Pol. J. Food Nutr. Sci., 2013, Vol. 63, No. 2, pp. 117-121
DOI: 10.2478/v10222-012-0070-8
http://journal.pan.olsztyn.pl

Original paper
Section: Nutrition

Dietary Fats and the Risk of Oxidative Stress in a Group of Apparently Healthy Women – a Short Report

Anna M. Witkowska, Małgorzata E. Zujko*

Department of Food Commodities Science and Technology, Medical University, ul. Szpitalna 37, 15–295 Białystok, Poland

Key words: FRAP assay, TAS assay, saturated fat intake, serum, healthy women

The study aimed to determine associations between diet's composition and serum antioxidant potential in 29 women aged 19–22 years. The participants completed self-report questionnaires concerning health condition, body measures, dietary habits and supplements taken, and 3-day detailed diet records. Fasting blood samples were collected to assess total antioxidant status (TAS) and ferric reducing antioxidant power (FRAP) in the serum. The women reported good health condition and lean body. The data concerning the TAS and FRAP methods demonstrated that the antioxidant potential was negatively correlated with saturated fat intake (r=-0.515 and r=-0.527, respectively), but not related to the intakes of protein, carbohydrate, mono- and polyunsaturated fats, cholesterol and antioxidant vitamins (vitamin C, β-carotene, α-tocopherol). The TAS antioxidant activity of the serum was significantly lower in the top *vs.* bottom quartile of the saturated fat intake, which corresponds to a consumption of at least 29 g saturated fat *vs.* intake below 19.7 g. The FRAP value in the highest quartile of the saturated fat intake was also reduced and close to significance. This study shows that the studied group of young women exposed to diets, which contained high amounts of saturated fat, are prone to risk of oxidative stress.

INTRODUCTION

Antioxidant protection of the organism against reactive oxygen/nitrogen species depends on various internal and external factors. In fact, antioxidant potential, as a result of biochemical processes and chemical transformations of exogenous substances, allows maintaining a balance between generation and inhibition of oxidative by-products emerging during metabolic reactions. Food is a vast source of chemical substances which are subject to complex transformations within the body. Many of them, like dietary fatty acids and glucose, are generally converted into energy. This process, taking place in the cellular mitochondria, is associated with free radical production in the electron transport system [Brand *et al*., 2004; Fariss *et al*., 2005]. Although the reactive oxygen species (ROS) life span is extremely short, they may impair fluidity of the biological membranes vital to normal functioning of the cell, resulting in the tissue degeneration and diseases [Mariani *et al*., 2005]. Free radicals can be removed from the environment provided sufficient access to antioxidant substances. Plenty of radical scavengers originate from food. Dietary flavonoids, β-carotene, antioxidant vitamins A, C, and E, are considered protective against degenerative diseases. The role of nutrients and biologically-active substances contained in food, as oils in marine food for instance, has to be elucidated with respect to the redox cycle [Chrysohoou *et al*., 2007; Hill *et al*., 2007].

Several assays were designed to determine total contribution of external and internal factors to the antioxidant activity of biological fluids. TAS (Total Antioxidant Status) and FRAP (Ferric-reducing Activity of Plasma) assays can measure antioxidant effects of albumin, bilirubin, urate, ascorbic acid and α-tocopherol [Cao & Prior, 1998]. The FRAP assay does not measure antioxidants containing thiol groups [Cao & Prior, 1998]. Internal antioxidant factors are also affected by the composition of a diet. Protein malnutrition, for example, is associated with decreased ability of antioxidant enzymes to scavenge reactive species [Feoli *et al*., 2006]. In fact, antioxidant activity of biological systems can be affected by a combination of dietary factors.

Earlier studies showed improper dietary habits in female adolescents [Przysławski *et al*., 2011]. Well-balanced nutrition seems to play a fundamental role for protection against atherosclerosis, diabetes, obesity and cancer development. In women, breast and ovarian cancers are in part diet-dependent [Willett, 2001; Bosetti *et al*., 2001]. Therefore the study aimed to establish dietary associations of the serum antioxidant activity in a group of young women to estimate the risk of oxidative stress and identify links between the diet's composition and the serum antioxidant activity.

MATERIAL AND METHODS

Subjects

The survey was approved by The Local Ethical Committee for Human Studies. A coherent group of 29 healthy women, students of the Dietetics Division at the Medical University in Białystok, age 19–22 years, were enrolled in the study.

* Corresponding Author:
E-mail: zttz@umwb.edu.pl (A. Witkowska)

The women reported good health condition, non-vegetarian dietary habits (except for one subject who was pesco-lacto-ovo vegetarian), two were occasional smokers. Of 29 women moderate physical activity level was found in 26, whilst 3 were intensive exercisers. Participants completed self-report questionnaires concerning health condition, body measures, food intake and supplements taken.

Dietary and supplement intakes assessment

The participants completed three-day detailed dietary records. Each participant determined portion size of food and dishes consumed according to a photographic album of food portion sizes [Szponar *et al*., 2000]. Energy and nutrient intakes were calculated with a Dieta 2.0 software (The National Food and Nutrition Institute, Poland) designed to use the national food composition tables [Kunachowicz *et al*., 1998, 2001; Nadolna *et al*., 1994, 2000]. Dietary supplements were included in the calculations. The participants did not consume fortified foods.

Body measurements

Subjects' height and body mass were taken from the questionnaires filled in by the participants. These data were basis for calculations of Body Mass Index (BMI) for each subject. Waist-to-hip ratios (WHR) were determined by measuring waist and hips circumference with a measuring tape. Skinfold thickness measurements were performed with the Lange caliper (Beta Technology Inc., USA) over the triceps muscle of the upper arm.

Blood samples collection

Fasting blood samples were drawn by venipuncture into the VACUTAINER Systems vacuum test tubes with a clot activator and gel for serum separation (Becton Dickinson, France). The samples were allowed to clot within 30 min, then were centrifuged at approximately 1000 ×*g* (MPW 350e centrifuge, MPW Med. Instruments, Poland). Serum was removed and assayed immediately.

TAS and FRAP measurements

Total antioxidant status of the serum was assayed at 37°C using "Total Antioxidant Status" kit, Product No. NX2332 (Randox Laboratories Ltd., United Kingdom). The principle of the assay consists in the suppression of the blue-green coloured radical cation $ABTS^{\bullet+}$ formation by the antioxidants present in the sample. Suppression of the colour production is measured colorimetrically at 600 nm (Spekol spectrophotometer, Carl Zeiss Jena, Germany) and calculated using concentration of the Trolox standard.

The ferric reducing ability of plasma (FRAP) was determined according to Benzie & Strain [1996]. The principle of this method consists in the reduction of ferric ions bound to 2,4,6-tripyridyl-s-triazine (TPTZ) (Sigma Aldrich, Switzerland), to ferrous ions by antioxidant substances present in the sample. A blue stain emerging during this reaction can be measured colorimetrically. In brief, freshly prepared 1.5 mL aliquot of FRAP reagent was warmed at 37°C and a reagent blank was read at a wavelength of 593 nm. Then, 200 mL of the diluted serum sample in deionized water (Simplicity UV System, Millipore, France) (1:4) was added to the FRAP reagent. The absorbance of the solution was measured on a Spekol spectrophotometer (Carl Zeiss Jena, Germany) after the 4-min incubation at 37°C. The antioxidant potential of serum was determined from a standard curve plotted against known $FeSO_4 \times 7H_2O$ concentrations.

Statistical analyses

Statistical analysis of the data set included basic statistical tests and correlation analysis performed using a Statistica 9.0 software (StatSoft, Inc.). Mean values for the body measures were reported together with standard deviations (±SD). Medians and minimal-maximal value intervals were reported for energy and nutrient intakes from food and supplements. Variables except the vitamin C and α-tocopherol intakes had normal distributions. Correlations between variables were analysed with the Pearson's correlation test for normal distributions and with the Spearman's rank correlation test for non-parametric distribution. P values below 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The subjects' body measures are given in Table 1 as means ±SD. The calculated mean body mass of the study participants was 57 kg. The average BMI was 20.2. None of the subjects was overweight or obese.

Daily energy value and nutrient intakes were calculated as median and min-max intervals (Table 2). Median energy – 1655 kcal was lower than the recommended 2400 kcal for women of age 19–22 years, and mean body weight 57 kg [Jarosz & Bułhak-Jachymczyk, 2008]. The WHO/FAO guidelines for the energy intake state that carbohydrate should provide 55–75% of energy in the daily diet, fat consumption 15–30%, and protein intake 10–15% [WHO/FAO, 2003]. Though total protein and carbohydrate intakes were higher, while fat intake was lower than the recommended values [Bułhak-Jachymczyk, 2008], the study showed that the median % contributions to energy value from protein, fats and carbohydrate were appropriate.

According to the WHO/FAO position [2003], the saturated fat intake should not exceed 10% of the total energy consumption, which is associated with a lower incidence of coronary heart disease (CHD). In this study, median % contribution to energy value from saturated fat was 12.4%, which means that it exceeded appropriate proportion, despite lower saturated fat intake (23.5 g/d) than the recommended 27 g/d [Jarosz & Bułhak-Jachymczyk, 2008].

TABLE 1. Subjects' body measures.

	Mean±SD
Body mass (kg)	57±9
Height (cm)	168±7
Body Mass Index (BMI) (kg/m^2)	20.2±2.3
Skinfold thickness of triceps muscle (mm)	16.4±7.0
Waist-to-Hip Ratio (WHR)	0.76±0.05

TABLE 2. Median energy and nutrient intakes*.

	Median (Min-Max)	Recommended Intake
Energy (kJ/d)	6953 (4270–13742)	10200[1)]
Energy (kcal/d)	1655 (1017–3272)	2400[1)]
Contribution to energy value (%): protein	14.7 (10.1–24.7)	10–15[2)]
Contribution to energy value (%): fat	29.6 (14.8–38.4)	15–30[2)]
Contribution to energy value (%): saturated fat	12.4 (2.79–17.2)	<10[2)]
Contribution to energy value (%): carbohydrate	58.1 (46.6–76.4)	55–75[2)]
Total protein (g/d)	65.1 (39.2–95.5)	48[1)]
Total carbohydrate (g/d)	244 (141–485)	130[1)]
Total fat (g/d)	56.7 (22.4–108)	67–80[1)]
Total cholesterol (g/d)	227 (95.8–429)	300[3)]
Saturated fat (g/d)	23.5 (5.10–43.0)	27[1)]
Monounsaturated fat (g/d)	21.0 (8.70–43.7)	-
Polyunsaturated fat (g/d)	6.2 (2.50–22.1)	-
Vitamin C (mg/d)	96.3 (26–259)	75[1)]
β-Carotene (μg/d)	3098 (734–14476)	-
α-Tocopherol equivalent (mg/d)	6.9 (4.1–208)	8[1)]

* from food and supplements; [1)]calculated according to Jarosz&Bułhak-Jachymczyk, 2008, for a woman of 60 kg body mass and moderate physical activity; [2)] according to WHO/FAO [2003]; [3)] according to AHA [2006].

Elevated dietary cholesterol is a known risk factor of CHD [Kromhout, 1995]. In the present study, the median dietary cholesterol was below the limit of 300 mg/d, though the cholesterol intake in the whole group of subjects ranged between 95.8 and 429 mg/d. These results show that some women consumed more cholesterol than it is recommended.

Vitamins are necessary for utilization of macronutrients and for antioxidant protection. Antioxidant vitamins are mostly constituents of plant foods. Vegetables and fruits are sources of vitamin C and β-carotene, while grains are main suppliers of α-tocopherol. Vitamins are also taken in as supplements. In this study, eight subjects supplemented their diets with additional dosage of ascorbic acid and/or α-tocopherol. Despite this, the median dietary α-tocopherol daily intake in this study was lower than 8 mg AI (Adequate Intake) recommended for women aged 19–22 years [Jarosz & Bułhak-Jachymczyk, 2008]. The daily diets provided 96 mg vitamin C, more than the RDA (Recommended Daily Allowance) 75 mg/d [Jarosz & Bułhak-Jachymczyk, 2008]. Water-soluble vitamins, however, are quickly eliminated from the organism, therefore higher intakes could be of benefit. Dietary fats are sources of fat-soluble vitamins and promote their intestinal absorption. In the extreme quartiles of the saturated fat intake, the α-tocopherol intakes did not differ significantly from each other (data not shown), though the α-tocopherol intake was the lowest in the bottom quartile of the saturated fat intake.

Statistical analysis showed that the TAS and FRAP values highly correlated with each other (r=0.588) (Figure 1). This finding suggests that the TAS and FRAP methods can measure antioxidant activity of similar antioxidant compounds present in the serum. Both methods, however, have some limitations. TAS assay can measure serum proteins, while FRAP method cannot, as well as it does not measure thiol antioxidants [Cao & Prior, 1998]. Earlier studies failed to find any link between both assays explaining the lack of correlation by the distinctive compounds measured in the serum and the influence of the technique of sample preparation [Cao & Prior, 1998]. Some reasons for this contradiction might have emerged during sample storage. Cao & Prior [1998] assayed the antioxidant activity in frozen, but unpreserved serum samples within a one-week storage period at a temperature of –80°C, while our samples were analysed immediately after blood collection. Our point was that any delay in the analysis may cause loss of antioxidant compounds in the sample, especially when it is not preserved against oxidation. Some small molecules are unstable and can be rapidly degraded in biological fluids, and may not be available for the assay [Hultberg & Hultberg, 2005].

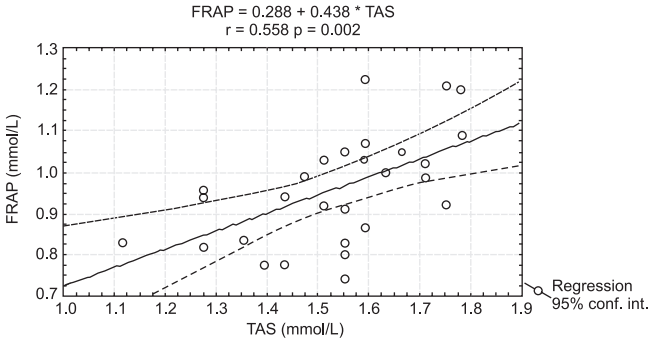

FIGURE 1. TAS and FRAP compliance in the correlation analysis.

Table 3 shows results of a correlation analysis for energy and nutrient intakes and the TAS or FRAP values in the serum of the study participants. The TAS and FRAP values were negatively correlated to the saturated fat intake (r=-0.515 and r=-0.527, respectively) (Table 3, Figure 2). The data collected in the present study indicated no associations between

TABLE 3. Correlations of TAS and FRAP values with energy and nutrient intakes*.

Energy and nutrient intake	TAS (mmol/L) coefficient r	FRAP (mmol/L) coefficient r
Energy (kJ/d)	-0.364	-0.266
Total protein (g/d)	-0.111	-0.017
Total carbohydrate (g/d)	0.067	0.023
Total cholesterol (mg/d)	-0.275	-0.197
Saturated fat (g/d)	**-0.515**	**-0.527**
Monounsaturated fat (g/d)	-0.363	-0.295
Polyunsaturated fat (g/d)	-0.310	-0.082
Vitamin C (mg/d)	0.025**	0.018**
β-Carotene (μg/d)	-0.164	-0.125
α-Tocopherol equivalent (mg/d)	0.003**	0.051**

*Calculated with the Pearson's correlation test. **Calculated with the Spearman's rank test.
Bold values are statistically significant.

antioxidant activity of the serum and the energy intake or cholesterol consumption in women taking part in the study. At the same time the antioxidant activity remained unaffected by intakes of protein, carbohydrate, mono- and polyunsaturated fat, and antioxidant vitamins (vitamin C, β-carotene, α-tocopherol).

The observation concerning associations between the saturated fat consumption and the antioxidant activity was supported by the analysis of quartiles of saturated fat consumption. The TAS value was significantly lower (p=0.042) in the highest quartile of the saturated fat intake (≥29 g/d) compared to the lowest quartile (≤19.69 g/d) (Figure 3). Similarly, a trend toward lower FRAP value was observed in the highest quartile of the saturated fat intake compared to the lowest quartile (p=0.052) (Figure 3).

The saturated fat consumption in the bottom quartile was below 19.7 g and 29 g and even more in the top quartile. These values represent 13.09% and 11.18% of energy provided by saturated fats in relation to energy value of diets in respective quartiles. These results do not meet requirements of WHO/FAO, who recommends saturated fat intake below 10% of the total daily energy [WHO/FAO, 2003]. Saturated fat consumption within this level is associated with a lower incidence of coronary heart disease (CHD) [Kromhout *et al*., 2000].

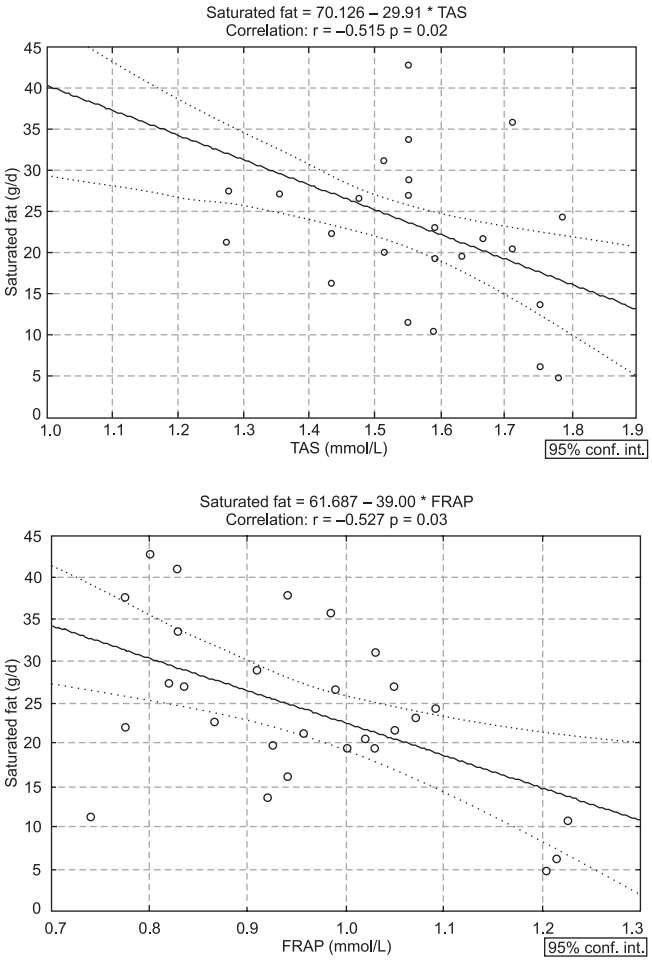

FIGURE 2. Correlations between saturated fat intake and antioxidant activity measured by the TAS and FRAP assays.

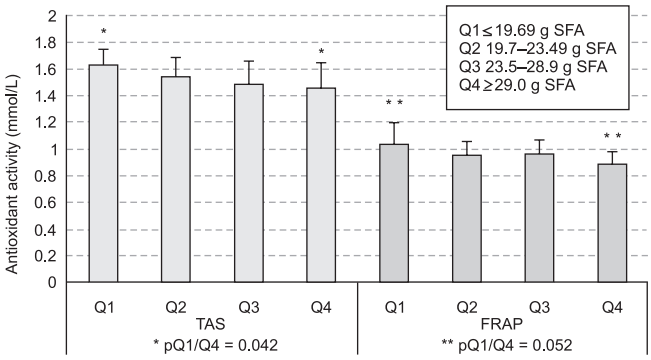

FIGURE 3. Antioxidant activity of the serum in the quartiles of saturated fat intake.

Epidemiological evidence points to a role of saturated fat, but not the total fat intake, in the etiology of coronary heart disease in women [Hu *et al*., 1997], increased risk of the primary breast cancer [Kallianpur *et al*., 2008], ovarian cancer [Risch *et al*., 1994], and lung cancer in non-smoking women [Alavanja *et al*., 1993]. In animal studies, saturated fat feeding caused symptoms of oxidative stress [Oliveros *et al*., 2004; Diniz *et al*., 2004], while in humans, reduction of the saturated fat intake was associated with a diminished LDL susceptibility to oxidation [Yu-Poth *et al*., 2000], and a saturated fat replacement for the monounsaturated or polyunsaturated fat lowered plasma cholesterol, LDL-cholesterol and HDL-cholesterol [Hodson *et al*., 2001].

CONCLUSION

The correlation analysis showed that the quality of dietary fat in the studied group of young healthy women appeared to be significant negative contributor to the antioxidant potential evaluated by two different methods. In the light of the results of correlation analysis, dietary doses of other nutrients seem to be of minor importance. This remark concerns mainly vitamins demonstrating *in vitro* antioxidant properties (vitamin C, β-carotene and α-tocopherol). The results of the study point to a role of saturated fat as a dietary factor which unfavorably modifies antioxidant potential of the serum. These findings show that the studied young females, exposed to diets containing saturated fat in the amounts higher than the recommended, are prone to a risk of oxidative stress and presumably to development of degenerative diseases. These results, however, need to be extended and verified in a larger population.

ACKNOWLEDGEMENTS

The Authors thank to Ms. Hanna Olechnowicz for the excellent technical assistance. The work was financed from the statutory funds of the Medical University in Białystok (project no. UMB 113-17566 P).

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Submitted: 5 March 2012. Revised: 10 May 2012. Accepted: 6 June 2012. Published on-line: 16th of November 2012.