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GENDER DIFFERENCE IN THE EFFECT OF SHORT-TERM CALORIE RESTRICTION ON BODY WEIGHT AND IMMUNOLOGICAL PARAMETERS IN ALBINO RATS

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Abstract : In the present study, we have assessed the effect of gender on short-term calorie restriction influencing body weight and immunological parameters in albino rats. Adult albino rats (12 females, 12 males) were taken for the study and randomly divided into control and experimental groups of both the genders (n=6 in each). Following the recording of their basal 24 h food intake and body weight, rats of experimental groups were allowed to undergo food restriction (eat every alternate day) for 21 days, whereas control rats were allowed to eat normally. 7 days before the completion of food restriction, all animals were immunized and different immunological parameters such as: $^{-}\log_2$ of anti-SRBC titer (Ab titer), liver weight/body weight ratio (LWBWR), spleen weight/body weight ratio (SWBWR), total globulin (Tg) and albumin/globulin ratio (AGR) were assessed. Following three weeks of intermittent calorie restriction, LWBWR was increased in both male ($p<0.05$) and female ($p<0.001$) experimental rats compared to their respective controls. Though the increase in SWBWR was not statistically significant in male rats, the increase was significant in female rats ($p<0.05$). The SRBC titer and total globulin concentration were increased significantly in both male ($p<0.01$) and female ($p<0.001$) experimental rats. The decrease AGR was more in female compared to male rats ($p<0.01$). The decrease in body weight following calorie restriction was significantly correlated with alteration in their immunological parameters. Short-term intermittent calorie restriction was found to facilitate immunity, especially in female rats, which promises future research on health promoting effects of calorie control.

Key words : Calorie restriction body weight immunological responses rats

INTRODUCTION

Evidences indicate that the process of degeneration is slower in females in comparison to males during their reproductive life (1, 2). It has been suggested that the gender difference in degeneration is due to the effect of estrogen during the active reproductive years of a woman, as this gonadal hormone is known to play important protective role against oxidative stress (1, 3). Fasting is a common tradition followed in various religions throughout the globe and it is generally believed to improve health. But, the scientific basis for health promotion following calorie restriction has not yet been fully elucidated. It has been proposed recently that intermittent calorie restriction

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prolongs the survival rate in animals (4) and long-term calorie restriction improves immunological responses in female non-human primates (5). Moreover, it has been reported that a gender difference exists in the process of inhibition of degeneration by calorie restriction (6). However, till date, no study has been conducted to assess the gender difference in immunological status in response to short-term calorie restriction.

Prevalence of obesity is more in females compared to males during and after their reproductive life (7). Report suggests that male-female difference exists in the control of food intake and body weight gain (8). Reports also suggest the gender difference in the effect of calorie restriction on body weight gain (9, 10). A recently conducted study on rural population in Poland indicates the prevalence of overweight in males and obesity in females, though in general occurrence of obesity increases with advancement of age (11). However, to best of our knowledge, no report is available to assess the gender difference on magnitude of body weight reduction in response to a short-term calorie restriction schedule. Also reports are inadequate to explain the mechanism of health improvement following such calorie restrictions. Therefore, in the present study we have assessed the difference in the effects of short-term calorie restriction on immunological responses and body weight reduction in male and female albino rats, and analyzed the association of weight reduction with immunological parameters.

MATERIALS AND METHODS

24 adult albino rats (12 females, 12 males) of Wistar strain were obtained from the central animal house of Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, after obtaining the clearance of animal ethics committee and research committee of JIPMER. In the animal research laboratory of Physiology Department, animals were kept individually in separate cages and food (rodent chow) and water were given *ad lib*, under a constant room temperature of 25°C, and 12 hours light-dark cycle. After one week of habituation of the rats to the cages, their 24 h food intake and body weight were recorded for seven days and the mean basal food intake and body weight were noted. The rats were randomly divided into control and experimental groups (n=6 in each) of both the genders. For rats of experimental groups, the food (only the mean basal 24 h quantity of food) was given every alternate day, for 21 days, whereas control rats were allowed to eat normally (without food restriction).

7 days before the completion of food restriction, animals were immunized and following immunological parameters were assessed:

1. $^{-}\log_2$ of anti-SRBC titer (Ab titer)
2. Liver weight/body weight ratio (LWBWR)
3. Spleen weight/body weight ratio (SWBWR)
4. Total globulin (Tg)
5. Albumin/globulin ratio (AGR)

Immunization

5% sheep red blood cell (SRBC) solution in normal saline was prepared from freshly collected sheep blood, following the procedure as described in our earlier report (12). 7 days before the completion of food restriction, 1 ml of 5% SRBC solution was injected intraperitoneally into each rat.

Sample collection

On 7th day of SRBC injection, the animals were anesthetized and blood was collected from the animals by cardiac puncture. Blood was immediately centrifuged at 3500 rpm for 10 minutes to collect serum. Serum was preserved at –20^{o}C and was analyzed next day for estimation of total antibody titer.

Estimation of antibody titer

Antibody titer was measured by direct hemeagglutination method as performed in our previous procedures (12) and followed by others (13).

Statistical analysis of data

‘ SPSS version 13 was used for statistical analysis. All the data were expressed as

mean±SE. One-way ANOVA with Tukey-Krammer post-hoc was used in analyzing the data across the three groups. Student's paired *t* test was used to detect the level of significance within the groups. The association between body weight and immunological parameters was assessed by Pearson correlation analysis. The P values less than 0.05 were considered significant.

RESULTS

The daily basal 24 h food intake and body weight in control and experimental rats have been depicted in Table I. There was a significant decrease in body weight following three weeks of intermittent calorie restriction in both male (P<0.01) and female (P<0.001) experimental rats compared to their controls, in which the decrease was more in females compared to the male rats (P<0.05) (Table II). The decrease in body weight was 6.85% and 10.2% in males and females respectively, compared to the body weight of control rats of their own group.

TABLE I: Comparison of basal food intake (FI) and body weight (BW) of male and female experimental and control rats (n=6, in each group).

	FI (g)	*BW (g)*
1.Male control	18.80±0.52	308.50±4.14
2.Male experimental	19.10±0.70	306.50±4.18
3.Female control	17.60±0.62	294.20±4.56
4.Female experimental		17.92±0.56
295.10±4.10		

Values are Mean ± SE

TABLE II : Comparison of alteration in body weight and immunological parameters in male and female experimental and control rats (n=6, in each group) following intermittent calorie restriction for three weeks.

	Male		*Female*			
	Control	*Experimental*	*Control*	*Experimental*	*P*	*F / dF*
1. Body weight (g)	310.80± 4.32	289.50±4.10**	302.40±4.08	271.54±3.90***, #	<0.001	17.362 / 3, 20
2. LWBWR (mg/g)	32.1±0.7	35.84±1.0*	30.6 ± 0.46	37.0± 1.0***	<0.001	15.148 / 3, 20
3. SWBWR (mg/g)	3.60±0.2	3.70±0.20	3.70±0.16	4.56±0.21*, #	0.0080	5.211 / 3, 20
4. SRBC titer	6.91±0.32	8.59±0.30**	7.06±0.28	9.98±0.32***, #	<0.0001	23.161 / 3, 20
5. Total globulin (g%)	1.46±0.10	1.92±0.12*	1.50±0.11	2.38±0.18***, #	<0.0001	13.870 / 3, 20
6. AGR	3.56±0.12	2.65±0.092***	3.33±0.10	2.14±0.90***, ##	<0.0001	40.970 / 3, 20

Values are Mean ± SE; LWBWR: Liver weight-body weight ratio; SWBWR: Spleen weight-body weight ratio; SRBC titer: This is the $^{-}\log_2$ of anti-SRBC titer; AGR: Albumin-globulin ratio; '*' mark indicates the difference when compared with their respective controls; '#' mark indicates the difference when compared with the male experimental; *P < 0.05; **P < 0.01; ***P < 0.001; #P < 0.05; ##P <0.01; There was no statistically significant difference between male control and female control rats for any of the parameters.

Following three weeks intermittent calorie restriction, liver weight-body weight ratio (LWBWR) was increased in both male (P<0.05) and female (P<0.001) experimental rats compared to their respective controls, and the increase in female experimental rats was more compared to the increase in male experimental rats (P<0.05) (Table II). Though the increase in spleen weight-body weight ratio (SWBWR) was not statistically significant in male rats, the increase was significant in female rats (P<0.05) compared to their controls. Also, increase in SWBWR in female experimental rats was more than the male experimental rats (P<0.05).

The SRBC titer was increased significantly in both male (P<0.01) and female (P<0.001) experimental rats compared to their controls, following three weeks intermittent calorie restriction, and the increase was more (P<0.05) in female experimental rats compared to the increase in male experimental rats (Table II). The total globulin concentration was increased significantly in both male (P<0.05) and female (P<0.001) experimental rats compared to their controls and the increase was more (P<0.05) in female experimental rats compared to the male experimental rats. Albumin-globulin

TABLE III : Correlation of decrease in body weight with alteration in immunological parameters in male and female experimental rats (n=6, in each group) following intermittent calorie restriction for three weeks.

		Male		*Female*	
		r	*p*	*r*	*p*
1.	LWBWR	– 0.36	0.048	– 0.72	0.000
2.	SWBWR	– 0.16	0.164	– 0.38	0.042
3.	SRBC titer	– 0.48	0.003	– 0.75	0.000
4.	Total globulin	– 0.33	0.045	– 0.70	0.000
5.	AGR	0.56	0.001	0.78	0.000

Liver weight-body weight ratio; SWBWR: Spleen weight-body weight ratio; SRBC titer**:** This is the **"**$\log_2$ of anti-SRBC titer; AGR: Albumin-globulin ratio; p <0.05 was considered significant.

ratio (AGR) was decreased (P<0.001) in both male and female experimental rats and the decrease in female rats was more than male rats (P<0.01).

The decrease in body weight in experimental rats was significantly correlated with alteration in their immunological parameters (Table III), following three weeks intermittent calorie restriction.

DISCUSSION

Intermittent (alternate day) calorie restriction for a period of three weeks resulted in significant decrease in body weight, which was more pronounced in female rats. This indicates that a short-term calorie-restriction could be an effective way of body weight reduction, especially in females. In general, obesity is more prevalent in women, at all the age groups (2). Exact cause for higher incidence of obesity in females is not known, though difference in eating behavior, working habits and hormonal influences have been proposed to be the contributing factors (14). Recently, it has been suggested that gender difference in adiposity is primarily due to the male-female difference in neurophysiological controlling mechanisms that influence satiety, behavioral aspects of food intake and fat distribution and deposition (adiposity) (7,8). Report indicates the gender difference in control of body weight by mesolimbic areas of the brain in animal model (15). Therefore, we presume that, the male-female difference in body weight reduction following short-term calorie restriction in the present study, could possibly be due to the difference in such neurohumoral mechanisms in males and females.

There was significant improvement of immunological responses following three weeks calorie restriction in both male and female rats indicating the immune-boosting effects of this short-term calorie control. For all the immunological parameters, improvement was more pronounced in female rats compared to their male counterparts. These data indicate that three weeks of alternate-day calorie restriction is highly beneficial in female rats. Though, generally it is believed that females have a better capacity to withstand food deprivation compared to males, the physiological basis of it has not yet been elucidated. The present study reveals that females not only sustain food restriction, but also get better health benefits from it. Among the immunological parameters assessed in the present study Ab titer, TSG and AGR mainly assess humoral immunity (13). Anti-SRBC titer developed in the present study is primarily the IgM antibody, as this immunoglobulin is usually formed as the primary immune response against SRBC antigen in its first exposure (17). Thus, increase in Ab-titer following intermittent food deprivation indicates enhancement of IgM response during the period of depivation. Also, there was increase in TSG, which indicates a general increase in immunoglobulin formation in plasma. Thus, increased level of these two parameters (Ab titer and TSG) reflects augmentation of humoral immunity in general following short-term calorie

restrction. SWBWR and LWBWR represent both cellular and humoral immune responses (18). Earlier reports indicate that long-term moderate calorie restriction suppresses humoral immunity, enhances cellular immunity and prolongs life span (19-22). However, to best of our knowledge, till date, no reports are available on effects of short-term intermittent calorie restriction on immunological parameters. Results of the present study indicates the augmentation of humoral immune responses following short-term calorie restriction. In male experimental rats, there was mild increase in LWBWR and no significant increase in SWBWR, which indicates that there was mild and nonspecific augmentation of cellular immunity in males following calorie restriction. Calorie restriction of same duration in female rats has resulted in highly significant increase in all immunological responses, indicating that immune augmentation could be more prominent in females following short-term food restriction. Reduction in AGR was more in females, as globulin content was more in females without significant alteration in albumin.

Interestingly, alteration in immunological parameters was highly correlated with the reduction in body weight. Moreover, the correlation was almost proportionate to the degree of body weight reduction in both male and female rats. To best of our knowledge, the report of the present study is the first of its kind to correlate the improvement of immune responses with reduction in body weight. Therefore, we propose that improvement of immunological functions in calorie restriction is primarily linked to the reduction in body weight. The exact cause of immunomodulation by change in body weight is not known. It has been suggested that obesity increases sympathetic activity and reduction in body weight promotes vagal activity (23), and improvement of vagal activity improves immunity (24). Therefore, in our future study we plan to assess the association of autonomic activities with immunological responses following weight reduction.

The animals taken for the study were adult rats during their active reproductive life. It has been proposed that estrogen plays a protective role against oxidative stress and degenerative diseases and also promotes immunity (5). Therefore, normally females during their reproductuve life are more protected from stress-related and degenerative dysfunctions like Alzhiemer's disease, Parkinson's disease, coronary artery diseases etc. than males. The present study reveals that female rats develop better immunological status following calorie restriction. As rats are mammals, we presume a similar response to calorie restriction in human beings. Therefore, future research works should assess the immunological responses following short-term calorie restriction in human subjects, involving more specific parameters to assess cellular and humoral immunities. Though, chronic and forced food deprivation stress like malnutrition is known to suppress immunity and increase the susceptibility of the individual to various diseases, present study reveals that a short-term food restriction is good for health. Presently, many diseases in the society are due to excess eating. Though we have not estimated very specific humoral and cellular immunological parameters, which is the limitation of the study, the present data ensures the possibility of exploring the beneficial effects of dietary restriction on the preventive aspects of health.

Conclusion

Present study reveals the health promoting effects of calorie restriction and its gender difference in rats. As the immunological responses following short-term calorie restriction linked to weight reduction were found to be more in females compared to males, present study throws light on the necessity for decreasing body weight in obese people, especially females. Presently, incidence of excess diet-induced disorders is

increasing in the society. Hence, the result of the present study may attempt to convince public for the necessity of intermittent calorie restriction in daily life for not only preventing the development of obesity, but also in maintaining a good immunity status of the body. The future human studies in this regard may reveal the details of gender difference in immunological responses following different schedules of calorie restriction.

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