Effect of Dietary Protein Level and Cholesterol Supplementation Prior to Acute Starvation on Serum and Liver Lipids in the Rat

By José Méndez

The effect of acute starvation on serum and liver lipids has been studied in rats receiving high and low protein diets, with or without cholesterol and cholic acid supplementation. The rats which had consumed the low protein diet alone, showed during starvation an increase in the concentration of all serum lipid fractions, while rats on the high protein diet had a marked and significant decrease. The groups on the diet supplemented with cholesterol and cholic acid had higher initial values that dropped rapidly during starvation. This change, however, was more pronounced in the group receiving the high protein-cholesterol supplemented diet. The animals on the low protein cholesterol supplemented diet maintained higher serum lipid levels throughout the experiment. Changes in liver lipids were also observed among the dietary groups studied. It was concluded, therefore, that serum and liver lipids change differently with starvation in animals on a low protein diet than in animals on a high protein diet. No mechanism for such differences can be postulated at present.

IN STUDIES OF LIPID METABOLISM the procedure and degree of fasting prior to the collection of blood or tissue samples may alter the results obtained. Although attention has been given to the effect of fasting on blood lipid levels, reports in the literature are controversial. It has been suggested, however, that the nutritional status of an animal might influence its response to starvation.[1-3] The following study will discuss the effect of acute starvation on serum lipid levels and liver composition in growing rats which are fed a 20 per cent casein diet as compared to those receiving a casein diet of only 5 per cent, supplemented or not with cholesterol.

Material and Methods

During a period of 4 weeks, 72 weanling male rats of the Sprague Dawley strain were fed ad libitum the laboratory chow used in the stock colony. They were then divided into 2 groups each having the same average weight of 82 Gm., placed in individual cages with raised screen bottoms, and were fed ad libitum either a 5 or 20 per cent casein diet during a 7-week period. The diets contained cottonseed oil 10 per cent, salt mixture 4 per cent, cellulose (Alfacel) 2 per cent, cod liver oil 1 per cent, and were made isocaloric by substituting the casein with an equivalent weight of cornstarch. All diets were further supplemented with 5 ml. of a complete vitamin solution[4] per 100 Gm. of diet. The

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average food consumption during the seventh week was 8 and 15 Gm. per day for the 5 and 20 per cent casein groups, respectively. At the end of this period, the rats on the 5 per cent casein diet had an average weight of 131 Gm., while the weight for those on the 20 per cent casein diet was 308 Gm. At this time each dietary group was divided into 6 subgroups containing 6 rats having the same average weight. Within each dietary group, 3 subgroups received, for 3 more weeks, the plain diet, while for the other 3 subgroups the diet was further supplemented with 1 per cent cholesterol and 0.5 per cent cholic acid. The rats in the subgroups were then starved for zero, 48, or 96 hours, respectively, before bleeding and sacrifice. Water was available at all times. The rats were decapitated. Blood for serum preparation was collected. Livers were removed, weighed, minced and prepared for chemical analysis.

Serum total lipids were determined by Bragdon's method,[5] lipid phosphorus by that of Chen et al.,[6] and total cholesterol by the method of Abell et al.[7] adapted for micromethods. Liver water was determined in a vacuum oven according to the AOAC,[8] liver total fat by the microsoxhlet continuous ether extraction, liver total cholesterol by the method of Abell et al.,[7] using an alcoholic potassium hydroxide autoclave hydrolyzate, and liver lipid phosphorus by the method of Chen et al.,[6] using Bloor's alcohol-ether mixture extraction. Liver composition was expressed on a fresh tissue basis.

Results

Table 1 shows the weight changes during the starvation periods. At the end of the feeding period the rats on the 20 per cent casein diets were 3 times as heavy as the rats on the 5 per cent casein diets. The net weight loss during starvation was significantly greater for the former groups than for the latter, although the weight loss expressed as per cent of body weight was significantly greater for the 5 per cent casein groups.

The serum lipid levels during the starvation periods are given in table 2. The rats which had consumed the plain diet containing 5 per cent casein showed, during starvation, an increase in concentration of all lipid fractions, while rats on the 20 per cent casein diet had a marked and significant decrease. The rats which received the cholesterol-cholic acid supplementation had higher initial values of all lipid fractions, with the exception of lipid phosphorus, and during starvation a rapid drop was observed in the first 48-hour period. It is important to point out that the rats receiving the 5 per cent casein-cholesterol diet maintained higher serum lipid levels throughout the experiment than rats receiving the 20 per cent casein-cholesterol diet.

The changes in liver composition are given in table 3. Although the animals on the 20 per cent casein diets showed heavier livers, the liver weights expressed as per cent of body weight were relatively greater for the 5 per cent casein groups. The net liver weight loss during starvation is much greater in the animals fed prior to starvation, either of the 20 per cent casein diets. Liver weight was greater in the group receiving the cholesterol supplementation within each protein level.

The rats receiving the diets supplemented with cholesterol and cholic acid showed higher liver fat content than those receiving the plain diets. All animals on the 5 per cent casein diet showed higher initial values of liver fat than those on the 20 per cent casein diet within each dietary treatment. During starvation, an increase in the first 48 hours, followed by a significant

Table 1.—*Effect of Dietary Protein Level and Cholesterol Supplementation Prior to Starvation on Body Weight Changes in the Rat*

Starvation hr.	Initial wt.		Final wt.		Net. wt. Loss		% wt. Loss	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
				5% Casein Diet				
0	139	38	—	—	—	—	—	—
48	138	29	123	29	14.2	1.7	10.7	2.5
96	137	23	111	21	25.3	3.6	18.7	2.4
				5% Casein-Cholesterol Diet				
0	138	28	—	—	—	—	—	—
48	143	32	126	31	17.5	0.8	12.6	2.2
96	132	24	107	22	25.5	2.2	19.6	2.4
				20% Casein Diet				
0	381	36	—	—	—	—	—	—
48*	368	56	343	54	25.6†	3.3	7.0†	0.8
96	382	40	336	39	46.2†	3.7	12.2†	1.3
				20% Casein-Cholesterol Diet				
0	366	38	—	—	—	—	—	—
48	376	35	348	34	27.8†	2.5	7.5†	0.7
96	373	35	326	32	47.0†	4.6	12.6†	1.2

*Only 5 rats were included; other groups included 6 rats. Body weight is given in Gm.
†Significant difference at $P < 0.01$ when 5 per cent casein groups are compared with 20 per cent casein groups. Only net weight loss and per cent weight loss were compared.

decrease, is seen in the groups receiving 5 per cent casein. This increase is only significant in the rats supplemented with cholesterol. During starvation, all groups receiving 20 per cent casein had a consistent and significant increase, but the major change took place during the first 48 hours of starvation.

Although the rats receiving 20 per cent casein diet showed higher initial liver lipid phosphorus values than those on the 5 per cent casein diet, the differences were not statistically significant. A highly significant increase was observed in both groups during the first period of starvation. The animals fed the 20 per cent casein-cholesterol diet, however, did not show this significant change of liver lipid phosphorus, but the animals receiving the 5 per cent casein-cholesterol diet had, during starvation, a consistent and highly significant increase in liver lipid phosphorus. It is interesting to observe the wide difference of initial values in the groups supplemented with cholesterol; the animals on the 5 per cent casein-cholesterol diet had a lower liver lipid phosphorus concentration.

The striking differences in liver cholesterol between the animals supplemented with cholesterol and those receiving the plain diets, are evident. There is about 17 times more liver cholesterol in the cholesterol-supplemented groups. The animals on the 5 per cent casein diets, however, had higher liver cholesterol than those on the 20 per cent casein diets within each

Table 2.—*Effect of Dietary Protein Level and Cholesterol Supplementation Prior to Starvation on Serum Lipid Concentration in the Rat*

Starvation hr.	Total Lipids		Lipid Phosphorus		Cholesterol	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
			5% Casein Diet			
0	426	119	7.0	0.9	86	12
48	580†	71	7.7	1.2	116†	23
96	510	73	6.6	1.7	103	38
			5% Casein-Cholesterol Diet			
0	710	129	8.4	1.8	221	52
48	578	122	6.1	2.1	142†	62
96	600	115	6.8	1.6	140†	45
			20% Casein Diet			
0	565	52	7.3	1.4	87	16
48*	403‡	28	4.4‡	1.3	54‡	17
96	349‡	82	3.2‡	0.9	42‡	11
			20% Casein-Cholesterol Diet			
0	630	59	6.0	0.5	170	16
48	412‡	53	3.4‡	0.4	76‡	16
96	379‡	51	3.1‡	0.9	66‡	18

*Only 5 rats were included; other groups included 6 rats. Serum lipid concentrations given in mg./100 ml.

†Significant difference at $P < 0.05$ and

‡Significant difference at $P < 0.01$, when compared with values at zero hours of starvation.

treatment. During starvation there is a consistent and significant increase, with the exception of the group on the 5 per cent casein-cholesterol diet which showed a marked and significant increase only in the first period of starvation.

Discussion

Although the effect of starvation on blood lipids has been extensively studied, the results using a great variety of animals are erratic. Keys et al.[9] have reviewed the literature in regard to the blood changes observed during starvation. The response to acute starvation of animals at different levels of nutrition has not as yet been fully explored.

From data here presented it is evident that the protein nutritional status of the animal prior to starvation influences its effect on serum lipid concentration. In this case the animals fed a 5 per cent casein diet showed a small increase in serum lipid levels while the animals fed the 20 per cent casein diet showed a marked decrease. The serum lipid changes observed in these experiments are consistent for the 3 lipid classes studied within the dietary treatments.

The results reported here may be explained by considering the two dietary groups as having opposite changes in: (a) hemoconcentration, (b) the rate

Table 3.—*Effect of Dietary Protein Level and Cholesterol Supplementation Prior to Starvation on Liver Composition in the Rat*

Starvation hr.	Liver wt.		Fat		Lipid Phosphorus		Cholesterol	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
			5% Casein Diet					
0	5.8	1.7	10.0	3.7	69	10	418	79
48	4.3	0.7	12.5	3.0	108‡	15	550†	114
96	4.0†	0.6	9.2	4.7	108‡	19	670	290
			5% Casein-Cholesterol Diet					
0	8.7	1.8	21.6	1.7	51	10	7670	614
48	6.5†	1.6	25.5†	3.0	76†	20	9671‡	1110
96	5.8‡	0.6	22.3	4.2	101‡	11	9652†	1630
			20% Casein Diet					
0	13.4	0.9	2.7	0.4	73	7	248	6
48*	8.5‡	1.1	7.7‡	0.9	121‡	26	425‡	24
96	8.0‡	1.0	8.4‡	1.3	119‡	11	509‡	47
			20% Casein-Cholesterol Diet					
0	15.8	1.9	15.4	1.6	97	28	5447	542
48	12.0‡	1.8	19.4‡	2.5	109	22	6601†	838
96	12.1†	2.6	20.5‡	2.9	97	24	7471‡	935

Liver weight given in Gm. Fat content given in Gm./100 Gm. of fresh tissue. Cholesterol and lipid phosphorus given in mg./100 Gm. of fresh tissue.

*Only 5 rats were included; other groups included 6 rats.

†Significant difference at $P < 0.05$ and

‡Significant difference at $P < 0.01$, when compared with values at zero hours of starvation.

of synthesis of the lipids studied, (c) their utilization, (d) their rate of excretion, and (e) changes in their distribution and mobilizaton.

Although it has been reported that the rat in acute starvation shows a decrease in plasma volume,[10] and lipid synthesis,[11,12] and an increase in lipid oxidation,[13] these findings explain only part of the results presented here.

The changes in total amount of fat per liver during starvation, as well as on a percentage basis, indicate a mobilization of fat to the liver in the 20 per cent casein fed group. This could be considered as being related to the state of body fat deposits, presuming that the protein-deficient rat had been largely depleted of body fat. Results from carcass analysis, however, showed no difference on per cent body fat between growing rats fed either 5 or 20 per cent casein diets.[14] The results of liver cholesterol during starvation showed an over-all increase in both groups, but the increase in the total amount per liver was greater in the 20 per cent than in the 5 per cent casein fed animals. This may indicate a better efficiency in the 20 per cent casein group for the removal of cholesterol from plasma, or a decrease in the rate of excretion into the gastrointestinal tract and/or discharge from the liver into the plasma.[15]

Although some of the explanations discussed could account for the findings

reported, the data throw no light on the mechanism for such differences. The fact that rats grown on diets of different protein content responded differently to starvation suggests further that the protein nutritional status of the animals as well as the state of fasting should be defined in the study of lipid metabolism.

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