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The Effect of Meal Frequency on Serum Immunoglobulins Profile, Insulin and Weight in Rat

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Abstract: Although consumption of three or less than three meals a day (gorging regimen) is usual in some communities, in western population there is an inclination towards many small meals a day (nibbling regimen). Up to now the effects of meal frequency on serum immunoglobulins have not been investigated in laboratory animals. There is also some controversy information about the effect of meal frequency on serum insulin and weight in human and animal studies. Therefore, the aim of current study is to investigate, for the first time the effects of meal frequency on immunoglobulins profile in rat. The effect of meal frequency on serum insulin and weight in rat are also surveyed. Thirty female Wistar rats aged 11 weeks (210 ±15 g), after 10 days of acclimation period, were weighed and randomly assigned into two equal groups. They were fed the same food as eight meals at 2-hours intervals starting from 6 p.m. (nibbling group) or as two meals at 9 p.m. and 6 a.m. (gorging group) for 60 days. Blood samples were taken before and after intervention. During the study period, the animals were weighed at the first and every 12 days. ELLISA method was used to the measurement of insulin and the same method was designed for the assay of immunoglobulins in serum. All values were expressed as Mean±SD using Student's t-test based comparisons. The research project was reviewed and approved by the Medical Ethics Committee of Tabriz University of Medical Sciences (IRAN). Diet regimens caused a non-significant reduction ($P>0.05$) in the serum insulin and IgA levels after sixty days of intervention. Although, in the two regimens serum insulin level was non-significantly decreased but the percentage of decreasing in nibbling regimen was more than gorging one (-5.3% vs -2.3%) . Serum IgM and IgG levels in the two regimens were significantly increased ($P<0.05$) but there was no-significant difference ($P>0.05$) between the two regimens before and after the intervention. Although, in both groups, serum IgM and IgG levels were significantly increased but the percentage of increasing was more in nibbling regimen than gorging one (56% vs 38% and 37% vs 28.7% for IgM & IgG, respectively). The body weight in the two groups of animals was significantly increased ($P<0.001$). The food and water intakes were non-significantly ($P>0.05$) lowered in the gorging group compared with the nibbling one. According to the obtained results, there is not a significant difference between nibbling and gorging dietary regimens with respect to serum IgM, IgG, IgA, insulin and weight in rat.

Key words: Nibbling, gorging, immunoglobulin, insulin, rat

Introduction

The function of the immune system is protection of the body against microorganisms and clearance of the target cells recognized as self (e.g., tumor cells or necrotic tissues). These mechanisms can be divided into two main categories; a nonspecific or innate immunity and a specific or acquired immunity (Hulsewe *et al*., 1999). Abnormalities of the innate immunity may be due to the dislipidemia and insulin resistance that finally accelerate arteriosclerosis (Pick Up *et al*., 1997). On the other hand, the results of some studies have shown that obesity and the increasing of some blood parameters such as insulin are the major risk factors for many life-threatening diseases (Reaven, 2004). In some communities, although consumption of three or less than three meals a day (gorging regimen) is usual, but in western population there is an inclination towards many small meals a day (nibbling regimen) (Drummond *et al*., 1996).

Several human studies have already provided evidence that nibbling regimen is associated with lower concentrations of serum insulin (Jenkins *et al*., 1989; 1992). Furthermore, according to some other authors, alteration of meal frequency does not result in significant differences in the serum insulin (Maislos *et al*., 1998; Arnold *et al*., 1994; Bertelsen *et al*., 1993; Segura *et al*., 1995). There is only one animal study that indicates increasing of serum insulin in gorging regimen (Fabry and Tepperman, 1970). The reason for these controversial results have been attributed to the variety of the methods used, lack of information about the foods consumed, exact nature of the dietary intervention on human subjects, limited period of experimental dietary regimens, deficiency in quantifying the number of meal frequencies, lack of an acceptable definition of an eating occasion and difficulty in conducting well-controlled

eating-frequency researches in human studies for a long period (Murphy *et al.*, 1996; Gatenby, 1997). One solution to overcome these problems could be the use of laboratory animals.

In spite of many reports on the effect of nutrition (qualitative and quantitative), fasting, and energy restriction or deprivation on the immune system in human and animal models (Wing *et al.*, 1983; Palmblad *et al.*, 1977; Kelley *et al.*, 1994), up to now, no studies have been conducted on the effect of meal frequency (the number, time, quality and quantity of foods eaten in each meal) on serum immunoglobulin parameters.

Therefore, in the present study, for the first time the effects of meal frequency (nibbling and gorging) dietary regimens on serum immunoglobulins profile in rat are investigated. The effect of meal frequency on serum insulin and weight in rat are also surveyed.

Materials and Methods

Thirty female Wistar rats (11 weeks old) with the mean initial body weight of 210±15g were obtained from the animal house of Tabriz University of Medical Sciences. They were fed standardized laboratory rat chow and water ad libitum for an acclimation period of 10 days. The room temperature was maintained at 22±2°C, at 50±10% of humidity, under a 12-hours light/dark cycle (07:00-19:00 as light hours and 19:00-07:00 as dark hours) with adequate ventilation. To obtain the average daily food intake of each rat, the daily food intake was recorded (20±0.74 g/day per rat) at the last three days of acclimation period. Then, the animals were weighted and randomly assigned into two equal groups. At the end of acclimatization period, the food was withdrawn at 18 p.m. leaving the animals with free access to water. Twelve hours later, the animals were lightly anesthetized with ether and blood samples were taken from the retro-orbital sinus. The blood samples were collected and centrifuged at 1200g for 15min at 2-8ºC. The serum was separated and stored at -80ºC until the assay of serum immunoglobulins and insulin as baseline. The first group received its diet as eight meals at 2-hour intervals starting from 18 p.m. (nibbling group) and was allowed to eat for half an hour at each meal. The other group received the same diet as two meals at 9 p.m. and 6 a.m. (gorging group) with one and half-hour allowance at each meal to eat their meals. To obtain the acclimatization period for these two dietary regimens, the amounts of daily food eaten by each animal in each group, were recorded. The corresponding value was 15 g, which was achieved at the 8th day after the beginning of intervention. This dietary regimen was continued for two months. At the 60th day, the blood samples were taken and the IgM, IgA, IgG and insulin contents of the serums were determined. During the study period, the animals were weighted at the first and every 12 days and the amount of food and water intakes were also recorded daily. ELLISA method was used to measure the insulin and the same method was designed for the assay of immunoglobulins in serum. All values were expressed as mean±SD and the comparison was made using Student's t-test. The research project was reviewed and approved by the Medical Ethics Committee of Tabriz University of Medical Sciences.

Results

The amounts of food intake in the two groups during the first 8 days after the beginning of intervention are presented in Fig. 1. The results indicated that, on the 7th and 8th days after the beginning of intervention, the amounts of food taken by the two groups were reached to a plateau level (15g/day/each rat).

The weight gain in the two groups of rats during the study period is illustrated in Fig. 2. In spite of the weight increase of the animals in both groups, however, the differences were found to be non significant ($p > 0.05$).

The average of body weight before and after the intervention together with the food and water intakes in the animals during the study period have been tabulated in Table 1. The body weight in the two groups increased significantly ($P<0.001$) during the study, but, the differences in the weights of animals in the two groups at the beginning and at the end of the intervention were not significant ($P>0.05$). Although the food and water intakes in the gorging group were lower than those of the nibbling group, these differences were not statistically significant ($P>0.05$).

Serum IgM, IgA, IgG and insulin levels in both groups of the animals, before and after the intervention, are tabulated in Table 2. It shows that, the diet regimens caused a non-significant reduction ($P>0.05$) in the serum insulin and IgA levels after sixty days of the intervention. There was no singnificant difference in the serum insulin and IgA levels before and after the intervention. Serum IgM and IgG levels in the two regimens were significantly increased ($P<0.05$) but the difference was not significant ($P>0.05$) before and after the intervention.

Discussion

High fasting or postprandial serum insulin levels are associated with subsequent myocardial infarction in non-diabetic men (Ducimetiere *et al.*, 1980). There is a controversial argument regarding the influence of meal frequency on serum insulin level. According to some human studies, serum concentration of insulin is reduced following the nibbling diet compared with the gorging one (Jenkins *et al.*, 1989; 1992). Jenkins *et al.*, 1989 have found a significant decrease in serum insulin following a nibbling regimen (seventeen snacks given hourly) compared to the gorging diet (three meals per day). The reduction in serum insulin levels during the nibbling diet may also have been contributed directly to

Table 1: The comparison of body weight before and after the study and food and water intakes between the two groups of animals*

Group	Body weight		Food intake (g/day/each rat)	Water intake (ml/day/each rat)
	Before	After		
Nibbling**	210.27±15.2 (195.2-225.3)	240.4±23.3 † (217.3-263.5)	14.69±0.52 (14.2-15.2)	30.15±3.28 (26.1-33.4)
Gorging**	213.8±14.28‡ (199.6-228.0)	234.87±19†,‡ (216.1-253.8)	13.95±1.07‡ (12.9-15.0)	29.52±3.42‡ (26.1-32.9)

*Values are expressed as mean ± SD. ** N=15 in each group. † Significant difference between after and before the intervention at P<0.05. ‡Non-significant difference between the two groups at P>0.05

Table 2: The serum of insulin, IgM, IgG and IgA levels before and after the intervention in two groups of animals*

		Nibbling**	Gorging**
InsulinPmol/L	Before	121.2±11.4 (109.9-132.5)	118.5±18.5§ (100.2-136.8)
	After	114.7±4 † (110.7-118.7)	115.8±8.2§,† (107.7-123.9)
IgM mg/dl	Before	5.3±0.8 (4.5-6.1)	5.2±1.4 § (3.8-6.6)
	After	8.3±1.8 ‡ (6.5-10.1)	7.2±2.2§‡ (5.0-9.4)
IgG mg/dl	Before	7.2±1.5 (5.7-8.7)	8±2.2 § (5.8-10.2)
	After	9.9±1.7 ‡ (8.2-11.6)	10.3±1.9 ‡§ (8.4-12.2)
IgA mg/dl	Before	7.7±4 (3.7-10.7)	7.9±2.6 § (5.3-10.5)
	After	7±3.5 † (3.5-10.5)	6.8±4 §,† (2.8-10.8)

*Values are expressed as mean±SD. **N = 15 in each group. †Non- significant difference between after and before the intervention P>0.05. ‡ Significant difference between after and before at P<0.05. §Non. significant difference between the two groups at P>0.05

the reduction of the risk of cardiovascular disease. Insulin stimulates lipogenesis in arterial tissue and enhances the growth and proliferation of arterial smooth-muscle cells (Sato *et al.*, 1989; Stout, 1968; Flodin, 1986). In the present study, diet regimens caused a non-significant reduction (P>0.05) in the serum insulin level after sixty days of intervention, but no significant difference in the serum insulin before and after the intervention was found between the two groups of animals. No significant difference in the serum insulin concentrations has been reported by other human studies (Maislos *et al.*, 1998; Arnold *et al.*, 1994; Bertelsen *et al.*, 1993; Segura *et al.*, 1995). The results of a study by Arnold *et al.*, 1994 have shown that a dietary regimen of three meals per day (gorging diet) or 9 meals per day (nibbling diet) does not influence the response of serum insulin, which is consistent with the results of our animal findings. Although, in the present study in two regimens serum insulin level was non-significantly (P>0.05) decreased but the percentage of decreasing of serum insulin in nibbling regimen was more than gorging one (-5.3% vs -2.3%) .

Although, human and animal studies on the effect of acute nutritional deprivation on serum immunoglobulins are too low and the results are also contraversing, but, yet no data exists on the effect of food frequency on the immunity of host defenses in animals. Kelley *et al.* (1994) reported that the serum IgA and IgG levels decreased significantly (P<0.005) but the serum IgM level was not affected by the energy restriction in overweight women. The result of a human study has also shown that the serum IgG level non-significantly increased by the fasting in obese subjects (Wing *et al.*, 1983). On the other hand, in an animal study, Umezawa *et al.* (1990) have shown a significant decrease in the serum IgA and IgM levels of the food-restricted group (fed 60% of energy intake of controls) compared with the control group (*ad libitum* fed). In the current study, diet regimens caused a significant increase (P>0.05) in the serum IgM and IgG levels and a non-significant decrease (P<0.05) in the serum IgA level after sixty days of intervention. There was no significant difference between the two regimens in the serum IgM, IgG and IgA levels before and after the intervention. Although, in current study in both groups serum IgM and IgG levels were significantly increased but the percentage of increasing of serum IgM and IgG levels were more than in nibbling regimen than gorging one (56% vs 38% and 37% vs 28.7% for IgM & IgG levels respectively).

It appears that short term fasting has multiple influences on the immune system function rather than a uniform effect.

Obesity is a major risk factor for some life-threatening diseases include: diabetes (Hegazi *et al.*, 2003), coronary heart disease (Yilmaz *et al.*, 2003), hypertension (Adair, 2004) and certain types of cancers (Engel *et al.*, 2003). The pattern of the food intake such as the number, quality and the time of the meal can impressed the weight gain besides of the amounts of nutrients intake (Bellisle *et al.*, 1997). The results concerning the effects of meal frequency on body weight are not consistent. Some authors (Miller *et al.*, 1973) have found a significant increase in body weight

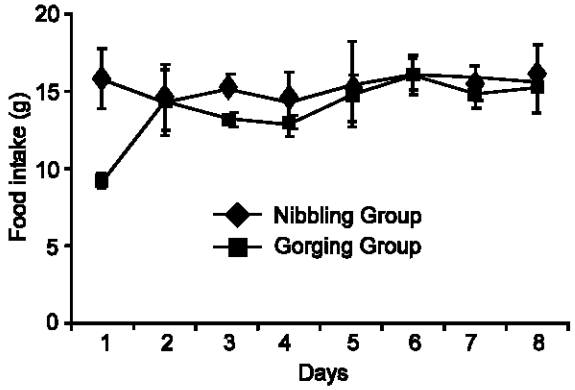

Fig. 1: The amounts of food intake by the nibbling and gorging groups of rats during the first 8 days of the intervention (mean ± SD, n = 15, in each group)

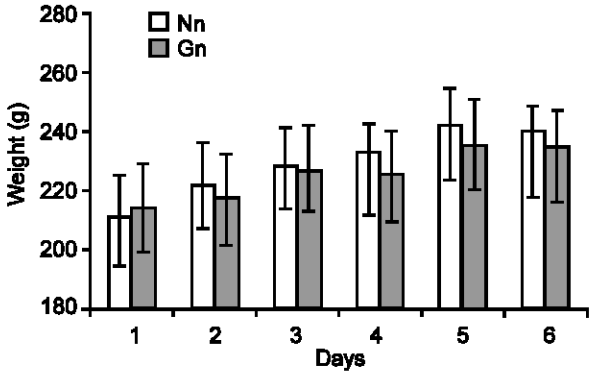

Fig. 2: The variation of weights in the nibbling and gorging groups of rats during the study period (mean ± SD, n = 15 in each group)

following a gorging regimen compared to a nibbling diet. On the other hand, some researchers have shown a significant decrease (Metzner *et al.*, 1997) to a significant increase (Edelstein *et al.*, 1992) in body weight during the nibbling regimen compared to the gorging one. The increase in body weight during the gorging dietary regimen has been attributed to the elevation of lipogenesis (fat synthesis and accumulation) or the storage of energy after consuming a large meal (Wilhelmine *et al.*, 1991). This controversy may partly result from the lack of a suitable control on the food intake and the shortage of study period. It is one major reason for using animal models for food frequency studies by some researchers (Juhel *et al.*, 2000; Ozelci *et al.*, 1977; Sitren and Stevenson, 1978). In the present animal study, it could be suggested that the body weight status is not influenced by meal frequency and the increase in the weights during the study in both groups could be due to the physiological growth of the animals rather than the influence of the dietary regimens.

In conclusion, according to the obtained results in the current study, there is not a significant difference between the nibbling and gorging dietary regimens regarding the serum immunoglobulins, insulin, and weight in rat. Although we do not advocate the use of the model of increased meal frequency described here, we believe that this model illustrates an important principle. Finally, further researches may be needed to confirm and explain this and to elucidate the mechanism of any alterations in animal subjects.

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