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The Effects of Walnut Consumption on Plasma Antioxidant Capacity

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ABSTRACT: Epidemiological studies have demonstrated the benefits of walnut consumption, attributed in part to their fatty acid profile, rich in unsaturated fatty acids, and also to walnut-derived antioxidant polyphenols, on human health. The purpose of the present study was to assess the acute effect of walnut ingestion on plasma antioxidant status in healthy volunteers. Thirteen healthy volunteers (24.3 ± 6.2 years of age) were participated in the study. After fasting blood was obtained, each volunteer consumed a certain amount of walnut and blood samples were taken at the 3rd hour. Total antioxidant capacity and lipid parameters were measured in the plasma. Total antioxidant capacity was significantly higher at the 3rd hour compared to the basal value. This result supports the hypothesis that walnut ingestion increases total antioxidant capacity in plasma. Conducting new epidemiologic studies with large a population consuming walnut would be useful to get more insight on this subject.

Keywords: Walnut, Antioxidant capacity, Reactive oxygen species

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INTRODUCTION

Reactive Oxygen Species (ROS), chemically reactive molecules containing oxygen, are mainly formed in mammalian cells as a consequence of aerobic respiration (Berlett and Stadman, 1997). They are generally very small molecules and are highly reactive due to the presence of unpaired shell electrons (Borek et al., 1991; Berlett and Stadman, 1997). Despite multiple antioxidant systems, a certain proportion of ROS continuously escape from the mitochondrial respiratory chain, being sufficiently potent to damage the structure of proteins, lipids and little cell molecules in various ways, including numerous carcinogenic DNA mutations (Berlett and Stadman, 1997). In living organisms, the levels of free radicals are controlled by a complex web of antioxidant defences, which minimize the oxidative damage to biomolecules. In human disease, this oxidant–antioxidant balance is tilted in favour of the reactive species, so that oxidative damage levels increase (Uttara et al., 2009). This makes a significant contribution to tissue injury, giving rise for examining the effects of various antioxidants.

Dietary factors play an important role in the development of diabetes, hypertension, thrombosis, hyperlipidemia, atherosclerosis, metabolic disorders and cardiovascular diseases (Ulbricht and Southgate, 1991; Bonerjee et al., 2002). Several studies found negative association between the consumption of walnut and diet-related disorders such as obesity and cardiovascular events (Sabate and And, 2009; Haddad et al., 2014). Healthy fatty acids of walnut by regular and frequent walnut intake have many beneficial effects such as preventing coronary heart disease, diabetus mellitus, and sudden cardiac death (Burns-Whithmore et al., 2014). Walnuts are rich in the polyunsaturated fatty acids and thus potentially susceptible to oxidation. These lipids are naturally protected by tocopherols in the pellicle or seed coat (Venkatachalam and Sathe, 2006 and Haddad et al., 2014). Other bioactive compounds present in walnut, including selenium, phytosteroller, squalene, micronutrients, fiber, and phytochemicals have high antioxidant capacity (Pellegrini et al., 2006; Ryan et al., 2006; Pappa et al., 2006 ; Kris-Etherton et al., 2008; McKay et al., 2010). Antioxidant vitamins contained by walnut are vitamin A and vitamin C (Blomhoff et al., 2006). The vitamin E composition of walnuts is also of special mention due to an unusual concentration of the gamma-tocopherol form of vitamin E (Amaral et al., 2005). Additionally, polyphenols isolated from walnuts, including ellagic acid monomers, polymeric tannins, and other phenolic compounds, are potent inhibitors of LDL oxidation in vitro, and have been found to act as an antiatherogenic agent (Anderson et al., 2001; McKay et al., 2010). Among common plant foods consumed worldwide, walnut was ranked the second after rose hip in their antioxidant activity (Halvorsen et al., 2002; McKay et al., 2010).

Oxidative stress which the body is exposed to, can be detected by measuring the Total Antioxidant Capacity

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(TCA) of body fluids such as plasma and serum. Low levels of TCA is an indicator of exposure of body to antioxidant stress. Walnuts and/or their constituents have been shown to decrease levels of oxidative stress in diabetic mice and increase serum antioxidant capacity in rats (Fukuda et al., 2004; Russel et al., 2005). In humans, in vivo antioxidant capacity levels after consuming walnuts have shown contradictory results, reporting either increases (Torabian et al., 2009; Berryman et al., 2013; Haddad et al., 2014) or absence of changes (Tapsell et al., 2004; Davis et al., 2007; McKay et al., 2010). Since it is well known that consumption of the food having antioxidant properties is very beneficial for health, the present study primarily aimed to investigate the acute effect of walnut ingestion on plasma antioxidant capacity and lipid parameters in healthy human volunteers.

MATERIAL AND METHODS

Study design

Thirteen healthy volunteers who were the laboratory staff at our hospital (five women and eight men, 24.3 ± 6.2 years of age) participated in the study. A written informed consent was obtained from each volunteer and the procedures were in accordance with the guidelines of the Helsinki Declaration on human experimentation. Subjects did not use any medication and antioxidant supplementation, and did not have any diseases. All subjects were nonsmokers. The dietary habits of the participants were not intervened before the study. On the day of testing, after a 12 hour overnight fast, baseline blood samples were drawn at 8:00 am, then each participant consumed 250 grams of walnut. It was warranted that the participants did not have any other food during 3 hours. Blood samples were obtained at the 3rd hour following the initiation of the test procedure.

Biochemical analyses

Serum TAC, total cholesterol, trigliseride and glucose were measured in an Abbott Aeroset Autoanalyzer. Serum TAC was measured using a novel colorimetric measurement method developed by Erel (2005). In this method the hydroxyl radical is produced by the Fenton reaction and reacts with the colorless substrate O-dianisidine to produce the dianisyl radical, which is bright yellowish-brown in color. Upon the addition of a plasma sample, the oxidative reactions initiated by the hydroxyl radicals present in the reaction mix are suppressed by the antioxidant components of the plasma, preventing the color change and thereby providing an effective measure of total antioxidant capacity of the plasma. The assay results are expressed as mmol Trolox eq/L. Calibration was performed with a ready-to-use commercial Clinical Chemistry-Abbott MC Calibrator (multiconstituent calibrator), except for HDL cholesterol for which a Clinical Chemistry HDL Calibrator was used. The total antioxidant capacity calibration was performed by diluting the commercial Trolox calibrator to five different concentrations of 0.25, 0.50, 1, 2 and 4 mmol/L.

Statistical method

Statistical analyses were performed using SPSS version 16.0. In order to detect the changes between the baseline and 3rd hour levels of biochemical parameters, Wilcoxon test was used. The results were expressed as the median (minumum-maximum) and the mean (±standard deviation). $P<0.05$ was considered statistically significant.

RESULTS

The antioxidant capacity and the biochemical variables levels at 3rd hours after walnut consumption are presented in table 1. At the 3rd hour, TAC levels were found to be significantly higher than the basal values (P=0.002) (Figure 1). The biochemical parameters including total cholesterol, triglycerides and glucose were compared before and after walnut consumption. Triglycerides levels were significantly increased by walnut ingestion (P=0.001). No significant difference was seen in the total cholesterol (P=0.058) and blood glucose (P=0.447) levels before and after walnut consumption.

Table 1. The results of biochemical variables before and after walnut consumption

Variables	Basal hour	3rd hour	p value
TAC [mmol trolox equivalent/l]	1.95 ± 0.31 2.04 (1.30-2.34)	2.09 ± 0.15 2.07 (1.86-2.40)	0.002*
Total cholesterol [mg/dl]	176.6±25.2 175.0 (138.0-223.0)	179.4±26.5 176.0 (133.0-230.0)	0.058
Triglycerides [mg/dl]	70.1±25.3 74.0 (34.0-110.0)	127.1±50.5 125.0 (55.0-229.0)	0.001*
Glucose [mg/dl]	100.8±11.3 103.0 (67.0-117.0)	101.3±6.2 101.0 (92.0-108.0)	0.447

TAC; Total Antioxidant Capacity

*Difference between medians within columns was significant (P<0.05)

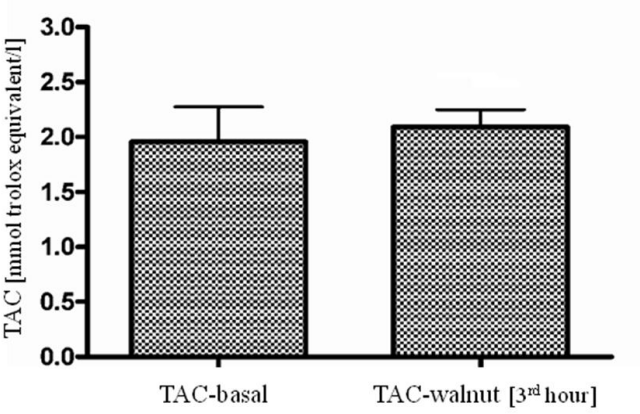

Figure 1. Mean and standard deviation of total antioxidant capacity at basal and 3rd hour after consuming walnut

DISCUSSION

Results of studies on the plasma antioxidant status of walnut-enriched diets have been remained controversial. A number of clinical studies showed reductions in lipid peroxidation measured with particularly malondialdehyde production associated with walnut consumption (Husain et al., 1987; Robak and Grglewski, 1998; Anderson et al., 2001; Torabian et al., 2009; Haddad et al., 2014), whereas others showed no change in any of the biomarkers of antioxidant status and oxidative stress (Tapsell et al., 2004; Davis et al., 2007; McKay et al., 2010).

In this in vivo study, we investigated the acute effect of consuming walnut on plasma antioxidant capacity in thirteen healthy subjects. Three hours after walnut ingestion, statistically significant increase in plasma antioxidant capacity was observed. This increase in TAC could have been due to the absorption and uptake into the circulation of antioxidative polyphenols from walnuts. Polyphenols as antioxidants reduce the generation of superoxide anions (Robak and Grglewski, 1998), hydroxyl radicals (Husain et al., 1987), and lipid peroxyl radicals (Torel et al., 1986; Torabian et al., 2009). Under normal conditions, the balance between the formation and removal of ROS is controlled by the antioxidant defence system, which includes superoxide dismutase, glutathione, thiols, antioxidant vitamins and other dietary micronutrients such as flavonoids and polyphenols (Vertuani et al., 2004). Moreover, total antioxidant activity in body fluids plays an important role against free radical attack and has been used for scientific purposes to examine the medical importance of free oxygen radicals and antioxidative defence (Koracevic et al., 2001). It was considered that phytosteroles and antioxidant vitamins (tocopherol- form of vitamin E, vitamin A and vitamin C) which are contained by walnut, increase the antioxidant capacity by reducing the damaging effects of free radicals (Ryan et al., 2006).

Recently some similar researches have been held relating to antioxidant and beneficial health effects of walnut consumption. Davis et al. (2007) reported that walnut consumption is associated with a protective effect against coronary heart disease, partly due to its high antioxidant content. Russel et al. (2005) also reported that melatonin is presented in walnuts and, when eaten, there is an increase in blood melatonin concentrations. The blood melatonin levels were compliance with increased antioxidative capacity. It was showed that walnuts could prevent oxidative damage in the tissues by inhibiting the lipid oxidation or the production of free radicals in an animal model (Bati et al., 2015). The beneficial effects of walnuts were direct diminution of the oxidative/nitrosative stress and the cell death (Choi et al., 2016).

In the current study, circulating triglycerides increased in response to the single high dose walnut meal, whereas plasma concentrations of total cholesterol did not change. Walnuts are typically high in fat. However, the high fat content of walnuts represents largely unsaturated fats, which have favourable effects on blood lipids in long term period (Sabate et al., 1993). It has been shown that the saturated fats in the meal less influence postprandial triglycerides compared to unsaturated fat, possibly due to their slower absorption rate (Tholstrup et al., 2001and Haddad et al., 2014).

Nevertheless, results from a previous study demonstrated that the higher triglyceride levels after the consumption of walnut was together with decrease in plasma Malondialdehyde (MDA) indicating possible antioxidant protection of walnut (Haddad et al., 2014). In another study, it was found that walnut polyphenol significantly decreased serum total triglycerides, cholesterol and MDA level and increased superoxide dismutase activity (Shi et al., 2014).

These results suggest that walnut intake can enhance antioxidant defenses and diminish oxidative stress and therefore retard the processes of free radical mediated disease such as atherosclerosis, cancer and diabetes mellitus. Walnut consumption is a natural way of antioxidant intake and it does not exert negative effect upon body weight for obese and overweight people if it is not consumed excessively. In conclusion, walnut has an antioxidant capacity

and when added to the diet could be protective against free radical mediated diseases.

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