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Effects of weight loss on enzymatic antioxidants and its correlation amongst overweight women

Abstract

The present study is a clinical trial carried out to evaluate the effects of weight loss on enzymatic antioxidants and their correlations.

Methods: Thirty obese women, between the ages of 19-50, were included in this study. A low calorie diet with a 500- 1000calorie deficit was recommended with the aim of 10% weight loss. To measure nutrient intake, general questionnaires and 24-hour recall forms were used three days prior and after intervention. A 10ml blood sample was taken from each subject before and after intervention for the measurement of enzymatic antioxidants in the red blood cells.

Results: Weight loss caused a significant increase ($p<0.01$) in the mean glutathione reductase (GR) and catalase (CAT). No statistically significant change occurred in the mean values of superoxide dismutase (SOD) or glutathione peroxidase (GPX). Based on the Pearson's correlation coefficient between the dependent variables (GPX, CAT, GR, SOD) of the participants after intervention, there was a meaningful correlation between the dependent variables SOD with GPX at $P<0.001$ and $r=0.618$ and between SOD with CAT at $P<0.05$ and $r=0.424$. Also between GPX with CAT at $P=0.003$ and $r=0.527$ and between CAT with GR at $P<0.05$ and $r=0.366$ there is a meaningful correlation after intervention.

Conclusion: A 10% reduction in body weight can have a significant effect on increasing the levels of enzymatic antioxidants in obese women. There is the possibility of greater increase in enzymatic antioxidants and their correlation with weight loss and the reduction of BMI to a normal range in obese people.

Keywords: women, obesity, enzymatic antioxidants, weight loss

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Introduction

Obesity (BMI>30) is considered as a major risk factor for many diseases such as type 2 diabetes (T2DM), hyperlipidemia, colon cancer, sudden death, gallbladder disease, arterial hypertension, atherosclerosis and arterial heart disease. The pathogenesis of these diseases is associated with increased oxygen-derived free radicals.[1,2] A Free radical is an atom or a molecule with an unpaired electron in its outer shell. This element tends to be chemically unstable and very reactive. Upon receiving electrons from neighbouring molecules it creates more free radicals which may initiate the beginning of a chain reaction and if not stopped can result in cell destruction.[3,4]

The body uses an antioxidant defence mechanism to reduce free radical damage. Antioxidants can prevent damage by donating electrons and neutralizing free radicals. Some Enzymatic antioxidants such as Glutathione peroxidase (GPX), Superoxide dismutases (SOD), catalase (CAT), Glutathione reductase (GR), and some elements such as (Se, Zn, Cu, Mg, Fe), and non-enzymatic antioxidants (Vitamin A, E, C) act as a cells natural defence mechanism against oxidative stress.[5] The removal of free radicals is performed by antioxidants through enzymatic and non-enzymatic reactions. Human studies have shown that increase in weight reduces plasmas antioxidant activity;[6] enzymes are the first and most important line of defence of cells.

Diet plays an important role in antioxidant protection against free radicals, and insufficient intake of foods containing antioxidants in malnourished people can result in oxidative stress. Recent studies have shown that obesity results in oxidative stress and has an important role in the prevalence of above mentioned diseases.[6,7] Likewise, adipose tissue was found to be an independent factor to produce oxidative stress.[8]

Studies performed on overweight children have revealed that obesity can result in oxidative stress.[9] Prevention and treatment of obesity with a weight management plan, physical activity and appropriate diet until the desired weight is achieved can be beneficial and can reduce the prevalence of death.[10,11] Diets rich in fruits and vegetables contain antioxidant that can protect the body from oxidative damage.[12,13] It was suggested that not taking enough antioxidant could increase oxidative damage.[14]

Based on the studies till now, there have been no research on the result of weight reduction on enzymatic antioxidants in Iran and most of the studies performed in other countries compare the enzymatic antioxidant activity in obese people with those who have a normal BMI. The aim of the present study is to review the changes in enzymatic antioxidants (Superoxide dismutases (SOD, Glutathione peroxidase (GPX), Glutathione reductase (GR), catalase (CAT)), and their correlation amongst obese women, after being on a low calorie diet for three months and achieving an approximate 10% weight loss.

Methods

This is a clinical trial carried out on 30 healthy obese women aged 19-50years attending a weight loss clinic in Tehran. The samples were randomly taken from a group of obese women who satisfied the requirements of the study. Individuals were included in the study if

they were older than 15yrs and before reaching menopause, had a BMI>30, were not suffering from arterial heart disease, kidney disease, liver disease, Diabetes, hyperlipidemia, irritating bowl syndrome, hypertension and were non-smokers. After signing a consent form for gathering information, general questionnaires and 24-h recall forms were used 3 days prior and after intervention. Micronutrients were measured using FP2 software. A 500-1000calorie deficit diet was recommended for three months to attain a 10% weight loss. A digital scale with 100 gram accuracy was used. Height was measured to 0.1cm accuracy. Body mass index (BMI) was measured by dividing the weight (kg) to the square of height (m^2). Triceps skin-fold (TSF) thickness was measured by the caliper to the nearest 0.1millimetre.

Biochemical measurements

For the measurement of haemoglobin and enzymatic antioxidants in 1gram haemoglobin, 10ml blood was collected before and after intervention.

Blood haemoglobin was measured with CaT No: 10-532 kit. In this method hemoglobin potassium ferricyanide and its derivatives were oxidized and converted to methemoglobin in a basic environment. This reaction was followed by the conversion of potassium cyanide methemoglobin to a constant composition, cyan- methemoglobin with maximum absorption at 540 nm. Superoxide dismutase was measured using Ransod kit made in the UK (CAT No: SD 125). This enzyme oxidized (dismutatized) semi-O_2 radical produced in oxidative processes to O_2 and H_2O_2.[15]

Catalase was examined by the method of Hugo Aebi in which the decomposition of hydrogen peroxide to water and oxygen was catalyzed. The decomposition of H_2O_2 in this reaction resulted in absorbance decrease at 240nm.[16] Glutathione reductase was assessed with the use of Sauberlich method in which glutathione reductase catalyzed the reduction of oxidized glutathione (GSSG).[17] And finally glutathione peroxydase was detemined using Lawrence method.[18]

Statistical analysis

Energy and nutrient intakes were determined using Food Processor 2 and the collected data were analyzed using SPSS software (ver.11.5). Paired t test was performed to compare quantitative independent variables before and after intervention. To evaluate the daily intakes of vitamins C, A, E and elements Zn, Fe, Cu, Mg, Se the subjects were classified into two categories: undernourished women with the intakes less than 75% of the Recommended Daily Allowances (RDA) and normal women with the intakes equal to or more than 75% of the RDA.[19] To examine the effects of changes in quantitative independent variables before and after intervention on antioxidant enzymes, we administered the regression analyses using the stepwise method. Changes in both independent variables and each enzyme (superoxide dismutase, glutathione reductase, glutathione peroxides and catalase) before and after intervention were included in this model. Pearson correlation coefficient was applied to determine the possible correlations between the measured enzymes.

Results

The mean energy derived from protein, carbohydrate and lipid before entering the intervention was measured and was recorded in sequence as following: 13±0.46, 51.9±1.08, 35.1 ±0.92. After intervention the results were as following: 22.8±0.6, 48.9±1.18 and 1.02±28.8. From statistical standpoint there is a considerable difference between the mean values before and after intervention with p<0/001, P<0/05 and p<0.001 respectively.

Table 1 show that there is a meaningful statistical difference between the measured variables (weight, BMI, TSF, calorie, carbohydrate, lipid, fiber, Cu, Zn, Mg, Se and Vitamin A derived from food) from before and after the intervention. There is no considerable difference between the mean values of Protein, Iron, Vitamin C and Vitamin E from before and after the intervention. Figure 1 shows the mean and error values of the measured parameters. Based on the results shown in the table there is a meaningful statistical difference between the values of glutathione reductase (GR) and catalase from before and after the intervention (P<0.01), so that the level of activity of these two enzymes are increased in the blood sample of the participants after intervention.

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

	Before	After
	(n=30)	(n=30)
Weight (kg)*	2.37±90.58	2.21±81.36
BMI (kg/m²)*	0.9±36.1	0.81±32.41
Triceps skin fold thickness in the arm (cm)*	0.15±4.35	0.15±3.5
Energy intake (kcal)*	76.1±1907.6	46.2±1107.6
Percentage of energy from protein**	0.46±13	0.6±22.8
Percentage of energy from carbohydrates***	1.08±51.9	1.18±48.9
Percentage of energy from fat***	0.92±35.1	1.02±28.8
Fiber (gr)*	1±18.28	0.97±28.84
Total vitamin A intake (mg)*	88.5±881.16	218.95±1797
Vitamin C intake (mg)	9.64±87.8	8.3±97.8
Vitamin E intake (mg)	1.06±7.76	0.68±8.86
Copper intake (mg)*	0.06±1.3	0.05±0.91
Zinc intake (mg)*	0.38±8.5	0.37±10.2
Iron intake (mg)	0.53±12.3	0.44±12.9
Magnesium (mg)*	12.08±239.4	12.3±192.07
Selenium (µg)*	6.64±131.2	4.64±105.12

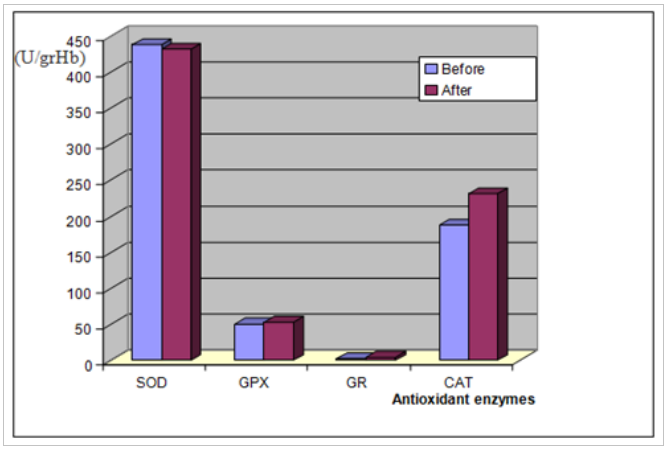

Figure 1 The antioxidant enzymes activities of participants before and after intervention.

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Table 2 shows the pearson dependent variables (GPX, CAT, GR, and SOD) of the participants after intervention. Based on this table there is a meaningful correlation between the dependent variables SOD and GPX at P<0.001 and r=0.618 and between SOD and CAT at P<0.05 and r=0.424. There is also a meaningful correlation between GPX and CAT with P=0.003 and r=0.527 and CAT with GR at P<0.05 and r=0.366 after intervention. In the stepwise analysis of the dependent variables (before and after intervention), only BMI and Vitamin E had an effect on GR and there was no meaningful relation between other variables.[20]

Table 2 Pearson correlation coefficient between dependent variables after intervention

GPx GR CAT			
*0.019(0.424)	0.062(0.038)	*0.001(0.618)	Superoxide dismutase(SOD)
*0.003(0.527)	0.078(0.202)	----------	(GPX) Glutathione peroxidase
*0.047(0.366)	----------	0.074(0.202)	(GR)Glutathione reductase
----------	*0.047(0.366)	*0.003(0.527)	(CAT)Catalase

For each of the variables in brackets is the correlation coefficient and the P value in parentheses.

*P<0.05 was considered statistically significant. Other variables showed no significant difference.

Discussion

Due to the recommendations made to consume a healthier diet by reducing the fat and carbohydrate intake and include adequate protein, fruits, vegetables and dairy products such as milk and yogurt in the diet there was a change in the nutrient uptake results of the participants after intervention.

The enzymatic antioxidants have a protein constitution and lack of adequate protein intake can have adverse effect on their activity. In this study there is no meaningful difference between the average protein intakes. Ana et al.,[21] in Spain to examine the effect of dietary antioxidants on improving the antioxidant capacity in obese women, weight loss study of 15 obese women (in two groups of 7 and 8 people) were assigned on two low-calorie diet with the following macronutrient breakdown (15% protein, 55% carbohydrate and 30% fat) With the difference that a group of 15% and another 5% energy from fructose fruits come, concluded with weight loss that with the weight reduction and change in BMI from 34.9±2.9 to 32.6±0.75 the group who obtains most of its energy from fruits has a better antioxidant status.[21] In the present study after weight loss, the BMI of the participants changed from 36.1±0.9 to 32.4 ±0.81 and TSF which is anthropometric index used to measure the skinfold body fat in the triceps area changed from 4.35±0.15 before intervention to 3.5±0.15 after intervention as a result of weight loss and body fat reduction. No further study on TSF index was found.

Dworschak et al.,[22] measured the enzymatic antioxidants GPX and SOD. Fifty four obese people (13 Male, 41 female) were assigned to a low calorie diet 5-6mj/day for threemonths. After weight loss SOD was reduced and GPX was unchanged.[22] This study confirms our present results, the average SOD was reduced after the intervention and average GPX was slightly increased with no meaningful connection.

In Bougoulia et al.,[23] a low calorie diet was recommended to 36 obese women with an average 35.4±9.2 age and a BMI of 38.5±7. After 6months the BMI of participants changed to 30.9±5.7 at P<0.001. The plasma GPX enzyme level was changed with a meaningful statistical value of P<0.001 from 22.3±9.5 to 48.9±14.1 nanogram/ml. In contrast to the present study, in the study by Maria the GPX value was increased, which can be as a result of increased BMI loss during the 6months period and a 20% weight loss. In the present study there was only a 10% weight loss. Bougoulia et al.,[23] in 2006 performed another study on 71 obese women with an average 36.7±8.3 age; they randomly assigned the participants in two groups of A1 and A2. Group A1 (n=35) included women who were on hypocaloric diet and received orlistat drug, and group A2 (n=36) included women who were only on a hypocaloric diet. In group A1 after six months they concluded that a meaningful reduction in BMI results in a significant increase in GPX at P<0.001. In A2 group a meaningful reduction in BMI results in a significant increase in GPX, there was no meaningful statistical difference between the GPX values of the two groups.[24] This study shows the effects of weight loss on the increase of GPX value. Compared to the present study weight reduction in the above study is 20% of initial weight.

Shih et al.,[25] performed a study by recommending a weight reduction regime and vigorous exercise plan on 62 obese people for two months. They confirmed that with weight reduction and a meaningful change in BMI, there is an increase in the value of SOD enzyme from 261.4±66U/ml to 302±30.9U/ml at P<0.001.[25] In the above study the weight loss was accompanied with vigorous exercise plan, whereas in the present study the physical activity was the same as before the participants entered the intervention. So in order to examine the effects of exercise on SOD level, more research is necessary.

On the other hand it is possible that the participants were under stressful condition during the intervention, or there might be a hormonal change in their body during intervention (the use or not using birth control pills). Other than this the accuracy of the equipments used for measurement might be low. Another factor contributing to the low level of meaningful difference between the values of SOD and GPX could be the slight weight reduction. The participants were still in higher than normal levels of BMI after 10% weight reduction. In similar studies performed on the comparison of the enzymatic antioxidants between obese people and those on the normal BMI range, like OLusi study, it was shown that obesity is an independent risk factor for lipid peroxidation and decreased activity of cytoprotective enzymes in humans. It was stressed that people with a normal BMI have a higher SOD and GPX value compared to obese people.[2] It is possible that in the present study with more weight loss and acquiring a normal BMI, there would be a meaningful increase in the difference between these two enzymes.

Zwirska et al.,[1] obtained a different result when they examined the oxidation status in obese women. In this research the value of enzymatic antioxidants CU/ZN-SOD and GPX was studied.

Compared to the witness group, in obese women the value of GPX in erythrocytes was higher and there was no considerable difference in the value of CU/ZN-SOD in plasma and erythrocytes. Therefore, based on the present study and other observations we can say that the value of GPX and SOD might not increase as a result of weight loss.

There were no studies found on the effect of weight loss on the values of the enzymes Glutathione reductase and catalase. Melissa et al.,[26] concluded that by replacing balloons in the stomach of obese people for six months and with the reduction of BMI and weight, the value of the plasmatic antioxidants increases considerably. This study also confirms the effect of weight loss on the increase of antioxidant capacity.

In the present study, by examining the Pearson's correlation coefficient between the dependent variables (enzymatic antioxidants, CAT, GPX, SOD, GR), there was no considerable correlation between the value of dependent variables of the participants before the intervention. Table 2 shows that after intervention and a 10% weight loss there is connection between the enzymatic antioxidants. Thus with weight reduction there is an increase in correlation of enzymatic antioxidants and their synergetic activity in the removal of free radicals. Therefore more research needs to be done to evaluate the effect of obesity on the enzymatic antioxidant activity. The study of a single antioxidant alone is not accurate, because antioxidants never function alone. Antioxidants are members of a family and they work synergically together.[27,28] An antioxidant becomes a free radical after donating an electron to another free radical and needs to be regenerated by another antioxidant which creates another free radical. So there needs to be a network of antioxidants to regenerate the free radicals.[27] In the present study the value of correlation coefficient of all the enzymes are positive. So we can say that as a result of increase of one enzymatic antioxidant, there is a possibility of increase in another enzymatic antioxidant. We can conclude that any increase or decrease of enzymatic antioxidant can result in increase or decrease of other enzymatic antioxidants. There is no study on the correlation of enzymatic antioxidants.

Lastly to evaluate the effects of weight loss or the nutrient intake on enzymatic antioxidants obtained from food on the difference between the enzymatic antioxidants before and after intervention. the regression analysis was performed between the studied variables such as weight, BMI, TSF, energy, Protein, carbohydrate, fiber, lipid and vitamin (A, C, E) and (Zn, Cu, Fe, Mg, Se). Initially difference between the above variables were added in to regression analysis stepwise, so that only the difference between BMI and Vit E with GR remained. This analysis shows that an increase in BMI difference results in an increase in GR difference and hence results in GR increase before and after intervention as a result of weight loss. In addition an increase in Vitamin E difference and an increase in vitamin E consumption results in the decrease of GR. No other correlation between weight loss and the remaining variables were found in the regression analysis.[20] It is important to say that the finding of obese participants who did not suffer from arterial heart disease, diabetes, hypertension, kidney and liver disease was a limiting factor. Also the calorie deficit diet was hard to follow by the participants. Therefore we tried to minimize the problem by weekly follow-ups.

Conclusion

In general, this study was performed to evaluate the effect of 10% weight loss on the increase of enzymatic antioxidants for the removal of free radicals which result in cell damage in the body, especially catalase and Glutathione reductase. In this study based on regression analysis weight loss had an effect on the GR value. On the other hand, the activities of these enzymes were influenced by nutrient intake and in the design of the diet attention was made to these nutrients. There is the possibility of greater increase in enzymatic antioxidants and their correlation with weight loss and the reduction of BMI to a normal range in obese people.

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None.

Conflict of interest

The author declares no conflict of interest.

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