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Effect of Dietary Protein Level Prior to Acute Starvation on Serum Proteins in the Rat [1,2]

JOSÉ MÉNDEZ AND MARÍA TERESA MENCHÚ
Institute of Nutrition of Central America and Panama (INCAP), Guatemala, Central America

ABSTRACT The effect of acute starvation and refeeding on serum proteins was studied in rats receiving high and low protein diets. The levels for total proteins, albumin and α_1-globulin were significantly lower in the group receiving the low protein diet before starvation. No significant differences were observed in α_2-, β- and γ-globulin fractions. During starvation, the rats that had consumed the low protein diet showed a significant increase in total protein and albumin, a decrease in α_1- and β-globulin, and no significant change in α_2- and β-globulin. The rats fed the high protein diet, on the other hand, showed a progressive and significant decrease in total proteins, α_1- and β-globulin, an increase in γ-globulin and no significant change in albumin and α_2-globulin fractions. During refeeding, the rats fed the low protein diet prior to starvation, showed a significant decrease in albumin, and an increase in α_1-globulin when refed either diet. The rats fed the high protein diet prior to starvation, showed during refeeding, an increase in total proteins and albumin when refed the high protein diet and an increase in α_1-globulin when refed either diet. The plane of protein nutrition of an animal, therefore, influences the response to acute starvation.

It has been shown that the biochemical response of an animal to acute starvation is closely related to its nutritional status. This was demonstrated in relation to serum lipids when rats were fed, prior to starvation, diets containing 5 or 20% casein. In this case, the rats that had consumed the low protein diet showed, during starvation, an increase in the concentration of all serum lipid fractions, whereas rats fed the high protein diet had a marked and significant decrease (1).

Although the effect of starvation on serum proteins has been studied in animals and humans (2), there is no specific information in the literature about the effect of starvation on serum proteins in animals on different planes of nutrition. It is possible, as in the case of serum lipids, that the protein nutritional status of an animal might influence the response of its serum proteins to acute starvation. Furthermore, changes in serum protein fractions, especially those related to lipid transport, may contribute to an explanation of the changes in serum lipids during starvation.

The following study was undertaken to investigate the effect of acute starvation on serum proteins in growing rats that were fed a 20% casein diet as compared with those receiving a diet with only 5% casein. Serum lipid changes were also studied as an indicator of the starvation effect.

MATERIAL AND METHODS

Experimental design. Seventy-two weanling male rats of the Sprague-Dawley strain were divided into 2 groups, each having the same average weight of 76 g, and placed in individual cages with raised screen bottoms. One group was fed ad libitum a diet containing 5% casein and the other group, 20% casein during 8 weeks. The composition of these diets is shown in table 1. Body weight was recorded weekly and food consumption was determined during 3 days at the end of the sixth week. The rats fed the 20% casein diet consumed an average of 15.2 g/day, whereas the rats fed the 5% casein diet consumed only 8.3 g/day.

At the end of the eighth week, each dietary group was divided into 6 subgroups of the same average body weight. These

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[2] INCAP Publication I-361.

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. G. Hart. J. Biol. Chem., *138:* 459, 1941; obtained from Nutritional Biochemicals Corporation, Cleveland.
[2] Alphacel, Nutritional Biochemicals Corporation.
[3] Each 100 g 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; and distilled water, to make 1 liter.

groups were treated in the following way: subgroups 1, 2, 3 and 4 within each dietary group were starved for periods of zero, 2, 4 and 6 days. Subgroups 5 and 6 within each dietary group were starved during 6 days and then refed during 8 days. In each dietary group, subgroups 5 were refed the 5% casein diet, and subgroups 6 were refed the 20% casein diet. Water was available at all times. The rats were decapitated at the end of each experimental period and blood for serum separation was collected.

Methods. Serum total lipids, cholesterol and lipid phosphorus were determined by micromethods adapted from those of Bragdon (3), Abell et al. (4), and Chen et al. (5), respectively. Serum total proteins were determined on the day of the sample collection, using the gradient tube method of Lowry and Hunter (6). Serum protein fractions were separated by paper electrophoresis by the method of Grassmann et al. (7) using 10 μliters of sample on Whatman no. 1 paper strips, and an acetate-barbital buffer, pH 8.6, with an ionic strength of 0.075. A current of 100 v was applied for 16 hours. The individual fractions were then determined colorimetrically after staining with Amido Black and eluting with 2 N sodium hydroxide solution. The method of Morrison and Slocum (8) was used for calculating the relative amounts of serum protein fractions. The absolute concentrations were obtained from the serum total proteins.

Analysis of variance (9) was applied in the statistical study of the results. The multiple range test of Duncan (10) was used in the comparison of individual means.

RESULTS

In table 2, the results in serum lipid fractions are shown. No significant differences in pre-starvation values were observed. Serum total lipids, cholesterol and lipid phos-

TABLE 2
Effect of dietary protein level prior to acute starvation and following refeeding on serum lipid concentration in the rat

Subgroup	No. days starved	Casein refed post-starvation	Body wt	Total lipids	Cholesterol	Lipid phosphorus
		%	*g*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*
				5% Casein group		
1	0	—	115±25 [1]	400±28	85±8	5.7±0.5
2	2	—	—	483±60*	127±25**	9.4±2.0**
3	4	—	—	421±53	97±18	6.9±1.7
4	6	—	78±14	369±104	96±18	5.8±1.0
5	6	5	93±18	444±69	111±17	7.1±1.5
6	6	20	97±18	476±74*	119±12*	8.3±1.2*
				20% Casein group		
1	0	—	332±42	399±80	79±15	6.0±1.5
2	2	—	—	335±97	65±17	6.1±0.7
3	4	—	—	280±33**	46±17**	3.7±0.9**
4	6	—	272±30	247±34**	55±16**	3.5±0.9**
5	6	5	304±42	405±81**	95±14**	7.6±1.8**
6	6	20	329±42	514±86**	96±8**	7.1±2.1**

* Significant difference at $P \leq 0.05$, and ** significant difference at $P \leq 0.01$, when serum lipid 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] Mean ± SD.

TABLE 3

Effect of dietary protein level prior to acute starvation and refeeding on serum protein concentration in the rat

Subgroup	No. days starved	Casein refed post-starvation	Total proteins	Albumin	Globulin α_1-	Globulin α_2-	Globulin β-	Globulin γ-
		%	g/100 ml	g/100 ml	g/100 ml	g/100 ml	g/100 ml	g/100 ml
				5% Casein group				
1	0	—	5.86 ± 0.14 [1]	2.45 ± 0.26	1.11 ± 0.16	0.68 ± 0.10	0.93 ± 0.12	0.67 ± 0.17
2	2	—	6.28 ± 0.66*	3.13 ± 0.35**	0.99 ± 0.16	0.69 ± 0.13	0.96 ± 0.16	0.50 ± 0.13
3	4	—	6.68 ± 0.28**	3.59 ± 0.27**	0.90 ± 0.21*	0.64 ± 0.11	1.01 ± 0.20	0.52 ± 0.11
4	6	—	6.28 ± 0.50*	3.33 ± 0.50**	0.87 ± 0.10*	0.67 ± 0.10	0.94 ± 0.09	0.47 ± 0.10*
5	6	5	5.78 ± 0.26	2.32 ± 0.08**	1.10 ± 0.19*	0.78 ± 0.18	0.85 ± 0.10	0.65 ± 0.26
6	6	20	5.99 ± 0.29	2.86 ± 0.19*	1.11 ± 0.14*	0.66 ± 0.03	0.87 ± 0.07	0.48 ± 0.04
				20% Casein group				
1	0	—	7.22 ± 0.32	3.28 ± 0.24	1.84 ± 0.36	0.60 ± 0.16	0.99 ± 0.14	0.51 ± 0.08
2	2	—	7.01 ± 0.27	3.20 ± 0.24	1.75 ± 0.13	0.56 ± 0.07	0.91 ± 0.24	0.59 ± 0.13
3	4	—	6.56 ± 0.24**	3.33 ± 0.19	1.33 ± 0.30**	0.57 ± 0.06	0.69 ± 0.10**	0.63 ± 0.22
4	6	—	6.37 ± 0.28**	3.19 ± 0.16	1.17 ± 0.11**	0.57 ± 0.06	0.72 ± 0.07**	0.71 ± 0.13*
5	6	5	6.53 ± 0.53	2.86 ± 0.19	1.41 ± 0.12*	0.65 ± 0.11	0.86 ± 0.20	0.74 ± 0.14
6	6	20	7.27 ± 0.42**	3.64 ± 0.32*	1.49 ± 0.27**	0.72 ± 0.06	0.86 ± 0.13	0.55 ± 0.19

* Significant difference at $P \leqq 0.05$, and ** significant difference at $P \leqq 0.01$, 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] Mean ± SD.

phorus concentrations during starvation in the low protein group increased significantly in the first periods, and at the end they decreased toward the initial values. The high protein group, in contrast, showed a marked decrease in all lipid fractions. The final levels at the end of starvation were significantly higher in the group fed the low protein diet prior to starvation. When the group receiving the low protein diet was refed with either of the diets, an increase in all values was obtained. This increase, however, was significant only when the diet containing 20% casein was fed. When the animals in the high protein group were refed, after starvation, with either of the diets, a significant increase was also observed. In this group the animals refed with the high protein diet showed significantly higher values in total lipids than those refed the low protein diet. The cholesterol levels at the end of the refeeding period were higher in the group fed the 5% casein diet prior to starvation.

Serum total proteins and the electrophoretic fractions expressed in absolute concentrations are given in table 3. The pre-starvation levels for total serum proteins, albumin and α_1-globulin were significantly lower in the group receiving the 5% casein diet. No significant differences were observed in α_2-, β-, and γ-globulin fractions, although the γ-globulin concentration tended to be higher in this group.

The animals fed the low protein diet prior to starvation showed, during starvation, a significant increase in total proteins and albumin, and a decrease in α_1- and γ-globulin. No significant change was observed in α_2- and β-globulin. The high protein group, however, showed a progressive and significant decrease in total proteins, α_1- and β-globulin and an increase in γ-globulin. No significant change was observed in albumin and α_2-globulin fractions. The final values at the end of the starvation period were significantly lower for α_1- and γ-globulin and greater for β-globulin in the group fed the 5% casein diet prior to starvation.

During refeeding, the animals fed the 5% casein diet, prior to starvation, showed a significant decrease in albumin and an increase in α_1-globulin when refed either diet. The animals fed the 20% casein diet, prior to starvation, showed during refeeding an increase in total proteins and albumin when given the diet containing 20% casein and an increase in α_1-globulin when refed either diet.

DISCUSSION

The data on acute starvation presented here, demonstrate that the plane of protein nutrition in the rat affects significantly the response to acute starvation in terms of serum protein concentration. In the two dietary groups studied, the different behavior of the serum protein fractions indicates that hemoconcentration does not explain the changes which occurred. If changes in hemoconcentration had been the only operative mechanism, then all serum fractions would have changed proportionally in the same direction.

Rutman et al. (11), on the basis of the incorporation of methionine-^{35}S by liver slices, have demonstrated that the effect of fasting on the rat previously fed a low protein or protein-free diet is to increase the rate of incorporation. Fasting of rats previously fed a diet of higher protein content, however, reduces the rate of incorporation. If incorporation is assumed to result from net protein synthesis, then fasting produces dissimilar effects in protein synthesis in animals fed low or high protein diets.

Kaufmann and Wertheimer (12) have described a "fasting effect" on total nitrogen release from liver slices of rats fed diets of different protein content. They observed a proportionally greater nitrogen release in rats fed a protein-free diet or a restricted intake prior to starvation than rats fed a stock or a high protein diet. If the discharge of nitrogen from liver slices can be assumed to represent a release of protein in vivo, then there is a marked difference in the net amount of protein liberated into the circulation.

From the above discussion, it could be inferred that the increase in serum proteins observed during starvation in the group of rats fed the low protein diet prior to this stress, is due to a selective increase in protein synthesis, and protein mobilization. The results following refeeding could also be attributed to the same mechanism of protein synthesis and mobilization as it has been observed that the total nitrogen release from liver slices of rats fed a low protein diet is lower than that of rats fed a normal one (12), and that the synthesis of proteins, as indicated by the incorporation of labeled methionine, is also greater in rats fed a normal or high protein diet (11).

The results on the serum lipid fraction presented in this paper confirm previous observations (1), which showed that the plane of nutrition of the rat affects the response to acute starvation. The fact that the 20% casein group of animals that had showed, during starvation, a marked decrease in all serum lipid fractions, also showed a decrease in α_1- and β-globulin concentration, is understandable in view of the association between both constituents in the blood plasma. Alpha- and β-globulin fractions are the main cholesterol carriers (13). Direct determinations of α- and β-lipoprotein concentrations during acute starvation may help to confirm this interpretation.

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