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Effect of Meal Frequency on Human Serum Complements C_3 and C_4

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Abstract

Objective
Despite numerous reports on the effects of meal frequency on biochemical parameters such as plasma lipid profile, glucose and insulin, there is almost no study to investigate the influence of meal frequency on immune system. In the present article, for the first time, the effect of meal frequency on complement components C_3 and C_4, as key components of the complement system, has been investigated.
Materials and Methods
The subjects of this study were fifteen healthy nonsmoker males aged 27.4±6 years. All subjects were placed on two identical diets, in which they consumed the same food either as nine snacks at 2 hrs intervals (nibbling diet) or three meals at 7 hrs intervals (gorging diet). Each diet was continued for fourteen days. At the end of each program, a fasting blood sample was obtained and its complements C_3 and C_4 levels were determined. The results were compared using Student's paired t-test.
Results
Nibbling diet led to a significant ($P<0.05$) decrease in the complement C_3 level compared to the normal dietary regimen (111.6±34.5 *vs*. 140.0±27.5 mg/dl). On the other hand, during gorging period, no significant change was observed in complement C_3 level compared with the control value 145.7±51.5 *vs.* 140.0±27.5 mg/dl). However, the level of complement C_4 increased significantly ($P< 0.05$) following gorging diet (25.6 ±15.5 *vs*. 37.6±11.5 mg/dl).
Conclusion
According to the results obtained, a change in the number of meals may alter the serum levels of complements C_3 and C_4 with a decrease in both complements levels and an increase in C_4 level during nibbling and gorging dietary regimens, respectively.

Keywords: Complements, Gorging, Meal frequency, Nibbling

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Introduction

The complement system is one of the important components of the immune system, which, as a system that integrates both innate and acquired immunity, has essential roles in functioning of these two immune systems (1). Complement system is involved in many processes that are essential to the body's defense and plays important role in the maintenance of host integrity and body defensive mechanisms.

The immune system, in general, can be influenced by many factors including nutrition. Nowadays, there is a large body of evidence indicating the importance of nutrient intake in the regulation of cellular and humoral immune responses, and the relationship between nutritional state and the function of the immune system has been well documented (1-7). The immune system, like any other living tissues, is dependent on nutrition for a variety of vital processes such as energy production, protein synthesis, proliferation, and other specific metabolic pathways (4). Accordingly, nutrients are considered as one of the major factors for the maintenance and well-functioning of immune system, as well as alongside therapeutics in different pathological conditions for correcting of immune deficits and malfunction (3). However, nowadays, it is well accepted that the importance of diet on body function is not limited only to the quality and quantity of nutrient intake and the pattern of food intake; including the number and time of foods eaten in each meal has also an important role in man biochemical parameters and metabolic processes such as glucose metabolism and lipid profile (8-11). It means that in spite of having the same food in terms of quantity and quality, it is possible that nutrients exert different effects on human biological, physiological and even psychological processes. Dietary intake pattern could be classified into two main classes of nibbling (dividing the daily food intake into several but small snacks) and gorging (receiving daily food intake as 2-3 the meals) diets with respect to meal frequency.

Despite many reports on the effect of nutrients, fasting, nutritional and energy restriction or deprivation on the immune system (12-17), to our knowledge, the pattern of food intake on immune system has not been investigated. Therefore, in the present study, for the first time, the influence of meal frequency on serum levels of C_4 and C_3 as the most important component of the complement system in healthy men is investigated.

Materials and Methods

The subjects of this interventional study were fifteen healthy normolipidaemic, non-smoker males with normal physical activity and an average age of 27.2±6.4 years ranging from 20 to 34 years and a mean body weight of 66.8 ±11.1 kg ranging from 59 to 85 kg. All subjects were in a good general health condition with no significant underlying illnesses and on no regular medication.

A blood sample was taken from all fifteen subjects at the beginning of the study after 12 hrs fasting at 6 pm. These samples were used as the control values. Then, the subjects were placed on two identical diets with a calculated energy intake of 2300 kcal/day. During the first diet, the subjects consumed their daily foods as nine snacks at 2 hrs intervals starting from 7 am (*nibbling diet*). Following a 3-week wash out period during which the volunteers followed their usual diets, all subjects switched to the second diet consisting of three meals with an equal energy consumed by each subject at 7 am, 2 and 9 pm (*gorging diet*). Each diet was of two weeks' duration. The summary of the study design is illustrated in Figure 1. Subjects were given the relevant information about the dietary protocols in the beginning of both diets and a diet sheet including the dietary tables was also given for all subjects. The food intake for both diets were planned and formulated by nutritionist to give 2300 kcal/day energy consisting 35% calories as fat, 55% calories as carbohydrate, and 12% calories as protein. The volunteers were asked to do ordinary physical activities during the study. Dietary intake during the study was assessed by three 24 hrs dietary recalls recorded for each subject and data analysis was carried out using Nutrition-III software. This study was approved by the

Medical Ethics Committee of Tabriz University of Medical Sciences and all subjects were given adequate information and the consent was obtained from each volunteer.

Blood samples (8 ml) were obtained after 12 hrs fasting at 6 pm on the last day of each dietary program and the serum levels of complements C_3 and C_4 were determined using SRID (Single Radial Immunodiffusion) method.

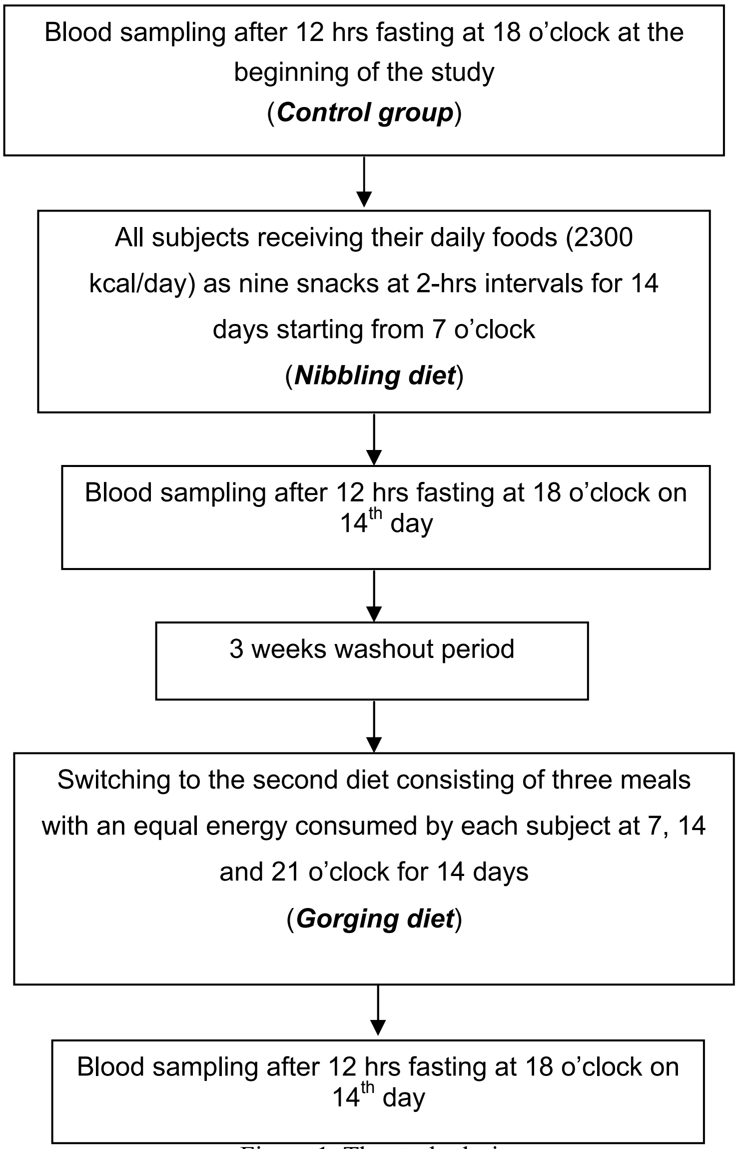

Figure 1. The study design

Statistical analysis

All values have been expressed as mean ± SD. The results obtained from two diets were compared with each other using Student's paired t-test. The difference was considered significant at level of $P<0.05$.

Results

All subjects showed a good adherence to the study and were able to complete the protocol and the timing of the meals and dietary tables were well followed by the subjects. In Table 1, the detailed composition of both diets followed by subjects has been tabulated. No significant difference was found between the two diets in terms of food composition and calorie intake, indicating that two diets followed by subjects can be considered identical apart from meal frequency.

Table 1. Characteristics of macronutrients intakes in the nibbling and gorging diets* no decimal point for SD values.

	Nibbling	Gorging	*P* value**
Energy (kcal/day)	2387.3 ± 206.2	2290.8 ± 201.3	0.169
- % Energy as fat	- 26.9 ± 5.2	- 24.5 ± 1.7	0.122
- % Energy as protein	- 11.2 ± 1.3	- 11.4 ± 1.5	0.381
- % Energy as carbohydrate	- 61.8 ± 5.1	- 64.4 ± 2.4	0.115
Fat (g)	74.4 ± 16.3	62.2 ± 7.4	0.155
Protein (g)	68.5 ± 11.1	64.4 ± 15.3	0.283
Carbohydrate (g)	381.8 ± 43.5	368.6 ± 40.7	0.263
Fiber (g)	22.3 ± 3.5	21.3 ± 4.5	0.289

* All date are expressed as mean ± SD (n=15)

** The comparison between both groups was made using Student's paired t-test.

Nibbling diet led to a marked (P<0.05) decrease in the complement C_3 level compared to the control group (111.6 ± 34.5 *vs*. 140.0 ± 27.5 mg/dl). However, although a small reduction in the C_4 level was observed after nibbling dietary regimen, when compared to the control group (27.1 ± 10.1 *vs.* 25.6 ± 15.0 mg/dl), this reduction was not significant. On the other hand, the complement C_3 level was not significantly affected during gorging dietary regimen compared to the control value (145.7 ± 51.5 *vs*. 140.0 ± 27.5 mg/dl). However, the level of complement C_4 increased significantly (P<0.05) following gorging diet (25.6 ± 15.5 *vs*. 37.6 ± 11.5 mg/dl). Figure 2 illustrates the variation in serum C_3 and C_4 levels following nibbling and gorging diets in comparison to the control group. The difference between the complement C_3 level in the nibbling and gorging groups was found to be significant (P<0.05), whereas, the levels of complement C_4 in the nibbling diet did not change significantly compared with the gorging diet.

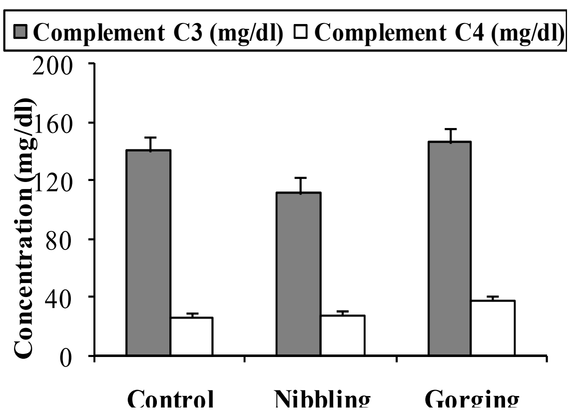

Figure 2. Basal, post-nibbling and post-gorging serum complements C_3 and C_4 levels in fifteen healthy men (n=15, mean ± SD)

Discussion

Nowadays, it is generally accepted that nutrition is an important determinant of immune functions and a large body of evidence has been published in literature concerning the effects of nutrition on immunity (2-7, 12-17). Almost all these studies have focused on the influence of energy restriction, malnutrition, macro- and micronutrients on immune functions. Although long-term fasting causes significant reduction in complement levels in serum, the effects of successive short-term fasting for a longer period, but with sufficient calorie intake, have not received enough attention. As both groups in this study received adequate and similar food intake, the differences observed can not be attributed to the other nutritional factors such as nutritional deficiency and calorie intake, influences of which on immunity have been well documented (4, 12-17). The results which were obtained in this study indicate that in addition to the quantity and quality of food eaten in daily life, the pattern of food intake in terms of meal frequency could also be important in immune responses as it has been shown for other biochemical parameters (8-11). At least two explanations may be suggested to account for the differences observed in two groups of the present study. Although malnutrition and reduction in energy intake due to long-term fasting or starvation have suppressive effects on immune response (12, 15, 17-19), successive short-term fasting (≈15 hrs per day) for a longer period (at least for two weeks) may act as an stimulator of some immune components providing that the body receives the nutritional requirements during this fasting.

There are some other evidences indicating that energy restriction in some conditions may improve some immunological parameters (13, 17, 20). According to Wing, *et al* (13), after a 14 days fast, blood monocyte bactericidal activity and natural killer cell cytolytic activity is enhanced in obese subjects. These authors have also shown an increase in the serum concentrations of IgG, IgM and IgA with a moderate decrease in lymphocyte blastogenic responses to the mitogen phytohemagglutinin (13). Weindruch *et al* have demonstrated that dietary restriction (undernutrition without malnutrition) can retard aging in mice (14). A 50% reduction of energy intake resulting in an increase in the number of cytotoxic T cells in overweight women has been reported by Kelley, *et al* (17). Wing and Barczynski have shown that starved mice for 48 or 72 hrs are resistant against *Listeria monocytogenes* (20).

Therefore, it could be possible that a successive daily mild energy restriction followed by a sufficient food intake which can occur during gorging dietary regimen may affect positively some components of the immune system. In contrast, a dietary regimen in which body receives its nutrition almost continuously with no daily experience of a significant reduction in energy as it is seen during nibbling diet may have some suppressive effects on immunity.

The differences observed in the complements levels of the two groups in the present study could be also attributed to the presence and transit of food in gastrointestinal (GI) tract.

One of the major changes during nibbling and gorging dietary regimens are the pattern of food transit throughout digestive tract and entrance of nutrients into blood stream. The fundamental and regulatory influences of nutrients on the immune response of the GI tract and, therefore, on the host defense have been mentioned by authors (21). The bacteria of the gut are one of the major components of GI tract and play important role in several functions related to the digestion of food and the establishment and maintenance of the gut immune defense barrier. It is likely that a change in intestinal transit following a change in meal frequency affects the survival and/or function of bacteria and also interferes with nutrient availability and impair beneficial stimulation of GI immune response (21).

Conclusion

In conclusion, according to the results obtained, a change in the number of meals may alter the serum levels of complements C_3 and C_4. This is the first experimental evidence for the influence of meal frequency on the human immune response. However, the exact mechanism and a clear explanation of the difference observed as well as the relation between meal frequency and immune system is subjected to further investigations.

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