Research article

Total antioxidant capacity of diet and serum, dietary antioxidant vitamins intake, and serum hs-CRP levels in relation to depression scales in university male students

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Objectives: Oxidative stress and inflammation have been reported to be higher in subjects with depression, but it is unclear whether this is due to inadequate dietary antioxidant intake or the pathophysiology of depression. The aim of this study was to assess the association between dietary and serum antioxidant status with depression scales in young male university students.
Methods: This research was a case–control study carried out on 60 male university students (30 students diagnosed with depression and 30 matched healthy controls). Beck Depression Inventory-II was used to assess the major depressive disorder (MDD) scales. A semi-quantitative food frequency questionnaire and 2-day 24-h recalls were used for dietary assessment. Dietary and serum total antioxidant capacity (TAC) and high-sensitive C-reactive protein (hs-CRP) concentrations were also measured.
Results: MDD subjects consumed less fruits ($P < 0.05$), legumes ($P < 0.001$), nuts and seeds ($P = 0.003$), vitamin C ($P = 0.005$), beta carotene ($P < 0.001$), lutein, and zeaxanthin ($P = 0.006$) than the controls. Moreover, the depressed group had lower serum TAC levels than their controls ($P < 0.05$). There were no significant differences in serum hs-CRP concentrations and dietary TAC levels between the study groups.
Discussion: Students with depression had significantly lower intake of dietary antioxidants. However, dietary TAC and serum hs-CRP levels were not significantly different between depressed and normal university male students. Intake of foods rich in antioxidants is encouraged in male students.

Keywords: Antioxidants, CRP, Depression, Oxidative stress, Students

Introduction

Depression is a debilitating mental disorder that affects 350 million people worldwide. It is estimated that depression is responsible for 50–70% of suicides.[1] The World Health Organization (WHO) predicts that depression will become the second most prevalent disorder (after ischemic heart disease) by the year 2020.[2]

Oxidative stress, the imbalance between the oxidant and antioxidant systems, induces damage to DNA, proteins, and fatty acids.[3] The brain is very sensitive to oxidative stress due to its modest antioxidant defense mechanisms, high oxygen consumption, high content of polyunsaturated fatty acids which are highly vulnerable to oxidative damage, the reducing potential of certain neurotransmitters, and the presence of redox-catalytic metals such as iron and copper.[4] The presence of oxidative stress in depression has already been established and more recent studies show that depression is accompanied by increased reactive oxygen species and significantly decreased antioxidant status.[3] However, it is not known whether this is due to inadequate dietary antioxidant intake or the pathophysiology of depression *per se*.

Recently, more papers have been published indicating the important role of inflammation in the

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pathophysiology of depression. Depression is accompanied by increased plasma levels of pro-inflammatory cytokines and altered levels of acute phase reactants such as hs-CRP.[3]

The total antioxidant capacity (TAC) is the capacity of enzymatic and non-enzymatic antioxidants required to reduce oxidants and its measurement provides useful information about the overall antioxidant status including those antioxidants not well measured or not well recognized.[5]

Recently, there has been increasing interest in the key role of diet in depression. More studies regarding the association between dietary antioxidants and depression have focused on individual antioxidants. For example, intakes of vitamin C and beta carotene have been shown to be lower in college students with depression compared with controls.[6] Another study showed lower intakes of vitamin C, beta cryptoxanthin, and lutein in older adults with depression compared with non-depressed subjects.[7]

However, studying these antioxidants may provide an incomplete picture of the relationship between dietary antioxidants and mental health. Measuring the TAC of plant foods rich in antioxidants such as fruits, vegetables, nuts, and seeds is a more practical approach because there are numerous antioxidants (certain vitamins and phytochemicals with antioxidant characteristics) in these foods need to be measured.[8]

This study was aimed to assess associations between depression scales and serum TAC, hs-CRP, and intake of dietary antioxidants in university male students. We hypothesized that a lower dietary intake of antioxidants would be found among subjects with depression than their healthy counterparts.

Methods

Subjects

In the Fall and Winter 2012–2013, subjects were recruited from students at the international division of the Ahvaz Jundishapur University of Medical Sciences and Azad University, City of Abadan, Iran, both located by the Persian Gulf. Inclusion criteria were being male students aged 18–25 years with no medical or psychiatric disorders except depression, non-smoking, and taking no antioxidant supplements. Participants were divided into two groups: the major depressive disorder (MDD) group including 30 male students who had Beck Depression Inventory-II (BDI-II) scores higher than 20 and the control group including 30 age-matched male students with BDI-II scores lower than 14 and no current or past history of depression symptoms. All subjects were selected in the same catchment area.

Anthropometric measurements and body composition

Subject's body height, weight, and waist and hip circumferences were recorded according to WHO standard procedures with the least amount of clothing. The body fat percent was measured using a Bodystat Quadscan 4000 (Bodystat Ltd, Douglas, UK).

Psychiatry assessment

The full 21-item version of BDI-II was used to assess symptoms of depression and the total scores were then calculated.[9] Validity and reliability of the Persian version of the BDI-II in non-clinical samples had been assessed before.[10] The following BDI-II cutoff values were applied for classifying the subjects: scores below 14, 14 to 19, 20 to 28, and 29 to 63 were regarded as normal, mild, moderate, and severe depression, respectively. MDD was defined as scores higher than 20 (moderate-to-severe depression), and the controls were defined as scores lower than 14 (normal). Subjects with mild depression (BDI-II scores of 14–19) were excluded from the study.

Dietary assessment

The semi-quantitative food frequency questionnaire (semi-FFQ) was used to assess the dietary intake during the preceding year. Validity and reproducibility of the semi-FFQ had previously been assessed.[11,12] The questionnaire included a list of foods with standard serving sizes typically consumed by Iranians. Participants reported their frequency of consumption of each food item on a daily, weekly, or monthly basis. The amount reported for each food item was converted to a daily intake value. The size of consumed foods was expressed in grams using household measures.[13] Each food item was then coded and analyzed for nutrients using Nutritionist IV software (version 3.5; First Databank Division, The Hearst Corporation, San Bruno, CA, USA), which has been corrected for Iranian foods. Participants also completed a 24-hour food recall questionnaire for 2 days to ensure the amount of nutrients consumed.

To assess dietary TAC, ferric reducing-antioxidant power (FRAP) and trolox equivalent antioxidant capacity (TEAC) values were calculated for each participant. To determine the amounts of each food FRAP and TEAC, we used the tables published in previous papers.[14–16] For analyzing similar food items (e.g. several types of oranges), we used the overall mean value. For each participant, the frequencies of consumption of each food were multiplied by their related FRAP and TEAC values and then summed to obtain dietary TAC. The FRAP assay measures the ability of antioxidants to reduce ferric ions to ferrous ions and the TEAC assay measures the

ability of antioxidants to quench a radical cation in both lipophilic and hydrophilic environments.[16]

Assessment of serum TAC and hs-CRP

Five milliliters of fasting venous blood samples were collected from each participant to measure serum TAC and hs-CRP levels. The samples were centrifuged within 1 hour, stored at −20°C, and analyzed within a week. The TAC of serum was measured using a Biorex kit (Biorex Diagnostics Limited, Antrim, UK). The TAC test was performed in accordance with the supplier's instruction. In brief, ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate)) is incubated with a peroxidase (metmyoglobin) and H2O2 to produce the radical cation ABTS+. This has a relatively stable blue-green colour, which is measured at 660 nm. Antioxidants in the serum cause suppression of this colour production to a degree, which is proportional to their concentrations. The assay results are expressed in mmol trolox equiv/l. Serum hs-CRP levels were measured using a fluorescence immunoassay i-CHROMA^{TM} kit (BoditechMed, Gangwondo, Korea) with the limit of detection of 0.10 mg/l.

Statistical analyses

The Kolmogorov–Smirnov test and independent sample t test were performed to show normal distribution of variables and to compare variable means between the two groups, respectively. Simple correlation analysis (Pearson's correlation coefficient) was applied to illustrate the linear correlation between continuous quantitative variables. Hierarchical multiple regression was used to investigate the influence of demographic, dietary, inflammatory, and antioxidant factors on depression. Independent variables were entered in three blocks. The first block contained anthropometric variables (height, weight, body mass index (BMI), waist-to-hip ratio, body fat percent, and also age), the second block contained dietary variables (energy intake, daily servings of antioxidant-rich food groups, antioxidant vitamins, and dietary TAC), and the third block contained inflammatory and antioxidant variables (serum hs-CRP and TAC levels). All statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS Inc., version 16, Chicago, IL, USA). A P value of less than 0.05 was considered significant.

Medical ethics

The study was approved by the Ahvaz Jundishapur University of Medical Sciences' Research Ethics Committee (no. B-91/013). All subjects gave their written informed consent.

Results

General characteristics of the participants are shown in Table 1. In both depressed and control groups, the average age, calorie intake, height, weight, BMI, waist-to-hip ratio, and body fat percent were not significantly different.

Dietary analysis revealed that the depressed group consumed less fruits ($P < 0.05$), legumes ($P < 0.001$), nuts and seeds ($P = 0.003$), vitamin C ($P = 0.005$), beta carotene ($P = 0.001$), lutein, and zeaxanthin ($P = 0.006$) than the control group (Table 2). No statistically significant difference was observed between the two groups in terms of dietary TAC.

Biochemical analysis showed that the depressed group had lower serum TAC than the controls ($P < 0.05$) (Table 3). No significant differences in serum hs-CRP concentrations were observed between the groups.

Pearson's correlation analysis showed negative association between Beck scores (severity of depression) and daily servings of legumes ($r = -0.408$, $P = 0.001$), nuts and seeds ($r = -0.383$, $P = 0.003$) (Fig. 1), dietary vitamin C ($r = -0.292$, $P < 0.05$), dietary total carotenoids ($r = -0.267$, $P < 0.05$) (Fig. 2), and serum TAC ($r = -0.296$, $P < 0.05$) (Fig. 3). However, these variables showed no significant correlations with depression scores in MDD subjects (figures not shown). There was no significant correlation between serum TAC, hs-CRP levels, and the intake of dietary antioxidant vitamins or TAC scores.

Table 1 General characteristics of the subjects (mean ± SD)

Variables	Control group ($n = 30$)	Depression group ($n = 30$)	*P* value
Beck scores	5.7 ± 2.2	27.8 ± 5.3	<0.001*
Age (years)	21.3 ± 1.6	20.6 ± 1.4	0.071
Energy intake in MJ (kcal)	10.5 ± 1.1 (2504.8 ± 266.4)	10.9 ± 1.3 (2615.0 ± 317.2)	0.151
Height (cm)	174.9 ± 6.2	175.2 ± 6.7	0.858
Weight (kg)	67.3 ± 10.7	71.5 ± 11.7	0.154
BMI (kg m^{-2})	22.1 ± 3.3	23.3 ± 3.4	0.159
WHR	0.84 ± 0.1	0.86 ± 0.1	0.198
%BF	12.8 ± 3.9	13.7 ± 4.4	0.406
Body fat weight (kg)	8.9 ± 4.4	10.1 ± 4.6	0.307

*Significant at the 0.001 level. ;
BMI, body mass index; WHR, waist-to-hip ratio; %BF, body fat percent.

Table 2 Intake of antioxidant-rich food groups and nutrients in the two study groups (mean ± SD)

Variables	Control group ($n = 30$)	Depression group ($n = 30$)	*P* value
Fruits (servings/day)	2.8 ± 0.7	2.4 ± 0.7	0.039*
Vegetables (servings/day)	3.2 ± 0.7	3.0 ± 0.6	0.408
Whole grains (servings/day)	1.2 ± 0.5	1.1 ± 0.5	0.848
Legumes (servings/day)	1.25 ± 0.3	0.96 ± 0.3	<0.001***
Nuts and seeds (servings/day)	0.87 ± 0.2	0.70 ± 0.1	0.003**
Tea and coffee (cups/day)	1.9 ± 1.3	2.2 ± 0.8	0.437
Chocolates (servings/day)	0.53 ± 0.3	0.52 ± 0.2	0.836
Seasoning (teaspoons/day)	0.74 ± 0.4	0.55 ± 0.4	0.095
Vegetable oils (servings/day)	1.6 ± 0.6	1.7 ± 0.7	0.377
Vitamin C (mg/day)	106.9 ± 15.3	94.9 ± 16.7	0.005**
Vitamin E (mg/day)	11.3 ± 3.6	11.6 ± 4.6	0.841
Alpha carotene (μg/day)	426.7 ± 96.3	394.8 ± 58.7	0.128
Beta carotene (μg/day)	2890.6 ± 475.5	2425.1 ± 539.0	0.001**
Beta cryptoxanthin (μg/day)	131.1 ± 38.9	129.0 ± 44.8	0.850
Lycopene (μg/day)	2913.6 ± 805.3	2761.0 ± 759.0	0.453
Lutein and zeaxanthin (μg/day)	1732.0 ± 451.6	1353.7 ± 572.9	0.006**
Total carotenoids (μg/day)	8094.0 ± 1444.8	7063.6 ± 1486.6	0.009**
Dietary FRAP (mmol Fe(II)/day)	10.07 ± 2.41	9.68 ± 2.48	0.536
Dietary TEAC (mmol trolox equiv./day)	5.34 ± 1.79	5.70 ± 2.40	0.513

* Significant at the 0.05 level.
** Significant at the 0.01 level.
*** Significant at the 0.001 level.
FRAP, ferric reducing-antioxidant power; TEAC, trolox equivalent antioxidant capacity.

Table 3 Serum TAC and hs-CRP of the two study groups (mean ± SD)

Variables	Control group ($n = 30$)	Depression group ($n = 30$)	*P* value
Serum TAC (mmol trolox equiv./l)	1.92 ± 0.34	1.69 ± 0.33	0.012*
Serum hs-CRP (mg/l)	2.05 ± 1.57	2.70 ± 2.04	0.175

*Significant at the 0.05 level.
TAC, total antioxidant capacity; hs-CRP = high-sensitive C-reactive protein.

In hierarchical multiple regression, demographic variables were entered at step one, explaining 10.6% of the variance in depression. Dietary variables entered in step two, explained an additional 46.8% of variance. After the entry of the inflammatory and antioxidant variables in step three, the total variance explained by the model was 59.3%. In this model, daily servings of legumes (beta = −0.34, $P < 0.05$) and serum TAC (beta = −0.29, $P < 0.05$) were shown as risk factors for depression.

Discussion

In this study, male university students with depression consumed less fruits, legumes, nuts, and seeds than their normal counterparts. These results are consistent with the findings of previous studies showing that depressed people are more likely to follow unhealthy diet compared with people without depression.[17,18] In accordance with this finding, several studies have shown that traditional dietary patterns characterized by high intake of fruits, vegetables, whole grains, and

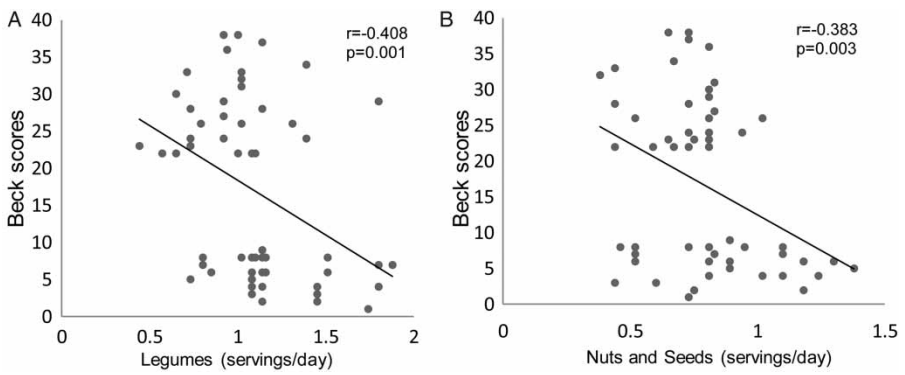

Figure 1 Correlation between Beck scores (severity of depression) and (A) daily servings of legumes, (B) daily servings of nuts and seeds in all subjects studied. The significant negative correlations suggest that subjects with depression are more likely to follow unhealthy diet compared with subjects without depression.

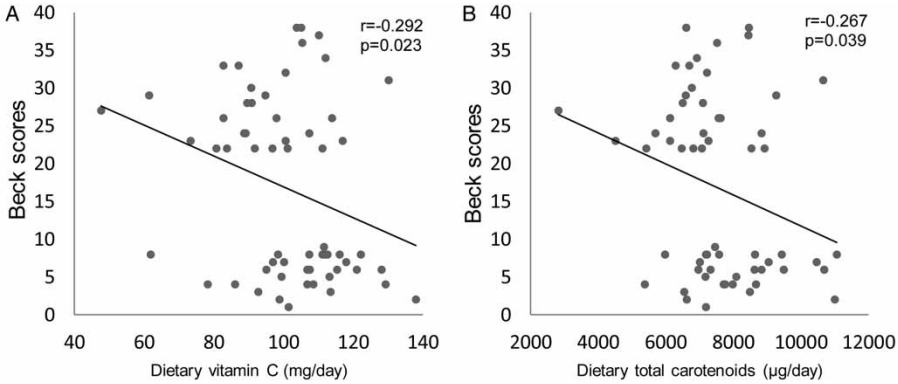

Figure 2 Correlation between Beck scores (severity of depression) and (A) dietary vitamin C intake (milligrams per day), (B) dietary total carotenoids intake (micrograms per day) in all subjects studied. The significant negative correlations between dietary antioxidant vitamin intake and depression scores suggest that subjects with depression had significantly lower intake of some foods that are high in antioxidants.

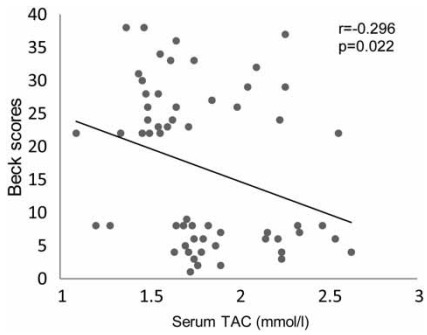

Figure 3 Correlation between Beck scores (severity of depression) and serum TAC (millimoles per liter) in all subjects studied. The significant negative correlation suggests that serum antioxidant defense is lower in subjects with depression.

fish were associated with lower risk of depression.[19–22] Conversely, Western dietary patterns rich in refined grains, sugary products, processed or fried foods, and beer were associated with higher risk of depression.[19,22] Furthermore, results from a meta-analysis suggest that antioxidant supplements have less beneficial effects, and even in some cases may be harmful.[23] These findings emphasize on the important role of antioxidant phytochemicals in plant foods.

In terms of antioxidant vitamins, the intake of vitamin C and carotenoids such as beta carotene, lutein, and zeaxanthin was lower in students with depression. Furthermore, there was a significant negative correlation between Beck scores (severity of depression) and dietary vitamin C or total carotenoids. Merrill *et al.*[24] demonstrated that dietary changes, which lead to increased vitamin C intake, may reduce depression symptoms. Khanzode *et al.*[25] and Beydoun *et al.*[26] showed that depressed people have lower serum levels of vitamin C and carotenoids, respectively. Our findings are consistent with Park *et al.*[6] and Payne *et al.*[7] as well. Park *et al.* have shown that depressed Korean female students consumed less vitamin C and beta carotene than controls. Payne *et al.* have also demonstrated that vitamin C, lutein, and beta cryptoxanthin intakes were lower in depressed older adults. In multivariable models controlled for sex, age, race, education, BMI, vascular comorbidity score, total dietary fat, and alcohol, vitamin C and beta cryptoxanthin remained significant. It is noteworthy that the intake of vitamins C and E was lower than the dietary reference intake in 23.3% and 70% of our subjects, respectively.

Regarding beta carotene, lutein, and zeaxanthin, our findings are in accordance with the findings on carotenoid profiles of the brain. Craft *et al.*[27] demonstrated that xanthophylls accounted for 66–77% of total carotenoids in the human brain. Studies have shown that total xanthophyll and total carotenoid concentrations in the frontal lobes decrease with age and may play an important role in the aetiology of depression.[7,27]

Regarding serum TAC levels, our findings are in agreement with Cumurcu *et al.*,[5] Galecki *et al.*,[28] and Sarandol *et al.*[29] who have suggested that serum antioxidant defense is lower in the depressed subjects. Furthermore, our findings showed a significant negative correlation between Beck scores and serum TAC levels. Lower serum TAC in the depressed group could be due to lower intake of some food groups such as fruits, legumes, nuts and seeds, or dietary antioxidant vitamins such as vitamin C or total carotenoids. However, there were no significant differences in dietary TAC scores between the groups. In addition, no significant correlation between dietary and serum TAC was observed. This finding is consistent with some studies suggesting that dietary TAC may not be related to serum TAC.[30,31]

Our findings are also in consistent with Steptoe *et al.*[32] showing no significant association between depressive symptoms and serum hs-CRP. In contrast to these findings, Ezat *et al.*[33] and Miller *et al.*[34]

showed that serum hs-CRP levels in the depressed group were significantly higher than the controls. This discrepancy may, in part, be due to differences in the sample size, the inflammatory and immune markers used and also the measures of depressive symptoms.[32]

To the best of our knowledge, this is the first study that examined the association between dietary TAC and depression in young university students. FRAP and TEAC scores represent a wide range of antioxidant nutrients in plant foods, including those antioxidants that were not well measured or characterized. Despite the fact that the depressed group consumed less fruits, legumes, and nuts and had lower intake of vitamin C and total carotenoids, dietary TAC showed no significant difference between depressed and apparently healthy controls. This could, partially, be due to higher intake of tea, coffee, and plant oils in the depressed group, although the differences did not reach the statistically significant level. These findings suggest that dietary TAC might be affected by phytochemicals with antioxidant properties other than vitamin C and carotenoids.

In conclusion, intake of dietary vitamin C and carotenoids and also serum TAC levels are correlated with depression scales in young university male students. Dietary TAC assessed by FFQ-based FRAP and TEAC scores did not show any significant association with depressive symptoms, serum TAC, and hs-CRP levels. The lower antioxidant status in students with depression suggests paying more attention to dietary modifications regarding consumption of antioxidant-rich food items. As a limitation, our study was carried out on male subjects suggesting further studies with higher sample size on both genders to determine factors affecting serum and dietary TAC and their relationship with depression.

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