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Effect of Hypocaloric Stress on Body and Tissue Composition of Rats

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ABSTRACT

Rats fed *ad libitum*, a ration comprising fresh foods (F ration) for 10 days, were switched over to another ration consisting of processed foods (P ration) for a similar period. Thereafter, the animals were subjected to a 50 per cent diet restriction for a period of 10 days and rehabilitated either on F ration or P ration for an equal period. The results showed a decline in growth rate, food consumption and food efficiency ratio in the group fed *ad libitum* on P ration compared to that on F ration. Fifty per cent diet restriction induced loss of body weight due to depletion of body fat. A fall in the gross weight of liver, kidney and epididymal fat pad along with a reduction in the contents of lipid in liver and fat pad were also observed. Refeeding of diet-restricted rats induced hyperphagia and super-normal weight gain with both rations. The data on liver triglycerides appeared to show an age-related rise which could be controlled by diet restriction to some extent. Feeding of P ration tended to predisperse to higher adipose tissue cholesterol attributable to higher fat content of this ration.

1. INTRODUCTION

Service personnel are exposed to stresses arising from newer diets and situations during the performance of special duties. In combat and patrol situations, where non-resupply of food is prevalent for short durations, the individual soldier must carry his field equipment and an adequate amount of food and water[1]. This is a considerable burden to him, who is otherwise heavily loaded with a battery of technical equipment. Sub-optimal feeding has been advocated in these situations, where logistic considerations demand optimisation of load[2].

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Defence Food Research Laboratory (DFRL) has been developing a number of pack rations to cater for similar situations faced by our Armed Forces, keeping the Indian palate in view. Suitability of these rations has been established by laboratory evaluation and also by field trials with soldiers engaged in diverse occupations[3].

Galloway and Spector[4] have used rat as an experimental model to provide guidance for the design of a suitable survival food packet for military operations. The effect of underfeeding on physical efficiency and cardiovascular changes has been studied in man and animals[5]. Several workers[6-9] have studied the effects of the calorie restriction of varying degrees and subsequent rehabilitation. They have used mostly synthetic and semi-synthetic diets to examine the effects of sub-optimal feeding in relation to obesity or protein-calorie malnutrition. No work has been reported, wherein a pack ration was employed exclusively for restricted feeding or refeeding. Therefore we wanted to study the metabolic effects of restricted feeding of a processed ration (P ration) and also to compare the effects of consuming the P ration vis-a-vis a fresh ration (F ration) designed on the pattern generally consumed by service personnel, on the tissue and body composition of rats.

2. MATERIALS AND METHODS

2.1 Diets

The F ration (Table 1) is based on the normal scale of ration for the Armed Forces. It was prepared as described in a previous paper[10] and stored in paper-aluminium foil-polythene pouches at –20°C. The various components of the P ration (Table 2) developed in DFRL for consumption by soldiers in situations where the F ration cannot be provided, have a shelf life of not less than six months. These items were prepared and stored for six months at ambient temperature before use.

Table 1. Composition of F ration employed for *ad lib.* feeding of rats

Items	Quantity (g)*
Wheat flour	600
Bengal gram flour	90
Whole milk powder	44
Sucrose	90
Hydrogenated oil*	70
Vegetable (Carrot)	180
Potato	110
Onion	60
Sweet lime	50
Salt	20

* Fortified with vitamin A, 25 I.U. and vitamin D 2 I.U. per g oil

Table 2. Menu for the one man ration, formulated out of processed foods (P ration) employed for *ad lib.* or restricted feeding of rats

Processed food	Quantity (g)
Bed tea	
Tea bar*	30
Break-fast	
Canned puri	240
Cabbage peas curry+	60
Tea bar*	30
Lunch	
Canned parotta	240
Curried potato-cauliflower+/Curried spinach-red gram dal+/Curried red gram dal+/green gram dal+/ Curried lentil dal+	80
After-noon tea	
Tea bar*	30
Dinner	
Pulav-vegetarian+,a	100
Pickle	40
Chikki (groundnut candy bar)b	100

Contained sugar : 20 g, whole milk powder : 5 g, and tea leaves 5 g. Tea leaves were omitted while compounding the diet.

Dehydrated foods requiring reconstitution and addition of salt to taste. Five times the one man ration was reconstituted at a time such that all the alternative dishes meant for lunch were included in preparing the diet for feeding of rats.

a Spiced rice with vegetables.

b Contains peanuts, roasted bengal gram dal and white til seeds with jaggery as base

The individual items were reconstituted by heating with water as much as 2-3 times in weight of the material, to boiling followed by simmering for 5-10 min. The reconstituted items were thoroughly mixed and minced in a meat mincer to obtain a homogenous mixture which was stored as above. The F and P rations were isoenergetic and isonitrogenous differing only in fat content (Table 3).

Table 3. Proximate compositions of the rations*

Ration	Fat	Protein	Carbohydrate	Crude fibre	Ash	Energy content kcal/g
	%, dry weight basis					
P ration	15.6	12.2	64.3	4.5	3.4	4.5
F ration	9.6	11.9	71.6	5.1	1.8	4.2

* Average values for three determinations

2.2 Experimental Protocol

Male young albino rats from the stock colony were exercised daily on a rodent tread mill for 15 min throughout the experimental period at a speed of 3 km per hour in order to maintain a minimum level of forced activity. When the animals reached a weight range of 190-220 g, they were divided randomly into six groups of 7-9 rats each. The rats were housed individually in wire-bottomed cages. They were placed on various dietary regimens during the experiment which lasted for 40 days and comprised three phases of 20, 10 and 10 days respectively. The experimental protocol is depicted in Table 4.

During phase I, designated as 'Stabilisation Phase' all the groups of animals were fed *ad libitum* on F ration for a period of 10 days for assessing the food intake and weight gain pattern. This was followed by feeding of P ration *ad libitum* to all the groups barring group 2 for the same period. Animals of group 2 received F ration *ad libitum* throughout the 3 phases and served as the age control. During phase II ('Restriction Phase') rats of groups 4, 5 and 6 were subjected to 50 per cent energy restriction for 10 days by offering half the amount of food (P ration) they consumed during the preceding 10 days. On the other hand, group 3 was continued on *ad libitum* feeding of P ration which also served as a control. During phase III ('Refeeding' or 'Rehabilitation' phase), the two hypo-calorie fed groups 5 and 6 were refed *ad libitum* on F and P ration respectively for 10 days. The food consumption and body weight records were maintained throughout the experimental period. During the last two days and prior to sacrifice, the animals were placed in metabolism cages for collection of urine and faeces. Urine was collected over 3 ml of 6 N hydrochloric acid and stored in a deep freezer and faeces were oven-dried and stored in a desiccator until analysed.

At the end of different phases various groups, as indicated in Table 4, were sacrificed after an overnight fast under light ether anaesthesis. The liver, epididymal

Table 4. Experimental protocol

Groups	Phase I Stabilisation (Days 1 – 10) diet	Phase I Stabilisation (Days 11 – 20) diet	Phase II Restriction (Days 21 – 30) diet	Phase III Rehabilitation (Days 31 – 40) diet
1. Base line	FR	PR*		
2. Age control	FR	FR	FR	FR*
3. Control	FR	PR	PR*	
4. Restricted	FR	PR	½PR*	
5. Rehabilitated	FR	PR	½PR	FR*
6. Rehabilitated	FR	PR	½PR	PR*

Group 1 provided base line data for comparison with other groups

FR = Fed *ad lib.* on F ration; PR = fed *ad lib.* on P ration; ½PR = fed at 50 per cent level of *ad libitum* intake of P ration during the preceding 10 days

* Sacrificed at the end of the respective phase.

fat pads and kidneys were quickly excised and weighed rapidly. Liver and fat pads were stored in tight fitting glass bottles at –20°C until analysed. The kidneys along with the ingesta-free carcasses were dried at 70°C in a flow-through oven to constant weight. The dried carcasses were minced in a blender and stored in a desiccator for analysis of fat and protein.

2.3 Chemical Analysis

The diets were analysed in duplicate for proximate composition by the method of AOAC[11]. The nitrogen in the urine, faeces and carcasses was determined by the micro-kjeldahl procedure. The carcass fat was determined by Soxhlet extraction. Total lipids in liver and fat pads were estimated by the method of Folch *et al*[12]. The total cholesterol (CH), phospholipids (PH) and triglycerides (TG) were determined in the aliquots of Folch's extract by the method of Searcy and Bergquist[13], King and Wootton[14] and van Handel and Zilversmit[15] respectively.

2.4 Statistical Analysis

All the data were analysed by Students 't' test. Individual values falling outside the mean by 2 standard deviations (SD) were rejected.

3. RESULTS

3.1 Growth Rate, Food Intake and Food Efficiency Ratio

The growth rate of the control and experimental animals are graphically represented in Fig. 1. Each point in the curve represents average values for animals of one or more number of groups depending upon whether they received the same diet or different diets *ad libitum* or in restricted amounts. The portion A'A of the curve represents average values for groups 1-6 receiving F ration *ad libitum* for the first 10 days. It will be seen from the curve (ABCD) that the growth rate of animals receiving F ration *ad libitum* (group 2) throughout is higher than those receiving P ration (AED') *ad libitum* (group 3). The weight gain in these two groups at the end of phase II (days 10 to 20) has been found to be 40.4 ± 11.6 and 22.8 ± 7.2 g respectively, the two values being significantly different at $P < 0.01$. Curtailing food intake to 50 per cent of *ad libitum* intake resulted in an average loss in the body weight (curve ER) equal to 34.1 ± 8.8 g in 10 days for the three restricted groups 4, 5 and 6. On the other hand the two *ad libitum* fed groups (groups 2 and 3) receiving F and P rations continued to gain weight during this period, but at different rate. Refeeding of the restricted rats for 10 days with either P or F ration resulted in a three-fold rise in weight gain compared to the stabilization values. The rate of regain in weight for the first 3 days was found to be similar for both the groups. On day 4 or 5 of rehabilitation, these animals assumed the pre-restriction weight. Thereafter the growth rate progressively decreased with time. Subsequently the differences in the weight gain became apparent, resulting ultimately in lower terminal weights, for rats fed P ration compared to the F ration.

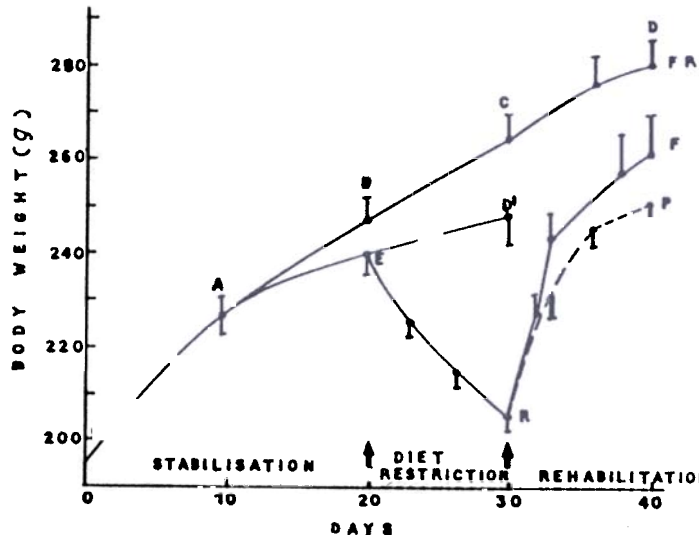

Figure 1. Growth curve for rats on various dietary regimens. Vertical bars denote S.E. A'A = *ad lib.*-fed on fresh ration (groups 1–6); ABCD = *ad lib.*-fed throughout on F ration (group 2); AE = *ad lib.*-fed on P ration (groups 3, 4, 5 & 6); ED' = *ad lib.*-fed on P ration (group 3); ER : food restricted (groups 4, 5 & 6); RF : rehabilitated on F ration (group 5); RP rehabilitated on P ration (group 6).

The food intake values for F ration during stabilization (Table 5) was found to be significantly higher than those for P ration. This difference persisted even during rehabilitation phase also, although it narrowed down to some extent. The food efficiency (FE) ratio (weight gain per unit weight of food consumed) generally followed the food intake pattern. During stabilization the FE ratio was significantly lower with animals fed on P ration than on F ration. Refeeding after calorie deprivation resulted in an elevation of this ratio in both the groups. However, there was no difference between the two groups.

3.2 Nitrogen Balance

Nitrogen balance varied as expected with the level of intake of nitrogen (data not presented). On reduced intake of food, the N intake was also reduced. The urinary and faecal nitrogen excretions during the 50 per cent dietary restriction declined almost

Table 5. Food intake and food efficiency ratio of rats maintained on various dietary regimens

	Stabilisation		Restriction	Rehabilitation	
	F ration[@]	P ration[£]	P ration[*]	F ration[**]	P ration[+]
Food intake in 10 days (g)	161.2±12.0[a]	140.7±13.1[b]	68.3±1.7[c]	178.5±4.6[d]	169.2±7.9[a]
Food efficieny ratio[e]	0.21±0.04[a]	0.13±0.03[b]	–	0.32±0.04[c]	0.29±0.05[c]

Values are Means ± SD for groups 1-6 (@), groups 3-6 (£), groups 4-6 (*), group 5 (**) and group 6 (+) respectively with 6-8 animals in each group

a,b,c,d – Values not sharing a same superscript in a row are significantly different ($P<0.05$)

e – Weight gain per g food intake

Table 6. Carcass composition of rats determined at the end of various dietary regimens

	F ration*	P ration+	Diet restriction	Rehabilitation	
				F ration	P ration
Moisture g/100 gb.wt.	55.6±2.5[b]	59.4±2.2[c]	59.2±1.2[c]	58.8±2.1[a,c]	60.6±1.8[a,c]
Fat g/100 gb.wt.	20.3±3.8[b]	14.9±1.1[e]	12.9±1.0[a]	18.0±1.4[b]	15.9±0.9[c]
Protein g/100 gb.wt.	18.6±1.0[a]	18.5±1.3[a]	20.5±0.5[b]	19.1±1.3[a,c]	19.1±2.0[a,b]

Values are Mean ± SD for 5–7 animals in each group

a,b,c,d,e The values not sharing a same superscript in a row are significantly different ($P < 0.05$)

Body weight

Age control fed *ad lib.* for 40 days

Control fed *ad lib.* for 20 days

to half the values obtained during stabilisation. Consequently, the nitrogen balance became significantly lowered but remained positive, viz. 50.1±11.8 mg/day. Refeeding resulted in an enhancement of nitrogen balance which at the end of 10 days was found to be identical with the pre-restriction values.

3.3 Carcass Composition

The carcass moisture content (Table 6) was similar in all the groups except in the case of age control (group 2) which was significantly low. The fat content in this group fed F ration (group 2) continuously for 40 days was higher than the group receiving P ration (group 3). Restricted feeding has resulted in a significant depletion of fat. On refeeding there was a significant increase in deposition of fat, over the restriction value, irrespective of the diets fed; refeeding with F ration, however, resulted in greater deposition. The body protein content on the contrary, in the two control groups (groups 2 and 3) was not different. The higher value for the carcass protein of the restricted group compared to the control groups was due to the lower body weights of these animals. Rehabilitation with either ration did not result in any change in the body protein content. Further these values were not different from the pre-restriction values.

3.4 Organ Weights

The weights of liver, kidney and epididymal fat pad in the control and restricted animals virtually followed the pattern of body weight (Fig. 2). Compared to the base line value, the liver suffered 13 per cent loss in its absolute weight against 15 per cent

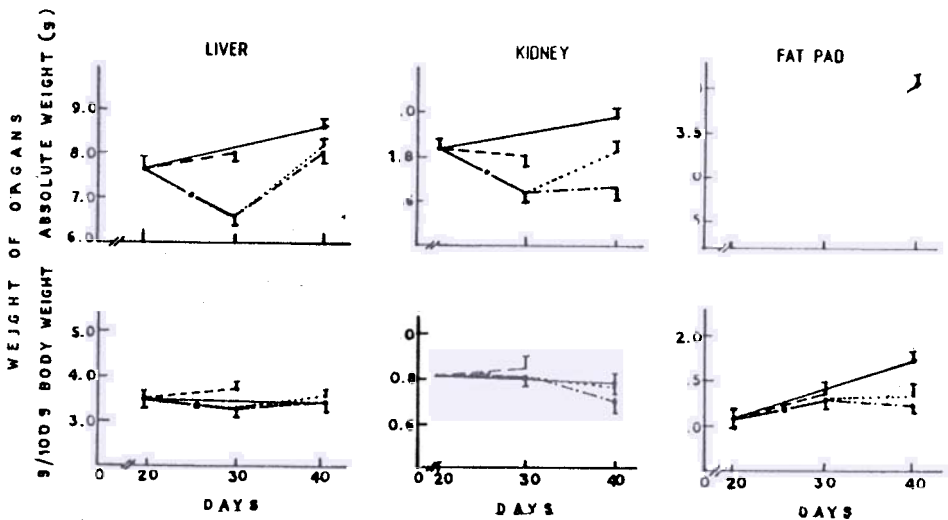

Figure 2. Organ weights of rats on various dietary regimens. Vertical bars denote S.E. *ad lib.*-fed throughout on F ration (——); *ad lib.*-fed on P ration (----); 50% diet restricted (–·–); rehabilitated on F ration (····); rehabilitated on P ration (–·–·). (for details see materials and methods).

observed in the body weight during restriction. The kidney, a smaller organ followed the pattern of liver, but the loss was comparatively of smaller magnitude. On the other hand, the fat pad representing about 15 per cent of the total adipose store, lost 20 per cent of its fat content. Refeeding with either ration produced significant increase in the absolute weight of the organs. The liver at the end of refeeding with P or F ration showed an increase of 21 and 24 per cent in weight respectively. The kidney, which showed negligible gain in weight with P ration, had 18 per cent gain with F ration. The fat pad had 16 per cent gain with P ration and 23 per cent with F ration. However, on camparing the data on 100 g weight basis these differences in weights merged and showed that the organ weights generally changed in a co-ordinated manner with the body weight.

3.5 Composition of Tissues

Liver

Animals maintained on F ration (group 2) had a higher lipid content (Table 7) compared to those on P ration (group 3). The total lipids were found to decline on calorie restriction. Refeeding resulted in raising this level almost to the control value. The hepatic TG values in the base line group (group 1) was found to be 100.4± 22.9 mg/Liver. Feeding of P ration for further 10 days elevated this value to 119.2± 20.3 mg/Liver. On the other hand, feeding of F ration from the beginning for the total period of 40 days enhanced the value to 167.5±7.0 mg/Liver indicating an age-related rise in the hepatic TG content. On restricted feeding, the TG content was drastically reduced while on refeeding with either ration, these values increased to levels intermediary to those of controls on F and P ration. The total CH content did not indicate any appreciable change consequent to feeding of low-calorie although there did exist a lowering trend. Refeeding values were more or less identical with those obtained for controls.

Table 7 Liver lipids in rats determined at the end of different dietary regimens

	F ration	P ration	Diet restriction	Rehabilitation	
				F ration	P ration
Total lipids mg/liver	387.7±10.4[a]	320.5±39.7[b]	256.4±20.4[c]	326.8±51.8[c]	324.0±38.8[b]
Triglycerides mg/liver	167.5±7.08[c]	119.2±20.3[b]	83.6±0.77[a]	132.2±43.8[a,b]	124.4±18.65[b]
Cholesterol mg/liver	23.2±3.42[a,b]	25.6±4.30[a,d]	17.8±3.93[a]	25.0±3.52[a,b]	28.3±3.53[b,d]
Phospholipids mg/liver	196.2±22.1[b]	169.4±24.2[a,b]	140.3±18.8[a]	176.3±22.2[a,b]	210.9±44.5[a,b]

Values are Mean ± SD for 4 or 5 rats in each group

Values not sharing a common superscript letter in a row are significantly different (P 0.05

Age control fed *ad lib.* for 40 days

\+ Control fed *ad. lib.* for 20 days

The PH content in the restricted group although appeared to be low, remained not significant from those for other groups excepting from the control group fed F ration (group 2).

3.5.2 Epididymal Fat Pad

Total lipid content (Table 8) was found to be higher with animals fed F ration (group 2) compared to those on P ration (group 3). Diet restriction caused a significant reduction in these values, which on rehabilitation rose to levels slightly less than those

Table 8. Lipid composition of epididymal fat pads in rats determined at the end of different dietary regimens

	F ration*	P ration	Diet restriction	Rehabilitation	
				F ration	P ration
Total lipids mg/g fat pad	814.0±7.5[b]	786.5±22.5[a]	703.8±12.3[c]	744.7±7.2[d]	747.5±12.2[d]
Triglycerides mg/g fat pad	573.1±8.9[b]	503.4±19.8[a]	467.4±9.5	495.8±38.4[a,c]	492.5±13.1[a]
Total cholesterol mg/g fat pad	90.1±4.4[b]	107.0±13.3[a,c]	96.5±9.8[a,b]	94.8±4.2[b]	109.3±7.4[c]
Phospholipids mg/g fat pad	14.0±1.3[a]	13.6±1.8[a]	14.6±1.5[a]	14.4±0.8[a]	13.8±1.4[a]

Values are Mean ± SD for 4 or 5 rats in each group

a,b,c,d Values not sharing a common superscript letter in a row are significantly different ($P < 0.05$)

* Age control fed *ad lib.* for 40 days

Control fed *ad lib.* for 20 days

for both the controls. The TG levels followed the pattern of total lipids. However, in this case the values obtained on rehabilitation were similar to the value for controls fed on P ration. Unlike total lipids and TG content the CH values for animals fed P ration were significantly higher than those fed F ration. This is true even on rehabilitation. Diet restriction did not induce any change. No difference was observed in the PH values of various groups.

4. DISCUSSION

The body can be considered a system in which body weight is a gross variable, reflecting a balance between energy inputs and outputs. From this perspective a net energy loss or gain by the system will be reflected by a concomitant depletion or accretion of body weight. The present experiments showed a differential pattern of weight gain with the feeding of P and F rations. The lower rate of weight gain in the case of P ration cannot be dispensed as a mere difference in energy, protein or fat intake, since the efficiency of utilization of food as reflected by the FE ratio has also been found drastically reduced. The alteration in quality of protein and reduction in availability of other nutrients as a result of processing and storage could presumably be the contributory factors. It is well-known that nutrients are lost during processing and subsequent storage. Loss of nutrients are by way of vitamins and limiting amino acids becoming unavailable or reducing considerably as a result of partial destruction during processing[16]. Evaluation of the vitamin content of the dehydrated foods such as vegetables, pulses and pulav, developed in the laboratory, has shown loss of vitamins of the B complex to varying extent[17]. The lower growth rate observed with this ration could be ascribed only to such factors.

The immediate response of short term dietary restriction imposed in the present experiments is the loss of body weights which can be traced to the loss of fat stores as evident from the carcass data. Restricted intake of energy and protein are known to cause these effects which are essentially dependent upon the degree and duration of deprivation, age, sex and species of the animal[18,19]. The maintenance need for energy of an adult rat is of the order of 75-80 per cent of *ad libitum* intake[20]. Since 50 per cent intake meets less than the maintenance need of the animal, a loss of weight was expected which was contributed by depletion of fat stores.

Another interesting point that has emerged from the present experiments is that the nutritionally-stressed animal when refed on P or F ration was not able to differentiate the quality of the diet till the animal had regained the pre-restriction weight. Until then the demand for energy apparently takes precedence over other nutrients. However, when the refeeding continued further, the differences in the quality of the diet became evident as reflected by their terminal body weights. Super-normal weight gain observed during refeeding of the malnourished animal has been described by several workers[21-24]. During this period a remarkable hyperphagia and a greater weight gain, disproportionately greater than that accounted by food intake has been shown to exist[7]. This compensatory growth depended mostly upon the lipogenic capacity of the animal as evident from the data on carcass fat. In the adult rat mostly the adipose tissue undergoes compensation[22].

The greater metabolic efficiency marked by a two-fold elevation in the FE ratio during rehabilitation could be related to increased gastrointestinal absorption[25] favoured by the hypertrophy of the digestive tract or/and also the increased capacity for lipogenesis in the adipose tissue[26]. It is felt that such stimuli for increased food efficiency exhibited by meal eating animal (having access to food limited to a single short period daily)[26] must exist in the restricted-refed animal also.

The present experiments demonstrated a weight-reducing and lipid-lowering effect of restricted feeding. This was noticed at the whole body as well as at tissue level. The liver and epididymal fat pad consistently showed loss of lipids on restricted feeding apparently in a co-ordinated manner with the body weight. This is expected since liver and adipose tissue form the major sites for synthesis and oxidation of fatty acids. Though the hepatic TG content was found to vary with energy deficit and surfeit conditions employed, a close examination of data revealed an age-related rise in hepatic TG of rats fed whether F or P ration. Such rise has been reported to be due to an increase in TG production and a decline in the efficiency of its removal[27,28]. Reaven and Reaven[8] have shown that the age-related rise in hepatic lipids of rats could be prevented by diet restriction coupled with exercise. This conforms well with our findings. Our experiments also showed that feeding of P ration predisposes to higher CH levels in adipose tissue compared to F ration. Thus could be related to the higher fat content of this ration.

The consumption of processed foods vis-a-vis fresh ration has brought about disparity in growth rate. Restriction of food intake has some definite effects in reducing the body weight and tissue lipids, a favourable effect in the case of obesity. However, rehabilitation especially with a high fat diet might prove counter-productive. Therefore, the composition of the rehabilitation diet is important in designing regimes for 'reducing diets'.

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