-Mendel mineral mix 5 00, comprehensive vitamin mix 2 20, nonnutritive fiber 2 00, liver powder 2 00, DL-methionine 0 25 and sucrose 3 55 The chow contained 4 3 kcal/g and the high-fat diet 7 3 kcal/g Consumption was measured three times a week throughout the study and was estimated as weight loss of the individual food cups corrected for spillage Bomb calorimetry of ingesta and excreta on eight rats during a preliminary metabolic trial established the absorbable energies at 3 34 ± 0 02 (standard error of 5 trials) and 6 89 ± 0 02 kcal/g for chow and high-fat diet, respectively Using these values we converted all our values for food consumption to kcals absorbed

The animals were killed by decapitation, bled out, sheared with animal clippers as closely as possible, and the content of the GI tract was removed and weighed The corrected live mass was calculated as weight after killing, plus weight of shed blood, less weight of fur and gut content The carcass was then dissected into nine components heart, liver, testes, gut, brain with spinal cord (CNS), skin, muscle, bone, and adrenals Each component was minced with scissors or ground and stirred thoroughly The summed weights of these processed components were within 2% of the corrected live mass Duplicate aliquots were freeze-dried to constant weight to obtain water content and extracted with petroleum ether (BP 30–60°C) by the Soxhlet apparatus to obtain fat content The word "fat" will refer to the fatty acid triglycerides (7) plus traces of other lipids extractable by our methods [1] FFBM was calculated as corrected live mass less weight of total extracted fat Further technical details have been presented elsewhere (21)

RESULTS

Effects on body weight Figure 1 presents mean live body weight by groups at 2- to 4-day intervals during the entire study The effects of the experimental variables on weight are progressively evident after the 70th day of life Obviously, exercise resulted in slower growth, and the high-fat diet in faster growth Others have reported similar effects of exercise (4, 27) and high-fat diet (28)

The trends and potentials of the groups represented in Fig 1 have been evaluated by longitudinal analysis of the growth of each individual, employing *Eq 1* presented in Table 1 The equation constants were estimated by the graphic method (3, p 524–543) with results typified by Fig 2 In several groups, our results corroborate those of Laird (10) in that one or more phases of late rectilinear growth (Table 2) prevented any apparent approach to an asymptote In Fig 2, rat 66 (*group II*) shows two cycles of exponential growth, one during the exercise period and one during the recovery period Rat 46 (*group III*) shows exponential growth changing into the first phase of rectilinear growth at about age 160 days and the second phase at age 245 days

In Fig 1 it can be seen that only those groups on the exercise regimen (*groups I* and *II*) appeared to approximate an asymptote in body weight Every other group, including *I* and *II* during the recovery period, showed late growth in the rectilinear mode which rose above the predicted asymptote However, there is reason to believe that the value for the asymptotic live weight (A) has physiological significance even if the trend of growth is diverted above or below it (3), and utilizing the data available before the diversion (as in Fig 2, rat 46) one can use *Eq 1* to predict A with considerable precision Thus it is valid to conclude from Table 1 that each group was significantly different from every other group with respect to A calculated from exercise-period data In other words, every possible combination of the two levels of activity and the two levels of diet has the potential for a different mature weight With respect to the rate of maturation (k) and the age at maturity (M), every group was significantly different from every other group with the exception of *I* vs *II* Thus, although these two exercising groups did not differ in rate or duration of growth, the asymptotic weights eventually accumulated were significantly different

The special case of a group which had retired from the exercise regimen is represented by *group IIb* in Table 1 Upon retirement a new cycle of exponential growth occurred (as in *rat 66*, Fig 2), with an A value not significantly different from that for *group IV* which had never exercised This suggests that the effect of the exercise regimen on mature body weight was completely reversible The same is probably true of *group I* after retirement from the exercise regimen but this could not be tested by this method [2]

All testable groups showed late rectilinear growth (Table 2) except those groups exercising (*I* and *II* before day 141) Apparently, treadmill exercise prevented late rectilinear growth

Summarizing the data on live weight, we have described late growth as a self-inhibiting exponential function changing, in most groups, to a rectilinear increase in mass before the exponential asymptote is reached Only those groups actually on the treadmill regimen showed a steady state of live weight near the asymptotic value After retirement from the exercise regimen there was a complete reversal of the exercise effects and also late rectilinear growth appeared Finally, A was different for each possible combination of the two levels of activity and the two levels of diet

Effects on rate of energy absorption During the last 20 days of the exercise period, mean food consumption (kcal absorbed per day ± SE) for *groups I–IV*, respectively were 72 ± 0 9, 67 ± 0 7, 94 ± 0 8, and 76 ± 0 4 The Student t-test showed each group to be statistically different ($P < 0\ 01$) from every other group Group energy absorption was in the same rank order as group body weight (Fig 1)

[1] Adrenals and CNS were not extracted, being considered free of triglycerides

[2] Because of irregularities (see DISCUSSION) in the growth curve of *group I*, neither the exponential nor the rectilinear model could adequately describe it during the recovery period Visual inspection suggests that exponential growth was initiated and sustained for only a few days immediately after termination of the exercise regimen An analysis of variance using growth (the last six successive weight values) vs group (*I* and *III*) yielded an F ratio for group which was not significant ($P > 0\ 05$) This suggests that *I* and *III* had merged into a single population with respect to body weight

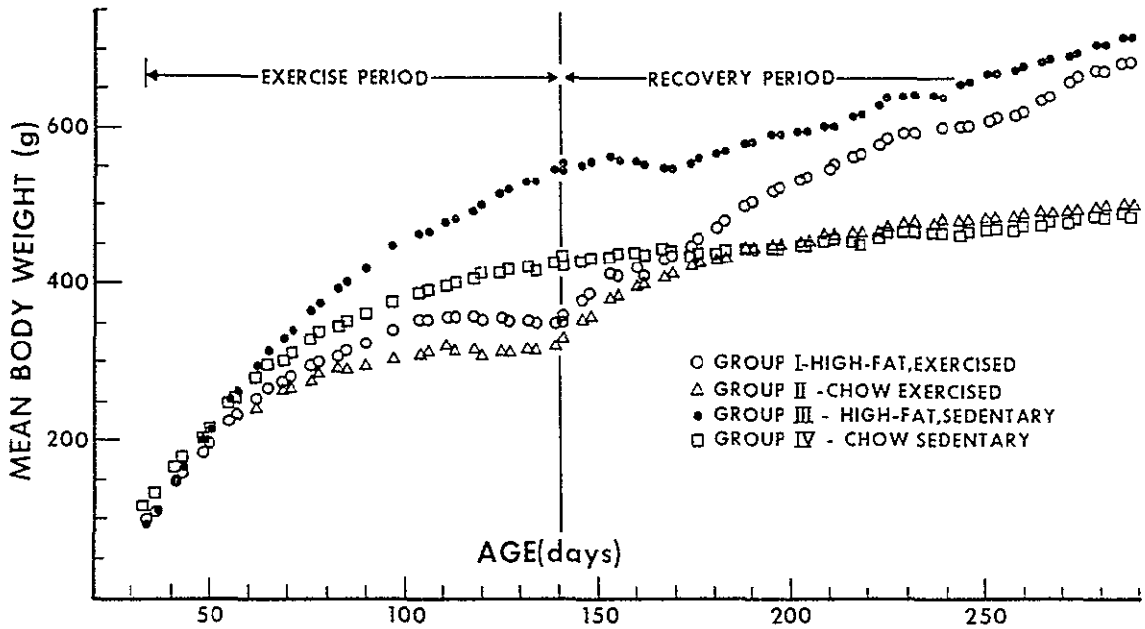

FIG 1 Mean growth in body weight by groups Standard errors were less than 10 g prior to *day 141* and increased progressively through *day 286* when they approximated 50 g for *group III*, 40 g for *I* and *IV* and 10 g for *II*

TABLE 1 *Means, standard errors, and results of t-test on constants for exponential growth*

Group	N	A g	$-k$, g/day	M days of age
I	23	$406 \pm 12^{1\ 2\ 3,4}$	$0\ 0279 \pm 0\ 0012^{2\ 3}$	$140 \pm 5^{1\ 2\ 3}$
IIa	18	$360 \pm 8^{1\ 5\ 6\ 7}$	$0\ 0299 \pm 0\ 0014^{1\ 4\ 5}$	$126 \pm 5^{4\ 5\ 6}$
b	9	$484 \pm 10^{2\ 5\ 8}$	$0\ 0239 \pm 0\ 0020^{1\ 6\ 7}$	$208 \pm 8^{1\ 4}$
III	25	$706 \pm 25^{3\ 6\ 8\ 9}$	$0\ 0143 \pm 0\ 0007^{2\ 4\ 6\ 8}$	$253 \pm 14^{2\ 5,7}$
IV	25	$491 \pm 11^{4\ 7\ 9}$	$0\ 0181 \pm 0\ 0006^{3\ 5\ 7\ 8}$	$190 \pm 5^{3\ 6\ 7}$

Exponential growth was described as

$$A - W = Be^{-kt} \qquad (1)$$

where A = asymptotic value for body weight, W = body weight at time t, $B = (A - W)$ when $t = 0$, e = base of natural logarithms, and k = maturation rate M is the age at maturity (by convention 0 95A) *Group IIb* values were calculated for the period after retirement from exercise All other values were calculated for the exercise period In a given column, pairs of values with the same digit in their superscripts differ significantly ($P < 0\ 01$) as tested by Student t (29)

During the 10-day period immediately following termination of exercise, the mean food consumption of *group I* rose to 97 ± 1 8 kcal absorbed per day and that of *group II* to 81 ± 1 7, both rises being statistically significant ($P < 0\ 01$) These increases were associated with the period of increased rate of gain in body weight (Fig 1)

Effects on body composition Our intent here is to determine which body components contributed to the differences observed in Figs 1 and 2 and Tables 1 and 2 and with which experimental variables the differences were associated

Column 2 in Table 3 presents body composition data obtained on the six weanlings killed at the start of the

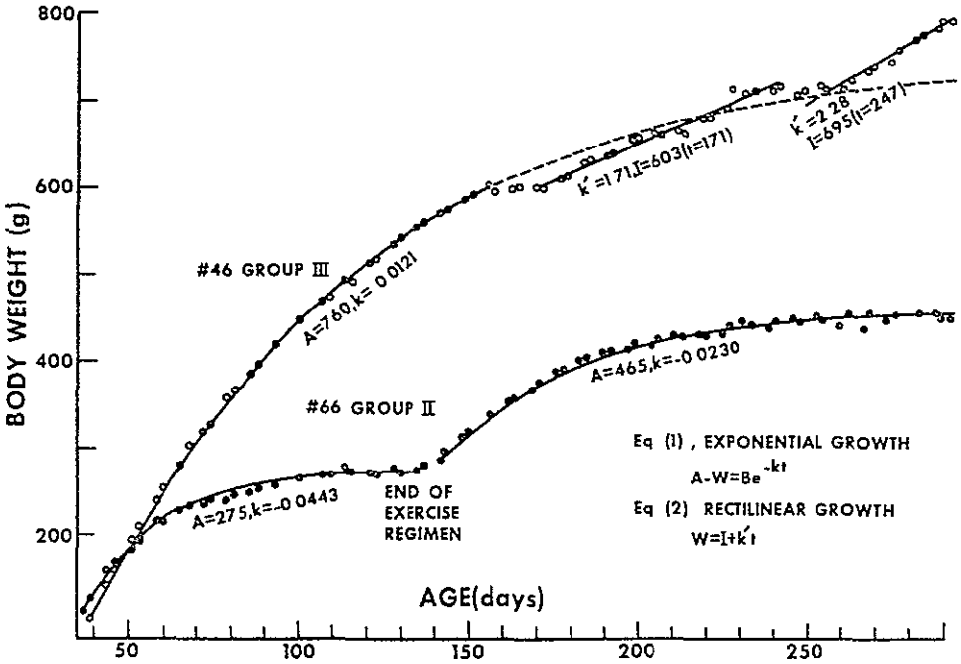

FIG 2 Representative growth curves fitted to individual animals

TABLE 2 *Means, standard errors and results of t-tests on constants for rectilinear growth*

Group	N	t Interval days	I g	k, g/day
II	9	185 to 291	439 ± 7[3 4 5]	0 66 ± 0 06[1 3 4 5]
IIIa	10	128 to 156	514 ± 16[2 3 6]	1 83 ± 0 14[2 4 6]
b	10	170 to 232	546 ± 18[1 4 7]	1 44 ± 0 10[2 5 7 8]
c	4	247 to 291	654 ± 44[1 5 8]	1 52 ± 0 29[2 7 9]
IV	11	144 to 291	424 ± 12[6 7 8]	0 52 ± 0 03[1 6 8 9]

Rectilinear growth was described as

$$W = I + k't \qquad (2)$$

where W = body weight at time t, I is the intercept on the ordinate at an arbitrary t (column 3), and k' is the slope In a given column pairs of values with the same digit in their superscripts differ significantly as tested by Student t (29) $P < 0\ 05$ for pairs with superscript 1 in column 4 and superscripts 1 and 2 in column 5 $P < 0\ 01$ for all other superscripts The subcategories under *group III* indicate separate periods of rectilinear growth

TABLE 3 *Body composition by groups*

Body Components	35 days old When Killed (N = 6)	141 days old When Killed (N = 10/group)			
		Group I High fat Exercised	Group II Chow Exercised	Group III High fat Sedentary	Group IV Chow Sedentary
Σ body fat	5 9 ± 0 7	46 ± 8 (55)	20 ± 2 (32)	172 ± 8 (15)	56 ± 4 (23)
FFBM*	92 8 ± 0 7	299 ± 11 (12)	282 ± 8 (9)	375 ± 6 (5)	343 ± 4 (4)
Skeletal muscle	44 9 ± 0 5	168 ± 7	157 ± 6	210 ± 4	194 ± 3
Skin	16 2 ± 0 6	37 9 ± 1 3	36 6 ± 1 1	49 2 ± 1 0	47 1 ± 0 9
Dry skeleton	2 93 ± 0 82	16 9 ± 0 6	16 7 ± 0 5	17 0 ± 0 2	17 1 ± 0 3
Liver	5 53 ± 0 12	11 3 ± 0 4	12 8 ± 0 5	15 0 ± 0 3	15 5 ± 0 3
Gut	5 49 ± 0 14	6 5 ± 0 2	9 2 ± 0 6	7 4 ± 0 2	9 3 ± 0 4
Testes	1 02 ± 0 07	3 18 ± 0 14	3 00 ± 0 18	3 31 ± 0 05	3 47 ± 0 09
CNS*	1 63 ± 0 01	2 38 ± 0 05	2 32 ± 0 05	2 52 ± 0 05	2 52 ± 0 03
Heart	0 57 ± 0 01	1 57 ± 0 08	1 35 ± 0 07	1 63 ± 0 05	1 36 ± 0 02
Adrenals mg	0 024 ± 0 00	74 ± 4	57 ± 2	60 ± 2	45 ± 1
H_2O/FFBM * %	78 6 ± 0 8	73 9 ± 0 2	73 5 ± 0 3	73 7 ± 0 4	74 3 ± 0 3

Values are means ± SE All components below the first are fat free Units are g except where noted Values in parentheses are coefficients of variation *FFBM = fat free body mass CNS = brain plus spinal cord

study The data in the succeeding columns of Table 3 on the four groups killed at the end of the exercise period were analyzed as a 2 × 2 factorial design for analysis of variance (29) with two conditions of diet and two of activity The results of this analysis are presented in Table 4 The component most sensitive to the experimental variables was fat, which was markedly increased by the high-fat diet and markedly reduced by treadmill exercise, with interaction between the two factors One aspect of this interaction is that the effect of exercise is much greater on rats eating the high-fat diet (Δfat = 126 g) than on those eating chow (Δfat = 36 g) Our rank order of fatness is identical with that reported by Bazzarre and Thye (2) using four similar groups but with spontaneous rather than forced exercise

The data on the FFBM in Tables 3 and 4 show it to be significantly greater in the groups on high-fat diet than in those on chow and smaller in the exercised groups than the sedentary ones Both of these observations have been reported previously (23, 28) Individual components of the FFBM which contributed to the activity effect included three of the largest skeletal muscle, skin and liver There were no statistically significant changes in skeleton Gut weight was not related to activity but was greater with chow than with the high-fat diet, possibly a response to the abrasive components of chow Weight of the CNS was less in exercised than in sedentary groups Hearts were larger in the groups on the high-fat diet than in those on chow If adrenal wet weight reflects the severity of environmental stresses, then our adrenal data suggest that treadmill exercise and the high-fat diet were comparably stressful Finally, H_2O as a percentage of FFBM has been included (Tables 3, 4, and 5) as an index of state of hydration and as an example of a parameter which is regulated with impressive precision It appears to be independent of regimen

Table 5 presents the results of body composition studies on rats killed at the end of the recovery period (293 days of age) Comparing this group with that killed at 141 days of age (Table 3) by t analyses with $P < 0\ 01$ as the criterion for rejecting the null hypothesis, we found evidences of growth in various individual components Body fat increased in all groups The FFBM showed continued growth in all groups except *IV* In most groups most of the individual components of the FFBM participated in this continued growth Two exceptions were CNS and testes, which showed no statistically significant changes in any group Finally the mass of the adrenals decreased in groups retired from exercise (*I* and *II*) and increased in the two sedentary groups

In the table there are no statistically significant dif-

TABLE 4 *F ratios obtained by analysis of variance of body composition data on rats killed at end of exercise (Table 3)*

Body Components	Main Effects		Interaction
	Diet	Activity	
Σ body fat	147 64*	195 93*	60 54*
FFBM	11 27*	83 91*	1 31
Skeletal muscle	6 85	58 25*	0 16
Skin	2 47	98 03*	0 13
Dry skeleton	0 00	0 46	0 09
Liver	7 03	63 58*	1 58
Gut	39 16*	2 10	1 15
Testes	0 01	6 60	2 15
CNS	0 34	12 43*	0 47
Heart	17 96*	0 31	0 27
Adrenals, mg	44 19*	30 89*	0 05
H_2O/FFBM, %	0 19	1 16	2 62

In each case the degrees of freedom between levels of a factor was 1 and for error, 36 *$P < 0\ 01$

TABLE 5 *Body composition by groups*

	Group I High fat Exercised N = 6	Group II Chow Exercised N = 9	Group III High fat Sedentary N = 4	Group IV Chow Sedentary N = 5
Total body fat	229 ± 23[1] (25)	75 ± 4[1] (10)	264 ± 27[6] (20)	82 ± 12[8] (33)
FFBM	432 ± 11[2] (6)	398 ± 7[7] (5)	429 ± 22 (10)	377 ± 25 (15)
Skeletal muscle	226 ± 6[3]	208 ± 4[3]	218 ± 11	201 ± 13
Skin	56 2 ± 2 4	55 6 ± 2 1	57 1 ± 3 2	50 4 ± 4 3
Dry skeleton	20 2 ± 2 7*	21 7 ± 0 8	21 1 ± 0 8	21 6 ± 0 9
Liver	14 8 ± 0 7	15 6 ± 0 5	14 9 ± 1 0	15 2 ± 1 8
Gut	8 3 ± 0 2[4]	12 7 ± 0 6[4]	8 9 ± 0 8	9 3 ± 1 8
Testes	3 35 ± 0 16	3 31 ± 0 33	3 27 ± 0 21	3 46 ± 0 08
CNS	2 44 ± 0 10	2 33 ± 0 07	2 58 ± 0 10	2 50 ± 0 05
Heart	1 78 ± 0 05[5]	1 58 ± 0 05[5]	1 78 ± 0 10	1 61 ± 0 11
Adrenals mg	60 ± 3	52 ± 3	80 ± 8[7]	51 ± 3[7]
H_2O/FFBM %	73 4 ± 0 5	73 2 ± 0 3	72 0 ± 0 6	72 8 ± 0 5

Values are means ± SE Rats killed at the end of the recovery period (293 days of age) All components except fat are fat free Units are g except where noted In any line pairs of values identified by superscript numbers are significantly different ($P < 0\ 01$) according to the Student s t test Values in parentheses are coefficients of variation *N = 3 for this value

ferences between exercised and nonexercised groups Thus, those effects of exercise on body composition which were seen at the end of the exercise period (Table 3) had disappeared by the 293rd day of age, leaving previously exercised groups and those which had never been exercised statistically alike in body composition The effects of diet which were statistically confirmed at 141 days of age (Tables 3 and 4) were sustained through 293 days of age (Table 5)

The analyses of body composition provided in Tables 3, 4, and 5 reveal changes in absolute mass but are incapable of delineating possible relationships between organ size and total body size These may usually be expressed as a power function (3, p 612–626)

$$Y = aX^b \qquad (3)$$

where Y = organ size, X = body size (both in mass units) and a and b are constants Such a relationship may be rectified by a double-log plot (Fig 3), frequently enabling one to distinguish organ growth related primarily to body size from that related primarily to age

Body size[3] appeared in Fig 3 to be the primary determinant of mass in three components water, throughout the weight range studied, and muscle and skin, at least in the adult groups Skin growth was directly proportional to growth in the fat-free compartment and growth in mass of water was nearly proportional (exponent = 0 96), so that body water content may be conveniently expressed as a percentage (Tables 3 and 5) Age was the primary determinant of skeletal mass in the adult groups The other components (liver, gut, heart, testes, and CNS) showed influences of more than one factor

Testes and CNS behaved similarly, increasing as a function of body size up to an abscissa value of approximately 2 54 (348 g), beyond which they appeared independent of the experimental variables If there were reason to ignore the value for *group I*, liver would be seen to behave similarly Gut mass responded to a combination of diet and body size The mean heart mass in the exercised groups was appreciably above that in the sedentary groups at any given level of FFBM, an effect not evident in the mass data of Table 4

In summary, the data on body composition suggested that exercise decreased the masses of both fat and the fat-free compartment below values for the sedentary groups, whereas the high-fat diet increased them above values for the chow groups The masses of individual components within the fat-free compartment were determined primarily by body size but in some cases by activity mode, dietary mode, and age Thus, the masses of water, muscle, skin, gut, and heart were positive functions of FFBM The masses of liver, testes, and CNS appeared to be positive functions of FFBM up to ~350 g above which they appear constant Exercise interacted with FFBM in determining mass of the heart Diet interacted with FFBM in determining mass of the gut Finally, age by itself appeared to determine mass of the skeleton

[3] Because of the lability of the fat fraction of total body weight we prefer FFBM as a measure of "body size"

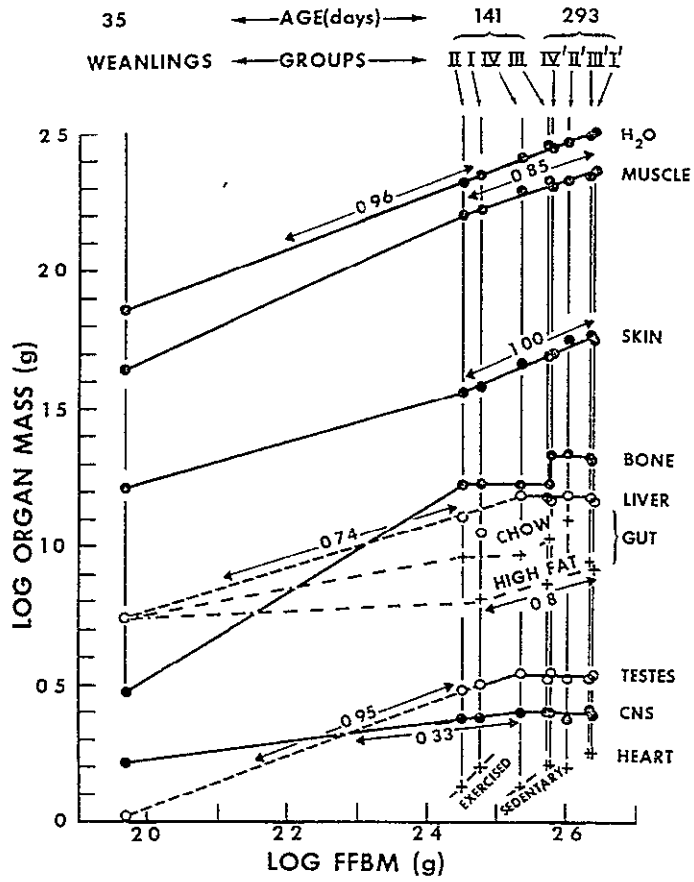

FIG 3 Relationship of mean log organ or component mass to mean log FFBM Curves were drawn by visual inspection since distribution of the data with respect to FFBM and age made statistical curve-fitting difficult *Groups I-IV* were killed at the end of the exercise period (141 days old) and *groups I'-IV'* at end of recovery period (293 days old) Values for b in *Eq 3* are given above the lines We have not estimated b for lines fixed by fewer than three data points

DISCUSSION

The two major body compartments, the fat and the fat-free, present a sharp contrast in mass stability Within apparently homogeneous samples the interindividual variation in fat content and in the FFBM are quite different For example, in Tables 3 and 5 the group coefficients of variation for fat content are 3–5 times those for FFBM In the adult rat, feeding ad libitum, various changes, including seemingly trivial ones, will alter body fat content, whereas the FFBM is virtually imperturbable For example, fatness in the adult rat changed in response to forced treadmill-running (23), voluntary wheel-running (unpublished data), combinations of forced exercise and force-feeding (25), surgical stress (26), changes in the daily light-darkness cycle (26) and fresh food daily versus a 3-day supply of food provided every third day (unpublished data), none of which altered the FFBM There is evidence for physiological regulation of the FFBM (24) Whether body fat content is regulated remains in contention (e g , 6)

The studies referred to above are uniformly cross-sectional in their approach There is little information on which to base an opinion of the relative stability of

fat and the fat-free compartment during longitudinal studies However, most physicians will agree that visually estimated fatness in clinically healthy patients evaluated at intervals may increase or decrease slowly or rapidly, and the changes in either direction may be sustained for periods of weeks or years These changes may be functions of various social factors (5) In any case, the variability of body fat content and the unpredictability of its time course forces us to agree with others (30) that it does not fit the usual concept of regulated growth

It is helpful to apply these conclusions on relative stability to our results For example, late rectilinear growth seen in all our groups during the recovery period (Fig 1) is probably due largely to fat accretion Such accretion probably results from the participation of a constant number of units (adipocytes), which should provide a rectilinar increase in mass However, recalling the documented instability of body-fat mass, it is not surprising to find that this late rectilinear accretion may be interrupted by dietary factors The breaks in the rectilinear growth mode (Fig 2 and Table 2, *group III*) were associated with transient reductions in food consumption and occurred when new lots of high-fat diet were opened, as though there were lot differences in palatability There is little reason to believe that FFBM would respond to such transient phenomena and strong reason to believe that body fat content would do so No such feeding response to lot or bag differences in the chow diet was observed

The exercising groups (*I* and *II*), while on the treadmill regimen, showed no rectilinear growth, all other groups did (Fig 1) The amount of treadmill running was 1 hr per day, a level at which rat food consumption equals expenditure, and below which consumption exceeds expenditure (13) Thus it appears highly probable that the absence of the rectilinear growth mode in exercising groups reflected an absence of fat accretion

The constants A and k in *Eq 1* have been termed "intrinsic or genetic characteristics of the animal under given environmental conditions" (3, p 544) We suggest that the qualification "given environmental conditions" was necessary because A and k are estimated from values of live weight and hence reflect the lability to environmental factors of the body-fat compartment A and k calculated from growth of the fat-free compartment would surely be less sensitive to environmental factors and hence a closer reflection of the genotype Unfortunately, current methods require killing small mammals to determine FFBM and consequently deprive us of longitudinal analysis as a tool

Our original purpose in initiating the experimental regimens as early during growth as possible was to determine whether pertinent regulatory systems might be inadequate or undeveloped in the young rat, allowing some plasticity in mass growth of the fat-free compartment, this proved to be the case We may now ask at what age the regulated level of FFBM is established The high-fat diet initiated before weaning (11), a few days after weaning (20, 28) or at 5 wk of age (Tables 3 and 4) produced larger FFBM's than in groups on chow However, initiated at 21 (8) or 34 (20) weeks of age, the diet had no effect on the FFBM although it still accelerated fat accretion Effective regulation must have been established between the 5th and the 21st wk of life

Forced exercise initiated at weaning (27) reduced growth in several linear dimensions Begun at age 5 (Tables 3 and 4) or 7 wk (4), exercise reduced the FFBM However, when exercise was initiated at age 21 (23) or 23 (19) wk, FFBM was unaffected [4] In this case, effective regulation must have been established between the 7th and 21st wk of life

The possibility remains that either activity or diet could have changed rate of maturation k without affecting asymptotic weight A of the FFBM In this case, at some point after termination of our observations, the more slowly growing and more rapidly growing groups would have converged at the same A value With respect to the high-fat diet, three published studies bear on this question By their 65th week of age slow-growing rat weanlings on chow had attained equality in grams of water, protein, and ash with a group on the high-fat diet (20) In another study of rats, the plane of nutrition during the suckling period was altered by adjusting litter size at birth The FFBM of the animals from large litters grew more slowly but, at the end of the study, was clearly converging on the mature level of FFBM for rats from small litters (31, fig 13) The third study is not in agreement, reporting that the body protein content of rats on the high-fat diet for 65 wk was continuing to diverge from that of animals on a grain diet (28) Nevertheless we think it probable that either a plethora of milk during the suckling period or a high-fat diet initiated by the 5th wk of life increases rate of growth in FFBM without changing A

It is difficult to determine whether the exercise regimen, if continued beyond 141 days of age, would have influenced the asymptotic values for FFBM The A values predicted for live weights of *groups I* and *II*, if exercise had continued, were 406 and 360 g, respectively (Table 1) If the percentage of body fat found in the groups killed at the end of exercise (12 and 6%) had persisted, then the respective asymptotic FFBM's would have been approximately 357 g and 338 g These appear well below the final values obtained for the FFBM's of sedentary *groups III* and *IV* (Table 5), but with no expressions available for dispersion about the means, statistical significance cannot be assessed

There is evidence that exercise can produce a smaller fat-free compartment in man too (12) On the other hand, physical training can cause an increase in FFBM in the young, growing human being (18) The latter is probably attributable to exercises of the overload type which produce muscular hypertrophy It is extremely difficult with the treadmill to achieve such overload exercise in the rat (25)

The effects of exercise on several items in the energy

[4] By contrast, swimming as an exercise reduces FFBM when initiated as late as 5 (8) or 11 (17) mo of age This difference from treadmill results may be attributable to a more intense psychic stress and/or opportunities for very rapid thermal exchanges both during and after the swim

budget may explain why exercise reduced FFBM Exercise probably increased daily energy expenditure despite some possible compensation (16), reduced food intake (4, 13, 17, 23, 25), and raised the thermic response to food, thereby increasing energy lost as heat (15) These factors could limit storage of energy as tissues of growth Thus, exercise may compete with growth for calories

It is of interest that Fig 3 reveals only two specific effects of the experimental variables on individual body components, i e , the effects of diet on gut, and activity on heart Figure 3 suggests that the statistically significant changes in other components seen in Table 4 are not specific responses to the respective experimental variables but rather secondary responses to the changes in FFBM which were evoked

In summary, our discussion suggests the following changes in mass of fat occurring during ontogeny are difficult to predict and detract from efforts to quantitate growth in mass of the fat-free compartment, late rectilinear growth is probably attributable to fat accretion, the observed influences on FFBM of exercise and high-fat diet are obtained only if the regimen is started at or before age 5–7 wk, there being no effect with a start at 21 weeks of age or later, and the high-fat diet probably increased rate of growth in FFBM without changing its asymptotic level

The authors are indebted to Mr John H Key, Jr , Mr E Darrell Morgan, Miss Anna K King and Mr George T Tillman, Jr for technical assistance We are particularly indebted to Dr A H Smith for suggestions on data analysis

This study was supported by Contract NAS2-1554 between the National Aeronautics and Space Administration and the University of Virginia

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Received for publication 14 January 1976

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B. Exercise and Body Composition

The following is a manuscript to be submitted to the Journal of Applied Physiology. Besides making contributions to the basic physiology of exercise in the rat, it makes the following general contributions which are useful in the context of NASA's program. The body composition response of the rat to three different exercise modes (forced swimming, forced treadmill running and voluntary wheel running) is characterized These modes will differ in their interactions with acceleration and in their potential convenience and effectiveness as a conditioning regimen for use during space flight. The response to exercise was found to be a function of sex, age when the exercise regimen starts and duration of the regimen. We suggest that the same variables will be important during exposure to weightlessness with or without exercise. Finally, the paper evaluates the role of psychic stress on the response to exercise and the role of the same variable must be evaluated in the weightless rat.

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EXERCISE AND BODY COMPOSITION IN THE RAT

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Running head Exercise and body composition in the rat.

In our earlier studies of the exercising rat (Pitts; Pitts & Bull; Pitts, Bull & Hollifield; Pitts, Bull & Wakefield, and unpublished) and in the publications of other investigators we have noted data suggesting that the body composition changes associated with exercise vary with the nature of the regimen and the condition of the subjects. Our published studies all involved forced treadmill running. Here we present results obtained on rats given the opportunity for voluntary wheel-running and we compare the results obtained with treadmills, running-wheels, and forced swimming. We also consider the effects on body composition of sex of the rats and their age when the regimen was initiated.

METHODS

Virgin female white rats of Sprague-Dawley origin were employed. In each experiment they were randomly seggregated into an exercise group allowed ad libitum access to running wheels propelled by the rat and a control group which remained within 8 x 8 x 24 cm. living cages. All animals were individually caged and given a ground chow diet (Ralston Purina Co.) and water ad libitum. In some cases, as indicated, food consumption was monitored by food cup weight. For the sedentary young adult rat it was established that 3.34 $\pm$ .02 kcals were absorbed from each gram of chow which passed through the gut (Pitts and Bull), and this value has been used to convert grams of chow ingested to kcals absorbed.

The running wheels employed (Wahmann Mfg. Co.) were ∿35 cm. in diameter and were equipped with revolution counters and living cages measuring 12½ x 15 x 25 cm.

Animal room temperature was regulated at 22 $\pm$ 1°C and relative humidity at 45-50%. Lights went off at 8AM and on at 8PM.

The rats were killed by decapitation, bled out, sheared as closely as possible with clippers and the content of the gastrointestinal tract was removed and weighed. Viscera and eviscerated carcass were separately weighed and ground. Water was determined by freeze-drying and fat content by Soxhlet extraction with petroleum ether (BP 30-60°C) on duplicate aliquots of each. Body mass after sacrifice, plus mass of shed blood, minus mass of fur and gut content minus the mass of extracted lipid yielded the fat-free body mass (FFBM).

RESULTS

Exercise initiated at 25 days of age. (Expt. I). Growth in live mass, energy absorption and activity are presented in Fig. 1. A composite model of growth (Brody) treats early growth (below the point of inflection) as parabolic,

$$W = W_o e^{kt} \qquad (1)$$

where W is body mass, W_o is mass when time (t) is 0 and k is the growth constant. Late growth is treated as hyperbolic

$$A-W = Be^{-k't} \qquad (2)$$

where A is the asymptotic body mass, k' the maturation constant (i.e., the rate at which W approaches A) and B is an integration constant. The mathematical model for early growth fits the data satisfactorily (Fig. 1). However, because of an unexplained flattening of the curves between 60 and 110 days of age the fit to late growth is poorer than can usually be expected (e.g., Pitts & Bull).

The mean constants for each group are entered into the equations in Fig. 1. The A values for exercised and sedentary groups respectively (mean $\pm$ SEM) are 250 $\pm$ 6 and 239 $\pm$ 5. According to the t test the difference between these two means is not statistically significant, implying that at maturity the two groups would be statistically alike in live mass.

However, body composition analyses of the individuals sacrificed at 133 days of age (Table 1) reveal significant differences between the groups. The exercised group had less fat and more FFBM than did the sedentary controls. The opposite effects of exercise on the two major compartments, fat decreasing and the FFBM increasing, is responsible for the closely similar live masses in the two groups both in Table 1 and in the A values calculated from equation (2).

It can be seen in Fig. 1 that after activity had reached a plateau at $\sim$ 15,000 revolutions per day, the mean group difference in energy absorbed was $\sim$ 25 kcals per day

Product-moment correlation coefficients were calculated for all combinations of activity, food consumption, total body fat and FFBM. The only significant correlation ($P < .05$) was that for the inverse relationship between activity and FFBM.

Exercise initiated during late growth. (Expts II, III and IV) Studies of the effects of wheel running on young adult female rats were initiated at ages of 11 weeks for 31 days duration (Expt. II), 16 weeks for 91 days (Expt. III) and 19 weeks for 43 days (Expt. IV). In Expt. IV body composition analyses were sacrificed in order to obtain in vivo data during a recovery period. The in vivo results, which are presented in Fig. 2, closely represent the results obtained in Expts. II and III also.

In Fig. 2 the exercise group shows changes in food consumption which are followed by changes in body mass, both lagging behind changes in regimen. On the 3rd and 4th day after running-wheels were made accessible food consumption and body mass passed through minima followed by recovery within $\sim$ 30 days. The food consumption plateau reached was $\sim$15 kcal/day above the sedentary group, a difference which probably approximates the energy cost of running activity. When the running-wheels were removed, food consumption required $\sim$25 days to subside to a steady state. During this period of overeating, exercise group body mass rose above, and then returned to, the sedentary level.

The body mass curves in Fig. 2 imply that if the wheel-running regimen lasts as long as 30 days, an exercising group will return to the same level as a sedentary group. However, in Expt. II during the last 10 days of the 31 day total duration the exercise group remained 5 to 10 g in body mass below the sedentary group and in Expt. III during the last 56 days of the 91 day total the exercise group remained 5 to 8 g below the sedentary group. These differences suggest that after recovery the live mass may still be slightly below that of controls

After a period of learning, activity reached 4,000 revolutions/day. This is much below the level attained in Expt. I (Fig. 1), probably because the rats in Expt. I were introduced to wheel-running at a much earlier age.

Using the data in each experiment after steady states were attained, we have made rough calculations of the energy cost of activity, obtaining 7.8 kcals/ 1000 revs/ kg body mass for Expt. I, 5.8 for Expt. II and 11.5 for Expt. IV. (Food consumption was not monitored in Expt. III.) Considering the uncontrolled variables (e.g., the lack of data on energy storage as fat) the range in values is not unreasonable.

The pertinent body composition data are presented in Table 1. Fat, expressed either as grams or percentage was reduced by exercise. The fat-free compartment (FFBM) did not respond to exercise in Expts. II and III. The impressive constancy in water fraction of the fat-free compartment suggests that state of hydration did not change much between groups. Finally, the larger adrenal mass in the exercise groups suggests that even volitional exercise may be accompanied by an increase in stress level

DISCUSSION

Our approach to the physiological effects of exercise involves in-vivo measurements of body mass on one hand and body composition analyses on the other. It is important to relate the two as far as possible.

Live body mass is a heterogeneous entity including fur, gut content, fluid compartments and multiple live tissue components. We have found no statistically significant differences between groups in mass of fur, mass of gut content or body fraction of water and will now direct our attention to two major compartments, fat and the FFBM.

The principal problem in interpreting live mass is indicated by Expt. 1 (Fig. 1) where two offsetting changes within the exercise group, a decrease in fat and an increase in FFBM (Table 1), are not reflected in live mass variety. Fat is primarily stored energy while the FFBM has a variet of functions, and a change in live mass which comprises unknown contributions from these two will yield limited information. However, with adequate nutrition the fat-free compartment is very stable in the adult, resisting perturbation by a great variety of factors, and shows a closely regulated increase in mass during growth (Pitts unpub.; Pitts & Bull). By contrast the body fat content is highly labile, changing in response to nearly any small change in regimen (Pitts unpub.; Pitts & Bull). In the discussion which follows it will be seen that these characteristics assist us in

interpreting data on live mass.

Studies of both sexes of rats exercised by three exercise modes and at a variety of ages are summarized in Tables 2,3 and 4. While these tables are extensive and, we believe, representative of reported results, they may not be exhaustive. Collectively the data are frustrating in that they show several gaps which prevent firm conclusions on the effects of any of the experimental variables of particular interest to us. For example, there are no body composition data on swimming females, none on wheel-running males and none on immature treadmill-running females. The tentative generalizations which follow may have to be modified when these gaps are filled.

In Fig. 2 the principal body compartments show results typical of our earlier observations (Pitts unpub.; Pitts & Bull). The labile fat compartment (g or %), wherever measured, was reduced in exercising animals. By contrast, the FFBM of the adult was protected from perturbation by either the running wheel or treadmill mode Although live body mass can only reflect the net result of changes in fat and the FFBM, these values where available are included because of their potential usefulness where body composition analyses were not performed.

There are some differences between the effects of the three exercise modes. In adult rats, swimming was the only mode which altered (↓) the fat-free compartment. The effect of swimming on body composition in the rat 12 weeks of age and younger is not documented. Wheel-running differed from the other two modes in two respects. It alone increased rate of growth of the FFBM (7 weeks of age or less), and while the wheel-running adult shows a loss of live mass, the loss was completely, or almost completely restored within 30 to 40 days of continued wheel-running. This loss and recovery is documented by our Fig. 2 which is representative of results obtained in Expts. II, III and IV. The changes involved are almost surely restricted to the fat compartment

The age at which exercise was initiated was important, with 7 to 11 weeks of age appearing to be a critical period As we have pointed out elsewhere (Pitts & Bull), the fat-free compartment is perturbable by exercise regimens initiated earlier than this. But the FFBM was successfully defended against regimens initiated later than this except in the case of swimming.

Sex of the exercised rats also proved to be important. At all ages females on a swimming regimen maintained their live mass whereas males lost mass. This distinction made elsewhere (Oscai, Mole & Holloszy) is documented more fully by Table 2. In contrast to the swimming data, adult females on treadmill regimens lost live mass. This appears paradoxical since the swimming regimens used were generally more arduous than the treadmill ones.

In conclusion, we suggest that the number of variables involved in most studies of exercise in the rat, and probably other small mammals, has become unwieldy. It would appear wise to evaluate fully the variables presently involved before new ones are added.

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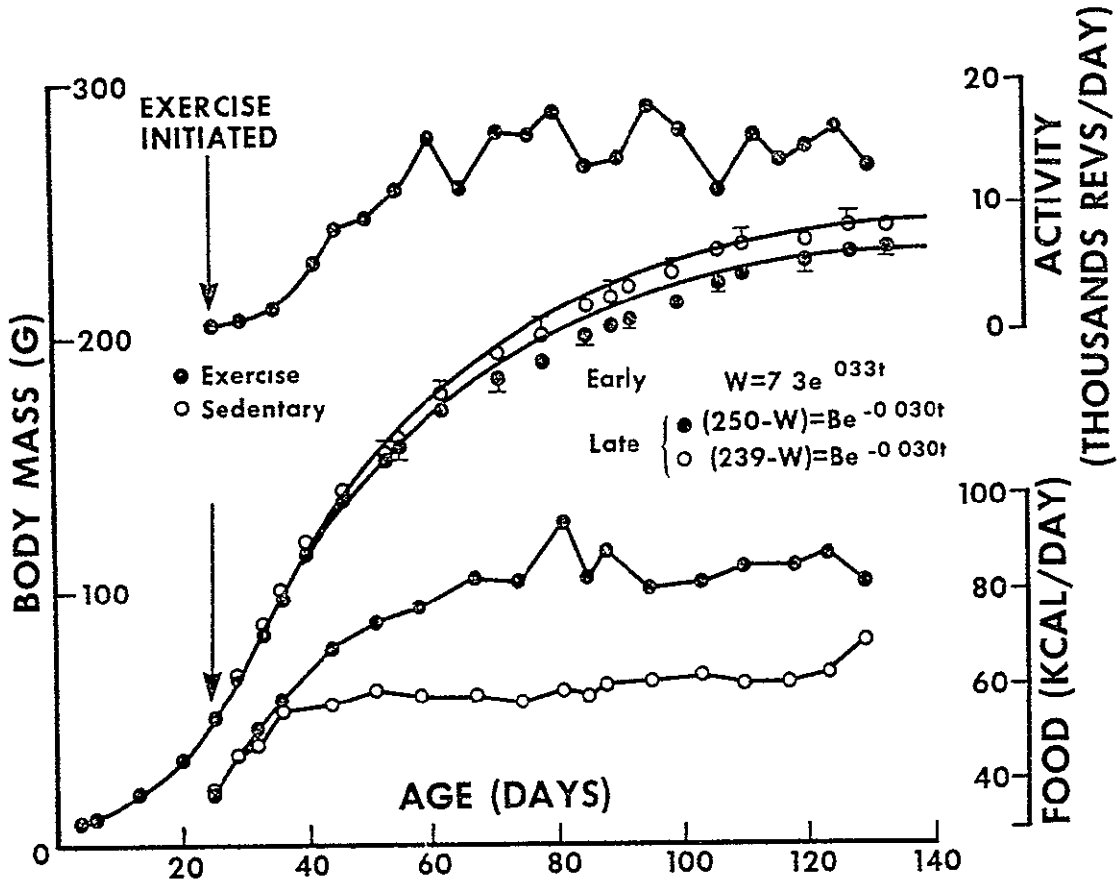

Fig. 1. Growth, activity and food consumption in weanling rats permitted voluntary running in a self-propelled activity wheel. (Expt. I.)

Table 1. Effects of voluntary wheel-running exercise on body composition.

Group (N)	Components (mean ± SEM)				
	Fat (g)	Fat (%)	FFBM (g)	H_2O/FFBM (%)	Adrenals (mg)
Experiment I: Regimen initiated at 24 days of age, duration 110 days.					
Exercise (6)	12 ± 2*	5 ± 1*	204 ± 7*	72.1 ± 0.3	
Sedentary (10)	24 ± 2	11 ± 1	186 ± 3	72.6 ± 0.4	
Experiment II: Regimen initiated at 11 weeks of age, duration 31 days.					
Exercise (8)	20 ± 2*	8 ± 1*	226 ± 5	73.6 ± 0.2	70 ± 3*
Sedentary (8)	41 ± 2	15 ± 1	224 ± 5	73.7 ± 0.2	58 ± 4
Experiment III: Regimen initiated at 16 weeks of age, duration 91 days.					
Exercise (8)	13 ± 3*	5 ± 1*	220 ± 4	73.3 ± 0.2	86 ± 2*
Sedentary (8)	28 ± 1	11 ± 1	225 ± 3	73.5 ± 0.2	73 ± 4

FFBM = the fat-free body mass

*Statistically significant ($P<.05$) difference between groups according to the method of t.

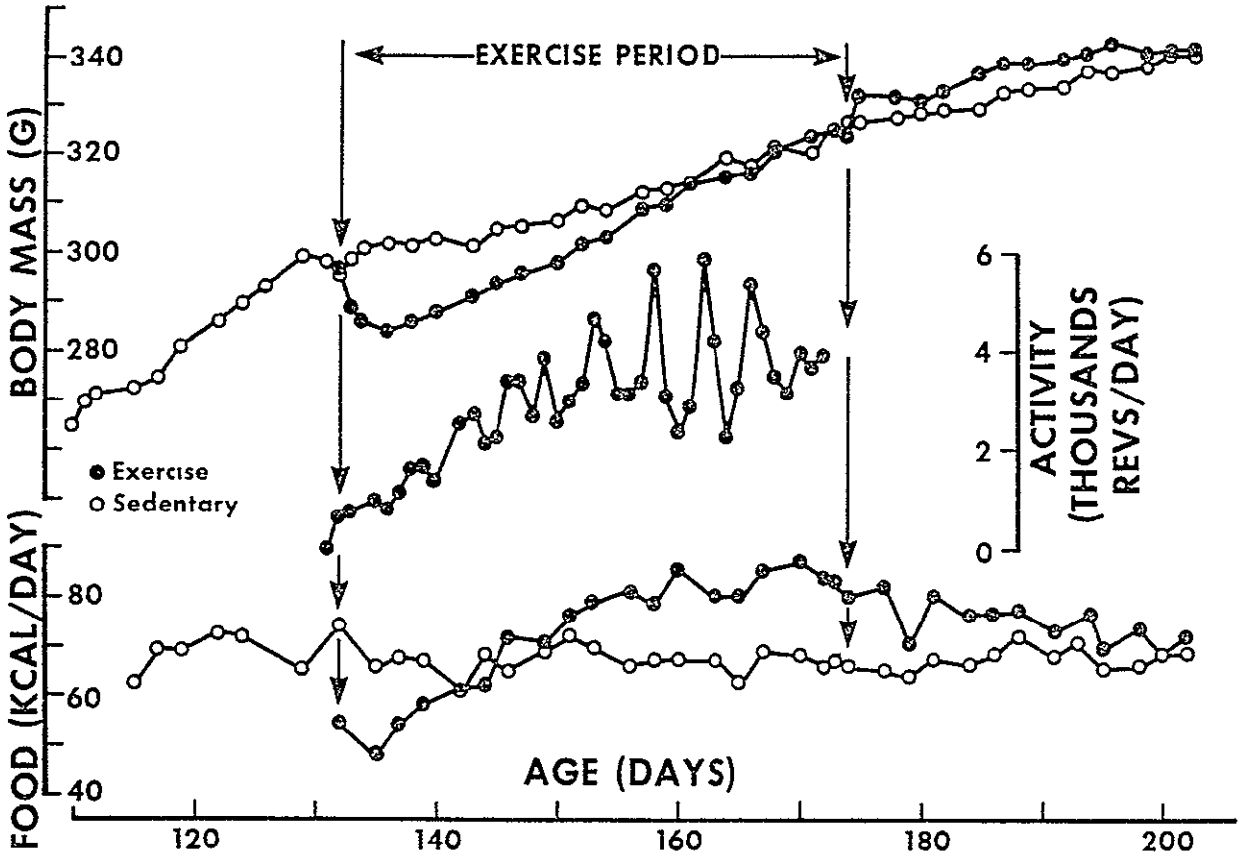

Fig. 2. Body mass, activity and food consumption in adult rats permitted voluntary running in a self-propelled activity wheel. (Expt. IV.)

Table 2. A summary of the effects of swimming on body size and composition in the rat.

A. MALES

Live mass	Fat	Fat-free compartment	Adrenals	Reference
1. Exercise initiated at 4 weeks of age.				
↓			→	Bloor, Pasyk & Leon
2. Exercise initiated at 6 weeks of age.				
↓				Oscai, Mole & Holloszy
3. Exercise initiated at 10 weeks of age.				
↓				Oscai, Mole, Brei & Holloszy
4. Exercise initiated at 12-16 weeks of age.				
→			↑	Bloor, Pasyk & Leon
5. Exercise initiated in the adult.				
↓				Stevenson, Feleki, et. al.
↓	↓	FFBM ↓	↑	Jones, Montoye, et. al.
↓	↓	H_2O↓, protein↓	↑	Hanson, et. al.
↓	↓	H_2O↓, protein↓, FFBM↓		Oscai & Holloszy
↓			↑	Bloor, Pasyk & Leon
→			↑	Eränko, et. al.
→				Tepperman & Pearlman

B. FEMALES

1. Exercise initiated at 6 weeks of age.				
	↓	FFBM ↑		Oscai, Mole, Krusack & Holloszy *
→				Oscai, Mole & Holloszy
2. Exercise initiated at 10 weeks of age.				
→				Oscai, Mole & Holloszy
3. Exercise initiated in the adult.				
→				Arcos, et. al.
→			→	Crews & Aldinger

*For the exercise group the investigators selected 13 of the 18 exercised rats. "Of the 18 swimmers, 13 increased their food intake sufficiently to gain weight at approximately the same rate as the sedentary animals with which they were paired." This probably created a bias.

Table 3. A summary of the effects of forced treadmill running on body size and composition in the rat.

A. MALES

Live mass	Fat	Fat-free compartment	Adrenals	Reference
1. Exercise initiated at 3-5 weeks of age.				
-	-	- - FFBM→	→ -	Parizkova & Faltova
↓	↓	↓	↑	Pitts & Bull
2. Exercise initiated at 6 weeks of age.				
- -↓ - -				Oscai, Mole, Brei & Holloszy
3. Exercise initiated at 7 weeks of age.				
- - ↓	↓	FFBM↓		Crews, et. al.
4. Exercise initiated in the adult.				
↓		-		Stevenson, Feleki, et. al.
	↓	FFBM→	→	Parizkova & Stankova

B. FEMALES

1. Exercise initiated in the adult.				
- - ↓	↓	FFBM→	↑	Pitts, Bull & Wakefield
↓	↓	FFBM→	↑	Unpublished
↓	↓	FFBM→		Pitts, Bull & Hollifield
↓		↓		Mayer, et. al.

Table 4. A summary of the effects of voluntary wheel running on body size and composition in the rat.

A. MALES

Live mass	Fat	Fat-free compartment	Adrenals	Reference
1. Exercise initiated in the adult.				
→				Tepperman & Pearlman

B. FEMALES

1. Exercise initiated at 24 days of age.				
→	↓	FFBM ↑		Table 1
2. Exercise initiated at 7 weeks of age.				
		Length ↑	↑	Ring, Bosch & Lo
3. Exercise initiated at 11 weeks of age.				
↺**	↓	FFBM →	↑	Fig. 2*
4. Exercise initiated in the adult.				
↓	↓	FFBM →	↑	Table 1
↺**				Fig. 2
→				Tepperman & Pearlman

*Fig. 2 represents results obtained on one 11-week old group and two adult groups.

**This symbol represents a fall in mass followed by a recovery.

IV. EXERCISE COMBINED WITH ACCELERATION

A. Exercise during Chronic Acceleration.

Body composition analyses on the carcasses from this study were completed very recently. Consequently, statistical and graphical analyses have barely started and this must be regarded as a preliminary presentation. Indeed, the entire study must be regarded as a preliminary study because exercise during centrifugation had not been tried before to our knowledge. In spite of careful planning and a large investment of time, the study was less successful than expected in data yield and more successful in revealing obstacles to be overcome in future experiments.

The plan for the study is presented in the experimental protocol which follows.

EXERCISE DURING CENTRIFUGATION
Ames Research Center
July-August, 1975

Objectives:

1. To evaluate the role of running activity in modifying the body-composition effects of centrifugation, thereby altering the rate of adaptation to Δ G.
2. To derive the quantitative relationships describing the body-composition response:
   a. with G-load constant and activity varying.
   b. with activity statistically constant and G-load varying.

General Plan:

1. Duration-tentatively 60 days depending upon dynamics of the observed changes in body mass.
2. Acceleration levels - approximately 2.76 G and approximately 4.15 G depending upon levels available in the repaired and recalibrated centrifuge.
3. Experimental animals - Simonsen Sprague-Dawley rats, virgin females approximately 250 g. at start of centrifugation.
4. Segregation into groups:

Group	Regimen	Number	Approx. Date of Sacrifice
I	Sacrificed at start of centrif.	8	July 11
II	Sedentary - 1 G	10	Aug. 8 or Sept. 8
III	Exercised - 1 G	8	"
IV	Exercised - 2.76 G	8	"
V	Exercised - 4.15 G	8	"

Data to be collected:

1. Number of wheel revolutions - whenever possible.
2. Body mass - daily for first week and twice weekly thereafter.
3. Food consumption - daily for first week and twice weekly thereafter.
4. Body composition - terminally.

Results: Deaths during centrifugation have been very rare in my experience (not counting deaths due to careless door closure by animal caretakers who service the centrifuge cages.) However, the 5 deaths out of 16 centrifuged rats which we encountered deserves analysis. These were distributed as follows.

G level	1.00 G		2.76 G		4.15 G	
Activity level	exercise	sedentary	exercise	sedentary	exercise	sedentary
Deaths	0 of 8	0 of 8	1 of 8	0 of 8	3 of 8	1 of 8

Exercise appears more hazardous than the sedentary condition and higher accelerations appear more hazardous than lower ones. There was an interaction between the two factors such that the greatest number of deaths occurred in the group exercised at the highest G level. One mechanism which was involved was as follows. At high G levels the rat cannot hold its tail elevated in the usual running posture. Consequently, the tail dragged and was occasionally skinned on the edge of the running wheel. If the area skinned on the tail was large enough, the rat usually died a few days later. It appears that this hazard can be greatly reduced by a simple alteration of wheel design.

The in vivo results are summarized in Fig. 1. Note that the centrifuged groups with the running wheel available lost masses comparable with those reported in earlier studies for groups centrifuged without an opportunity to run.

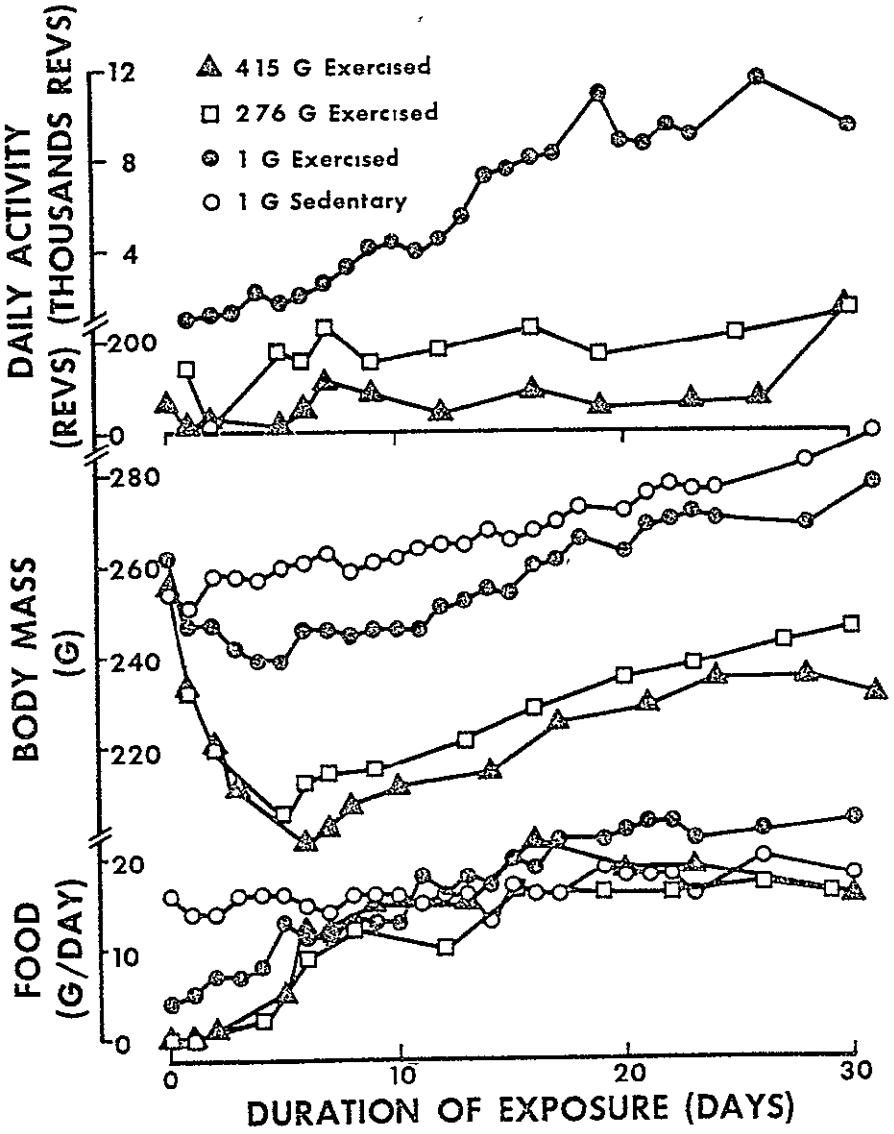

Fig. 1. Body mass, activity and food consumption in rats permitted voluntary access to self-propelled running wheels during chronic centrifugation.

Sedentary rats at 1 G showed a near steady state in food intake. However, either wheel-running at 1 G or the initiation of centrifugation caused an abrupt reduction in food intake followed by a slow recovery to and above the level of the 1 G sedentary group.

The activity levels for centrifuged groups were disappointing, the highest mean value being 270 revs/day as compared to the highest mean value of 11,700 revs/day for the group at 1 G. There is some doubt that the level of wheel-running obtained was sufficient for a meaningful test of exercise as an experimental variable. Daily activity varied among the individuals of each group and we expect to analyze the individual data for correlations among the parameters measured. Also, in future studies we suggest allowing rats to go through a learning period in the running wheels before centrifugation is initiated.

The body composition data by groups are presented in Table 1. The following points should be observed. As has been demonstrated often before, either exercise or centrifugation reduced body fatness (in g or %) and the greatest reduction occurred in the exercised group at the highest G level.

With respect to the fat-free body mass (FFBM) it showed a rectilinear relationship to G level in exercised rats just as it does in sedentary ones. These data must be compared statistically with data previously obtained on sedentary rats at 2.76 and 4.15 G in order to determine whether exercise during centrifugation altered the acceleration effect.

Note that in the table only 3 individuals are listed as sedentary during centrifugation. These were placed in running cages rendered stationary after dead animals had been removed from them. Limited space on the centrifuge and reductions in our manpower prevented us from planning full-sized sedentary groups on the centrifuge.

Table 1. Body composition by groups (mean ± SEM)

CLW	Fat	Fat / CLW	FFBM	Skinned FF Mass	Evisc. Carc. H_2O	Adrenals
(g)	(g)	(%)	(g)	(g)	(g)	(mg)
Before centrigugation or exercise - Day 0 (N=8)						
235 ± 3	35 ± 2	15 ± 1	200 ± 3	141 ± 2	103 ± 2	58 ± 2
1 G sedentary - Day 31 (N=8)						
265 ± 5	40 ± 2	15 ± 1	224 ± 5	161 ± 4	118 ± 3	58 ± 4
1 G exercised - Day 31 (N=8)						
246 ± 4	20 ± 2	8 ± 1	226 ± 5	161 ± 4	118 ± 3	70 ± 3
2.76 G exercised - Day 31 (N=7)						
224 ± 6	14 ± 1	6 ± 1	210 ± 6	149 ± 5	109 ± 4	62 ± 4
2.76 G sedentary - Day 17 (N=1)						
213	10	5	202	146	107	62
4.15 G exercised - Day 31 (N=5)						
211 ± 10	7 ± 2	3 ± 1	204 ± 9	144 ± 6	105 ± 5	65 + 3
4.15 G sedentary - Day 17 (N=2)						
199	13	6	186	131	95	83

CLW = corrected live weight, FFBM = fat-free body mass.

V. RESTRAINT STUDIES

Hypokinesia simulates weightlessness in several respects. Consequently, the members of the Animal Physiology Consortium (a group of investigators representing diverse physiological disciplines of which I am a member) are exploring the possibility that the rat rendered hypokinetic by suspension in harness might constitute an adequate model on which to collect background data in preparation for an eventual study of the rat in earth orbit.

Besides the above application hypokinesia has special significance for me because of my program of investigations in exercise physiology. Hypokinesia represents the ultimately mild exercise regimen and should be included in a program of study of exercise physiology.

While awaiting the availability of the rat harness being developed by the Consortium for use by its members, I have completed a study of the rat rendered hypokinesic by cage restraint.

The protocol for the study follows.

Body Composition and Cage Restraint In the Rat

Objectives:

To determine whether the restraint resulting from continuous residence in cages of very small volume has an effect on body composition of young adult rats.

Animals:

Virgin female albino rats of Sprague-Dawley origin purchased from Hilltop Lab Animals, Inc. Weight specified 200 - 210g. Received Wednesday, June 30, 1976 at which time the mean weight was 191g.

Two groups:

Restrained - N = 12 - housed singly in cages 17 X 18 X 8 cm.

Unrestrained - N = 12 - housed singly in cages 45 X 23 X 20 cm.

Measurements:

Total body mass at appropriate intervals (see Chronology)

Terminal body composition:

Chemical components - H_2O, fat, ash. Determined on each anatomical component.

Anatomical components -

Total muscle (by estimation from water)

Total bone (by difference, i.e., FF MS syst. - FF muscle)

Skin

Heart

CNS

Remaining viscera pooled.

Chronology:

Day 0 - 24 rats received in good condition.

Day 1 - weight and divide into 2 weight-adjusted groups.

Day 2 - weigh

Day 5 - weigh

Day 8 thru 61 - weigh twice a week.

Day 62 - Remove food at beginning of light period.

Sacrifice all animals at end of light period.

The effect on body mass of cage restraint for slightly more than one month is presented in Fig. 1. The restrained rats (∿8 wks. old at the start of the study) were kept in cages approximately 4 to 6 times their body volume (judged by eye). The only point of interest in the figure is that restraint did not change late growth from that of the unrestrained group. Because restraint appeared to have no effect we decided not to perform body composition analyses on the carcasses.

As has been shown in the reprint included under III.A and the manuscript included under III.B, the response of the rat to either an exercise regimen or a high fat diet is determined by the animal's age. For example, the FFBM is altered by either regimen initiated at 5-7 wks. of age or earlier but is unchanged by the same regimen initiated at an age older than the 5-7 wk. critical period. Consequently, we are repeating the cage restraint study starting with rats 3-4 wks. of age. At this writing the study is not yet completed, but it is already clear that the restrained animals are smaller and are growing less rapidly. Body composition studies will be carried out on these animals.

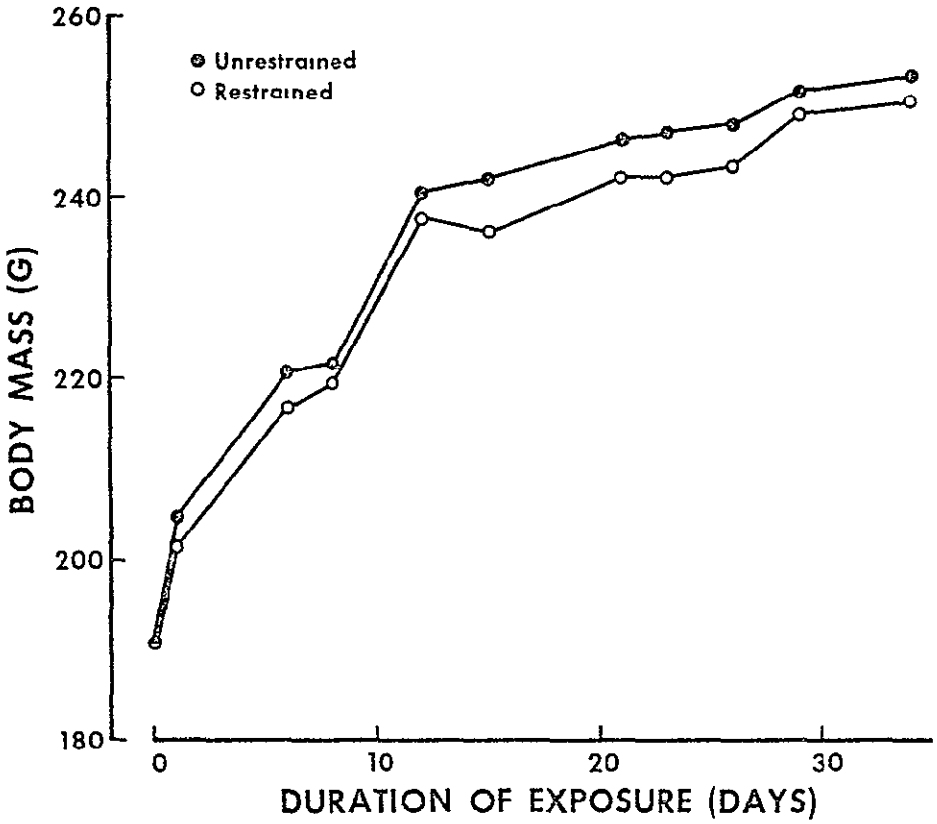

Fig. 1. Effect of cage restraint on body mass in the rat.

VI. PHYSIOLOGICAL REGULATION

A. Physiological Regulation and Energy Balance.

The following manuscript has been submitted for publication to the American Journal of Physiology. Besides making several contributions to basic physiology, it makes the following general contributions which are useful in the context of NASA's programs. Some body components were found to be vigorously defended against perturbation by environmental factors while others changed readily. As a result of this manuscript it has begun to be clear which body components are likely to change in response to ΔG and which are not, even before the actual observations are made. The results of this study together with that presented under III.A give us a new confidence in predicting the results on body composition of exposures to new acceleration environments.

(Submitted to Am. J. Physiol. on 12/5/76.)

Physiological Regulation and Energy Balance

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Running head: Physiological Regulation and Energy Balance

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ABSTRACT

Mean body fat mass and fat-free body mass (FFBM) for 53 samples of rats, 28 samples of human beings, 17 of mice and 16 of wild mammals (all adults) were gleaned from the literature. These individually homogeneous samples collectively showed wide differences in experimental variables. The criterion of regulation was a central tendency (bell-shaped curve) as opposed to wide scatter. The frequency distribution for FFBM was comparable to those for resting body temperature and systolic blood pressure. However, the fatness data were widely scattered and suggested that with selected regimens (all allowing feeding ad libitum) one could produce a continuous spectrum in body fatness from the lean to the obese. Because hypothalamic lesions produce animals which defend specific levels of body fat which are either very high or very low while the middle range is characterized by lability, an hypothesis of regulation which is effective at the range extremes but less rigorous in the mid-range is suggested. Advantages and complications of such an arrangement are discussed.

Fat, the fat-free compartment, body composition.

Numerous investigators from several different scientific disciplines have studied the possibility that body energy balance is regulated. Some of these efforts have been directed at the "regulation of body weight" or the "setpoint of body weight" (12, 44, 72). Because total body mass is a complex and, we believe, inappropriate entity from the standpoint of regulation, we have directed attention to data on fat and the fat-free compartment considered separately. Because there are difficulties in identifying and characterizing regulatory phenomena we have made an effort at clarification by redefining a few terms and concepts. We have applied these distinctions to a reexamination of data in the literature previously unevaluated from the standpoint of regulation[1] and we believe that the results have provided some new perspectives.

MATERIALS

Our materials are largely in the form of sample means for body fat content and the fat-free body mass (FFBM) obtained with standard body composition techniques by indirect in vivo methods on man and direct terminal methods on other species. Most of these are from papers published by other investigators. While each sample was homogeneous with respect to the experimental variables of interest to the particular investigator, collectively the samples represent a variety of experimental variables as well as unrecognized variables characteristic of the particular laboratory environments. Because of the nearly universal absence of data on individuals, the sample means were treated as data units. In some cases the desired data were not provided by could be calculated by simple arithmetic from other values which were provided.

Our literature search was extensive but probably not exhaustive. All of the discovered samples which met the following criteria were used.

1. The subjects should be adults thereby ruling out growth as a variable and eliminating immature animals in which control of food consumption was not yet established (35). We have defined adult laboratory rats as being 24 weeks of age or more, laboratory mice as being 12 weeks of age or more, human beings as being 18 years of age or more and wild mammals as meeting the published weight and dimension description for adults of each species.
2. They should be subsisting on a nutritionally balanced diet available ad libitum
3. They should have no recognized pathology.
4. They should be intact prior to sacrifice. The sole exception was the use (in Fig. 2) of rats with hypothalamic lesions.
5. Each sample should supply values for both fat mass and the FFBM.[2] A comparison of these two components is important for our purposes and this precaution insured that the sample means for the two compartments represented the same constellation of known and unknown variables. Data on fat[3] and the fat-free compartment[3] yield information about different processes. Since body fat is primarily an energy store, in an adult with a constant FFBM the mass of fat is a useful index of the net imbalance between energy intake and expenditure summated over the period of study. By contrast, the FFBM is responsible for virtually all of the processes essential to daily living. We must keep in mind that the FFBM is not a physiological entity but a collection of entities (muscle, skin, bone, etc.) which may be subject to independent control.

The wild mammals used (61) are identified in Table 1. One sample was available for each of the 16 species. The individuals were taken with snap traps which killed at capture With the exception of the shrews these species are all plant eaters and they were collected during the season when their natural foods occurred in greatest abundance. In each case post-mortem examination revealed a lean animal but with no suggestion of emaciation. Thus, their dietary regimens were not restricted and may reasonably be termed ad libitum.

Data from 25 published papers plus 2 unpublished ones from this laboratory are qualified by these criteria. These 27 papers provided the 114 separate statistical samples which are plotted one or more times in Figs. 1, 2, and 3. Of these, 53 were samples of rats (5, 8, 28, 38, 43, 50, 54, 56, 58, 59, 66, 67, unpublished), 28 of men and women (6, 10, 11, 15, 16, 39, 51, 53, 71, 73, 74), 17 of mice (8, 34, unpublished) and 16 of wild mammals (61). Sample size varied from 3 to 98 with most falling in the 5 to 10 range.

METHODS, DEFINITIONS AND CONCEPTS

There is a variety of anatomical, physiological and behavioral parameters which are controlled primarily by the organism although they may respond to environmental factors. Most of these controlled parameters may be adjusted by the organism to levels anywhere within the viable range as is momentarily appropriate in serving the economy of the body. However, a particular subset of these parameters is controlled in a more rigorous manner so as to resist or minimize displacement of its value from a narrow, presumably optimal, range and this is designated "regulation" (14). This distinction is real,

useful and biologically significant since it spotlights an important difference between living and non-living systems Treatment of control and regulation as synonyms will usually result in confusion.

Brobeck (14) pointed out that a regulated parameter is typically coupled with one or more controlled parameters (effectors). As applied to energy balance:

A		B		C
Energy intake	$\longrightarrow$	Energy storage	$\longrightarrow$	Energy loss

Any constancy in B, assumed to be regulated, is made possible by control of A and C which may vary as appropriate between lower and upper limits of viability. If measured repeatedly under a variety of environmental circumstances, the regulated term B will be found within one or a very few narrow ranges which are frequently called regulatory set-points. The existence of many such set-points is unlikely. Difficulties associated with the set-point concept should be noted (22, 29).

The essence of regulation lies in its response to perturbation. A change in A to $(A + \Delta A)$ or C to $(C + \Delta C)$ will displace B to $(B + \Delta B)$. If the system were in physicochemical equilibrium, $(B + \Delta B)$ would be maintained as long as $(A + \Delta A)$ or $(C + \Delta C)$ was sustained and would be restored to B only when the former balance between A and C was restored. However, physicochemical equilibria are so infrequent in living organisms that they rarely cause confusion in studies of regulation If B is indeed regulated, ΔB will be promptly reduced even while the changed value in A or C is sustained. In a slow response system one could observe the perturbed B value followed by a return toward the regulated range. In this study such transients are noted

only in the few cases where longitudinal data on individuals are examined. The more numerous group means employed are believed to represent steady state conditions because of the durations of the respective regimens. They may nevertheless reflect the influence of regulatory systems when judged by the following criterion.

Multiple measurements of an unregulated parameter made under a wide variety of experimental conditions are likely to be widely distributed throughout the available range However, if the parameter is regulated, the values should show a central tendency (bell-shaped curve) about the mean which should be close to the regulatory set-point. It is unlikely that any factor other than regulation would yield a clear central tendency from data involving a variety of exercise regimens, dietary regimens and others. In other words, a very heterogeneous data sample will show wide scatter unless regulatory mechanisms act to confine the parametric value within a narrow range. While the criterion may be poorly suited for absolute quantitative applications, it serves adequately in the comparative uses to which we have put it. This criterion has been applied to the study of the behavioral regulation of body temperature in desert reptiles (68) and the regulation of gross chemical composition in rabbits (26).

RESULTS

Fig. 1A shows the frequency distribution of sample means for FFBM and fat mass in our predominant species the rat. The curves for fat have maxima at approximately 60g but 12% of the values occur far to the right in each sex. By contrast the curves for FFBM suggest bell-shaped distributions with some possible skewing.

In Fig. 1B the distribution of FFBM is placed on a common ordinate with distributions for two parameters generally regarded as regulated, i.e. those

for resting systolic blood pressure (37) and resting oral temperature (32) both in young men. In comparing the curves two things should be kept in mind. First, the curve for blood pressure as well as that for oral temperature was obtained by plotting individual values from a relatively large sample whereas the FFBM curve was obtained by plotting 26 sample means. Second, the blood pressures and oral temperatures were measured under standardized conditions whereas the sample means for FFBM represent variations in location, investigators, diet, amount and kind of exercise etc. Certainly this comparison suggests that regulation may play a role in the distribution of rat FFBM but in the case of body fat there is room for considerable doubt.

Fig. 2 has fat as a percentage of total body mass on the abscissa. It presents both sexes of rats, mice and human beings in a single plot, thereby increasing the size of the total sample with no apparent loss in the resolution of peaks. The combined curve shows a distribution which is bimodal or possibly trimodal. It should be remembered that this curve represents the frequency of occurrence of these abscissa intervals in our data but not necessarily their frequency of occurrence in the natural populations. Unless it is possible to interpret the three peaks as different regulatory set-points, this plot suggests more clearly than does Fig. 1 a deviation from what is expected of regulated parameters.

In Fig. 2 note that all the samples with experimental or genetic obesity were located above 40% fatness. However, 16 samples of "normal" animals occurred in the same high range. This suggests that while hypothalamic lesions and specific genes may facilitate the attainment of very high or

very low levels of fatness, these extreme ranges are also available to normal intact animals, an interpretation in accord with (45) but not (33).

In Fig. 3 are plotted frequency distributions in percentage body fat for four subsamples:

A. 16 means, each for a different wild species as identified in Table 1 (61). Mode 2-6%.

B. 77 individual females of an inbred strain (MSD) of Sprague-Dawley rats, born within a 15 day span, individually housed in a constant (temperature, humidity, light, sound) environment (unpublished). This sample represents our ultimate reduction of genetic and environmental variability. Mode 8-12%.

C. 32 samples of random-bred laboratory rats fed on chow-type diets (38, 50, 54, 56, 57, 58, 59, unpublished). Mode 16-20%.

D. 17 samples of random-bred laboratory rats fed on high fat (40-60%) diets. (28, 38, 54, 56, 59, 66, 67). Mode 36-48%.

The overlapping population ranges extend from nearly 0 to 56% fatness and suggest the existence of a continuous spectrum.

DISCUSSION

The results on the fat-free compartment are relatively easy to interpret and will be disposed of first. Within the scope of our limited criterion it appears very likely that the FFBM is subject to regulation (Fig. 1B) but perhaps not as a unit. Its major components (e.g., skeletal muscle, bone) may be regulated individually and the observed near-constancy of the whole could be the result of these several interacting systems of regulation.

The near imperturbability (except by inanition) of the fat-free compartment in the adult rat (28, 33, 54, 57 and Fig. 1) may suggest that it is qualitatively

different from the control of body fatness. However, that it is perturbable and behaves like various other regulated parameters is demonstrated by its response to altered gravity simulated by chronic centrifugation (55, 58, 59). The FFBM shows an inverse rectilinear relationship to simulated G force and the effect is promptly and completely reversible upon returning to the original level of acceleration. Furthermore, the adult rat being chronically centrifuged can approach its new lower set-point for FFBM from either above or below (59). But the fact remains that the fat-free compartment shows an impressive constancy under a variety of circumstances which result in altered fat mass

With respect to body fat content our results (Figs. 1, 2, and 3) do not provide frequency distributions such as are expected with regulated parameters but also do not exclude a regulatory interpretation. However, there are other data which suggest regulation and we shall attempt to resolve the apparent conflict.

The results reported in greatest abundance agree with ours presented above in supporting the conclusion that body fat content is labile whether expressed in mass units or in percentage. It changes in response to changes in: age (36, 57), forced exercise (57), volitional exercise (unpublished), combinations of exercise and/or force-feeding (60), surgical stress (62), circannual rhythms (48, 49), circadian rhythms (62), environmental temperature (7), chronic acceleration (58, 59), dietary composition (56), apparent palatability of the diet (21, 33, 45) and social factors (23, 42). In our samples the body fatness levels are nearly as numerous as the regimens employed. Fig. 3 implies that by utilizing appropriate regimens and/or species, one could produce a continuous spectrum of fatness from perhaps 2 to 60%.

While fatness undeniably responds to changes in regimen, a constant regimen may not insure constant body fatness even in the long term. For example, annual records of body mass were kept for 7 years on 24 healthy long-term prisoners

(24 - p. 20) during which time some individuals showed a net gain and others a loss but there was no mean trend upward or downward. The constant regimen made it highly probably that the observed changes in total mass reflected changes in mass of fat primarily. If one assumes a mean group fatness of 20%, it may be calculated that the average individual altered his energy stored as fat by approximately 50%.[4]

Besides the extensive evidence of lability in body fatness there are two lines of evidence which suggest regulation: first, the evidence for the presence of central nervous mechanisms and effector mechanisms such as one would expect to participate in a system of physiological regulation and, second, the evidence that in some cases specific levels of body mass and/or body fatness are defended.

The central mechanisms, termed hunger and satiety, have been studied perhaps most effectively by experimental production of hypothalamic lesions. They appear to have separate central representation with dynamic interaction between the two (33) and are believed to represent a central integrating function.

Among the effector mechanisms likely to participate in a regulation of energy balance are ingestive behavior and the dissipation of energy as heat. Ingestive behavior, when averaged for periods of a week or more, is usually altered in a direction appropriate to the maintenance of energy balance (24-p. 11). The other effector mechanism, the dissipation of excess energy as heat, termed "luxuskonsumption," has been recently reviewed (24-p. 132). Several studies now support the conclusions that prolonged hyperphagia and hypophagia are associated with an increase and decrease respectively of energy dissipated as heat (4, 40, 41, 63, 24-p. 134).

Central integrator and peripheral effector mechanisms like those described appear to defend specific levels of body mass against perturbation in rats made very fat or very lean by appropriate hypothalamic lesions (21, 33). Body composition studies indicating that changes in mass of the fat compartment are largely responsible for these changes in total mass (33) suggest strongly that in these extremes of fatness there is regulation of fat at specific levels.

Finally, there are infrequent data suggesting that individuals may not be regulated or may break away from regulatory influences. In an unpublished study of 8 female rats eating a chow diet ad libitum, when first allowed ad libitum access to a vertical running wheel, 7 individuals showed small reductions in food intake and body mass followed by recovery to the previous levels within a very few days. By contrast, the eighth rat, always appearing normal and well groomed, voluntarily fasted for the first 11 days of wheel running during which its body mass dropped to ~190g (Fig. 4). This nearly rectilinear drop is unlike a regulated approach to a set-point. Upon reaching 190g the body mass turned sharply upward as though it had encountered a limiting condition. It then approached a new steady state via an apparently exponential curve such as is typical of "homing" in regulated parameters (70).

In another study (19) rats force-fed to approximately twice the control level of body mass followed by ad libitum feeding, reduced their intakes until their body mass approached control levels. However, one individual (their Fig. 6) showed a response nearly identical with that presented in Fig. 4.

In a study of the control of food intake in 15 human subjects (69) 6 regulated effectively through a period of dietary dilution, 3 were questionable

and 6 termed "nonregulators". The only nonregulator presented in detail (their Fig. 2) showed a rectilinear weight loss throughout the 21 experimental days. Observations like those in this and the two preceding paragraphs suggest that regulation of body fatness is frequently not rigorous enough to suppress non-regulatory responses in apparently normal individuals with intermediate levels of fatness.

In spite of this scanty basis of facts an hypothesis of the physiological mechanism involved is desireable because of its possible heuristic effect. However, we should resist any temptation to explain the evidence of lability in body fatness by assuming that the new values observed after perturbations represent changes in set-point. By increasing the number of assumed set-points at which the organism is equally fit for survival, one must eventually render the concept of regulation meaningless and must concede that regulation no longer supplies a need which cannot be filled by simple control. The hypothesis which we favor is probably only one of several which might serve at least as well. It is prompted by the following trends in the data reviewed above:

a. The abundant evidence of lability in body fat was obtained on animals in the mid-range of body fatness.

b. Individuals seen to drift across this mid-range and eventually reach extremely low values responded as though regulatory forces were evoked to move them back.

c. The best evidence for rigorous defense of specific fatness levels is seen in individuals with hypothalamic lesions where the absence of adequate hunger or satiety pushes the individual against one

or the other extreme level of fatness.

The above observations suggest that the system operates more effectively at extreme values of fatness than in the mid-range. That regulation is probably not absent from the mid-range is suggested by several lines of evidence including the effective compensation for dietary dilution seen in rats which are neither very fat or very lean (2). However, the evidence for lability of fat mass in the mid-range suggests that there it is readily suppressed or overridden by a variety of influences. But as fatness approaches extreme values in either direction, effector mechanisms operating to pull it back into the mid-range become increasingly evident.

The proposed mechanism could not be designated a "long-term" system since the length of its time constant would not be a critical consideration. Presumably, if the female rat represented in Fig. 4 had a very low body fatness such as is seen in wild rodents (Table 1), she might have started her decrease in body mass from a steady state value of 200g and one day later the upward movement resembling regulation would have been initiated. Probably the descriptive name "broad-band regulation" would be satisfactory.

Broad-band regulation has certain substantial advantages, for example, it provides a simpler interpretation of age-associated changes in fatness. Over a period of years individual fatness may creep up, down or stay nearly the same (variations seen in most normal populations) and so long as the changes remained within the broad band, they would not require a regulatory explanation. Also this hypothesis frees body fatness to change in other cases. For example, independent control of protein intake (20, 46, 52, 65) would allow adjustments in body protein to proceed unimpaired by any resulting

changes in fat mass. Changes in fatness (usually increases) required by events in the life cycles of various species might be explained as controlled changes within the broad band, for example, increases in fatness preparatory for: hibernation, migration, a mating season (seals) or brooding season (penguins) without feeding opportunitites, etc. Finally and incidentally, it should be noted that changes in dietary palatability produce altered intake of food, body mass, and probably body fatness which appear independent of, and additive with, even the extreme levels of fatness seen with lateral or medial hypothalamic lesions (21, 64).

Some retrospective observations which have improved our perspective may be helpful to others. For example, it appears probable that energy balance comprises several distinguishable processes including the following:

a. control of the release of chemical energy so that it matches expenditure for an appropriate unit of time.

b. control of the proportionate energy contributions from endogenous stores of glycogen, fat and protein.

c. adjustment of energy metabolism to changes in physiological condition, e.g., growth, gestation, lactation, egg-laying, inanition, physical activity.

d. control of the mass of body fat.

It is unlikely that a single system could control or regulate all of these, suggesting the probability that more than one regulatory system is involved.

Adolph's warning that the living organism is characterized by complex interactions between multiple systems of regulation according to established priorities (1) is highly pertinent in this case. A low priority on the maintenance of constant fatness would mean that it would probably yield at nearly every conflict with another system. Also, these multiple regulatory

systems must frequently share elements. For example, feeding behavior as an effector mechanism controlling the ingestion of calories, water, amino acids, vitamins, minerals, electrolytes and others is shared by several regulatory systems. The resulting necessity for interactions between the systems must be a serious obstacle to the quantitative understanding of feeding behavior.

Regulation of the mass of a body compartment must involve monitoring that compartmental mass, either as the extensive property of weight ("ponderostasis") or as some intensive property (9) which is an index of mass or weight. Since the weight of a constant body mass can be increased by chronic centrifugation and decreased by insertion into earth orbit (weightlessness), these techniques provide a test for the existence of ponderoceptors. If regulation of the FFBM or body fat is based upon ponderostasis, the regulatory response should be a decrease in the compartmental mass during centrifugation and an increase during weightlessness. Decreases occur in both components during centrifugation but weightless astronauts and cosmonauts also showed decreases (55). Thus, FFBM appears to pass through a maximum at terrestrial gravity and ponderostatic regulation is not supported by these results.

Alternatively, if the blood concentration (an intensive property) of some metabolic substrate or product (for example) is a dependable index of the mass of a regulated compartment, then that concentration might be the regulated parameter (30). The problem then becomes the more familiar one of regulation of a concentration in the body's internal environment. We have no name exclusively for this class of regulations. Homeostasis, coined for physiological usage, is now applied to the regulation of intensive properties in a variety of systems, for example, ecosystems (47) and social systems (17).

A unique term for the regulation of quality of the body's internal environment could be "Bernardian regulation" in honor of the experimental physiologist who did much of the early work upon it (3).

FOOTNOTES

1. Several other efforts to interpret existing data have been based on the design of models for systems analysis (13, 18, 31).
2. Note that measurements based on entities short of the total animal (e.g., the eviscerated carcass) could not provide values for total body fat or the total fat-free compartment.
3. We prefer "fat", defined as the fatty acid esters of glycerol (27) to the generic "lipid" which does not distinguish between storage and structural moieties. In practice the FFBM presumably lacks small amounts of structural lipids which are removed by the fat extractives commonly used.
4. The mean weight for each year could have been computed and the seven values would show a 5 pound range. This constitutes ~3% of total body mass and might support the assumption that total body mass is precisely regulated in the long term. The misleading character of group means used for this purpose has been pointed out (25).

ACKNOWLEDGMENTS

This study was supported in part by National Aeronautics and Space Administration research grant NGR47-005-213.

I would like to acknowledge the technical assistance of Mr. John H. Key, Jr.

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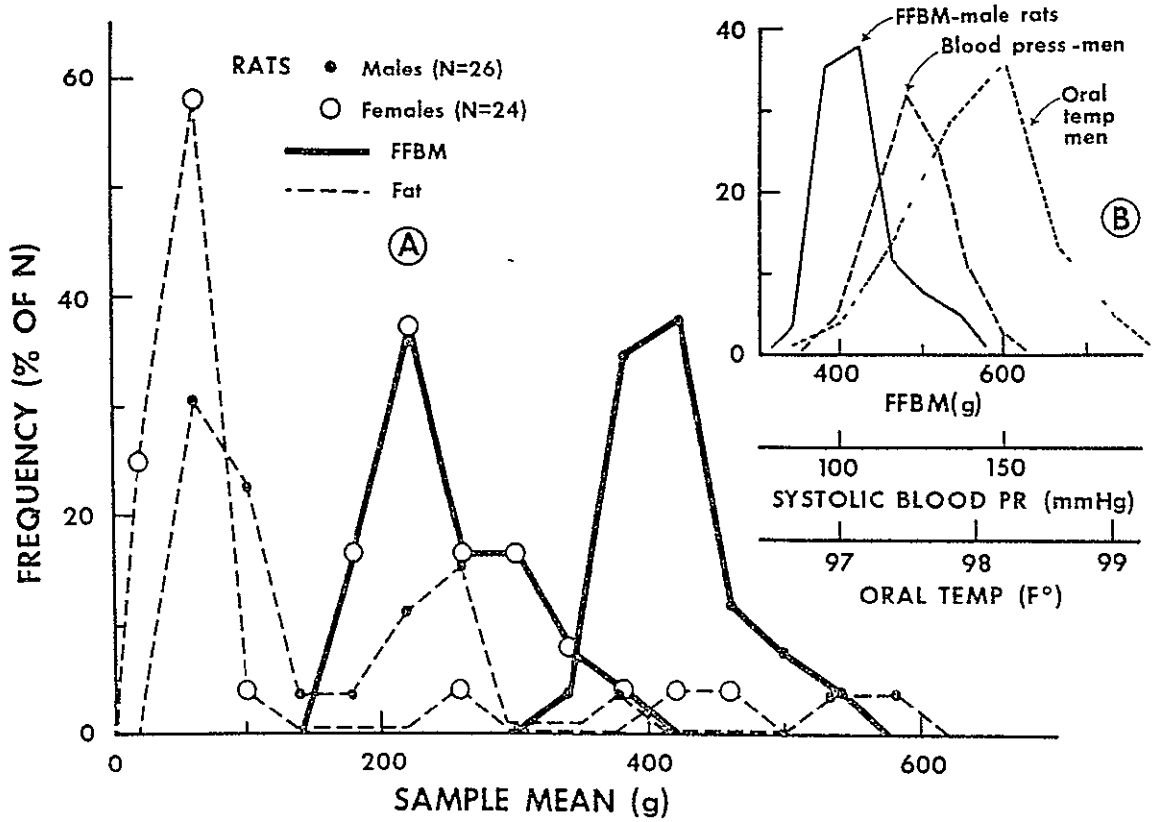

Fig. 1A Frequency distribution of statistical samples of rats. Both fat and FFBM are expressed in mass units. Each point represents the percentage frequency of occurrence within an abscissa band 40g wide. Rats which were not intact or had genetic or experimental obesity were omitted.

Fig. 1B. The frequency distribution of FFBM of male rats (from 1A) compared with that for resting systolic blood pressure of men 20-24 years of age (37) and resting oral temperature of men (32). The tails of the curves below 1% were not plotted. In each case the frequency interval on the abscissa was chosen to enable presentation of the curve with 5 to 6 plotted points.

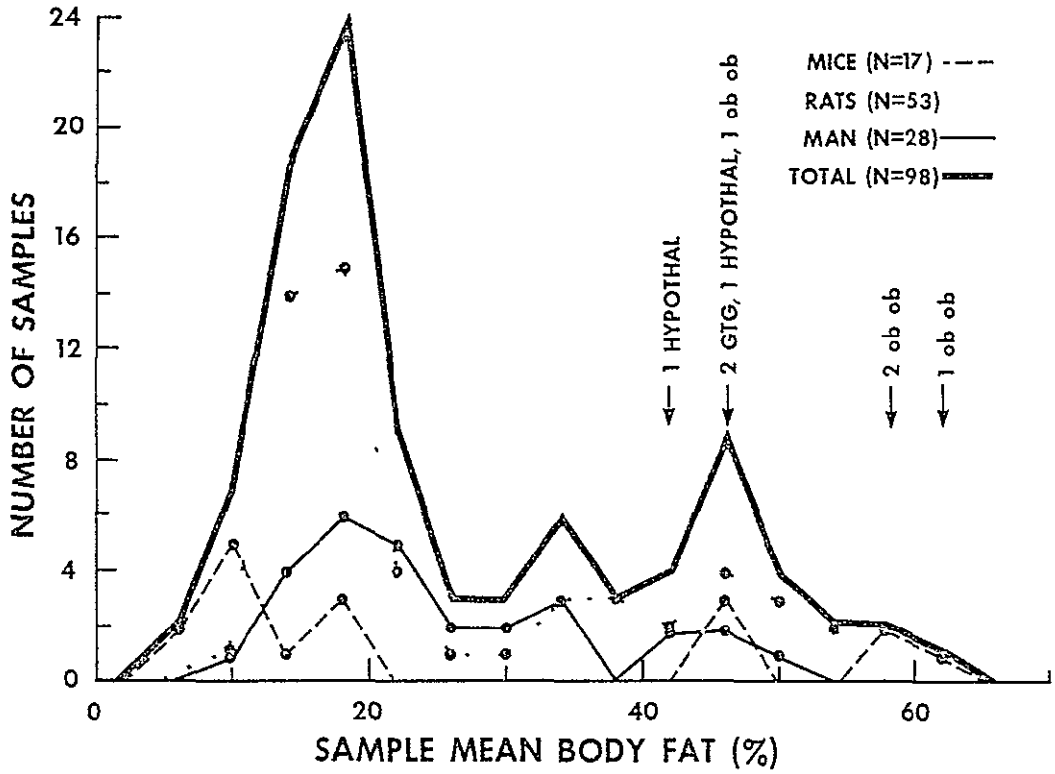

Fig. 2. The frequency distribution (by species and combined) of mean percentage body fat in statistical samples of rats, mice and human beings. Each point represents the number of samples occurring within an abscissa band 4% wide. The abscissa locations of samples with experimental obesity are indicated at upper right. Hypothal = rats with hypothalamic obesity. ob ob = obese hyperglycemic mice and GTG = mice with gold thioglucose obesity.

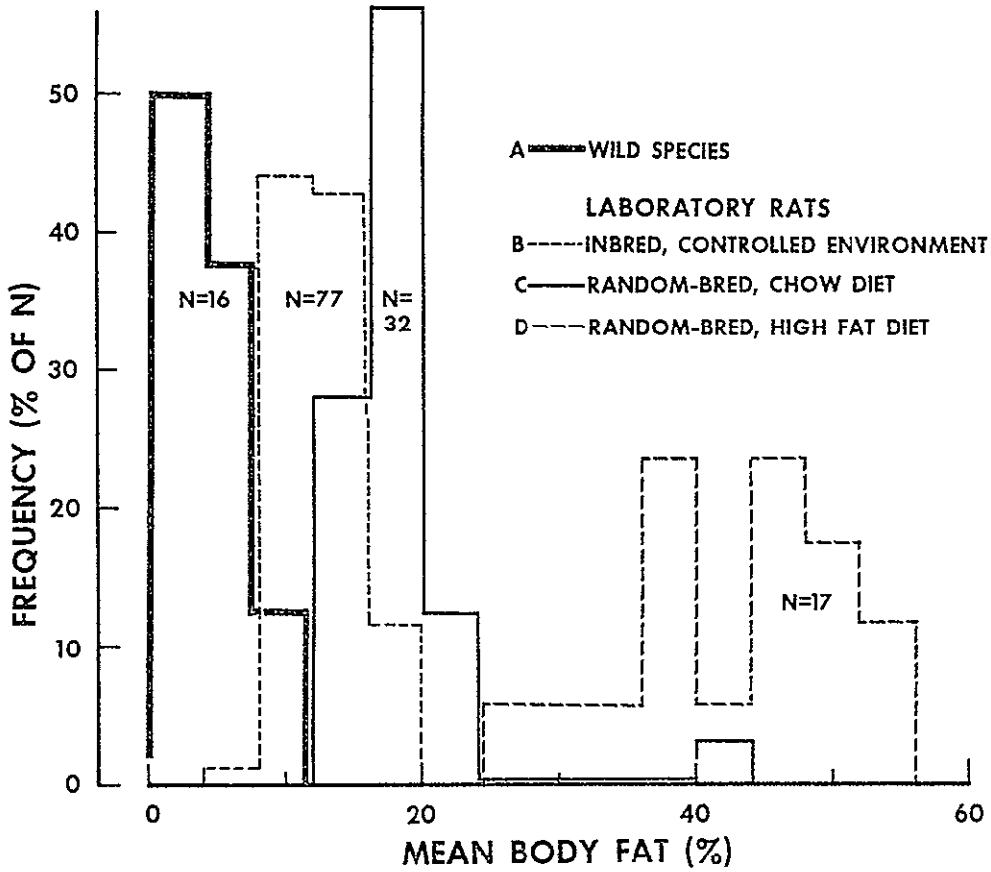

Fig. 3. A comparison of the frequency distributions of body fat in four groups: A - wild species, B - inbred Sprague-Dawley rats in a controlled environment, C - random-bred laboratory rats on a chow-type diet, D - random-bred laboratory rats on a high-fat diet. The bar graph for B is based on the frequency distribution of individuals. In the other three groups it is based on the frequency distribution of samples.

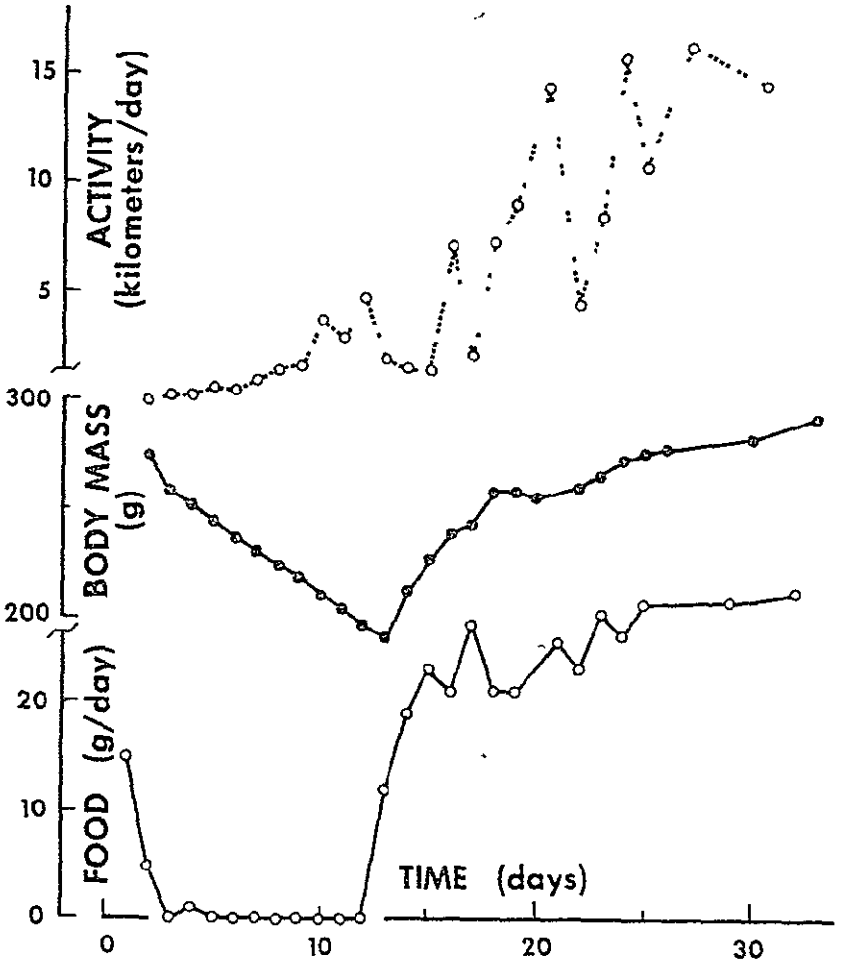

Fig. 4. Body mass, activity and food consumption on one female rat. Activity mode was voluntary running in a vertical wheel.

Table 1. Body Composition in Wild Mammals from Reference (61).

Species	Number	Means ± SD		
		Fat (g)	Fat (%)	FFBM (g)
Shrew:				
Sorex cinereus	7	.16 ± .08	4.54 ± 1.92	3.56 ± 1.01
Bats:				
Artibeus jamaicensis	14	3.79 ± 2.55	9.13 ± 5.78	36.18 ± 3.85
Artibeus lituratus	5	6.22 ± 2.37	9.56 ± 2.80	57.19 ± 6.39
Phyllostomus discolor	7	2.38 ± 1.96	6.60 ± 4.87	32.20 ± 2.25
Marmoset:				
Callithrix jacchus	4	8.77	4.40	176.2
Chipmunk:				
Eutamias minimus	22	1.08 ± 0.63	1.86 ± 1.03	59.08 ±12.51
Squirrels:				
Citellus undulatus	4	21	4.4	458
Tamiasciurus hudsonicus	11	3.8 ± 2.4	1.97 ± 1.13	189.0 ± 18.9
Mice:				
Peromyscus leucopus	9	.59 ± .14	3.41 ± .78	16.40 ± 2.03
Lemmus trimucronatus	5	.75 ± .38	1.86	40.87 ±15.88
Clethrionomys rutilus	20	.72 ± .32	2.90 ± 1.40	24.55 ± 4.93
Microtus pennsylvanicus	7	1.20 ± .47	3.76 ± 1.02	30.18 ± 5.89
Microtus oeconomus	9	0.45 ± .01	2.08 ± .89	24.38 ± 7.07
Microtus pinetorum	7	0.45 ± .22	2.36 ± 1.21	18.96 ± 2.69
Mus musculus	4	0.96	5.55	14.92
Musk rat:				
Ondatra zibethica	8	86 ± 17	7.35 ± 1.41	1094 ± 114

VII. GENERAL CONCLUSIONS

The following conclusions are somewhat more general than the detailed conclusions prompted by each reprint and manuscript presented above.

A. Acceleration

1. In a comparative study (mice, rats, monkeys, and men) at accelerations ranging from 0 to 4.75 G the mass of the fat-free compartment appeared to pass through a maximum at 1 G with lower values at both higher and lower G levels.

2. Nearly any change in chronic acceleration is followed by a reduction in body fat content. However, the fat-free compartment appears to be defended against perturbation at any one level of chronic acceleration. Indeed, changing the level of chronic acceleration is the only method we have found to change the fat-free body mass in the adult rat.

3. Growth with respect to both total body mass and the masses of individual body components is normal at accelerations greater than 1 G. However, the mature masses attained at G>1 are almost always lower than those attained at 1 G.

4. Early retirement from chronic G's>1 is followed by complete recovery to the 1 G condition. The effects of late retirement on body components are not known.

B. Exercise Studies.

1. Seven weeks of age appears to be a critical period in the life of the albino rat. The exercise (and dietary) regimens which we have studied are capable of perturbing the growth of the fat-free compartment when initiated at or before that age but have no effect when initiated after that age.

2. The body fat compartment is so labile and unpredictable that it interferes with the quantitative characterization of rat growth. A quantitative treatment of growth of the fat-free compartment would be much more significant and useful.

3. The effects of exercise on body composition are a function of exercise mode (swimming, treadmill, running-wheel), sex and age at which the regimen is initiated.

4. The composite entity live body mass is of little help in evaluating the response to exercise of individual body components, e.g., fat and the fat-free compartment.

5. Voluntary wheel-running starting at or before 7 weeks of age is the only exercise mode which increases FFBM.

C. Exercise Combined with Acceleration

1. Our preliminary study indicates that this combination is somewhat hazardous and allowance for mortality must be made in experimental plans.

2. The amount of voluntary wheel-running done by centrifuged rats is extremely low. This may be improved by letting rats become accustomed to wheel running and selecting good runners before centrifugation begins.

D. Restraint Studies

1. Restraint should be included in any complete program for the study of activity.

2. Early results on the effects of cage restraint upon weanling rats appear interesting and potentially informative.

E. Physiological Regulation

1. It has become clear that a knowledge of whether or not regulation is involved will be of great help in studying physiological responses to ΔG.

VIII. RECOMMENDATIONS

The following are recommendations for investigations which flow from the results presented above and which we believe to be highly desirable.

A. Our preliminary study of exercise during centrifugation convinces us that there is much to be gained in this line of research. We strongly urge a study of body composition involving the following groups:

1. Sedentary -- 1 G
2. Wheel-running -- 1 G
3. Restrained -- 1 g
4. Sedentary -- 2.76 G
5. Wheel-running -- 2.76 G
6. Restrained -- 2.76 G

B. We think it urgent to study weightless rats for several reasons. From the stand point of body composition effects it is highly desirable to corroborate for a single species the principal finding of our comparative study (II.A). Does the rat (or man) actually pass through a maximum of fat-free body mass at 1 G? We could answer this for the rat is we added data collected on weightless rats to those in hand on rats at 1, 2.76 and 4.15 G.

C. It is urgent to study stress in the rat. To what extent are the responses to ΔG (as well as to exercise and restraint) simply non-specific responses to stress ? A study of this sort should involve an endocrinologist experienced in adrenal studies.

D. Further studies should be made of the physiological regulation of the fat-free body mass. It is difficult to propose an effective approach to this topic at this stage of our knowledge. However, as a start it would be desirable to use intermittent exposure to ΔG (e.g., alternation between 1 G and 2.76 G for several cycles) and see whether the changes in FFBM which appear to be regulatory wane or persist undiminished.

E. Hypodynamia appears most promising, both as a model of weightlessness and as a toolin basic research. Various types of hypodynamia, e.g. harness restraint and cage restraint, should be studies comparatively.

IX. PUBLICATIONS

Pitts, G. C., Are centrifugation-induced changes in energy balance the result of physiological regulation? Life Sciences and Space Research. vol. 11, 1974. (Abstract)

Pitts, G. C., L. S. Bull and J. A. Wakefield. Exercise with force feeding in the rat. American Journal of Physiology 227:341-344, 1974.

Pitts, G. C., L. S. Bull and J. Oyama. Regulation of body mass in rats exposed to chronic acceleration. American Journal of Physiology 228:714-717,1975.

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