Defence Science Journal, Vol 49, No 2, April 1999, pp. 123-133

Changes in Body/Tissue Composition of Rats in Relation to Dietary Protein Levels during Rehabilitation

K.R. Viswanathan, M. Siddalinga Swamy and N. Narayan Prasad
Defence Food Research Laboratory, Mysore-570 011.

ABSTRACT

Effect of rehabilitation with marginally sub-optimal and adequate levels of dietary protein following a 50 per cent diet restriction for 10 days was studied in adult rats. The data revealed hyperphagia, supernormal weight gain and greater food efficiency in rehabilitated animals which progressively tapered off on days 9 and 10, irrespective of the dietary protein level. However, the values remained slightly higher than their respective controls. The food efficiency ratio and nitrogen balance which followed the above pattern, on the other hand, returned to control levels in the group that was refed 20 per cent protein diet. The weights of liver and muscles of 20 per cent protein diet group were higher than those of 10 per cent protein diet group, while the fat pad weight showed a reverse trend. This was observed only in the case of control and rehabilitated animals. The liver lipid and protein concentrations were less in rehabilitated rats as compared to their *ad libitum* fed counterparts. The carcasses of control as well as rehabilitated animals on 10 per cent protein diet had less moisture and more fat content than those on 20 per cent protein diet. The carcass in 20 per cent protein diet group had a higher protein content. A linear correlation was observed between body weight and body fat, while a reciprocal relationship existed between the body fat and body water regardless of whether the rats were rehabilitated or restricted fed.

1. INTRODUCTION

Restricted food intake is practiced for weight reduction by the obese[1], as a ritual by Muslims during Ramadan[2] and for meeting logistic and operational requirements by the troops[3]. Our earlier studies[4-6] in both human subjects and animals on food restriction have revealed that a loss of body weight occurs invariably in all such cases. Contrary to the observations on obese individuals who always aim at reduction in body weight, the military planners are concerned about the loss of weight suffered by the troops during restricted food intake. However, a speedy recovery is desirable from the weight loss by adopting suitable nutritional measures during rehabilitation. The main objective is to ensure regain of health and physical fitness of the soldiers to enable them to undertake combat operation, if warranted. To achieve this objective, composition of the rehabilitation diet plays a vital role, and as such requires in-depth study. Further, very little information is available wrt the role of diet composition and efficiency of such diets in restoration of health during rehabilitation.

The changes occurring both at gross and tissue levels in rats subjected to 50 per cent food/calorie restriction have been reported[6]. In the present study,

Received 20 May 1997, revised 22 January 1999

an attempt has been made to examine two different isocaloric synthetic diets varying only in the level of protein–one marginally sub-optimal (viz., 10 per cent protein) and the other adequate (viz., 20 per cent protein) for their ability in accelerating the process of recovery of body weight and regeneration of tissue constituents during rehabilitation. Only protein was kept as a variable in the diets since any increase in dietary fat is not without any adverse effect on health. Such data would help in formulating different rations out of the products hitherto developed, especially for use by the Armed Forces deployed under diverse environmental conditions.

2. MATERIALS & METHODS

2.1 Experimental Diets & Protocol

The composition of the two types of isocaloric, synthetic diets containing 10 per cent protein (diet A) and 20 per cent protein (diet B) employed and the experimental protocol adopted for the present study were similar to that reported earlier[6], except for inclusion of a rehabilitation phase of 10 days after the food restriction phase, wherein the control and experimental rats were fed *ad libitum* on the respective diets. Thus, the experiment lasted for 30 days and was divided into three phases of 10 days each, viz., stabilisation, restriction and rehabilitation (Fig. 1).

Thirty-two young adult male albino rats (180 g) of *Wistar* strain were divided initially into groups I and II of 16 animals each, and fed diets A and B, respectively for 10 days (phase I) as shown in Fig 1. At the end of phase I, the animals of each group were further sub-divided into two groups, viz., groups 1 and 2, and 3 and 4 of eight animals each. Groups 1 and 3 were continued on *ad libitum* feeding and served as controls to groups 2 and 4 which were maintained on their respective diets at 50 per cent level for 10 days (phase II). On completion of this phase, the animals of restricted-fed groups 2 and 4 together with the controls (groups 1 and 3) were fed *ad libitum* with their respective diets for another period of 10 days (rehabilitation: phase III). During rehabilitation,

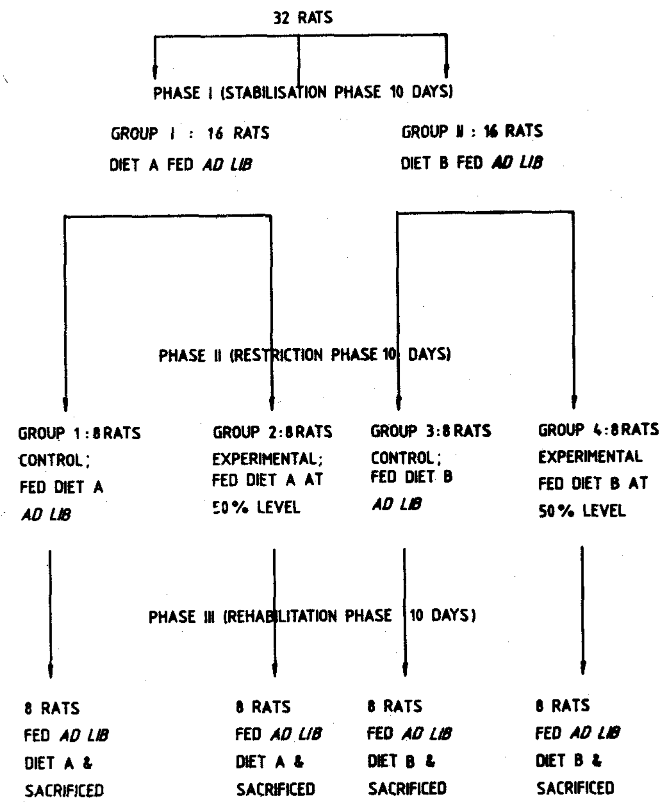


Figure 1. Schematic representation of experimental protocol

individual food intake and weight gain were recorded for days 1, 2-3, 4-8 and 9-10. The urine and faeces were separately collected on days 2-3 and 9-10 by placing the animals individually in metabolic cages. The collection system adopted prevented coprography and mixing of urine and faeces. Water was provided *ad libitum* throughout the experiment. The samples of 9 and 10 day pertaining to group 2 rehabilitated with diet A were lost inadvertently and hence not analysed. At the end of phase III, the animals of all the groups were sacrificed and the various tissues/organs and carcasses were processed/analysed for different parameters as described earlier[6].

2.2 Chemical Analysis

The analytical parameters included: (i) protein in diets A and B, (ii) nitrogen in urine and faeces, (iii) moisture in various organs/tissues and carcass (carcass of only four animals from each of the four groups were analysed), (iv) gastrocnemius

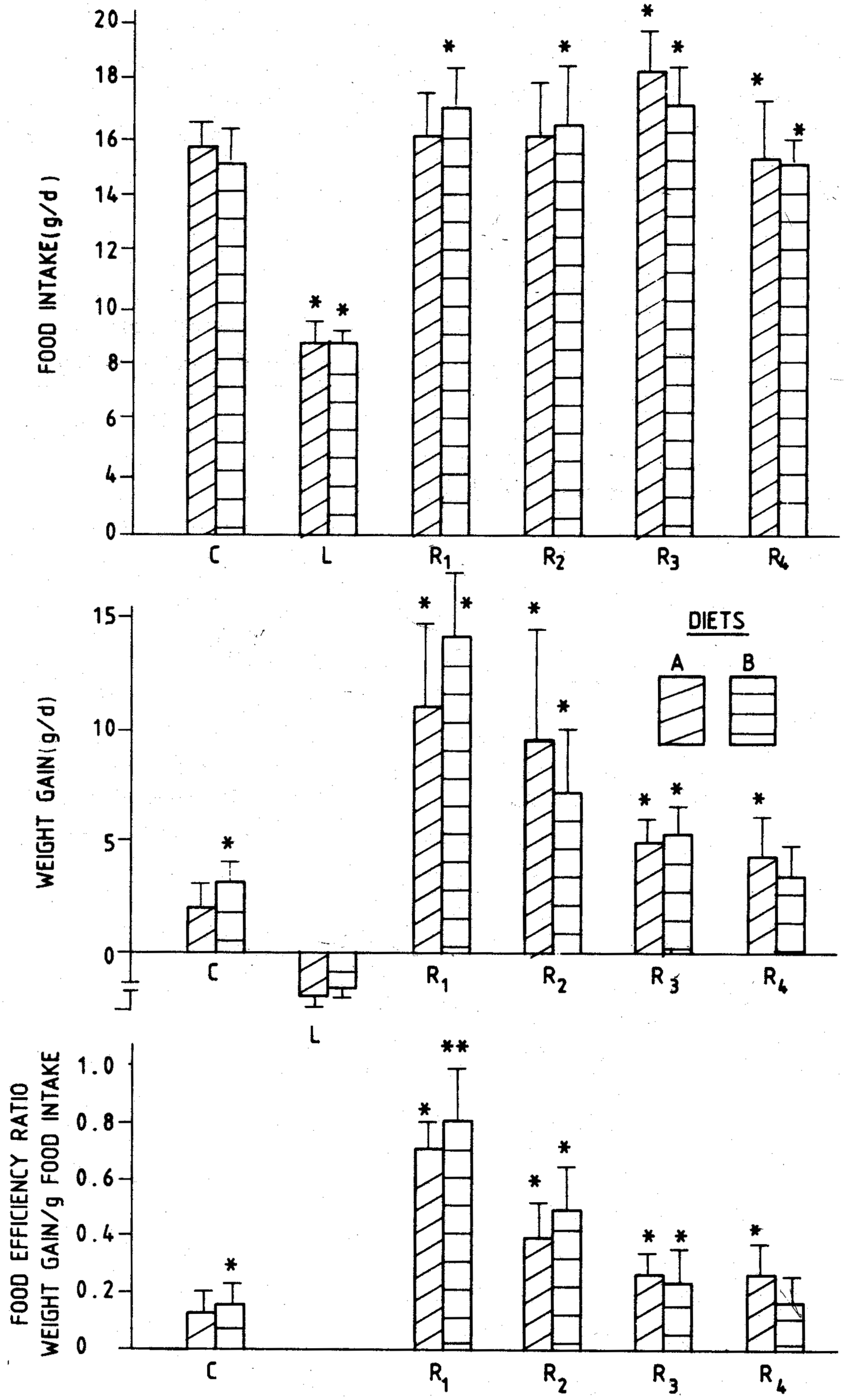


Figure 2. Food intake, weight gain and food efficiency ratio of *ad libitum*-fed control (C), food-restricted (L) and rehabilitated rats on day 1 (R_1), days 2-3 (R_2), days 4-5 (R_3) and days 9-10 (R_4) of rehabilitation. Values are mean ±S.D. for 6-8 rats per group. Vertical bars denote S.D. Values for food-restricted rats have been reproduced[6]. * Significantly different ($P < 0.05$) from control values; + significantly different ($P < 0.05$) from diet A group.

muscle protein fractions, viz., sarcoplasmic and myofibrillar in groups 1 and 2, (v) protein content in these fractions and liver, (vi) lipids in liver and epididymal fat pads, and (vii) carcass composition.

2.3 Statistical Analysis

The data were statistically analysed by using Student's *t* test. The values presented in the tables are mean ± S.D. while in the figures, mean ± S.E has been used.

3. RESULTS

The values for all the parameters evaluated during/at the end of food restriction phase (phase II) have been reproduced from our earlier publication[6] for comparison and to draw a meaningful interpretation of the results obtained in the present study.

3.1 Food Intake, Weight Gain & Food Efficiency Ratio

During *ad libitum* feeding, following 50 per cent energy restriction (phase III), the rehabilitated rats of both the protein groups showed hyperphagia (Fig. 2). The food consumption in group 2 (receiving 10 per cent protein diet) was found to increase steadily from day 1 (14.04 ± 1.55 g) till day 8 of rehabilitation (15.77 ± 1.62 g) which was nearly 20 per cent higher than that of control [group 1 (13.27 ± 0.72 g: P < 05)]. The food intake subsequently declined on days 9 and 10 to a value (15.07 ± 1.85 g) which was still 15 per cent higher than that of control (P < 0.05). The group 4 (receiving 20 per cent protein diet) on the other hand showed a 23 per cent higher (P < 0.05) food consumption on the 1 day of refeeding itself, which progressively decreased until days 9 and 10 to a value (14.93 ± 1.36 g) which still remained nearly 20 per cent higher than that of control [group 3 (12.53 ± 1.34 g: P < 0.05)].

Although the pattern of weight gain during rehabilitation (phase III) was essentially similar to that of food intake, the magnitude of increase was markedly higher. Thus, the rate of weight gain showed a five-fold increase at the end of first day of refeeding in both the protein groups; thereafter, it declined till days 9 and 10 but still remained

Table 1. Nitrogen balance in *ad libitum* fed control, food-restricted and rehabilitated rats

Diets	Control	Food restricted[@]	Rehabilitated	
			Days 2-3	Days 9-10
		Nitrogen intake		
A	206.2 ± 15.1	103.7 ± 7.2	232.1 ± 21.8*	-
B	461.1 ± 33.6	216.1 ± 4.1	512.8 ± 24.6*	514.5 ± 22.7 *
		Nitrogen in urine		
A	102.7 ± 14.9	76.7 ± 13.0	86.8 ± 12.5*	-
B	197.5 ± 33.0	134.0 ± 27.3	227.5 ± 31.5	199.8 ± 34.7
		Nitrogen in faeces		
A	31.6 ± 2.6	17.6 ± 1.7	32.9 ± 4.9	-
B	36.4 ± 6.5	22.0 ± 5.9	37.9 ± 9.7	43.0 ± 5.9
		Nitrogen balance		
A	72.0 ± 11.5	9.6 ± 14.0	112.4 ± 17.6*	-
B	227.2 ± 22.7	64.4 ± 14.0	240.3 ± 30.2	271.6 ± 46.6

Values expressed as mg/day are mean ± S.D. for six animals in each group.
Values under this column are reproduced from our earlier publication[6].
* Significantly different from control values at P < 0.05
Group A : 10 % protein diet; group B: 20 % protein diet.

significantly higher (4.07 ± 1.33 g per day, for group 2 and 2.5 ± 0.96 g per day, for group 4 compared to controls {(2.09 ± 0.45 g per day (for group 1) and 2.52 ± 0.31 g per day (for group 3) (P < 0.05)}. Consequently, the food efficiency ratio exhibited an initial spurt in the refed animals compared to controls {(0.92 ± 0.18 g (for group 2) and 0.78 ± 0.10 g (for group 4) versus 0.20 ± 0.1 g (for group 1) and 0.16 ± 0.03 g (for group 3)} on day 1. On day 10, the food efficiency ratio returned to control levels in the rats of group 4 fed diet B while it still remained significantly elevated (P < 0.05) in the low protein group 2 diet A (0.26 ± 0.08).

3.2 Nitrogen Balance

The nitrogen balance which had declined drastically during diet restriction increased over control levels on *ad libitum* feeding on days 2-3 of rehabilitation in both the refed groups 2 and 4 (Table 1); this increase was, however, significant (P < 0.05) only in group 2 and was found to be related to the increased nitrogen intake. The urinary nitrogen output continued to be low in group 2 even after refeeding for 2-3 days, resulting in better nitrogen balance, whereas the values showed a slight increase in group 4. There was no change in faecal nitrogen output. The nitrogen balance in group 4 tended to remain high even after 9-10 days of refeeding compared to the control group 3. It is possible that the nitrogen balance in group 2 also would have remained higher than that in the control animals (group 1).

3.3 Organ Weight & Composition

The relative weights (expressed as g/100 g body weight) of different tissues are shown in Fig. 3. The weights of livers of control as well as rehabilitated animals, fed diet B, were significantly higher (P < 0.05) than those fed diet A. The liver of rehabilitated rats fed *ad libitum* after restricted feeding, generally appeared to be heavier than their control counterparts. In the case of group 2 (refed with diet A), this increase was significant (P < 0.05). The weights of epididymal fat pads in rats of groups 1 and 2 were heavier than those of groups 3 and 4, although the differences were not statistically significant. Muscle weights were not reduced during diet restriction as is seen from the values reported earlier[6]. The weight of gastrocnemius muscle was insignificantly higher in groups maintained on diet B during phase I and III. During diet restriction, a reverse trend was observed due to the lower terminal weights of the rats receiving 50 per cent of diet A compared to those on diet B. There was no difference in the muscle weights between the control, restricted and the refed rats of both protein groups.

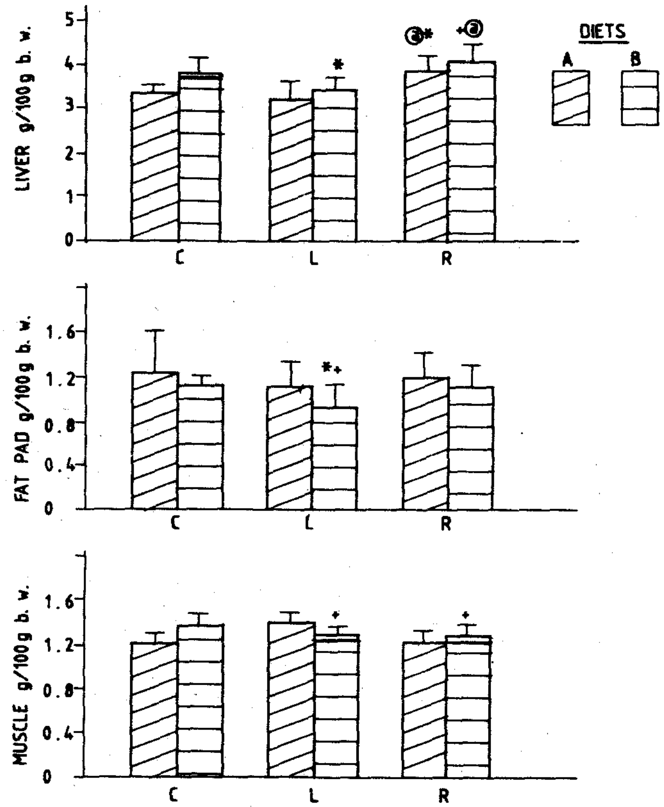


Figure 3. **Relative weights of organs (mean ± S.D.) for 7 rats in *ad-libitum*-fed control (C), food-restricted (L) and rehabilitated (R) rats. Vertical bars denote S.D. values for food-restricted rats have been reproduced[6].**
*** Significantly different (P < 0.05) from values for group fed diet A.**
+ Significantly different (P < 0.05) from values for control group
@ Significantly different (P < 0.05) from values for food-restricted group.

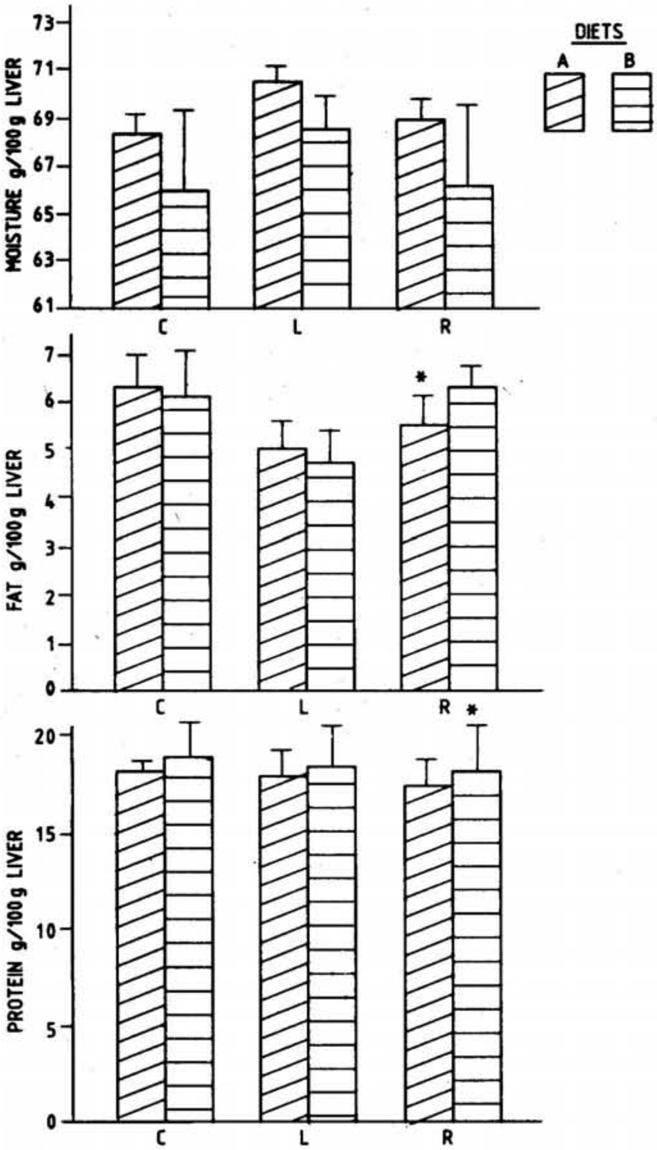


Figure 4. Composition of liver (mean ± S.D.) for 5-7 rats expressed as g/100 g liver) in *ad libitum*-fed control (C), food-restricted (L) and rehabilitated (R) rats. Vertical bars denote S.D. Values for food-restricted rats have been reproduced[6].

*** Significantly different (P < 0.05) from control.**

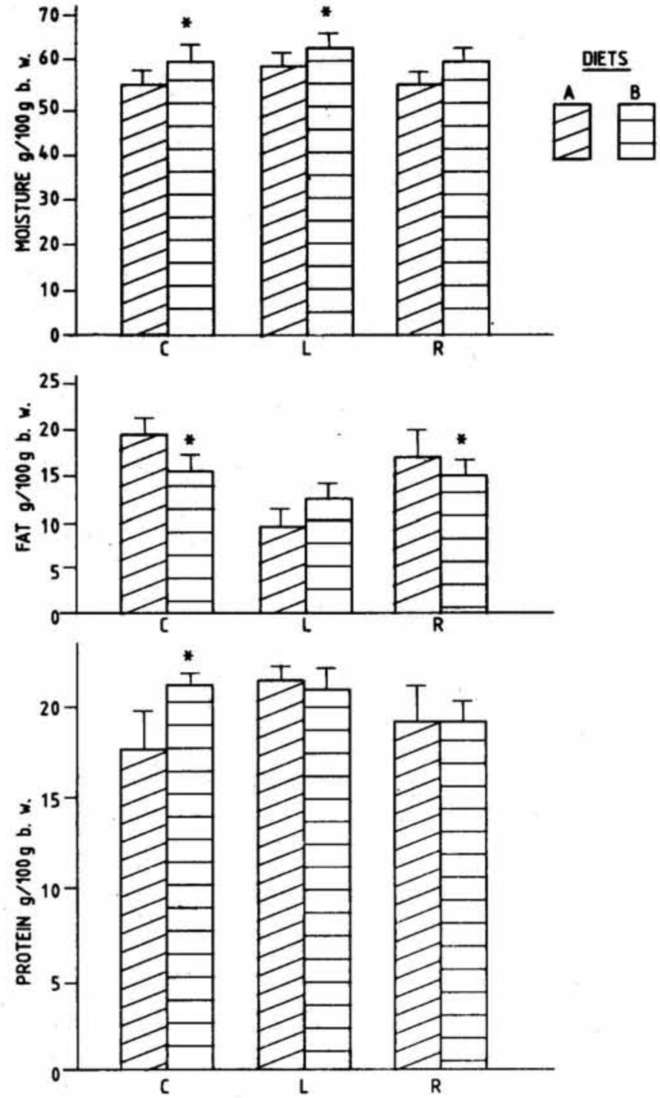


Figure 5. Carcass composition of (mean ± S.D.) for 4 rats expressed as g/100 g body weight) in *ad libitum*-fed control (C), food-restricted (L) and rehabilitated (R) rats. Vertical bars denote S.D. Values for food-restricted rats have been reproduced[6].

*** Significantly different (P < 0.05) from group fed on diet A.**

The moisture content in livers of rats fed diet A was not significantly diffe·ent from those of diet B, irrespective of whether they were fed *ad libitum* throughout or restricted-fed or rehabilitated after food restriction (Fig. 4). The liver lipid concentration was more or less similar in the control and the food-restricted rats, but was significantly lower in animals rehabilitated on diet A (group 2). The liver protein concentration in the rehabilitated rats on diet A also did not reach the levels prevalent in the corresponding control animals, even after 10 days of rehabilitation. The concentration of the intracellular muscle protein fractions (sarcoplasmic and myofibrillar) in the gastrocnemius muscle of rats refed with diet A were nearly identical (data not presented).

3.4 Carcass Composition

Although the carcass moisture level was found to be lower in the groups fed diet A than in groups fed diet B, regardless of whether the animals were continuously fed *ad libitum*, restricted-fed or rehabilitated after food restriction (Fig. 5), the differences were statistically significant only in the control animals ($P < 0.05$). On the contrary, the carcass fat was higher in all the groups fed diet A, irrespective of different dietary treatments. However, the differences were significant only in cases of control and rehabilitated groups ($P < 0.05$). The carcass protein level in the groups fed diet B tended to be higher, both in the control and refed groups, but the difference in the latter was not significant. The carcass constituents in the restricted-refed animals (groups 2 and 4) viz., moisture, fat and protein reached more or less the levels of control animals.

Figure 6 shows a reciprocal relationship between per cent body fat and per cent body water, obtained when data for the 16 animals of groups fed diet B, irrespective of the level of feeding, viz., four rats from each of control and restricted groups (phase II) of our earlier publication[6] and four rats from each of control and rehabilitated groups (phase III) of the present experiment. The linear correlation coefficient $r = -0.79$, is highly significant. A regression line was fitted between per cent body water (x) and per cent body fat (y) based on the regression equation, $y = 70.35 - 0.937x$. Similarly, a correlation was also established between body weight and per cent body fat (Fig. 7) for the above mentioned set of 16 rats. The linear correlation coefficient $r = 0.82$, is also highly significant.

The correlation coefficient was also similarly calculated using various data obtained with the

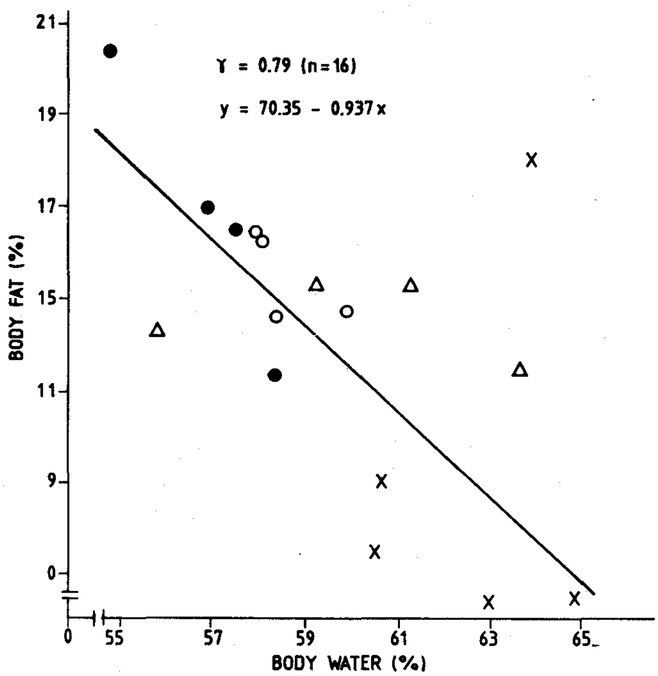


Figure 6. Relationship between body fat and body water in rats fed diet B. n = total number of carcasses analysed; ϒ = correlation coefficient; y = per cent body fat; x = per cent body water; • = control for food restricted group[6]; × = food restricted group[6]; ° = control for rehabilitated group 3 (n = 4) and Δ = rehabilitated group 4 (n = 4).

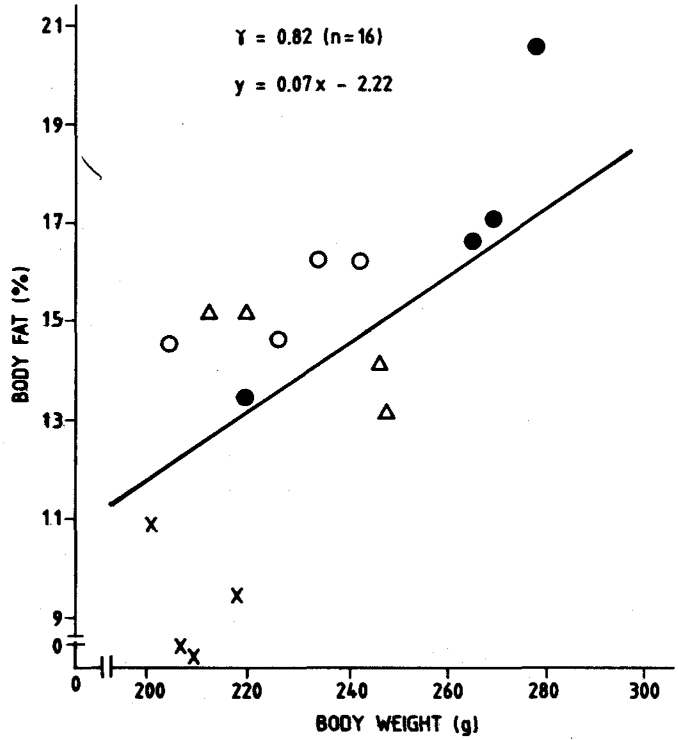


Figure 7. Relationship between body weight and body fat in rats fed diet B. n = total number of carcasses analysed; ϒ = correlation coefficient; y = per cent body fat; x = per cent body weight; • = control for food-restricted group (n = 4)[6]. × = food-restricted group (n = 4)[6]. ° = control for rehabilitated group 3 (n = 4); Δ = rehabilitated group, group 4 (n=4).

animals fed diet A. A linear correlation coefficient $r = -0.74$ and $r = 0.69$, (both $n = 16$) obtained for carcass fat versus per cent body water and body weight versus per cent body fat respectively, was also found to be significant [$P < 0.05$ data not presented].

4. DISCUSSIONS

The body can be considered a system in which body weight is a gross variable reflecting a long-term balance between energy input and output. A net gain/loss in energy results in gain/loss of body weight. A supernormal weight gain, observed during rehabilitation of food-restricted rats, irrespective of the dietary protein level, has been described by several workers as 'compensatory weight gain' or 'catch up growth'[7-9] resultant of a marked elevation in food intake[10]. Such responses for which food restriction is a pre-requisite and provides the necessary stimulus, often resemble 'metabolic memory'; the effect of a past nutritional event on a future adaptation of starvation[11,12].

The mechanism of increased efficiency of food utilisation observed during rehabilitation is not clear. Such an increased metabolic efficiency has been reported to exist also in the meal-eating animals (animals trained to eat food daily in a single short period) compared to nibbling animals. This phenemonon attributed to the hypertrophy of the digestive tract[12] and also to the increased lipogenic capacity of the adipose tissue[13], may be existing in the restricted-refed animals of the present experiment at the initial stage and progressively tapering off with time.

The faster than normal weight gain found in animals during rehabilitation phase, in the present experiment, could be due to fat deposition as a result of increased lipogenic capacity of the fat tissue and this is in close agreement with the observations reported earlier[11,12,15]. The contribution of tissue protein during 'catch up growth' is minimal, since there was no loss of body protein in the rats during diet restriction. The phenomenon of compensatory growth assumes importance in veterinary practice because it economises the food in winter by food restriction to produce animals with more fat[16]. However, in human beings, the problem of obesity (overweight) afflicts many and is not desirable.

The anomaly of a lower weight gain in animals fed diet A containing sub-optimal level of protein, despite a greater food intake can be explained in terms of an elevated 'diet-induced thermogenesis' resulting in lower food efficiency. The loss of body fat observed during food restriction was found to be accompanied by an increase in body water and vice versa. Thus, there was a reciprocal relationship between body fat and body water. It is not known whether the moisture retention following fat depletion in tissues is a phenomenon of redistribution of water in tissues or is correlated with sodium pump, and in turn to mineral and water retention.

Another significant finding is the marginal increase in fat content in the carcasses of animals fed diet A compared to those on diet B. Reduction in protein concentration in the diet has been reported to be associated with decreased growth rate and efficiency of food utilisation with a concomitant increase in body fatness[17]. This effect was not reflected in the metabolically-active organ, such as liver of rehabilitated animals, unlike the food-restricted animals. The causative factors for the differential behaviour of this tissue is not clear. However, lack of an increase in the fat content in liver of rehabilitated animals is presumed to be due to the alteration in the rate of production and removal of triglycerides from the liver.

The liver does not show any tendency for over-compensation. The liver protein and lipid depletion that occurred during food restriction was restored on subsequent surfeit feeding during the

10 day rehabilitation in the animals of group 4 on diet B but not fully in group 2 on diet A, which shows that dietary protein has a definite role in the regeneration of tissue constituents.

The present study demonstrates that rehabilitation following 50 per cent restricted feeding is characterised by hyperphagia, supernormal weight gain and increased metabolic efficiency of food. The reversion of these variables to control levels depends upon the composition of the hypocaloric and refeeding diet and the duration of refeeding. The organs, such as liver, fat pad and muscles respond differently during such dietary manipulations depending upon whether the tissue is predominantly a promoter of synthesis of tissue lipid, or protein, or both. The incomplete recovery of tissue constituents in animals of group 2 fed diet A indicates that dietary protein is an important constituent that needs serious consideration, while designing rehabilitation diets. Further work on the effect of quality and quantity of protein on the efficiency of a rehabilitation diet and protein turnover would yield interesting results.

ACKNOWLEDGEMENT

The authors wish to thank Dr S.S. Arya, Director and Dr K. Santhanam, Biochemistry and Nutrition Group of Defence Food Research Laboratory, Mysore, for their keen interest and valuable guidance.

REFERENCES

1. Garrow, J.S. Energy balance in man – an overview. *Am. J. Clin Nutr.*, 1987, **45**, 1114-19.
2. Gumaa, K.A.; Musthafa, K.Y.; Mahmoud, N.A. & Gader, A.M.A. The effect of fasting in ramdan. *Br. J. Nutr.*, 1978, **40**, 573-81.
3. Crowdy, J.P.; Haisman, M.P. & Mc Gavock, H. The effect of a restricted diet on the performance of hard and prolonged physical work. Armed Forces Personnel Research Establishment, U.K. 1971, Report No. 2/71.
4. Viswanathan, K.R.; Narayan Prasad, N.; Siddalinga Swamy, M. & Rama Rao, M.V. Effect of hypocaloric stress on body and tissue composition of rats. *Def. Sci. J.*, 1988, **38**(3), 261-72.
5. Viswanathan, K.R.; Narayan Prasad, N.; Ramanuja, M.N. & Narayanan, V.A. Evaluation of low energy pack ration by short-term feeding to soldiers. *Def. Sci. J.*, 1991, **41**, 263-75.
6. Viswanathan, K.R; Narayan Prasad, N. & Siddalinga Swamy, M. Effect of short-term energy and protein restriction on tissue and body composition of rats. *Def. Sci. J.*, 1997, **47**, 159-66.
7. Widdowson, E.M. & Mc Cance, R.A. The effect of finite periods of under-nutrition at different ages on the composition and subsequent development of the rat. *Proc. Roy. Soc. B*, 1963, **158**, 329-42.
8. Werner, S.C. Comparison between weight reduction on a high calorie, high-fat diet and on an isocaloric regimen high in carbohydrate. *New Eng. J. Med.* 1955, **252**, 661-65.
9. Mc Anulty, P.A. & Dickerson, J.W.T. The development of the weanling rat during nutritionally-induced growth retardation and during early rehabilitation. *Brit. J. Nutr.*, 1974, **32**, 301-12.
10. Szepsi, B. & Vojnik, C. A model for the study of nutritionally-induced overweight. *Nutr. Rep. Inter.*, 1975, **11**, 305-11.
11. Szepsi, B.; Vogers, R; Michaelis, O.E. & De Mony, J.M. Long-term effects of starvation-refeeding in the rat. *Nutrition & Metabol*ism, 1975, 19-45.
12. Szepsi, B. Metabolic memory: effect of antecedent dietary manipulations on subsequent diet-induced response of rats. Part 1. Effect of body weight, food intake, G6PD and malic enzyme. *Can. J. Biochem.* 1973, **51**, 1604-16.

13. Leveille, G.A. & Chakraborthy, K. Absorption and utilisation of glucose by meal-fed and nibbling rats. *Journal of Nutrition*, 1968, **96**, 69-75.

14. Leveille, G.A. Lipogenic adapatations related to pattern of intake. *Nutritional Review*, 1972, **30**, 151-55.

15. Hegsted, D.M; Gallagher, A. & Hanford, H. Reducing diets in rats. *Am. J. Clin. Nutr.*, 1975, **28**, 837-40.

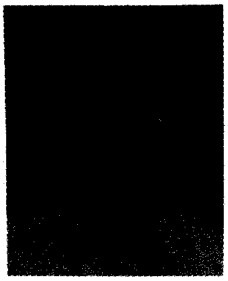
16. Wilson, P.N. & Osbourn, D.F. Effects of different pattern of allocation of restricted quantity of food upon growth and development of cockerels. *J. Agric. Sci.* 1960, **54**, 278-79.

17. Zhao, X.Q.; Jorgensen; Gabert, V.M. & Eggum, B.O. Energy metabolism and protein balance in growing rats housed in 18 °C or 28 °C environments and fed different levels of dietary protein. *Journal of Nutrition.*, 1996, **126**, 2036-43.

Contributors

Dr KR Viswanathan obtained his PhD (Biochemistry) from the University of Mysore in 1984. He is a recognised guide for MSc and PhD in Food Science at Mysore University. Presently, he is working as Scientist F at the Defence Food Research Laboratory (DFRL), Mysore. His areas of work include: nutrition under diverse stress conditions, hypocalorie feeding of man and animals in relation to diet and physical efficiency, nutrient content of processed foods and nutritional quality and storage stability of textured soya protein. Currently, he is working on the antioxidant properties of fruits and vegetables in relation to cancer preventive potential in experimental animals. He has published several papers in national/international journals.

Mr M Siddalinga Swamy obtained his MSc (Food Science) from the University of Mysore, in 1984. Presently, he is working as Scientist at DFRL, Mysore. His areas of research include: nutrition, biochemistry, hypocaloric stress in relation to tissue and body composition of animals, safety evaluation of processed foods in rats, nutritional quality and storage stability of textured soya protein and nutritional evaluation of processed foods with special reference to the proximate score, energy, dietary fibre and mineral contents. He has several publications to his credit.



Mr N Narayan Prasad obtained his MSc (Food Science) from the University of Mysore, in 1980. Presently, he is working as Scientist at DFRL, Mysore. His areas of research include: chemical and biological evaluation of nutritional quality of foods, hypocaloric stress in relation to changes in the tissue and body composition, nutritional changes associated with storage quality of textured soya protein and evaluation of processed foods, particularly for the content of vitamins, minerals and dietary fibre. He is a recognised guide for MSc (Food Science) at Mysore University. He has published several papers in national/international journals.