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Effect of Short-Term Energy & Protein Restriction on Tissue & Body Composition of Rats

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ABSTRACT

The effect of 50 per cent restriction in energy and protein intake in young adult rats has been studied. Two groups of rats were fed for 10 days (Stabilisation, Phase I) an isocaloric diet with two levels of protein: 20 per cent (20 P) or 10 per cent (10 P). At the end of Phase I, each protein group was sub-divided into two groups—a control group and an experimental (Restricted) group - and fed *ad libitum* or at 50 per cent level of the respective diet for a further period of 10 days (Phase II). The animals were then sacrificed and organs and carcasses preserved for analysis. The results showed higher food consumption and lower weight gain in the *ad libitum* fed 10 P group compared to 20 P group during Phase II. Fifty per cent diet restriction resulted in nearly identical reduction in weight gain in both the groups. Though nitrogen (*N*) balance was reduced drastically during diet restriction, it remained clearly positive in the 20 P restricted group, while it was just maintained in the 10 P restricted group. The carcass and tissue composition data showed that the loss in weight was due to extensive depletion of lipids in both the food restricted groups without degradation of the protein component. The study thus demonstrates that short- term 50 per cent diet restriction did not result in protein degradation when maintenance need of protein is met.

1. INTRODUCTION

Hypocalorie feeding is a form of dietary stress experienced by several groups of population the worldover for various reasons. Low-calorie diets coupled with exercise were employed as a weight reducing regimen by the obese people[1]. During Ramdan, the Muslim community observes dawn-to-dusk fast[2]. On the other hand, military personnel, while on long-range patrol operation, are advocated 50 per cent food restriction from logistic considerations demanding optimisation of load contributed by ration packs, besides a battery of technical equipment[3].

The nutrient composition of rations meant for sub-optimal feeding plays an important role in overcoming the effects of malnutrition. During underfeeding, although the body tries to economise the need for protein and energy [4,5], inadequate amounts of protein in low-calorie diets could result in degradation of body proteins for energy purposes[3,6,7]. The present study was undertaken to investigate the optimal level of dietary proteins required to maintain body nitrogen status in rats under condition of 50 per cent food restriction, for short periods. The results obtained hitherto would provide a guideline to formulate satisfactory diet for sub-optimal feeding of military personnel.

2. MATERIALS & METHODS

2.1. Experimental Diet & Animals

The composition of the diets used in the study are given in Table 1. Thirty-two young adult albino

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e 1. Compositions of experimental diets (values are in g/kg diet)

Constituent	10 P	20 P
		207.0
Glucose	20.0	20.0
Refined groundnut oil	90.0	90.0
Vitaminised starch**	10.0	10.0
Shark liver oil***	10.0	10.0
Mineral mix****	20.0	20.0
Corn starch edible	746.5	643.0

* High protein casein

** Prepared as per Indian Standard (1975) I.S. 7481-1974[8]

Fortified with α-tocopherol acetate (10 mg per g oil):1g shark liver oil provided vitamin A 1500 I.U. vitamin D 100 I.U.

**** Prepared as per Hubbell, *et al*[9].

rats (≈180 g) from the laboratory animal facility were divided into two Groups, A and B, of 16 animals each and fed *ad libitum* diet 10 P and 20 P respectively for 10 days (Stabilisation, Phase I). On the eleventh day, each of these Groups (A and B) was sub-divided into Groups 1, 2, 3 and 4. These groups were matched with respect to their weight gain and food intake patterns during the Stabilisation period. By this process, animals of Group A were distributed into Groups 1 and 2 and those of Group B into Groups 3 and 4. Groups 1 and 3 were continued on *ad libitum* feeding of 10 P and 20 P diet respectively for a further period of 10 days. On the other hand, Groups 2 and 4 were fed respectively 10 P and 20 P diet in amounts equal to 50 per cent of the quantities consumed by them during Phase I. This experimental protocol is schematically represented in Fig. 1. The body weight and food consumption records of all the groups were maintained throughout the experimental period.

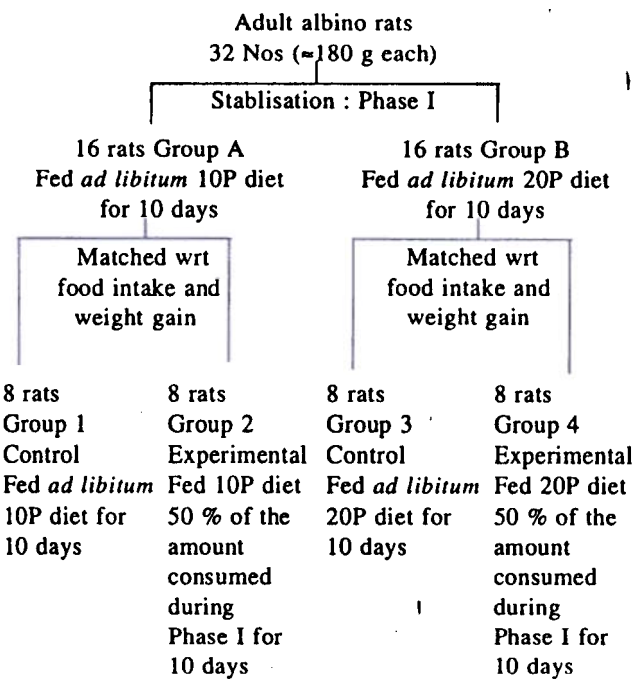

Figure 1. Schematic representation of the experimental protocol

2.2 Collection of Urine, Faeces & Various Tissues

On days 8 to 10 of Phase I and on days 4,5,9 and 10 of Phase II, animals of all the four groups were placed individually in metabolic cages for collecting urine and faeces for analysis of *N* content, as described earlier[10].

At the end of Phase II, the animals were killed under anaesthesia by injecting nembutal (50 mg/kg b.w.) intraperitonially. Organs/tissues, such as liver, gastrocnemius muscle and epididymal fat pads were quickly excised, weighed and stored in a deep freezer at -20 °C, until analysed. The carcasses made ingesta-free were autoclaved in cans at 15 psi for 15 min and stored as above.

2.3 Chemical Analysis

The nitrogen (*N*) in urine, faeces and diet was determined by the micro-Kjeldahl method. Protein in diet was calculated as *N* x 6.25. Urinary urea and creatinine were estimated by the method of Geyer and Dabich[11] and Mitchell[12], respectively. Frozen carcass samples were processed according to the method of Mickelson and Anderson[13]. The carcass and the various organs/tissues were analysed for moisture content by vacuum drying at 70 °C. Muscle proteins were fractionated into sarcoplasmic, myofibrillar and stromal proteins, as

described by Helander[13]. Protein content in these fractions and liver tissues was determined by the method of Lowry, *et al*[15]. as modified by Munro[16]. Total lipids in liver, muscle and epididymal fat pads were determined by the method of Folck, *et al.*[17], and the carcass fat by Soxhlet extraction using petroleum ether (40-60 °C). The carcass composition was calculated by combining the respective values for organs/tissues with those for residual carcass.

2.4 Statistical Analysis

The data expressed as mean ± SD were analysed by Students 't' test.

3. THE RESULTS

3.1 Food Intake, Weight Gain & Food Efficiency Ratio

The food consumption and weight gain of all the animals were similar during Phase I; irrespective of the protein level in the diet fed (Table 2). On the other hand, during Phase II, the control groups fed *ad libitum* either 10 P or 20 P diet showed significant ($P < 0.05$) differences in

Table 2. Food intake, weight gain and food efficiency ratio of rats fed 10 P or 20 P diets during various dietary regimens

	Diet	Stabilisation Phase I	Diet restriction Phase II	
			Control	Experimental
Food intake g/day	10 P	13.51±1.16		
	20 P	13.37±0.84		
Weight gain, g/day	10 P	3.04±0.63		
	20 P	3.31±0.55		
Food efficiency ratio[1]	10 P	0.23±0.04		
	20 P	0.26±0.03		

Values are mean ± S.D. for 15-16 rats in each group in Phase I and for 7-8 rats in each group in Phase II.

a,b,c Significantly different from the corresponding values during Stabilisation Phase I at $P < 0.05$, 0.01 and 0.001, respectively.

\+ Significantly different from the corresponding values for 10 P diet fed group at $P < 0.05$.

1 Weight gain/g food intake.

Table 3. Nitrogen balance in rats fed 10 P or 20 P diet during various dietary regimens

Diet	Stabilisation Phase I days 8 - 10	Diet restriction : Phase II			
		Days 4 & 5		Days 9 & 10	
		Control	Experimental	Control	Experimental
		Nitrogen intake (mg/day)			
10P	214.0±15.1	205.0±12.6	103.8±7.1	200.3±10.9	103.7±7.2
20P	427.9±46.5	441.7±60.5	216.1±4.1	412.6±57.8	216.1±4.1
		Nitrogen in urine (mg/day)			
10P	108.4±13.2	98.3±18.1	$80.4±9.1^{+}$	102.0±12.6	$76.7±13.0^{*}$
20P	$168.4±30.6^{a}$	$192.9±46.9^{a}$	$130.1±34.3^{+**a}$	$165.6±47.7^{a}$	$132.0±26.7^{a}$
		Nitrogen in faeces (mg/day)			
10P	34.6±4.7	31.6±5.6	$17.1±3.0^{**+}$	35.0±2.0	$17.6±1.7^{***}$
20P	38.6±6.6	36.5±12.7	$21.8±7.2^{*+b}$	36.9±6.5	$20.5±5.4^{*b}$
		Nitrogen balance l(mg/day)			
10P	70.9 ±20.9	75.1±17.2	$6.2±11.5^{**}$	62.6±12.4	$9.6±14.0^{***}$
20P	$223.1±52.5^{a}$	$216.7±34.3^{a}$	$53.2±47.0^{**a}$	$212.0±50.0^{a}$	$62.9±24.2^{***a}$

Values are mean ± S.D for 11-12 rats in Phase I and for 6-7 rats in each group in Phase II

*,**,*** Significantly different from the corresponding control values at $P < 0.05$, 0.01and 0.001, respectively

\+ Significantly different from the corresponding values during stabilisation Phase I ($P < 0.05$)

a,b Significantly different from the corresponding values for 10 P diet fed group at $P < 0.001$ and 0.05 level, respectively.

food intake and weight gain. Animals of Group 1 fed 10 P diet *ad libitum* consumed larger amount of food and showed lesser weight gain compared to Group 3 fed 20 P diet *ad libitum*. As a result, the food conversion ratio is lower in Group 1 animals. The loss in body weight observed in food restricted Groups 2 and 4 during Phase II was nearly identical.

3.2 Nitrogen Balance & Urinary Urea & Creatinine Output

Fifty per cent diet restriction caused a concomitant reduction in *N* intake, which, in turn, resulted in 20-30 per cent reduction in urinary *N* and 40-50 per cent in faecal *N* in both the restricted Groups 2 and 4 (Table 3). Consequently, the *N* balance fell by 85-90 per cent in 10 P group and by 70-75 per cent in 20 P group. Although, one animal out of seven in Group 2 showed marginally negative *N* balance throughout Phase II, the overall *N* balance in the group was just maintained.

The changes in urinary urea (Table 4) followed the pattern of urinary nitrogen, the output being lower in Group 1 than in Group 3. The urea output declined in both Groups 2 and 4 as a result of restricted feeding. On the contrary, the urinary creatinine values (Table 4) of both dietary protein groups (10 P and 20 P) showed the trend of a gradual increase over the 20-day *ad libitum* feeding period, irrespective of the level of feeding. Food restriction had no effect on these values.

3.3 Weights & Compositions of Tissues

The gross weight of liver and gastrocnemius muscle of animals of Group 3 fed 20 P diet were significantly greater than those of Group 1 maintained on 10 P diet (Table 5). The fat pad weight, on the other hand, showed an increasing trend (not statistically significant) in Groups 1 and 3, irrespective of their level of feeding. As a result of diet restriction, the different organs of Groups 2 (10 P) and 4 (20 P) lost weight to varying degrees. The liver exhibited the maximum loss (29 per cent in 10 P diet group and 37 per cent in 20 P diet group) followed by fat pads (25 per cent in 10 P diet group and 31 per cent in 20 P diet group) and muscle (9 per cent in 10 P diet group and 13 per cent in 20 P diet group). Analysis of liver showed that restricted feeding led to the depletion of absolute amounts of moisture, lipid and protein in liver (Table 6). The increase observed in the lipid concentration, when expressed as g/100 g tissue, is ascribed to the lower body weight of these animals. The liver protein content was not different in both the diet-restricted Groups 2 and 4, whether expressed on whole tissue basis or per cent-wise. The moisture content of muscle did not show any change due to various dietary treatments. On the

Table 4. Changes in urinary urea and creatinine excretion of animals subjected to different dietary treatments

Diet	Stabilisation Phase I days 8 - 10	Diet restriction : Phase II			
		Days 4 & 5		Days 9 & 10	
		Control	Experimental	Control	Experimental
		Urea (mg/day)			
10P	105.6±16.5	98.7±17.4	98.1±16.2	120.0±17.4	98.5±14.5[a]
20P	252.3±43.6[a]	276.3±52.3[a]	223.6±14.9[b]	252.9±85.9[a,b]	195.3±19.4[c]
		Creatinine (mg/day)			
10P	3.00±0.54[c]	3.50±0.74[a,b]	3.91±0.89[b]	3.89±0.38[b]	3.53±0.65[b,c]
20P	4.51±1.28[a+]	4.84±1.66[a]	4.45±1.34[a]	4.90±0.71[a+]	5.14±0.96[a+]

Values are mean ± S.D. for 12-14 rats in Phase I and for 6-7 rats in each group in Phase II

a,b,c Values not sharing a common superscript in a row are significantly different at $P < 0.05$ level

\+ Significantly different from the corresponding values for 10 P diet fed group at $P < 0.05$ level

Table 5. Organ weights of *ad libitum* fed control and diet restricted animals

Organs	Diet	Diet restriction : Phase II	
		Control	Experimental
Liver, g	10P	8.37+0.84 (3.57+0.26)	5.92+0.66* (3.06+0.34)*
	20P	10.30+1.16+ (4.08+0.29)+	6.48+0.40* (3.14+0.20)*
Fat pad, g	10P	3.61+1.05 (1.53+0.38)	2.72+0.43* (1.41+0.20)
	20P	3.32+0.86 (1.27+0.25)	2.29+0.55* (1.11+0.27)
Muscle, g	10P	2.69+0.21 (1.15+0.09)	2.46+0.61* (1.28+0.09)*
		3.03+0.19+ (1.28+0.07)	2.63+0.16*+ (1.19+0.05)*

Values are mean ± S.D. for not less than 6 rats in each group

* Significantly different from the corresponding control values at $P < 0.05$

\+ Significantly different from the corresponding values for 10 P diet fed group at $P < 0.05$

Values within parentheses indicate relative weights, viz., g/100g body weight

contrary, the lipid content showed a marginal decline due to diet restriction. The various muscle protein fractions also did not indicate any alteration as a result of diet restriction (data not presented).

Although, the carcass moisture did not undergo any change in absolute amounts, an increase was observed when expressed as percentage on account of food restriction, which is attributable to the lower body weight of these animals. Diet restriction resulted in drastic depletion of carcass fat. The carcass fat content of both control and experimental groups on 10 P diet appeared to be higher (although not significant) compared to their counterparts on 20 P diet. The carcass protein content did not exhibit any change.

4. THE DISCUSSION

The metabolic responses of animals to food restriction depend on several factors, such as energy and protein content of diet and degree and duration of deprivation[18,19]. A lower weight gain was observed in the control group on a lower

Table 6. Compositions of liver, muscle and carcass of *ad libitum* fed control and diet restricted experimental animals

Trait	Diets	Liver[1]		Muscle[2]		Carcass[3]	
		Control	Experimental	Control	Experimental	Control	Experimental
		g/100g liver		g/100g muscle		g/100g body weight	
Moisture	10P	67.54±1.48 (5.91±0.59)	70.49±0.52* (4.16±0.32*)	75.89±0.97	75.41±1.74	54.77±3.94	59.75±2.06*
	20P	65.98±0.96 (6.93±0.73*)	68.02±1.42* (4.38±0.37*)	74.24±1.73	74.68±0.77	57.58±1.60	63.04±2.11**
Total lipids	10P	6.22±1.02 (0.54±0.09)	5.07±0.49 (0.30±0.03*)	3.90±0.45	3.36±0.21**	19.34±3.56	11.83±1.53***
	20P	4.94±0.54+ (0.44±0.04)	4.79±0.59 (0.26±0.04*)	2.48±0.15+	2.30±0.19*+	16.86±2.88	9.24±2.88**
Protein	10P	18.54±1.59 (1.61±0.07)	18.34±1.64 (1.08±0.14*)	20.58±0.56	19.46±0.72	20.08±1.89	20.87±0.70
	20P	17.94±2.34 (1.84±0.21)	18.68±2.12 (1.21±0.17*)	20.25±0.35	20.24±0.61	22.56±2.81	20.55+1.49

Values are mean ± S.D. 1) 4 animals in 10P groups and 6-8 animals in 20P groups. 2) 4 animals in each group. 3) 5 to 6 animals in each group.

Values within parentheses represent absolute weights in g.:

*,**,*** Significantly different from the corresponding control values at $P < 0.05, 0.01$ and 0.001 levels respectively

\+ Significantly different from the corresponding value for 10P diet fed group at $P < 0.05$ level.

protein diet, despite a higher food intake, because the animals were able to meet the marginal deficiency of protein by increasing their food intake. But the excess energy thus ingested does not contribute to gain in body weight but is dissipated as heat by diet-induced thermogenesis[20,21].

Nitrogen balance studies showed a significant reduction in body weight in both the diet-restricted groups. Animals on a 10 P protein diet were able to maintain *N* equilibrium on a 50 per cent diet restriction because they received maintenance level of protein. National Research Council[22] has recommended a diet with 4.4 per cent protein as the maintenance need for adult rats. Examination of *N* excretion pattern showed an immediate decrease in *N* excretion following diet restriction, which depicts the adaptive mechanism of the body to low *N* intakes. A corresponding reduction in the output of urea indicates, *inter alia*, a diminished hepatic gluconeogenesis, a step towards protecting the body protein from breakdown[23]. Urinary creatinine, an index of muscle mass, did not decline as expected in restricted animals, indicating absence of breakdown of muscle. The higher value observed in the high protein group is in agreement with the findings of Fischer[24].

The data on carcass and tissue composition illustrate a drastic depletion of lipids at both whole body and tissue levels in diet-restricted groups. On the other hand, the protein fraction, especially of the muscle, which is considered the largest reservoir of the body protein, did not undergo any alteration. This is supported by the *N* balance data and indicates that the body has conserved the tissue protein, mobilising lipids for energy purpose. Our finding is supported by the fact that the body protein does not undergo degradation until the adipose stores are exhausted[25,26].

Interestingly, the carcass, liver and fat pads of animals of low protein diet groups (1 and 2) had marginally higher levels of fat compared to their respective high protein diet groups (3 and 4). Although the higher lipid content in Groups 1 and 2 is within the normal range, it could not have been due to deficiency of lipotropic factors, as our diet contained adequate amounts of choline. This could be attributed to factors like increased production of triglycerides to levels which surpass the ability of liver to metabolise/interfere with lipoprotein formation and release, etc.[27,28]

The present study demonstrates that young adult rats are able to maintain *N* equilibrium when subjected to restricted feeding for short periods, even on a low protein diet. This finding suggests that during energy/protein restriction, the body assumes a metabolic adaptive trend similar to that which occurs when the body begins to shift from a 'fed to a fasted economy'. The non-protein calories supplied during food deprivation markedly help reduce urinary loss of *N* (protein sparing action) as reflected in urinary urea output. Thus, the study also suggests that the adipose tissue has a major role to play in short-term energy and protein restriction and that the body protein is protected from degradation when maintenance level of dietary protein is provided.

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