

## ASSOCIATION OF VEGAN DIET WITH RMR, BODY COMPOSITION AND OXIDATIVE STRESS

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### ABSTRACT

**Background.** There is increasing evidence to suggest that a vegetarian diet low in fat and high in complex carbohydrates offers the potential for decreasing the risk of chronic disease. However, there is little information about the effect of vegetarian diets on resting metabolic rate (RMR). The objective of this study was to determine the association of vegan diet with RMR and body composition and oxidative stress.

**Material and methodology.** This research is a cross-sectional descriptive analytic study in which two groups of vegetarians and non vegetarians were compared. RMR was determined by indirect calorimetry, the amount of body fat mass (FM), the percentage of free fat mass (FFM), the markers of oxidative stress (MAD), proteins (PCO) and total anti-oxidant capacity were measured in 20 vegetarians and 20 non-vegetarians. The two groups were matched with regard to body mass index, sex and menstrual cycle. Energy and macronutrient intakes were determined using a 3-day food record and body composition was determined by bioelectric impedance.

**Results.** VEG reported a lower relative intake of protein ( $40.45 \pm 19.41$  g,  $56.96 \pm 11.94$  g,  $p = 0.04$ ), whereas no differences were observed in daily energy, carbohydrate or fat intakes and body composition. NVEG exhibited a higher absolute RMR ( $1354.7 \pm 192.6$ ,  $1569.10 \pm 348.24$  Kcal/24 h,  $p = 0.02$ ). PCO plasma density was seen significantly higher among non-vegetarians ( $1.09 \pm 3.6$ ,  $0.81 \pm 0.42$ ,  $p = 0.02$ ). No significant differences were seen in plasma density of TAC between two groups and MAD was higher among vegetarians.

**Conclusion.** These results suggest that the lower RMR observed in VEG is partially mediated by differences in dietary macronutrient composition.

**Key words:** basal metabolic rate, body composition, free fat mass, malondialdehyde, oxidative stress, vegetarian diet

### INTRODUCTION

Basal Energy Expenditure (BEE) is the amount of energy consumed in 24 hours by one person who is located in mental and physical resting (bed resting) in

the inactive state regarding to heat causes to prevent producing heat like body vibration [Frary and Johnson 2008]. Basal Metabolic Rate (BMR) is measured

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in the morning before starting every activity and after 10-12 hours without consuming any meal, liquid and nicotine. Basal Metabolic Rate includes 60-70% of total energy expenditure [Shetty 1996]. Basal Metabolic Rate usually was not measured but Resting Metabolic Rate (RMR) will be measured. Resting Energy Expenditure (REE) is the requirement energy for necessary action of body which would be necessary for normal body functioning and body hemostasis includes: breathing, blood pressure, ion pumping from cell wall, the energy requirement for central nervous system and protection of normal body temperature. When body organs work, it produces 40% heat of REE including heat produced in liver, and kidney [Gallagher 1998]. Vegetarian diet uses high complex carbohydrates and low fat in comparison with omnivorous people [Select Committee... 1977]. There are a lot of studies about vegetarians, but in order to describe differences in macronutrient intakes in vegetarians to compare with omnivorous people and RMR, a few studies have been done.

The reports show that vegetarians have a low body weight and fat mass comparing with omnivorous people [Elis and Montegriffo 1970, Burslem et al. 1978]. They intake contains lower fat, that is why they intake lower energy. In addition, RMR is an effective factor in weight that rarely is considered in non-vegetarians. Also, some studies have shown that protein intakes in vegetarians are lower than the others [Spencer et al. 2003] and can affect RMR or other hormonal factors.

Oxidative stress can directly affect the sources of anti-oxidants such as glutamine, vitamin E, carotenoids and other anti-oxidative molecules via inducing or suppressing of proteins in anti-oxidative systems [Thomas 2006]. Oxidative stress is a factor in chronic diseases, Also free radicals are important factors in old age processes [Harman 1956].

Studies have shown direct relation between RMR and oxidative stress and indirect relation with life time. With due attention to increase vegetarian diet in order to lose weight, it seems necessary to study the effects of vegetarian diet on weight and RMR since there is a direct relation between them and it oxidative stress.

## **MATERIAL AND METHODOLOGY**

### **Samples**

This case-control study was performed on 20 vegetarians and 20 non-vegetarians as case and control groups, respectively. In each group, there were 10 men and 10 women. They were introduced by the department of health education to enter the study. The participants had to fill up consent form and all of them had 20-27 body mass index (BMI), low or moderate physical activity, aged 21-55 and minimum one year of vegetarian practice. Those who had lost weight, smoked or used drugs, supplements and alcohol or excessive exercise and weight changes in last 6 months were excluded from the study. Also two groups were matched with regard to ages, BMI, sex and menstrual period.

### **Gathering basic, anthropometric data and intakes**

For gathering data, first, people who had qualified for the study were asked about their weight changes in recent 6 months, history of diseases, consumption of drugs and supplements, smoking and women the last time of menstrual period. Then measurement of their height, weight, waist and hip circumference were performed according to standard methods.

The weight of each person was measured using seca scale with accuracy of 5 gr and for the height; we had used the instrument connected to seca scale with accuracy of 0.5 cm. For the waist and hip circumferences, a tape meter was used with an accuracy of 0.1 cm in standing position. For evaluation of food consumption, we requested from every participants to record their food consumption for 3 days (2 week days, one weekend). In addition, we used FP2<sup>1</sup> software to record and evaluate 24 hours consumption and International Physical Activity Questionnaires (IPAQ) was used for physical activity assessment.

### **RMR**

Resting Metabolic Rate was measured via indirect calorimetry method (Fitmate, Cosmed Company, Italy). In this study, participants were tested after 10-12 hours fasting and 10 minutes after resting.

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<sup>1</sup> Food processor 2.

### Body composition

Participant's body composition was measured after fasting and urination via body STAT 1500 (version 2002). Before testing, participants had to stay in bed for 5 minutes. The results were body fat mass (FM), free fat mass (FFM) and body water content.

### Oxidative stress

5 cc blood was taken from participants and the below test was done on them.

Plasma malondialdehyde (MDA) was done via spectrophotometry. Total capacity of serum antioxidants were measured via ABTS<sup>2</sup> method. Also carbonyl protein was measured by spectrophotometry Cecil 8000.

### Statistical methods

In order to analyse the data, software package was used for statistical analysis (SPSS, version 11.5). Mann-Whitney U test was applied for variants that had no normal distribution. In order to compare the variants with normal distribution, independent t-test was used.

## RESULTS

In Table 1, anthropometric measurements of vegetarians and non-vegetarians are given. The means of age, weight, height, BMI and waist/hip ratio (WHR) were not significantly differentiated in two groups. In Table 2, macronutrients intakes are compared in two groups. Differences of carbohydrate, fat and energy intakes were not significant in these groups but protein intakes in vegetarians were significantly lower than non-vegetarians ( $p = 0.04$ ). There were no significant differences in the means of FM<sup>3</sup> and FFM<sup>4</sup> between two groups.

The mean of RMR in vegetarians was lower than in non-vegetarians ( $p = 0.02$ ). Moreover, RMR/body weight (kg) in vegetarians was lower than in non-vegetarians, but about RMR/non-fat mass (kg), no significant differences were seen in two groups (Table 4).

Two factors of oxidative stress including MDA and carbonyl protein, and one anti-oxidative factor

<sup>2</sup> ABTS: 2-2 Azino-bi (3-E thy Benz Thiazolin-6-Sulfonic acid).

<sup>3</sup> Fat Mass.

<sup>4</sup> Free Fat Mass.

**Table 1.** Means of age, weight, height, BMI, waist/hip in two groups

Variant	Vegetarian (20)	Non-vegetarian (20)
Age**, year <sup>a</sup>	37.4 ±11.28	37.45 ±6.86
Weight**, kg	63.49 ±10.09	65.5 ±11.60
Height**, cm <sup>a</sup>	165.93 ±10.2	166.12 ±11.54
BMI*, kg/m <sup>2</sup>	23.05 ±1.87	23.62 ±1.85
Waist/hip*	0.81 ±0.11	0.79 ±0.07

Mean ±standard deviation.

\*Mann-Whitney U test.

\*\*Independent sample T-test.

<sup>a</sup> $P \leq 0.05$ .

**Table 2.** Daily energy, carbohydrate, fat and protein in two groups

Variant	Vegetarian (20)	Non-vegetarian (20)
Carbohydrate**, g <sup>a</sup>	249.78 ±17.28	227.61 ±61.03
Fat*, g <sup>a</sup>	55.36 ±25.53	58.28 ±10.95
Protein**, g <sup>b</sup>	40.45 ±19.41	56.96 ±11.94
Energy**, kcal <sup>a</sup>	1 592 ±461.80	1 622 ±306.59

Mean ±standard deviation.

\*Mann-Whitney U test.

\*\*Independent sample T-test.

<sup>a</sup> $P \leq 0.05$ .

<sup>b</sup> $P \geq 0.05$ .

**Table 3.** Means of fat mass (FM) and free fat mass (FFM) in two groups

Variant	Vegetarian (20)	Non-vegetarian (20)
Fat Mass** (FM) <sup>a</sup>	26.46 ±7.54	26.85 ±9.33
Free Fat Mass* (FFM) <sup>a</sup>	73.54 ±7.54	70.59 ±15.36

Explanations as in Table 1.

**Table 4.** RMR, RMR/body weight and RMR/FFM in two groups

Variant	Vegetarian (20)	Non-vegetarian (20)
RMR*, kcal/24 hrs <sup>b</sup>	1 354.7 ±192.96	1 569.10 ±348.24
RMR/body weight*, kg <sup>b</sup>	21.60 ±3.01	23.94 ±2.99
RMR/FFM*, g <sup>a</sup>	29.69 ±5.64	32.76 ±5.26

Explanations as in Table 2.

**Table 5.** Carbonyl protein, MDA and total anti-oxidant in two groups

Variant	Vegetarian (20)	Non-vegetarian (20)
Carbonyl protein*, ngr/mg <sup>b</sup>	0.81 ±0.42	1.09 ±3.6
MDA**, nmol/ml <sup>b</sup>	3.93 ±1.07	2.74 ±0.84
Total anti-oxidant**, gr/dl <sup>a</sup>	3.09 ±0.54	2.74 ±0.84

Explanations as in Table 2.

**Table 6.** Regression between RMR and carbonyl protein, MDA and total anti-oxidant

Variant	Vegetarian	Non-vegetarian
Carbonyl protein, ngr/mg	R = 0.12 P = 0.58	R = 0.34 P = 0.14
MDA, nmol/ml	R = 0.39 P = 0.08	R = 0.2 P = 0.41
Total anti-oxidant, gr/dl	R = 0.12 P = 0.58	R = 0.48 P = 0.03

including total anti-oxidant were measured (Table 5). The mean of carbonyl protein was higher in non-vegetarians. The mean of MDA in vegetarians was higher than in non-vegetarians ( $p = 0.002$ ), but there were no significant differences in total anti-oxidant between two groups ( $p = 0.13$ ). Also the relation between oxidative stress factors and RMR was considered (Table 6). There was no significant relation between MDA and carbonyl protein with RMR in two groups, but there was reverse relation between total anti-oxidant rate and RMR in non-vegetarians ( $p = 0.03$ ).

## DISCUSSION

In this study, RMR and RMR/body weight were different in two groups and were significantly higher in non-vegetarians, but the difference in RMR/FFM (kg) was not significant. Comparing RMR in vegetarians and non-vegetarians that are different in macronutrients intakes, helps us understanding the effect of macronutrients changes in diet on RMR. In our study, protein intakes were significantly lower in vegetarians, but fat and carbohydrate intakes were not significantly different in two groups.

### Relation between vegetarian diet and RMR

There are limited studies about this subject; one of them was carried out by Poehlman et al. [1998]. 12 vegetarians were compared with 11 non-vegetarians. In this study, there was no significant difference in RMR between two groups, but the mean of RMR in non-vegetarians was higher than in vegetarians, also in our study RMR was higher in non-vegetarians, that shows us the same result in two studies. Yet in our study, the differences were significant, and the reason could be a larger sample size and matched weight in two groups. In the other study, RMR/body weight (kg) in vegetarians was higher than in non-vegetarians which is different from our study, but there was no significant difference which can be caused by not matching of two groups in weight and BMI [Poehlman et al. 1988]. Otherwise, our study shows some difference with another study done by Toth et al. [1994]. In their study, RMR in vegetarians was higher than in non-vegetarians, and two groups had the same weight, height, BMI and FFM, but in the study of Poehlman et al. [1988], there was no difference in weight between two groups [Toth and Poehlman 1994]. These differences in comparison with our results may be caused by different consumption patterns of macronutrients. In their study, fat intakes in vegetarians were significantly lower than in non-vegetarians and there is no significant difference in other macronutrient intakes.

The results of Bissoli [Bissoli et al. 1999] were different from our study, too. This study included 16 vegetarians and 16 non-vegetarians that were matched in BMI, weight and age. In their study, energy and protein intakes in vegetarians were lower than in non-vegetarians, but carbohydrate intakes in vegetarians

were higher. The different results in their study in comparison with ours can be the results of differences in patterns of energy and macronutrients consumption, also vegetarian diet was compared with Mediterranean diet which was similar to vegetarian diet [Bissoli et al. 1999].

As can be seen in the above studies, sample sizes are lower than in our study. We do not know sufficient mechanisms for relation between vegetarian diet and RMR yet, but changes in macronutrients intakes and lower protein intakes with direct effect on hormonal and non-hormonal factors that cause low RMR in vegetarians probably are the mechanisms. Some of the studies show that low protein high-carbohydrate diet leads to low RMR [Kerksick 2009]. Another reason probably is changing in thyroid hormones function which can change RMR. Low protein diet in new borns causes a decrease of T3, T4 and increase of TSH which is compensated with high consumption of protein [Ramos et al. 1997]. Also another reason may be difference in RMR between vegetarians and non-vegetarians. All of these mechanisms are presumptive and there is no clear mechanism that shows that RMR in vegetarians is lower than in non-vegetarians.

### **Interrelation between vegetarian diet and body composition**

We considered body composition in two groups. The mean of fat percentage was very close between two groups, also the mean of FM did not have significant difference in two groups. BMI was matched in them and WHR was not significantly different. The results of the studies are controversial. The study of Ho-Pham et al. [2009] shows the same results with our study; however protein intakes in vegetarians was lower than in non-vegetarian but FM and FFM do not have differences in the groups. The results of Lee and Krawinkel [2009], were different from our study and show that FFM, body weight and BMI are higher in vegetarians and FFM has direct relation with duration of vegetarian diet. Therefore, the results of their study may be caused by a longer duration of the vegetarian diet. Yet, the results of Saxe et al. [2008], were different and indicated the vegetarian diet in a period of 6 months, leads to reduction in WHR and BMI. In their study, there are differences in weight between the groups that could lead to reduction in WHR.

The study of Siani et al. [2003], was much closer to our study. In their study, 20 Italian vegetarians and 10 non-vegetarians were considered and no significant differences in FM and FFM were seen between the groups.

If the groups were matched in BMI, weight and age, vegetarian diet could not affect body composition.

### **Relation between vegetarian diet and oxidative stress**

In our study, carbonyl protein levels in non-vegetarians and MDA levels in vegetarians were higher, but total anti-oxidant showed no differences in the groups. Similar results were taken in the study of Hal-dar et al. [2007], that was performed on 36 vegetarians and 58 non-vegetarians, and it indicates anti-oxidative levels were not different in the groups, but glutathione peroxidase and superoxide dismutase were different in comparison with our study.

In the study of Calombo. Reactive Oxygen Species (ROS) levels were considered in vegetarian diet which showed us ROS levels decreased in vegetarians. The results of Krajcovic et al. [2008], were close to our study and showed carbonyl protein levels in vegetarians aged 20-69, lower than in non-vegetarians, but pyridine oxide levels decreased in vegetarians. Hipkiss et al. [2006], showed available carnosine in animal tissues prevented glycation and oxidation in proteins and decrease carbonyl protein production in non-vegetarians. However, anti-oxidants consumption may lead to low carbonyl protein in vegetarians. In our study, levels of total anti-oxidative capacity were not different in two groups, but total anti-oxidants did not include all anti-oxidants; they included anti-oxidants soluble in water such as uric acid, albumin and ascorbic acid [Yeum et al. 2004]. Therefore, probably in vegetarians anti-oxidant levels that are soluble in fat are higher than in non-vegetarians which causes some decrease in carbonyl protein.

Consumption of unsaturated fats in vegetarians increases MDA. The study of Herrero et al. [2001], showed that unsaturated fats in mitochondria and cell walls of mice on diet with unsaturated fats were higher. Moreover, lipid oxidation levels in these mice were higher than in mice with standard fatty acids on a diet. Unsaturated fatty acids, having double bonds, are more exposed to oxidative stress. Vegetarians

consume more unsaturated fats with vegetable sources; therefore these people are more vulnerable to attack of free radicals and production of MDA.

### Relation between RMR and oxidative stress

Some studies show that decrease in RMR, has a direct relation with increase in ROS and free radicals in mice [Barja and Herrero 2000]. In our study, this relation was considered in two groups and there was no significant relation between carbonyl protein and MDA with RMR, but there was inverse relation between total anti-oxidants and RMR in non-vegetarians. The results of Barja and Herrero [2000], showed that there is a direct relation between oxidative stress production and metabolic rate, but there is an inverse relation between those who live long. This study had some differences from our study; the first difference was the use of mice. In mice, segregation of RMR and energy or thermogenesis is difficult and usually total daily energy expenditure (TDEE) is measured [Ravussin et al. 1988]; moreover, free radical production is higher in resting condition and low energy expenditure [Boveris et al. 1972]. Therefore, it seems that free radical production is more related with total daily energy expenditure and RMR. In their study, factor of DNA oxidation (pyridine oxide) was considered that was different from our factors. Frisard et al. [2007] considered 3 age groups (20-34, 60-70, 90<) and carbonyl protein is used for protein demolition and isoprostane for fat demolition. In their study, there is no significant relation between RMR and these two factors. The study of Topp et al. [2008], was performed on 44 men 3-18 ages, and it was shown there is a direct relation between levels of exoguanosin-8 excretion and RMR. The study of velthnis-wierik et al was close to our study and shows that in people, who had limited calorie diet, RMR was decreased and RMR did not have significant relation with MDA. This result was the same with our study but it showed that excretion of oxidative DNA demolition (8-hydroxydeoxyguanosine) had a direct relation with RMR. Also, there was no significant relation between MDA and carbonyl protein with RMR. Therefore, there is a relationship between RMR and DNA oxidation that needs more consideration. Total antioxidant capacity has not been measured in any of the previous studies, but in our study, there is an inverse relation between RMR

and total antioxidant capacity levels in non-vegetarians, the reason may be an increase of DNA oxidation in RMR that increase requirements of anti-oxidants [Topp et al. 2008], but this relation is not seen in non-vegetarians and it is probably because vegetarians consume more fat soluble oxidants and the measured result was lower in total antioxidants and therefore compensated the stress condition.

### CONCLUSION

In this study, RMR and RMR/body weight (kg) was higher in non-vegetarians, but MDA levels in non-vegetarians were lower than in vegetarians. Also, carbonyl protein levels in vegetarians were lower than non-vegetarians and total antioxidant capacity levels have inverse relation with RMR in non-vegetarians.

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