

The effect of weight reduction on antioxidant enzymes and their association with dietary intake of vitamins A, C and E

Efeito da redução de peso sobre as enzimas antioxidantes e associação destas com a ingestão de vitaminas A, C e E

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ABSTRACT

Objective: Our goal was to assess the effects of weight loss on antioxidant enzymes of red blood cells and its relation with vitamins A, E and C intake in 30 obese women. **Subjects and methods:** General information, anthropometric measurements, 3-day food recall, and fasting blood samples were collected from 30 obese women at the beginning of the study and after 3 months intervention. Weight loss was set at about 10% of their weight before the intervention. **Results:** Glutathione reductase and catalase activities showed a significant increase ($P < 0.01$) after weight reduction, but no significant changes were seen in the superoxide dismutase and glutathione peroxidase activities. There was a positive linear correlation between daily vitamin C intake with superoxide dismutase enzyme after intervention ($P = 0.004$, $r = 0.507$). There was a negative linear correlation between vitamin E intake and glutathione peroxidase activity before intervention ($P = 0.005$, $r = -0.5$). A negative correlation was found between daily vitamin A intake and glutathione reductase enzyme before and after intervention ($r = -0.385$, $r = -0.397$, $P < 0.05$) respectively. No significant correlation was observed between vitamins A, C, E amounts and catalase activity. **Conclusions:** Ten percent weight reduction can have a significant role in increasing antioxidant enzymes activities, especially glutathione reductase, and catalase enzymes in obese women. However, it is important to take into consideration a balanced amount of certain nutrients while administering a diet with limited energy. *Arq Bras Endocrinol Metab.* 2014;58(7):744-9

Keywords

Obesity; enzymic antioxidants; weight reduction

RESUMO

Objetivo: Nosso objetivo foi avaliar os efeitos da perda de peso sobre as enzimas antioxidantes de eritrócitos, e a relação destas com a ingestão das vitaminas A, E e C. **Sujeitos e métodos:** Foram coletadas informações gerais e medidas antropométricas, registro alimentar de três dias e amostras de sangue em jejum de 30 mulheres obesas no início do estudo e depois de três meses da intervenção. A perda de peso determinada antes da intervenção foi de 10% do peso. **Resultados:** As atividades da glutathione reductase e da catalase mostraram aumento significativo ($P < 0,01$) depois da perda de peso, mas não houve mudanças significativas nas atividades da superóxido dismutase e da glutathione peroxidase. Foi observada uma correlação linear positiva entre a ingestão diária de vitamina C e a enzima superóxido dismutase após a intervenção ($P = 0,004$, $r = 0,507$). Houve uma correlação linear negativa entre a ingestão de vitamina E e a atividade da glutathione peroxidase antes da intervenção ($P = 0,005$, $r = -0,5$). Foi observada uma correlação negativa entre a ingestão diária de vitamina A e a enzima glutathione reductase antes e depois da intervenção ($r = -0,385$, $r = -0,397$, $P < 0,05$), respectivamente. Não foram observadas correlações significativas entre as vitaminas A, C, E e os níveis e a atividade da catalase. **Conclusões:** Uma redução de 10% no peso pode ter um papel significativo no aumento da atividade das enzimas antioxidantes, especialmente na glutathione reductase e catalase em mulheres obesas. Entretanto, é importante levar em consideração uma ingestão equilibrada de certos nutrientes ao se recomendar uma dieta com níveis de energia restritos. *Arq Bras Endocrinol Metab.* 2014;58(7):744-9

Descritores

Obesidade; enzimas antioxidantes; redução de peso

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INTRODUCTION

Obesity, which is defined as having a body mass index (BMI) of 30 kg/m² and above, has been on the increase in many countries in recent decades (1-3). It is a risk factor for many diseases including *diabetes mellitus*, hyperlipidemia, colon cancer, sudden death, illnesses related to gall bladder, high blood pressure, arteriosclerosis, and cardiovascular diseases. Obesity pathogenesis is accompanied with the increase of oxygen derived free radicals (1-3). Recent studies show that obesity results in oxidative stress even in the absence of other risk factors including cardiovascular diseases (4) and therefore plays a role in development of the above-mentioned diseases (5-7). Likewise, adipose tissue was found to be an independent factor to produce oxidative stress (8).

The removal of free radicals by antioxidants happens through enzymic and non-enzymic reactions, but research on human has shown that an increase in weight can reduce the antioxidant capacity of plasma (5,9). Therefore, enzymic and non enzymic antioxidants can constitute one of the most important defense barrier of cells (9,10).

Obesity-associated oxidative stress could occur because of increased oxygen utilization and consequent radical formation through mitochondrial respiration, more fat deposition and cell damage (12). Studies on human and animals show controversial results. Some studies have shown that antioxidant enzymes increase in obesity (13,14). Other studies have identified no significant difference in antioxidant enzymes concentrations in obesity (1,15).

Dietary intake can play an important role in antioxidant defense system of the body by reducing the oxidative damage occurred in the presence of free radicals (16). Diets rich in fruits and vegetables contain antioxidant that can protect the body from oxidative damage (16,17). It was suggested that not taking enough antioxidant could increase oxidative damage (18,19). Most of the research done in this regard is related to the comparison of antioxidant enzymes activities in overweight people compared to people within a normal BMI range. In this survey we studied the effects of the 10% weight loss (achieved through a limited energy diet) on antioxidant enzymes activities of red blood cells; glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and its relation with the intakes of vitamins A, E and C in obese women.

SUBJECTS AND METHODS

This study is a quasi experimental trial conducted on 30 obese women aged between 19 to 50 years, who visited a diet therapy clinic in the city of Tehran. The sample was chosen from women who frequented the diet therapy clinic and who met the requirements for participating in the study. The inclusion criteria were being women aged between 19 to 50 years and having a BMI of or above 30 kg/m². Exclusion criteria were being menopausal, smoking, having diseases related to the heart, kidneys, liver, cancer, *diabetes mellitus*, and hyperlipidemia. Other exclusion criteria were having intestinal inflammatory diseases, taking supplements, taking medication, being pregnant or being breastfeeding women. After attaining written consent, general information, anthropometric measurements and 3-day 24 hour food recall were completed by a nutritionist. Subjects went on a weight loss diet with an energy deficit of 500-1,000 kcal/day through lower intake of macronutrients and higher intake of fiber. For achievement of the recommended calories by the nutritionist, the carbohydrate, fat and protein were set at 55%, 25% and 20% of the total amount of energy required for approximately 10% weight loss in 3 months, respectively. During the study participants were monitored every two weeks for weight changes and for compliance with the diet and the study protocol.

The participant's weight was measured with digital scale (Seca GmbH & co.KG, Germany) with a precision of 100 grams and height was measured with non-stretchable tape (Seca GmbH, Germany) with a precision of 0.1 cm. BMI was obtained by dividing weight by the square of height. Triceps skin fold (TSF) was measured with a caliper with an accuracy of 0.1 ml. TSF, BMI and weight measurement were used to intensify that weight loss was achieved after the intervention.

Dietary intake

Three 24-hour dietary recalls for 2 weekdays and one weekend day were asked from all subjects both before and after the intervention and the collected data of before and after the intervention was separately analyzed by Food Processor 2.

The records comprised of one weekend and two non-consecutive weekdays. The amount of food eaten was estimated from photographs of portion sizes and household measures. After three months of following

a recommended diet, the information was gathered again. The amount of the recommended calories by the nutritionist was 500 to 1,000 kcal/day less than the calorie intake before intervention. In accordance, for achievement of the recommended calories by the nutritionist, the carbohydrate, fat and protein were set at 55%, 25% and 20% of the total amount of energy required for approximately 10% weight loss in 3 months, respectively.

Biochemical measurements

In order to measure hemoglobin and antioxidants enzymes activities per gram of hemoglobin, 10 mL fasting blood sample was drawn from the saphenous vein before and after intervention. The hemoglobin was measured according to Zistshimi kit method (Cat No: 10-532, Zistshimi company, Tehran, Iran).

SOD enzyme activity was measured according to Ransod Kit method (Cat No: SD 125, Ransod-Ransod, UK) In this method SOD activity was assessed by measuring the dismutation of superoxide radicals generated by xanthine oxidase and hypoxanthine (20). Catalase activity in erythrocytes was assessed by the method described by Hugo Aebi (21). In this method, activity of CAT was determined by following the decomposition of H_2O_2 in phosphate buffer pH 7.2 spectrophotometrically at 230 nm. GR activity was measured according to Sauberlich method (22) and GPX activity was measured according to Paglia and Valentine method with modifications according to Lawrence and Burk (23).

The present study was conducted according to the guidelines laid down in the declaration of Helsinki and all procedures involving human subjects were approved by the Ethics Committee at Tehran University Medical Sciences. Written informed consent was obtained from all subjects.

Statistical analysis

The Food Processor II, nutrient analysis program software, was used to determine the calorie and nutrients intake of the participants. SPSS software (ver.11.5) was used for the analysis of the data. The paired t-test was used for the comparison of quantitative variables before and after intervention.

The percentage of energy from carbohydrates, fat and protein and the intake of fiber before and after the intervention were examined. Furthermore, the association of vitamins A, C and E with antioxidant enzymes

was assessed since these vitamins were likely to influence the antioxidant enzymes activities. To explore the effect of vitamins A and E independent of dietary fat intake, stepwise regression analysis was performed.

In order to determine the amount of daily intake of A, E and C vitamins from food, participants were divided into two groups: the group that received 75% less than the recommended daily allowance (RDA) and the normal group who received equal or more than 75% of the RDA (24). The stepwise regression analysis was applied to assess the changes in quantitative variables before and after intervention on antioxidant enzymes activities. In order to measure the correlation between enzymes activities, the Pearson's correlation coefficient was used.

RESULTS

As it is shown in table 1, on the basis of the paired t-test, there was a significant difference between the weight, BMI, TSE, energy intake, dietary fibers, total vitamin A intake ($P < 0.01$), and the achieved energy percentage from the received carbohydrates and fat ($P < 0.01$) of the participants before and after intervention. Also the achievement of the energy percentage from the received protein of the participants showed a statistically significant difference between before and after intervention ($P < 0.05$). No statistical significant difference was observed between the intake amounts of vitamin E (tocopherol) and vitamin C before and after intervention.

Table 1. Mean and standard errors of independent variables before and after intervention

	Before (n = 30)	After (n = 30)
Weight (kg)*	90.58 ± 2.37	81.36 ± 2.21
BMI (kg/m ²)*	36.1 ± 0.9	32.41 ± 0.81
Triceps skin fold thickness in the arm (cm)*	4.35 ± 0.15	3.5 ± 0.15
Energy intake (kcal)*	1907.6 ± 76.1	1107.6 ± 46.2
Percentage of energy from protein**	13 ± 0.46	22.8 ± 0.6
Percentage of energy from carbohydrates***	51.9 ± 1.08	48.9 ± 1.18
Percentage of energy from fat***	35.1 ± 0.92	28.8 ± 1.02
Fiber (gr)*	18.28 ± 1	28.84 ± 0.97
Total vitamin A intake (µg)*	881.16 ± 88.5	1797 ± 218.95
Vitamin C intake (mg)	87.8 ± 9.64	97.8 ± 8.3
Vitamin E intake (mg)	7.76 ± 1.06	8.86 ± 0.68

Data are shown as mean and standard errors.

* $P < 0.01$, ** $P < 0.05$, and *** $P < 0.001$ were considered statistically significant. Other variables showed no significant difference.

Table 2 shows the antioxidant vitamins intake status of the participants before and after intervention. Vitamin intake level less than 75% of RDA was classified as deficient and intake level equal to more than 75% of RDA were classified as normal. By applying the Mc Nemar test, daily intake status of total amounts of the vitamins A and E showed a significant difference ($P < 0.05$) between before and after intervention. There was no significant difference between the vitamin C intake status of the participants before and after intervention.

Table 2. Daily vitamins A, C and E intake status of participants before and after intervention

		Before	After
		(n = 30)	(n = 30)
		n (%)	n (%)
Vitamin A (mg/day)**	Deficient*	11 (36.7)	1 (3.3)
	Adequate	19 (63.3)	29 (96.7)
Vitamin C (mg/day)	Deficient	5 (16.7)	2 (6.7)
	Adequate	25 (83.3)	28 (93.3)
Vitamin E (mg/day)*	Deficient	9 (30)	1(3.3)
	Adequate	21 (70)	29 (96.7)

Mc Nemar test was used to assess before and after the intervention.

* $P < 0.05$ and ** $P < 0.01$ were considered statistically significant. Other variables showed no significant difference.

+ Deficient: $< 75\%$ of RDA & Adequate: $\geq 75\%$ of RDA.

Table 3 shows the mean and standard error of the antioxidant enzymes activities before and after intervention using paired t-test. GR and CAT activities showed a significant increase after the intervention ($P < 0.01$).

Table 3. The antioxidant enzymes activities of participants before and after intervention

	Before	After
	(n = 30)	(n = 30)
Superoxide dismutase (U/grHb)	438.1 \pm 32.4	431.8 \pm 34.7
Glutathione peroxidase (U/grHb)	50.6 \pm 2.52	53.02 \pm 2.44
Glutathione reductase (U/grHb)*	2.47 \pm 0.33	4.73 \pm 0.51
Catalase (U/grHb)*	188.3 \pm 8.95	231.4 \pm 10.8

Data are shown as mean \pm standard errors.

Paired t-test was used to assess before and after intervention.

* $P < 0.01$ was considered statistically significant. Other variables showed no significant difference.

In order to assess the effect of the weight reduction or nutrient intake on the antioxidant enzymes activities, stepwise regression analysis was conducted (Table 4). In the present study, a positive linear correlation was found between vitamin C intake and SOD activity before intervention ($P = 0.004$, $r = 0.507$). A negative linear correlation was identified between vitamin E intake and

GPX activity after intervention ($P = 0.005$, $r = -0.5$). A negative linear correlation was also found between vitamin A intake and GR activity both before and after the intervention ($r = -0.397$, $r = -0.385$, $P < 0.05$), respectively. There was no significant correlation between vitamins A, C and E intake with CAT activity.

Table 4. Stepwise regression analysis of the GR activity changes and the alteration of the independent variables before and after intervention

	Unstandardized coefficient		Standardized coefficient	T
	Beta	Std Error	Beta	
Constant	-5.22	1.94	-	-2.7
Difference in BMI*	2.01	0.51	0.64	3.9
Difference in vitamin E**	-0.21	0.08	-0.4	-2.45

* $P < 0.001$ and ** $P < 0.022$ were considered statistically significant. Other variables showed no significant difference.

DISCUSSION

This study, complying with a restricted energy diet which is low in fat and carbohydrate and rich in protein and vegetables and dairy products, resulted in changes in the nutrient intake of participants after intervention. Therefore, besides the effect of weight loss on antioxidant enzymes activities, the changes in vitamins A, C and E intake can affect the amount of antioxidants as well.

Crujeiras and cols. conducted a study to assess the effect of weight loss and diet modification on antioxidant capacities of obese people. Total antioxidant capacity improved in those who consumed more fruits and vegetables (25). A similar study showed that obesity was an independent risk factor in draining protective enzymes in erythrocytes; individuals with normal BMI had higher SOD and GPX activities in comparison with obese people (1). Thus, weight loss can result in an increase in antioxidant enzymes activities. Melissas and cols. observed that weight loss and decrease in BMI obtained through placing a balloon in the stomach of extremely obese individuals for 6 months, increased the plasma antioxidant capacity (26). Bougoulia and cols. prescribed a calorie restricted diet to 36 obese women for 6 months. They showed that the reduction of BMI from 38.5 to 30.9 is accompanied with a significant increase in the GPX enzyme activity from 22.3 to 48.9 ng/mL (27). Contrary to present study, GPX activity had a significant increase after 20% weight reduction

In an interventional study conducted by Dworschak and cols., on obese individuals, in which they were given

a restricted energy diet of for three months, SOD activity was reduced, whereas the GPX did not change (28). In the present study mean of SOD activity decreased somewhat (but not significantly) after intervention. Contrary to Bougoulia and cols.'s study, the GPX activity in Dworschak and cols. study did not change. In the present study there was a slight increase after intervention, although it was not significant. This could be because of the lower amount of weight reduction compared with Bougoulia and cols. study, where weight loss was 20%.

We found a positive, albeit statistically weak, correlation between vitamin C intake status and SOD activity after intervention (Table 2). Even though, no significant statistical difference was seen between mean vitamin C intake before and after intervention, most participants had normal intakes of vitamin C after intervention. Standard deviation for intake of vitamin C was high among participants. This could be because the power of the study was not enough to show a significant difference before and after intervention for this vitamin or to show a weak association between vitamin C status and SOD activity. Vitamin C as an antioxidant could be effective in maintaining and increasing SOD activity. This needs further investigation.

We observed a significant negative correlation between vitamin E intake and GPX activity before intervention. Although mean intake of vitamin E did not show any significant difference before and after the intervention, vitamin E status differed significantly after the intervention. This means the number of individuals with adequate intake of vitamin E increased after the intervention. This can be the reason for the decrease in GPX activity after intervention.

In the present study, GR activity increased significantly after the intervention and a significant negative correlation was observed between vitamin A intake and GR activity before and after the intervention. As a result of dietary intervention, the number of individuals with adequate intake of vitamin A increased after intervention. It is possible that if vitamin A intake had not increase after intervention, the increase of GR activity would have been higher.

The number of those that had adequate intake of vitamin A increased after the intervention. Also, a significant negative correlation between vitamin A intake and GR activity was observed before and after the intervention. An increase in intake of this vitamin can result in a decrease of GR activity. In the present study, GR increased significantly after the intervention. It is

possible that if vitamin A intake did not increase after intervention, the increase of GR activity after intervention would be higher.

The results of the stepwise regression analysis showed that more weight loss causes an increase in GR activity and an increase in vitamin E intake causes a decrease in GR activity. However, the amount of increase in GR activity after intervention was almost twice as before the intervention. Perhaps if there had been more weight loss among the participants, other enzymes would have been increased and the effects of weight loss would have been more apparent.

The first limitation of our study was small sample size. Thus, when the intakes of vitamins A, E and C were analyzed in subgroups of the study, the power of the research might decrease. Also, the study group was limited to only women and therefore our findings can be generalized only to women. We did not observe significant changes in activity of some enzymes after intervention. Besides the changes in antioxidant vitamin intake during intervention, one explanation could be that weight reduction was not enough in our study. We suggest further studies with more weight reduction, longer duration of intervention, and different percentages of weight reduction. Another reason that we did not observe significant correlation between intake of vitamins such as C could be that the sample size was not large enough for a high standard deviation for intake of this vitamin in our population. Furthermore, we did not assess the status of other oxidative stress factors. We recommend conducting studies which assess other oxidative stress factors.

In conclusion, a weight loss of about 10% of original weight can increase antioxidant enzymes especially CAT and GR activities. Also, activity of these enzymes is influenced by certain nutrients in the diet. Because a correlation was observed between antioxidant vitamins amounts and antioxidant enzymes activities, daily intake of these vitamins may induce significant changes in the antioxidant enzymes activities. The weight loss and BMI reduction might induce an increase of antioxidant enzymes activities in the obese individuals.

Author contributions: Masoud Ramezanipour designed and conducted the study, performed the statistical analysis and drafted the paper. The research was done under supervision of Mahmood Jalali, Haleh Sadrzade-Yeganeh and Seyed Ali Keshavarz who were involved in all parts of the study including study design and data interpretation, critical revisions of the paper and provided approval for its publication. Mohammad Reza Eshraghian advised on the study design and statistical approach. Minoos Bagheri assisted in conducting the study and drafting the paper and Sara

Seyed Emami assisted in conducting the study. All authors have read and approved the final manuscript.

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