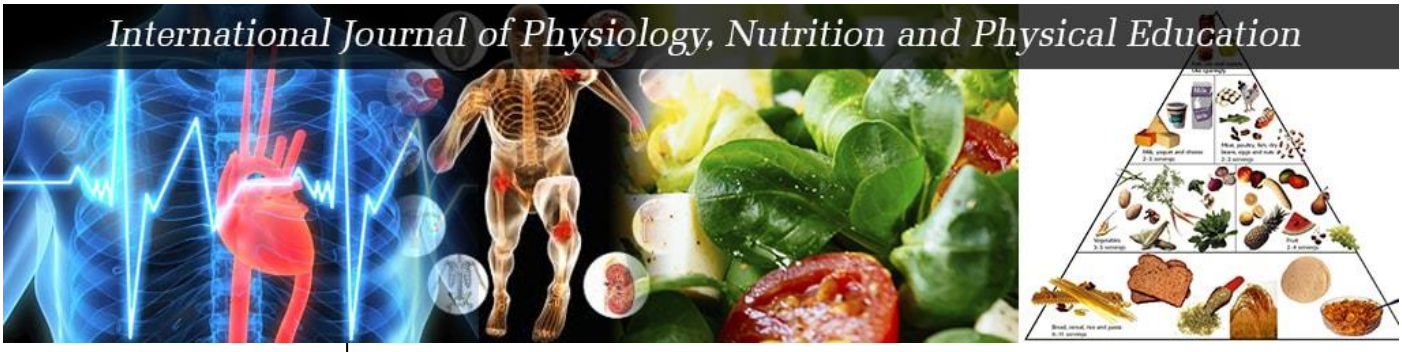

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Controlled study on short term calorie restriction as possible nutritional approach to sustain and survive during emergency situations

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Abstract
Many studies indicate that Calorie Restriction (CR) may have role in life span extension and other beneficial metabolic health effects. But the application of CR as a possible strategy for maintaining metabolic homeostasis under emergency situations remains unexplored. Assuming a situation of emergency/natural disasters such as flood, earthquake, thunder storm, other relief operations etc. when affected individuals have to sustain and survive on ready to eat packed food, a study on short term continuous calorie restriction using ready to eat packed food was carried out on 40 participants comprising (20 males and 20 females). Two different restricted calorie intake levels were used 2000 kcal/day for males and 1600 kcal/day for females respectively. The energy expenditure of males and females were 2877 ± 527 and 2427 ± 184 kcal/day respectively. Therefore, calorie restriction of about 30-35% was done. The menu planning was made using commercially available ready to eat products. The nutritional status was assessed by analyzing changes in body composition, biochemical indices before and after calorie restriction. There was no adverse effect of short term calorie restriction except the feeling of hunger in case of few participants. It was concluded that during emergency situations lasting 3-7 days, a diet containing 1600-2000 kcal/day using ready to eat products may be provided during relief operations.

Keywords: Calorie restriction, body composition, energy expenditure, lipid profile

1. Introduction
Nutritional adequacy is a basic component of healthy life. The nutritional requirements of humans are influenced by body composition, activity level, Climatic conditions and also by dietary habits [1, 2]. Optimum nutrition is sometimes hindered due to several factors affecting food intake, emergency situation such as flood, earthquake etc. is one of the factor which influences the normal food intake. Food supply is a basic priority and essential for the Survival as well as sustenance in such emergency situations. The importance of food supply increases manifold and maintenance of normal food supply becomes difficult in initial days. Moreover, nutritional, health and logistics (weight, space and transportation) aspects becomes more critical for handling such emergency situations. Assuming the such situations, when the routine life of population is disrupted, logistics & supply chain management geared up for handling such situations, data on the minimum calorie requirement for sustenance and survival of affected individuals will be highly beneficial in better planning and logistics management for food supply. So, Calorie restriction as the possible strategy may be explored for application under such circumstances. Calorie restriction (CR) refers to a dietary regimen low in calories without under nutrition. Calorie restriction is well reported for increasing life span in variety of species and its mechanism through which it extends the life span has also been explored well. CR has not only emerged as a most robust non genetic intervention for life extension but it also provides healthy span by significantly reducing the occurrence of chronic diseases. CR has been found to be beneficial for weight management, cardiovascular health, prevention of cancer, diabetes, hypertension, inflammatory diseases etc. [3-16]. The published reports focussed mainly on the positive health effects of calorie restriction during aging and clinical conditions such as obesity and interventional studies.

Very few studies on effect of CR on performance and metabolic homeostasis [15], using intermittent fasting or restricted intake are available [17, 18] but none of the study has so far reported effect of sudden continuous CR on performance and metabolic homeostasis. Hence, a study on sudden short term continuous calorie restriction (30- 35%) for 7 days was conducted with an aim to explore it as a strategy for possible use under emergency situation.

2. Materials and Methods

2.1 Study volunteers and diet plans

A total of 40 volunteers from our institute comprising 20 males and 20 females participated in the study. Two different calorie restricted plans were made for females and males based on their habitual dietary intake and consisted 1600 kcal/day and 2000 kcal/day respectively. Subjects were explained about study procedure and written consent for participation in study was obtained.

All study variables were recorded initially (Pre) and after one week (post) of calorie restriction. During calorie restriction participants took calorie restricted diet made up of ready to eat food recipes. Only the food intake was restricted and however, the routine work was continued as per participant's schedule throughout the week.

The energy intake was calculated based on the energy value mentioned over the ready to eat products package. The seven days' meal was planned according to the restricted calorie intake levels.

2.2 Assessment of energy expenditure

To evaluate energy expenditure two different methods were used

2.2.1 Activity records: The time spent in different activities was recorded and energy cost of different activities on the basis of oxygen consumption was used to compute energy expenditure.

2.2.2 Accelerometry based Actical system: Actical devices were worn on wrist of by subjects for a period of 7days. Minute by minute data for energy expenditure was recorded. This system is a good tool to give reliable measure of energy expenditure in case of subjects following a set pattern of activities [19].

2.3 Analysis of body composition and nutritional status

2.3.1 Body composition: Body composition *viz*. body fat, body water and lean body mass were measured by bioelectrical impedance analysis (BIA) using Tanita body fat analyser (BC420 MA) and Martin's Anthropometer for height.

2.3.2 Hematological profile: Hematological variables i.e. hemoglobin, leucocytes counts (lymphocytes, neutrophils), red blood cell, platelet counts, hematocrit, RBC indices [mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC)] were determined using Automatic Hematology Analyzer (MS_4Se, Melet Scholoesing laboratories, Pontoise, Cedex – France).

2.3.3 Biochemical variables: Venous blood samples were drawn in the morning after overnight fast. Samples were collected in heparinized tubes, centrifuged at 1000g for 10 min to recover plasma and stored in aliquots in cryovials. An aliquot of 200µl of each plasma sample immediately after recovery was mixed with 10% metaphosphoric acid for estimation of Vitamin 'C'. Erythrocytes were washed three times with 150mM KCl and 10% (w/v) lysates were prepared with distilled water for estimation of enzyme markers of vitamin status. Samples were brought in frozen condition to laboratory where they were stored at -70^0 C until assayed. Total plasma protein was estimated using modified method of Lowry *et al*. 1951 [20]. Plasma albumin was estimated using colorimetric method. Globulin levels were obtained by subtracting albumin level from total proteins. A/G ratio was also calculated. Ascorbic acid (Vitamin C) in plasma using αα dipyridyl [21]. Lipid profile by estimating total cholesterol, HDL cholesterol and triglycerides in plasma using commercially available kits. LDL was calculated using Freidwalds formula i.e. LDL cholesterol = Total Cholesterol – (HDL cholesterol+ Triglyceride/5). One fifth of triglycerides were taken as VLDL (very low-density lipoproteins) content. Plasma hydroperoxides were estimated using FOX-1 assay [22]. Uric acid, creatinine and MDA were estimated using standard biochemical procedures [23]. Hormones (Leptin, Cortisol, testosterone) and NEFA were estimated using commercially available kits.

2.4 Handgrip strength

Passive Handgrip Strength test for muscular strength was carried out using Grip Strength Dynamometer (TKK5001, Grip A, Takaki Scientific Instruments Co. LTD, least count 0.5kg). Subjects were explained the technique before conducting the measurement and encouraged to press dynamometer by their nonworking hand (i.e. left hand for right hand users) with full grip strength and readings were recorded.

2.5 Feedback from subjects on CR

Subjects were given a questionnaire to give their feedback after following calorie restricted regime.

2.6 Statistical analysis

Comparison of data obtained initially and after one week of calorie restriction was made using Student 't' test and p value <0.05 was considered as a significant change.

3. Results

3.1 Demographic profile of study participants

The mean age, dietary and life style i.e. vegetarian, non-vegetarian, smoking, drinking habits of the study volunteers are summarized in Table1. The volunteers represented various states of the country, the trend of population from different state was Delhi (25%) > West Bengal &Uttar Pradesh (12.5%)> Rajasthan (10%), Haryana, Bihar& Uttarakhand (7.5%)>Tamil Nadu, Jharkhand & Punjab (5%) > Himachal (2.5%).

Table 1: Age, Dietary habits and life style of study volunteers

Study group	Age (Years)	Vegetarian/ Non-Vegetarian	Alcohol use/Non -Alcohol	Smoker/ Non Smoker
Males (n=20)	34.1± 10.4	8/12	1/19	1/19
Females (n=20)	30.2± 9.1	13/7	0/20	0/20

Values are Mean ± SD

3.2 Energy expenditure, energy intake and calorie restriction

The working schedule of volunteers were different, therefore analysis of time spent in different grades of activities was made on the basis of metabolic equivalents (METs) using Actical Devices. Energy expenditure (Kcal/day) was calculated for different days and the mean expenditure was found to be 2877 ± 527 and 2427 ± 184 for males and females respectively.

Seven days menu was planned using Ready to eat meals (Table-2), the calculated energy of the menu was1600 kcal/day for females and 2000 kcal/day for males.

Table 2: Calorie restricted menu sample using ready to eat food items

Meal	RTE food Item	Quantity (g) (Females/ Males)	Energy (kcal)	
			Females	Males
Break fast	Parantha (No. S)	2/2	300	300
	Navratan Kurma	150/200	142.5	190
	Tea/Coffee	1 cup		
Lunch	Mutter Paneer	150/200	255	340
	Chapati No. S	2/3	189	270
Evening tea	Biscuit No. S	3/3	120	120
	Tea/Coffee	1 cup		
Dinner	Dal Makhani	150/200	328.5	438
	Chapati No. S	3/4	270	351
Total Energy			1605	2009

3.2.1 Calorie Restriction

Estimated energy expenditure and calculated energy intake resulted in calorie restriction in the approximate range of 30-35% (Table-3) for total population and higher percentage of calorie restriction was applied to females. Since, females are more vulnerable in emergency situations and the impact of calorie restriction will become more meaningful with such cases.

Table 3: Energy intake, energy expenditure and % calorie restriction

Study group	Energy Expenditure (kcal)	Energy intake (kcal)	% Calorie restriction
Males (n=20)	2877 ± 527	2000	30
Females (n=20)	2427 ± 184	1600	34

Values are Mean ± SD

3.3 Body Composition

Changes in body weights and body composition during study are given in Table-4. There was a significant decrease in mean body weight of males and females. The decrease in fat mass and lean body mass was observed which was not significant. BMI and BMR was decreased marginally.

Table 4: Changes in body composition before and after calorie restriction

Body Composition Analysis	Males		Females	
	Initial	Final	Initial	Final
Weight (Kg)	75.22 ± 10.61	73.8 ± 11.89**	60.72 ± 14.05	59.69 ± 13.99*
BMI	25.72 ± 3.08	25.45 ± 3.14**	24.54 ± 5.25	24.22 ± 5.20*
BMR (Kcal)	1725 ± 115	1701 ± 180**	1387 ± 113	1382 ± 113**
Fat (%)	21.15 ± 5.19	20.85 ± 5.67 (n.s.)	27.46 ± 10.24	26.76 ± 10.04 (n.s.)
Fat Mass (Kg)	16.2 ± 5.65	15.65 ± 5.50 (n.s.)	17.90 ± 10.44	17.18 ±10.3 (n.s.)
FFM (Kg)	59.02 ± 6.84	58.14 ± 8.59 (n.s.)	42.82 ± 3.95	42.49 ±3.90 (n.s.)
TBW (Kg)	43.22 ± 5.10	42.57 ± 6.30 (n.s.)	31.38 ± 2.91	31.11 ± 2.85 (n.s.)

Values are Mean ± SD, * significant, *(p<0.05), ** (p<0.01), n.s. not significant.

3.4 Hematological profile

Hemoglobin, blood cell counts and RBC indices were analysed. All values were within normal range both pre and post calorie restriction and no significant difference was observed. Hb (mg/dl) value was found to be 13.1 ± 1.6 and 12.9 ± 2.0 pre and post CR respectively.

3.5 Changes in biochemical markers of nutritional status

3.5.1 Lipid profile

As shown in table - 5, significant decrease in cholesterol, LDL, VLDL and triglycerides was observed in males and females.

Table 5: Changes in lipid profile pre and post calorie restriction

Biochemical Parameter	Males		Females	
	Initial	Final	Initial	Final
Cholesterol (mg/dl)	206.29 ± 41.74	178.30 ± 31.44*	187.04 ± 47.29	149.11 ± 41.90**
HDL(mg/dl)	49.16 ± 9.22	49.72 ± 11.13 (n.s.)	52.29 ± 6.94	49.72 ± 6.96 (n.s.)
Triglycerides (mg/dl)	119.46 ± 50.45	98.1 ± 50.76*	154.73 ± 69.75	105.43 ± 79.0 (n.s.)
LDL (mg/dl)	133.24 ± 23.81	108.96 ± 10.5**	103.8 ± 25.8	78.31 ± 14.2**
VLDL (mg/dl)	23.89 ± 12.8	19.62 ± 8.9*	30.95 ± 16.3	21.09 ± 12.7*
NEFA (mg/dl)	0.56 ± 0.25	0.53 ± 0.17 (n.s.)	0.46 ± 0.020	0.55 ± 0.21 (n.s.)

Values are Mean ± SD, * significant, *(p<0.05),** (p<0.001),, n.s. not significant.

3.5.2 Protein
Changes in total protein concentration, albumin, Globulin and A/G ratio are shown in table-6.

Table 6: Changes in total protein concentration, albumin, globulin and A/G ration pre and post calorie restriction

Biochemical Parameter	Males		Females	
	Initial	Final	Initial	Final
Protein (g/dl)	8.16 ± 0.61	7.31 ± 0.60**	8.08 ± 0.65	7.80 ± 0.60 (n.s.)
Albumin (g/dl)	5.13 ± 0.80	4.60 ± 0.60*	4.73 ± .50	4.69 ± 0.29*
Globulin (g/dl)	2.96 ± 0.61	2.70 ± 0.79 (n.s.)	3.28 ± 0.67	3.10 ± 0.52 (n.s.)
A/G ratio	1.87 ± 0.62	1.92 ± 0.89 (n.s.)	1.55 ± 0.52	1.55 ± 0.28 (n.s.)

Values are Mean ± SD, * significant, *(p<0.05),** (p<0.001), n.s. not significant.

3.5.3 Hormones
As shown in table-7, significant changes in testosterone and cortisol levels were observed in males and females, however leptin decrease was not significant in males.

Table 7: Changes in hormones pre and post calorie restriction

Biochemical Parameter	Males		Females	
	Initial	Final	Initial	Final
Testosterone (ng/dl)	4.14 ± 1.23	3.11 ± 0.94**	0.57± 0.36	0.37± 0.25*
Cortisol (nMol/L)	426.67 ± 190.69	309.53 ± 71.02*	351.47 ± 178.11	344.11 ± 168.08*
Leptin (ng/ml)	19.02 ± 9.38	16.16 ± 10.68 (n.s.)	50.19 ± 25.82	42.21 ± 23.18 *

Values are Mean ± SD, * significant, *(p<0.05),** (p<0.001), n.s. not significant.

3.5.4 Metabolic by products
No significant changes in creatinine and uric acid were observed (table-8).

Table 8: Changes in creatinine and uric acid concentration pre and post calorie restriction

Biochemical Parameter	Males		Females	
	Initial	Final	Initial	Final
Creatinine(mg/dl)	1.01 ± 0.46	1.12 ± 0.86 (n.s.)	0.98 ± 0.15	0.83 ± 0.41 (n.s.)
Uric Acid (mg/dl)	6.89 ± 0.62	6.91 ± 0.81 (n.s.)	8.1 ± 0.21	7.6 ± 0.32 (n.s.)

Values are Mean ± SD, n.s. not significant

3.5.5 Vitamin and Antioxidant status
Changes in Vitamin C, MDA and Hydro peroxides concentration are shown in table-9.

Table 9: Changes in Vitamin C, Hydroperoxides and MDA concentration pre and post calorie restriction

Biochemical Parameter	Males		Females	
	Initial	Final	Initial	Final
Vitamin C (mg/dl)	1.377 ± 0.58	1.023 ± 0.291 *	1.389 ± 0.33	1.134 ± 0.346 *
Hydroperoxides (µ mole/ml)	12.60 ± 2.00	12.87 ± 4.53 (n.s.)	12.01 ± 2.23	13.19 ± 3.96 (n.s.)
MDA (µ mol)	0.93 ± 0.39	0.66 ± 0.21 *	1.15 ± 0.39	0.67 ± 0.30 **

Values are Mean ± SD, * significant, *(p<0.05),** (p<0.001), n.s. not significant.

4. Changes in performance status
4.1 Handgrip strength
As shown in table-10, significant increase in handgrip strength of males was observed, however no significant changes in hand grip strength of females was observed.

Table 10: Changes in hand grip strength pre and post calorie restriction

Performance status	Males		Females	
	Initial	Final	Initial	Final
Hand Grip Strength	42.88 ± 7.10	44.83 ± 7.10 *	25.41 ± 4.48	27.08 ± 5.10 (n.s.)

Values are Mean ± SD, * significant, *(p<0.5), n.s. not significant, p value is given in parenthesis.

5. Feedback from CR subjects
Qualitative analysis of questionnaire described that there was no adverse effect of CR on routine activities, however feeling of hunger was mentioned by participants and it was observed as expected.

6. Discussion
The present study was under taken to study the effect of short term calorie restriction on nutritional status, metabolic homeostasis and performance on males and females. The study design was a pre and post CR intervention design without a control group. Normal participants were taken randomly without any inclusion criteria from research institute. The calorie restricted regime reduced the daily specific micronutrient intake to values lower than 90% of recommended RDA. Energy intake was reduced to about 30-35%. There are several reports on energy restriction which point out that with an energy expenditure of 3800 kcal/day and an intake of 2000-2200 kcal the work efficiency is not adversely affected [24-29]. Under energy restriction certain amount of adaptation in basal metabolic rate takes place to minimize energy expenditure [35-37]. Calorie restriction is also

evidenced well for changes in circulating hormones, better lipid profile and other biochemical changes [30, 31]. Changes in anthropometry were also observed as weight loss, loss of fat mass and some loss of lean mass was also observed which doesn't result in any decrease in performance, similar observations have been reported earlier in different studies. CR didn't show any decrease in performance as observed by hand grip strength and literature has shown that CR results in enhancement of physical performance [17, 33-34]. In short term CR the nutritional deficiency didn't manifest but long duration CR may result in micronutrient deficiency which needs to addressed by supplementation with micronutrients as reported in earlier studies [17, 35].

7. Conclusion

During emergency situation and for relief operations lasting 3-7 days, a diet with 1600-2000 kcal/day using ready to eat products may be supplied safely without any adverse effect on health.

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9. References

1. Mahan LK, Escott-Stump S. Krause's Food, Nutrition, & Diet Therapy. 11th Edition. Elseviers USA, 2004.
2. ICMR. Nutrient Requirements and Recommended Dietary allowances for Indians, National Institute of Nutrition, Hyderabad, 1989.
3. Heilbronn LK, Ravussin E. Calorie restriction and aging: review of the literature and implications for studies in humans. Am J Clin Nutr. 2003; 78:361-369.
4. Varady KA, Hellerstein MK. Alternate-day fasting and chronic disease prevention: a review of human and animal trials. Am J Clin Nutr, 2007; 86:7-13.
5. Fontana L, Partridge L. Promoting health and longevity through diet: from model organisms to humans. Cell. 2015; 161(1):106-118. doi:10.1016/j.cell.2015.02.020
6. Fontana L, Partridge L, Longo VD. Extending healthy life span-from yeast to humans. Science. 2010a; 328:321-326.
7. Fontana L, Coleman RJ, Holloszy JO, Weindruch R. Calorie restriction in non-human and human primates. In Handbook of the Biology of Aging, E.J. Masoro and S.N. Austad, eds. (Academic Press), 2010b, 447-461.
8. Ikeno Y, Lew CM, Cortez LA, Webb CR, Lee S, Hubbard GB. Do long-lived mutant and calorie-restricted mice share common anti-aging mechanisms?-a pathological point of view. Age (Dordr.) 2006; 28:163-171.
9. Bodkin NL *et al*. Mortality and morbidity in laboratory-maintained rhesus monkeys and effects of long-term dietary restriction. J Gerontology A Biol. Sci. Med Sci. 2003; 58(3):212-9.
10. Colman RJ, Beasley TM, Kemnitz JW, Johnson SC, Weindruch R, Anderson RM. Caloric restriction reduces age-related and all cause mortality in rhesus monkeys. Nat. Commun. 2014; 5:3557.
11. Mattison JA, Roth GS, Beasley TM, Tilmont EM, Handy AM, Herbert RL *et al*. Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. Nature. 2012; 489:318-321.
12. Heilbronn LK, de Jonge L, Frisard MI, DeLany JP, Larson-Meyer DE, Rood J *et al*. Pennington CALERIE Team Effect of 6-month calorie restriction on biomarkers of longevity, metabolic adaptation, and oxidative stress in overweight individuals: a randomized controlled trial. JAMA. 2006; 295:1539-1548
13. Cava E, Fontana L. Will calorie restriction work in humans? Aging (Albany, N.Y. Online) 2013; 5:507-514.
14. Mercken EM, Crosby SD, Lamming DW, JeBailey L, Krzysik-Walker S, Villareal DT *et al*. Calorie restriction in humans inhibits the PI3K/AKT pathway and induces a younger transcription profile. Aging Cell. 2013; 12:645-651.
15. Mattson MP, Wan R. Beneficial effects of intermittent fasting and caloric restriction on the cardiovascular and cerebrovascular systems. J Nutr. Bio chem. 2005; 16(3):129-37.
16. Omodei D, Fontana L. Calorie restriction and prevention of age-associated chronic disease. FEBS Lett. 2011; 585(11):1537-1542. doi:10.1016/j.febslet.2011.03.015
17. Pons V, Riera J, Capo X *et al*. Calorie restriction regime enhances physical performance of trained athletes. J IntSoc Sports Nutr. Mar 2018; 15(12). https://doi.org/10.1186/s12970-018-0214-2
18. Ferguson LM *et al*. Effects of caloric restriction and overnight fasting on cycling endurance performance. J Strength Cond Res. 2009; 23(2):560-70.
19. Singh SN, Shukla V, Dutta A, Singh VK, Vats P, Banerjee PK. Assessment of free living energy expenditure using an omnidirectional accelerometer based activity monitoring system. Procc 92nd Indian Science Congress, section of Medical Sciences (including Physiology), Jan 3-7, 2005, Ahmedabad, 2005, 20.
20. Lowry OH, Rosebourgh NJ, Far AL, Randal RJ. Protein measurement with fool in phenol reagent. J Bio. Chem. 1951; 193:265-275.
21. Zannoni V, Lynch M, Goldstein S, Sato P. A rapid micro method for the determination of ascorbic acid in plasma and tissues. Biochem Med. 1974; 11:41-48.
22. Wolff SP. Ferrous ion oxidation in presence of ferric ion indicator xylenol orange for measurement of hydroperoxides Methods Enzymol. 1994; 233:182-185.
23. Saroj Kumar Verma, Vandana Kirar, Vijay Kumar Singh, Gurseen Rakhra, Daisy Masih, Annu Vats *et al*. Energy Expenditure and Nutritional Status of Sailors During One Month of Extensive Physical Training. Defence Life Science Journal, 2018; 3(3):216-223, DOI: 10.14429/dlsj.3.12907
24. Viswantahan KR, Prasad NN, Ramanuja MN, Narayanan VA. Evaluation of low energy pack ration by short-term feeding to soldiers. Def Sci J. 1991; 41:263-275.
25. Consolazio CF, Moutoush LO, Johnson HL, Krzywicki HJ, Isaac GJ, Witt NF. Metabolic aspects of calorie restriction: hypohydration effects on body weight and blood parameters. Am J Clin Nutr. 1968a; 21:793-802.
26. Consolazio CF, Moutoush LO, Johnson HL, Krzywicki HJ, Isaac GJ, Witt NF. Metabolic aspects of calorie restriction: nitrogen and mineral balances and vitamin excretion. Am J Clin Nutr. 1968b; 21:803-12
27. Friedl KE, Moore RJ, Hoyt RW, Marchitelli LS, Martinez-Lopez LE, Askew EW. Endocrine markers of semistarvation in healthy lean men in multistressor environment. J Appl Physiol. 2000; 88:1820-1830.
28. Rai RM, Sridharan K *et al*. Effect of repeated strenuous exercise under low energy in take on physical performance. DIPAS/6/83, 1983.

29. Carlson MG, Snead WL, Campbell PJ. Fuel and energy metabolism in fasting humans. Am J Clin Nutr. 1994; 60:29-36.
30. Trexler ET, Smith-Ryan AE, Norton LE. Metabolic adaptation to weight loss: implications for the athlete. J Int. Soc. Sports Nutr. 2014; 11(1):7.
31. Dungan CM, Li J, Williamson DL. Caloric restriction normalizes obesity induced alterations on regulators of skeletal muscle growth signalling. Lipids. 2016; 51(8):905-12.
32. Ishihara H *et al*. Effects of dietary restriction on physical performance in mice. J Physiol. Anthropol. Appl Hum Sci. 2005; 24(3):209-13.
33. Balliett, Mary, Burke, Jeanmarie. Changes in anthropometric measurements, body composition, blood pressure, lipid profile, and testosterone in patients participating in a low-energy dietary intervention. Journal of chiropractic medicine. 2013; 12:3-14. 10.1016/j.jcm.2012.11.003.
34. Kouda K, Nakamura H, Kohno H *et al*. Metabolic response to short-term 4-day energy restriction in a controlled study. Environ Health Prev. Med. 2006; 11(2):89-92. doi:10.1007/BF02898148
35. Mettler S, Mitchell N, Tipton KD. Increased protein intake reduces lean body mass loss during weight loss in athletes. Med Sci Sports Exec. 2010; 42(2):326-37.