Effect of Acute Starvation and Refeeding on Body Composition of Rats Fed Previously at Different Levels of Dietary Protein [1,2]

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ABSTRACT The effect of acute starvation and refeeding on body composition was studied in rats receiving high and low protein diets. For this purpose, weanling Sprague-Dawley male rats were fed a high protein diet (20% casein) or a low protein diet (5% casein) during an 8-week period, at the end of which the animals were starved for 2, 4, and 6 days. Rats in both dietary groups were treated similarly and after 6 days of starvation, each group was refed for 8 days with 5% or 20% casein diets. Body composition was somewhat similar at both protein levels. During starvation, the 2 groups of rats used their body fat reserves efficiently, although the malnourished animals utilized them at a more rapid rate. Higher body protein content was observed in all animals receiving the 20% casein diet. Although some differences among the groups studied are evident when the comparison is made in terms of average percentages, these differences are not as great as expected if the level of protein in the diet, the age of the animals, the length of time fed the experimental diets and the stress of acute starvation and refeeding are considered. When total body water, protein, and ash were related to fat-free weight, highly significant correlations were obtained. This information suggests, therefore, that the composition of the lean body remains relatively constant throughout all the experimental conditions, and depends on body size in growing animals.

The effect of acute starvation in the rat at different levels of protein nutrition has been studied in relation to serum lipids (1) and serum proteins (2). These observations have shown that the status of protein nutrition in the rat produces a disparity in the course of response of serum components to acute starvation.

Direct chemical analysis of experimental animals under a variety of conditions of growth and suboptimal nutrient intake have indicated a remarkable constancy of body composition (3–6).[3] It has been shown, for instance, that protein nutrition had little or no effect on percentage body composition, and that total body proteins appear to be weight-dependent (6).

Although there are some questions in extrapolating the results from experimental animals to humans, the basic information which supported the derivation of many of the equations used in calculating body composition by indirect methods, was obtained from laboratory animals.

The constancy of the composition of the lean body mass has been an accepted assumption in most of the body composition work. The concept of "chemical maturity" has been the basis of the different indirect methods involving the lean body mass (7).

Although the chemical composition of different animals under various dietary conditions has been studied, there is no information on the changes in body components of protein-malnourished animals subjected to the stress of acute starvation nor on the effect of refeeding after starvation.

The experiments to be described were designed to test whether the composition of the lean body mass remains relatively constant in rats at different levels of protein nutrition when they are submitted to acute starvation and refeeding with diets containing 5 or 20% casein.

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[2] INCAP Publication I-363.

[3] Wallace, W. M. 1959 Nitrogen content of the body and its relation to retention and loss of nitrogen. Federation Proc., *18:* 1125 (abstract).

TABLE 1

Composition of diets

	5% Casein	20% Casein
Casein, g	5	20
Salt mixture,[1] g	4	4
Cellulose,[2] g	2	2
Cottonseed oil, ml	10	10
Cod liver oil, ml	1	1
Cornstarch, g	78	63
Vitamin mixture [3]		

[1] Hegsted, D. M., R. C. Mills, C. A. Elvehjem and E. B. Hart. J. Biol. Chem., *138:* 459, 1941.

[2] Alphacel, Nuritional Biochemicals Corporation, Cleveland.

[3] One hundred grams of diet were supplemented with 5 ml of a vitamin solution containing thiamine·HCl, 0.6 g; riboflavin, 0.6 g; nicotinic acid, 1 g; Ca pantothenate, 2 g; pyridoxine, 0.6 g; biotin, 2 mg; folic acid, 4 mg; vitamin B_{12}, 0.6 mg; inositol, 8 g; choline·HCl, 30 g; *p*-aminobenzoic acid, 6 g; menadione, 0.2 g; ethyl alcohol, 842 ml; distilled water to make 1 liter.

The experimental design was described in a previous report (2). The composition of the diets is shown in table 1. At the end of 8 weeks of feeding the experimental diets, 2 levels of protein nutrition were obtained: one group which had consumed the 5% casein diet and the other the 20% casein diet. As presented, each dietary group was divided into 6 subgroups of the same body weight. These groups were treated in the following way: subgroups 1, 2, 3, and 4 within each level of protein nutrition were starved for zero, 2, 4, and 6 days, respectively. Subgroups 5 and 6 were starved for 6 days and then refed for 8 days. Subgroups 5 within each dietary group were refed the low protein diet, while subgroups 6 were given the diet containing 20% casein. The animals were placed in individual all-wire screen cages with raised screen bottoms and were fed ad libitum. Water was available at all times. The rats were decapitated at the end of each experimental period, bled and the carcass was opened and carefully eviscerated.[4] The weight of the eviscerated animal was recorded.

The carcass and head were frozen, chopped and then ground in a meat grinder. This preparation was ground twice more in order to obtain a homogeneous sample. Aliquots of the samples were taken for water content determination. The dried samples were ground further in a laboratory-type Wiley mill and dried again to constant weight.

All methods used in determining the proximal composition were those given by the AOAC (8). Water content was determined in a vacuum oven at 70°. Fat was determined by continuous ether extraction in a micro-Soxhlet apparatus. Total nitrogen was obtained by the micro-Kjeldahl method using the modification of Hamilton and Simpson (9). Total proteins were calculated by the factor 6.25. The ash content was determined by calcination in a muffle furnace.

Analysis of variance was applied in the statistical study of the results. The multiple range test of Duncan (10) was used in the comparison of individual means. These statistical methods, and others used in the evaluation of the results, are those given by Snedecor (11).

RESULTS

The statistical comparisons shown in the following tables are those found within each dietary group. The comparisons between groups are not given in the tables.

In table 2, the body composition of the animals is expressed as the percentage of fresh tissue. No significant difference in initial pre-starvation values in water and fat content were observed (subgroups 1). Protein percentage was higher and ash lower in the group fed the 20% casein diet.

During starvation, the low protein group had an increase in water and protein, a decrease in fat, reaching very low values, and no significant changes in ash concentration. The high protein group, however, did not show any significant change in the percentage of the different body components. At the end of starvation, water and ash percentages were higher and fat lower in the 5% casein group.

During refeeding there was no significant change in water concentration when animals of both dietary groups were refed either of the diets. The water content of the animals in the low protein group, however, when refed the diet containing 20% casein after starvation, (5% casein group, subgroup 6), was higher than that of the other dietary group when refed the same

[4] The carcass was completely emptied of all viscera. Blood was not included in the body materials analyzed.

TABLE 2

Effect of acute starvation and refeeding on body composition of rats previously fed different levels of dietary protein [1]

Sub group	No. days starved	Casein refed post-starvation	Carcass wt		Water		Fat		Protein		Ash	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
		%	*g*		*% wet wt*		*% wet wt*		*% wet wt*		*% wet wt*	
					5% casein group							
1	0	—	82	20	59.07	1.44	14.99	2.17	19.68	1.13	4.24	0.76
2	2	—	72	16	59.67	3.21	12.70	4.23	21.25	1.01*	4.28	0.32
3	4	—	69	15	60.83	1.44	10.06	1.89**	20.82	1.06	4.58	0.33
4	6	—	61	10	64.92	1.80**	5.83	2.11**	21.91	1.04**	4.64	0.41
5	6	5	73	15	63.68	1.80	11.35	1.52**	18.86	0.75**	4.42	0.33
6	6	20	106	9	64.84	1.00	12.35	1.35**	18.21	0.78**	3.56	0.29**
					20% casein group							
1	0	—	267	35	61.43	2.85	12.40	3.63	22.26	1.27	3.20	0.31
2	2	—	250	34	63.37	1.96	9.60	2.14	21.74	1.07	3.09	0.62
3	4	—	234	32	59.79	4.08	11.72	4.44	21.96	1.10	3.42	0.20
4	6	—	231	27	61.48	2.70	9.52	2.55	23.07	1.30	3.40	0.37
5	6	5	250	32	61.32	1.69	13.23	2.11*	21.53	0.84*	3.64	0.41
6	6	20	265	30	61.47	2.84	12.75	3.35*	20.71	1.23**	3.19	0.27

* Significant difference at $P < 0.05$, and ** significant at $P < 0.01$, when starvation values are compared with initial subgroups no. 1 and refeeding values are compared with final starvation subgroups no. 4 within each dietary group.

[1] N = 6 in all subgroups, except in subgroup no. 6 of the 5% casein group, where N = 4.

20% casein diet (20% casein group, subgroup 6).

The fat concentration increased significantly during refeeding, although the increase in the group previously fed the low protein diet was more pronounced. This group was also the one which during starvation had had the most drastic decrease in fat content.

The protein concentration during refeeding decreased in both dietary groups. The groups previously fed the low protein diet showed lower protein percentages than the other dietary group when they were refed either of the diets.

Finally, the ash content decreased significantly only in the low protein group when refed the 20% casein diet.

From these results, it appears that the main effect produced during starvation and refeeding is the disproportional change in the fat content that makes the other components vary in their percentage concentration.

If instead of comparing group percentages, the individual total weight body components are related to the fat-free carcass weight (FFW), then highly significant correlations are obtained. These relationships are shown in the following regression equations:

$$\text{Total water} = 0.29 + 0.6942\ (\text{FFW})$$
$$r = 0.9986;\ s_{yx} = 2.963.$$
$$\text{Total protein} = -1.81 + 0.2554\ (\text{FFW})$$
$$r = 0.9945;\ s_{yx} = 2.154.$$
$$\text{Total ash} = 0.94 + 0.03356\ (\text{FFW})$$
$$r = 0.9623;\ s_{yx} = 0.763.$$

The linear regressions account for 99.7, 98.9 and 92.6 of the variation for the total body water, protein and ash relationships, respectively.

Because of the different groups involved in the regressions, covariance analysis was necessary in order to determine whether all the lines of the individual subgroups were parallel; that is, if there were no significant differences in the slopes of the different subgroups. According to this analysis, a proof of the homogeneity of the variances is necessary before the comparison is made. Therefore, the Bartlett test for the homogeneity of variances was performed with the total weight of body water, protein, ash and fat-free body weight data. The results of the test gave chi-square values of 13.58, 19.49, 45.44 and 14.34, respectively, with 11 degrees of freedom. Only the chi-square test for the ash content was significant. On this basis, therefore, the analysis of covariance technique can be adequately applied only in the relationships water/fat-free weight and protein/fat-free weight. Because of the close association of total water and total protein to the fat-free weight, the correlation between water and protein was calculated. This correlation was also highly significant and the regression equation was:

$$\text{Total protein} = -1.70 + 0.3658\ (\text{total water})$$
$$r = 0.9905;\ s_{yx} = 2.847.$$

The analysis of covariance of the different regression lines in which the variances were homogeneous and also in the case of ash content is shown in table 3. The regression coefficients for the individual subgroups were not significantly different and the regression lines did not deviate significantly from the common regression. The adjusted means of the subgroups showed significant differences. Table 4 shows the average total weight body components for the different groups studied. They summarize the individual values used in the calculation of the different regressions given above.

DISCUSSION

If the level of protein in the diets, the age of the animals and the length of time they were fed the experimental diets were considered, it could be logically expected that the animals in the low protein diet group would show marked differences in body composition. The results presented in this paper, when expressed on a percentage basis, either in terms of fresh tissue or in fat-free weight, give no indication of marked differences in body components. The significant differences pointed out, although of statistical value, failed to give evidence of a profound effect produced by the level of the protein fed. During starvation, there is a marked reduction in the percentage of fat in the group previously fed the 5% casein diet, indicating a faster depletion of their fat reserves. The differences in the percentage of ash throughout the experimental

TABLE 3

Analysis of covariance with total body components [1]

	df	Fat-free weight			Water-protein
		Water	Protein	Ash	
Within	46	3.845	3.632	0.408	5.355
Regression coefficient	11	4.816	2.274	0.657	3.630
Common	57	4.033	3.370	0.456	5.022
Adjusted means	11	33.369*	11.022*	1.239*	24.082*

[1] Mean squares.
* Significant at $P < 0.01$.

TABLE 4

Effect of acute starvation and refeeding on total body components of rats previously fed different levels of dietary protein

	Starvation, days								Refeeding			
	0		2		4		6		5% casein		20% casein	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	Fat-free wet weight, g											
LP [1]	69.7	15.4	62.4	13.1	62.1	14.1	57.1	9.4	64.9	12.2	92.9	6.8
HP	232.8	22.8	225.5	27.1	205.4	22.4	208.7	23.6	216.6	27.7	230.8	22.9
	Total fat, g											
LP	12.7	4.8	9.5	4.2	6.8	1.0	3.6	1.3	8.4	2.5	13.4	2.5
HP	34.0	13.9	24.5	8.3	28.3	13.6	22.2	7.1	33.2	7.2	34.2	11.6
	Total water, g											
LP	48.5	10.9	42.6	9.0	42.0	9.6	39.3	6.3	46.5	8.4	68.7	5.1
HP	163.2	15.9	158.0	17.8	138.9	14.1	141.5	13.5	153.1	19.6	162.6	16.4
	Total protein, g											
LP	16.1	3.8	15.1	2.9	14.4	3.4	13.3	2.2	13.9	3.2	19.5	2.4
HP	59.2	6.2	54.4	7.9	51.3	7.3	53.3	7.1	53.9	8.0	54.7	4.8
	Total ash, g											
LP	3.4	0.3	3.0	0.6	3.2	0.6	2.8	0.4	3.2	0.6	3.8	0.3
HP	8.5	0.7	7.8	2.1	8.0	0.8	7.9	1.3	9.2	2.2	8.5	1.2

[1] LP indicates low protein; HP, high protein.

periods appear to be valid and consistent. The groups fed the 5% casein showed higher values than those fed the diet containing 20% casein.

The results presented here confirm the conclusion of Weil and Wallace (6) who have shown that animals under a variety of conditions of growth and suboptimal nutrient intake have a remarkably constant body composition per unit of body size. Widdowson and McCance (3) have shown that neither rats maintained with poor protein diets nor well-nourished rats subjected to starvation appear to have significant changes in their concentration of body protein on a fat-free basis. It can be added from the present work that animals with different levels of protein nutrition, especially malnourished animals during starvation, also showed a remarkable constancy of body composition, particularly on a fat-free basis.

The expression of body composition in terms of percentages or ratios has been criticized and the comparison of groups in terms of such averages may be misleading (12). In this respect, the comparison of the average percentages suggested some differences among the groups studied. However, the correlation coefficients showed a very close association between grams of fat-free weight and grams of total body water, protein or ash regardless of the dietary group or experimental condition. Also, the single linear regression lines account for more than 98% of the

variation for water and protein to fat-free weight, and more than 92% for ash to fat-free weight. The extreme closeness of fit suggested that the differences indicated by group comparisons might be "random phenomena or of questionable biological significance" (12).

It thus appears that the constancy of the composition of the lean body mass holds for animals in extreme levels of protein nutrition and also in cases of superimposed stress of acute starvation or refeeding after starvation. These relationships show that the body composition of the growing animals is size-dependent and that the dietary treatments and experimental conditions imposed had no effect on the relative distribution of body components, independent of its body size. The relationship of body composition and size has been proposed previously for ruminants by Reid et al. (13) and for the rat by Weil and Wallace (6). This conclusion, however, does not apply to animals after maturity, when growth has stopped and body fat becomes the only component dependent on diet.

The data presented here preclude the possibility that animals which consumed diets low in protein content and whose daily intake is lower would accumulate less body fat, assuming that the low food intake would not satisfy their caloric requirements. The percentage of body fat of the protein-malnourished animals was as high as that of the animals given the high protein diet. This observation has been indicated previously by Wallace.[5] It appears, therefore, that rats under dietary restriction during growth, adapt their metabolism to the diet, stopping their somatic growth. As a consequence, this results in small bodies with a harmonic distribution of body components which did not differ greatly from that in animals which had been maintained under optimal dietary conditions whose body size is three to four times greater.

Although body weight has been widely used as a measure of body size in nutrition, it has been severely criticized on the basis of its poor definition of body components. The information presented here, as well as the evidence given by others (6, 13), however, supports very strongly the use of body weight in growing animals as one of the best indicators of nutriture. Body weight, therefore, should be used with more assurance in the evaluation of the nutritional status in man and for the classification of malnutrition in children (14).

Although the results from animal experimentation should be extrapolated to humans only with caution, the possibility exists that the population groups in many of the developing areas which have subsisted under restricted diets, especially in quantity and quality of their dietary protein, could have a similar mechanism of adaptation to that discussed above. This would result in decreasing the rate of growth, giving small bodies but with a relatively similar composition to that of populations consuming adequate diets. It has been reported in this respect that the rural Guatemalan Indians (15) are shorter and lighter than the well-nourished urban population in Guatemala City. The weight-to-height ratio, however, is similar in both population groups when compared at the same heights, probably indicating a decrease in the rate of growth. The clinical impression is that rural Guatemalan Indians are well-endowed with muscle mass, although their skinfold thickness is small. These observations are also confirmed by different anthropometric measurements (15).

In the rat, protein nutrition or starvation of animals having different levels of protein nutrition, or refeeding after starvation, does not produce drastic changes in the composition of the lean body mass. This lends a good degree of confidence for the application of methods involving the assumption of the constancy of the lean body mass, to the study of body composition in population groups of different nutritional status or racial origin.

In addition, the close association of body size and body components in the growing rat also supports the use of body weight as a valuable index in the biological assay of protein quality. In this way not only the values derived from body weight per se can be calculated, but all other indexes involving body components as well.

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[5] See footnote 3.

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