Total serum antioxidant capacity in healthy normal weight and asymptomatic overweight adults

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Abstract

Obesity and overweight are major contributors to the burden of chronic disease. Both are defined as abnormal or excessive fat accumulation and by increased production of free radicals leading to oxidative stress. The aim of the present study was to evaluate whether overweight and fat accumulation is associated with serum total antioxidant capacity (TAC) in men and women, irrespective of nutritional habits, nutrient intakes, physical activity, smoking, and other confounders, which may be responsible to modifying the association between serum TAC and overweight/obesity measures. This cross-sectional study was conducted on 48 normal weight and 48 overweight adults aged 25-49. All participants underwent standard anthromorphological measurements of body composition, blood pressure and biochemical measurements, aerobic capabilities assessment and dietary intake evaluation. TAC was measured using photochemioluminescence method. All data were analysed using SPSS software. Men had higher values of TAC than women and concentrations of TAC were significantly higher in overweight subjects compared to normal weight subjects. In the present study TAC tended to be increased with various metabolic risk factors, especially overweight/obesity parameters (body mass index, body fat), inflammation and increased serum levels of Cysteine, irrespective of nutritional habits, nutrient intakes, physical activity and smoking. Overweight and obesity in early stage may stimulate TAC. Therefore, the elevation of TAC in overweight adults may be a compensatory response to oxidative stress, generated by reactive oxygen species.

Keywords: antioxidant capacity, overweight, obesity, oxidative stress, metabolic risk factors, asymptomatic population

Introduction

Obesity and overweight are chronic disorders of multifactorial origin which can be defined as an increase in the accumulation of body fat (BF) (Fernandez-Sanchez et al. 2011). Obesity is increasing at an alarming rate worldwide and it is known that obesity has a significant impact on mortality and morbidity of people of all ages. Obesity is correlated with systemic inflammation, and accompanied by high-oxidative status (D'Archivio et al. 2012). Therefore, high oxidative status is a principal causative factor in the development of various diseases, such as metabolic syndrome (MetS), dyslipidaemia, atherosclerosis, cardiovascular diseases (CVDs), type-2 diabetes mellitus (T2DM) and others (Freedman et al. 2001; Olusi 2002).

Oxidative stress results from redox imbalance, characterized by condition when the production of reactive oxygen species (ROS) compromises the action of endogenous antioxidant system (Wolin 2009). The damage induced by oxidative stress only occurs when the antioxidant defences are unable to counteract the production of ROS; in that occasion it leads to oxidative damage of lipids, proteins and DNA and might have a significant impact on the pathophysiology of obesity-related complications (Martin-Gallan et al. 2007). The human body has developed several mechanisms to protect biomolecules from the deleterious effects of ROS. These include the antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase and glutathione peroxidase (GSH-Px), as well as water and lipid-soluble antioxidants, such as GSH, ascorbate (vitamin C), α-tocopherol (vitamin E) and β-carotene (Gutierrez-Salinas et al. 2013). The sum of endogenous and food-derived antioxidants represents the total antioxidant capacity (TAC) of extracellular fluids.

TAC assessment is an established methodology to measure the overall antioxidant defense system; in recent years, several methods have been proposed to determine the TAC.

An increasing number of studies focus on the role of ROS in the pathogenesis of numerous civilization diseases, such as obesity (Karaouzene et al. 2011). It has been suggested that higher antioxidant potential can protect the organism against undesirable ROS activity and thus prevent disease incidence (Briasoulis et al. 2009). However, the present state of knowledge on such dependence is still not complete (Munzel et al. 2010). In addition, the knowledge of the antioxidant potential in asymptomatic, healthy, overweight subjects warrants confirmation, because the previous studies provided confusing results on the relationship of the presence of excess of BF to TAC. TAC has been reported to be higher or lower in different studies (Kim et al. 2000; Bae et al. 2006; Kwak and Soon 2007; Simao et al. 2008; Faienza et al. 2012).

Therefore, the aim of the study was to investigate whether overweight, obesity at early stage and fat accumulation is associated with serum TAC in men and women, irrespective of nutritional habits, nutrient intakes, physical activity, smoking, and other confounders, which may be responsible for modifying the association between serum TAC and overweight/obesity measures.

Materials and methods

Subjects and Protocol of measures

The initial study population consisted of 182 individuals (70 males and 112 females). Subjects age range from 25 to 49 years old. The subjects were recruited by the use of flyers, internet forums, e-mail, and newspaper advertisements and by personal contact. Sample size (124 subjects) was calculated based on the confidence level (95%), confidence interval (5%), on the population size (182 individuals), and on the number of participants in related previous studies. But the final analytical sample was confined to 96 asymptomatic subjects (32 males and 64 females). Among the initial subjects, subjects with BMI higher than 35 or lower than 19, taking medications for lipid disorder or taking anti-inflammatory drugs (NSAID), taking nutritional supplements, having CVDs, endocrine and acute or chronic inflammatory disease, T2DM, not completing questionnaires, and more than 3% change of body mass within the last three months were excluded. The final analytical sample was confined to 96 asymptomatic subjects (32 males and 64 females). The study protocol was approved by the Slovenian National Medical Ethics Committee and informed consent was obtained from each study participants. Measurements were performed in standardized conditions: quiet thermally neutral environment (20 to 22°C), overnight fast (at least 12 h from the last meal and drink consumption (except from water), 12 h without physical exercise and smoking, empty bladder.

Dietary and lifestyle assessment

Participant's nutritional intake was assessed by analysis of written food records as described previously (Jenko-Pražnikar et al. 2013). Briefly, all subjects enrolled in the study were instructed to record their daily dietary intake for three days, including a weekend day Records were reviewed by a trained dietitian and analyzed using a web application for analysis of food

diary, named OPEN (Open Platform for Clinical Nutrition), accessible through the web site http://www.opkp.si/en_GB/cms/vstopna-stran. Hand-held indirect calorimeter MedGem® (Medical Home Solutions, Inc., Golden, CO, Microlife) was used for measuring resting metabolic rate (RMR) in standardized conditions and 10 min rest prior the measurements. Fitness index (FI) was assessed to predict maximal oxygen uptake and to measure the ability of brisk walking. The ability to perform high intensity walking on 2 km was measured. The walking time was recorded; the pulse rate was measured at the cervical aorta for 15 s and multiplied by 4. The FI was calculated on the base on the UKK walk test program developed by the UKK Institute for health promotion research, Tampere, Finland, accessible through the website http://www.ukkinstituutti.fi. Gender-specific FI was calculated based on age (A), BMI, walking time (T; in minutes, seconds) and pulse (P): for males: [I= 420 +A*0.2 –T*11.6 – P*0.56 – BMI*2.6] and for females: [I= 304 + A*0.4 – T*8.5 – P*0.32 – BMI*1.1]. The interpretation of FI measurements is: 1) FI < 70 significantly low; 2) FI 70-89 under average; 3) FI 90-100 average; 4) FI 111-130 above average; and 5) FI > 130 significantly high according to the UKK walk test program.

The Physical questionnaire AMA accessible through the web site (http://www.ama-assn.org) was used to assess the general information of physical activity (time spent performing physical activity (number of sessions and average time per session), sedentary pursuits; essentially sitting, and physical activity barriers). The questionnaire was created and funded by the American medical association and the Robert Wood Johnson foundation in November 2003. The whole questionnaire is accessible through the website: http://www.afhaz.com/images/obesityamaphysicalacitvity.pdf

Anthropometric measurements

137 All measurements were carried out following an overnight fast by the same examiner. Subject
138 height was measured to the nearest 0.1 cm in a standing position, without shoes, using
139 stadiometer Leicester Height Measure (Invicta Plastics Limited, Oadby, England). Participant
140 were asked to stand on the stadiometer, facing forwards and straight as possible with their arms
141 hanging loosely at their sides. Their feet were flat on the base plate of the stadiometer and
142 positioned slightly apart, in line with their hips, to aid balance. Their knees were straight and
143 their buttocks and shoulders touched the stadiometer. The participant's head was in the
144 "Frankfort plane". Body weight of the participants wearing usual light indoor clothing without
145 shoes and standing in a stable position without bending their knees and standing motionless
146 with their arms at their sides was measured with a 0.1 kg precision by using bioelectrical
147 impedance analysis (BIA) Tanita BC 418MA (Tanita Corporation, Arlington Heights, IL) and
148 data analyzed with the software provided by the same producer. Waist circumference (WC) was
149 measured midway between the lowest rib and the iliac crest with the subjects in a standing
150 position. Hip circumference (HC) was measured as the maximum circumference around the
151 buttocks. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Body composition
152 (total percentage body fat (% BF) and percentage trunk fat (% TF)) was assessed using
153 bioelectrical impedance analysis (BIA) Tanita BC 418MA (Tanita Corporation, Arlington
154 Heights, IL) and data analyzed with the software provided by the same producer. Overweight
155 was defined by $25 < BMI < 30$ kg/m^2 and obesity by two of the following BMI ≥ 30 kg/m^2, WC
156 ≥ 94 cm for male and ≥ 80 cm for female and/or % BF ≥ 21.5 for male and ≥ 32 for female. In
157 male group 16 % were obese, 34 % were overweight and 50 % were normal weight. Similarly,
158 in female group 17 % were obese, 33 % were overweight and 50 % were normal weight.
159 Systolic and diastolic blood pressure was monitored in the left arm. MetS was defined as having
160 ≥ 3 of criteria based on the Harmonization definition (Alberti et al. 2009).
161

Total antioxidant capacity (TAC)

The antioxidant capacity of serum samples was measured by Photochem® instrument (Analytic Jena, Jena, Germany) using ACW kits (Water Antioxidant Capacity) to detect the activity of hydrophilic compounds. The ultra-sensitive PCL assay was carried out with the procedure described by Popov and Lewin (1999). 1.5 mL of reagent 1 (solvent and dilution reagent), 1 mL of reagent 2 (buffer solution), 25 μL of reagent 3 (photosensitizer), and 0 to 30 μL of standard (ascorbic acid) or 10 μL sample (diluted serum) solution were mixed and measured. In ACW studies, the presence of ascorbic acid or any other antioxidants from the extracts, retarded luminescence for a period; hence, a lag time was noted before a signal was measured. The duration of the lag, calculated by the computer software from the first derivative of the detector signal at its turning point and intersection with the x-axis, was plotted against the concentration of ascorbic acid added to the assay medium. The results were expressed as nanomoles equivalents of ascorbic acid per μL of serum.

Laboratory measurements

Blood samples were collected after an overnight fast in 4 mL vacuum test tubes (Beckton-Dickinson, Rutherford, USA). The blood samples were centrifuged and the separated serum samples were immediately frozen and stored at -20 °C. Biochemical analyses have been described (Jenko-Pražnikar et al. 2013). The serum interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) levels were measured in duplicate on microplate reader (Tecan, Männedorf, Switzerland) with an enzyme immunoassay kit for IL-6 and TNF-α (both Thermo Fischer Scientific Inc., Rockford, USA). Assay sensitivity was <1 pg/mL for IL-6 and <2 pg/mL for TNF-α. Serum concentrations of glucose, TAG, total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol and C-reactive protein (CRP) were measured with the use of Olympus reagents and performed on an AU 680 analyzer (Beckman

Coulter). Serum insulin concentrations was measured with the use of Abbott reagents and performed on a 2000 iSR analyzer (Abbott Architect). The homeostasis model assessment (HOMA) was used as a measure of insulin resistance HOMA-IR. HOMA-IR was calculated according to the following formula: fasting insulin (μU/L) x fasting glucose (mmol/L) / 22.5 (Matthews et al. 1985).

Serum levels of Cysteine (Cys) were analyzed by gas chromatography-mass spectrometry (GC-MS), as previously described (Biolo et al. 20089). Briefly, known amounts of $^{2}H_{2}$-Cysteine (Cambridge Isotope Laboratories) were used as internal standards. Serum samples (200 μL) with a known amount of added internal standard were treated with sulphosalicylic acid, then centrifuged and purified on a cationic resin (AG50W-X8; Bio-Rad, Hercules, CA). After that, samples were lyophilized and Cys was derivatized by the addition of acetonitrile and MTBSTFA (N-methyl-N- (tert-butyldimethylsilyl)-trifluoroacetamide) and by heating at 90°C for 45 min. After derivatization, samples were injected into a GC-MS system (HP 5890, Agilent Technologies, and Santa Clara, CA, USA). Serum concentrations of Cys had his own internal standard (Cys m/z 406/408).

Statistical analysis

Variables are presented as means ± SD. Descriptive statistics were tested before statistical analysis as being normally distributed; data that was not normally distributed (serum levels of LDL cholesterol, TAG, CRP, insulin, HOMA-IR and TNF-α) was logarithmically transformed for subsequent analysis. In order to investigate the metabolic profile and TAC the subjects were divided in two different groups according to the BMI, WC and body fat (in normal weight or overweight group). Anthropometrical, nutritional, physical characteristics and serum metabolites were compared between two groups using an independent-sample t-test. A chi square (χ^2) was used to investigate whether distributions of categorical variables differ from

one another. Pearson's correlation analyses (crude and adjusted for age, physical activity, total energy intake, and common cardio metabolic risk factors (blood pressure, glucose, LDL/HDL-cholesterol, TAG) were performed to detect different associations between serum TAC and anthropometric and biochemical parameters in men and women. SPSS software (IBM SPSS version 19.0, Chicago, IL) was used for all analyses. A *P* value of less than 0.05 was considered statistically significant.

220 **Results**

221

222 Table 1 present the baseline characteristics of normal weight group and overweight group,
223 separately for females (64 subjects) and males (32 subjects). No significant differences were
224 found between the tested groups with respect to age. The differences were found in
225 anthropometric parameters, i.e. in body weight, BMI, WC and % BF in both sexes. A wide
226 range of lean and overweight subjects were included in our study (Table 1); thus, the % BF
227 ranged from 5 % to 30 % in men, and from 17 % to 45 % in women. Participants of both groups
228 were either normotensive or pre-hypertensive (average SBP < 140 and average DSP < 90). In
229 men, weight, WC, systolic blood pressure and RMR, were found to be significantly higher and
230 values of BF significantly lower when compared with women (data not shown). In addition, the
231 biochemical analyses, fasting insulin, HOMA-IR, TAG, CRP, TNF-α, LDL cholesterol of
232 overweight participants of both sexes were found to be statistically significantly higher when
233 compared to normal weight participants (Table 1). Moreover, male subjects were characterized
234 by significantly higher values of fasting glucose, HOMA-IR, TAG and lower values of HDL-
235 cholesterol, and CRP in comparison with the women (data not shown).

236

237 >> **Table 1**

238

239 Aerobic capabilities of participants were assessed by general fitness level, using calculated FI
240 values from the applied UKK Walk Test. No significant differences were found in FI between
241 female and male groups. However, overweight subjects obtained statistically significantly
242 lower values for the aerobic times, meaning they had lower aerobic fitness levels compared to
243 the normal weight subjects. In particular, normal weight participants were in the range of
244 average fitness level (average male FI was 101 ± 13, and average female FI was 111 ± 15),

while overweight participants were classified as below average (average male FI was 82 ± 18, and average female FI was 83 ± 15) (Table 1). Moreover, there were also some differences in nutrient intake between men and women; significantly higher intake of proteins and fatty acids were found in men than in women (data not shown). On the other hand, there were no significant differences in nutrient intakes between normal and overweight groups (Table 1). Moreover, in the normal weight group we found four underreporters, while in the overweight group we found nine underreporters, where energy intake was less than 0.96*RMR. However, overweight male and female participants consumed more meat and meat products and less vegetable than normal weight male and female participants (Table 2).

>> **Table 2**

Measured serum TAC levels in our study, expressed as nanomoles of ascorbic acids per μL of serum, were lower in the normal weight group in comparison to the overweight participants, in both men and women (Fig. 1). In addition, the TAC was significantly higher in men in comparison to the women (Fig. 1).

>> **Fig. 1**

Pearson's correlation analyses were performed to investigate the possible associations between serum TAC levels and the studied parameters (Table 3). Age was not a determinant affecting the antioxidative barrier, regardless of the gender. In both studied groups, TAC was positively related to several anthropometric overweight/obesity measures BMI, BF and WC (Table 3, Fig. 2). Positive correlations were observed between serum TAC levels and fasting serum triacylglycerols and CRP in women; on the other hand positive correlations between serum

TAC levels and fasting serum glucose, insulin, HOMA-IR and amino acid Cys were found in men (Table 3).

>> **Table 3**

>> **Fig. 2**

Partial correlation analysis were performed between TAC and obesity measures to investigate whether obesity and fat accumulation is associated with serum TAC in men and women, irrespective of nutritional habits, nutrient intakes, physical activity, smoking, and other confounders, which may be responsible to modifying the association between serum TAC and overweight/obesity measures. Adjustments included age, physical activity, total energy intake, blood pressure, fasting glucose, LDL/HDL-cholesterol and TAG in men and women. Indeed, in both groups TAC was positively related to BMI and BF, irrespective of mentioned confounders. In addition, positive association remained significant between TAC and CRP after adjustments in female subjects and between TAC and Cys in male subjects (Table 4).

>> **Table 4**

289 **Discussion**

290 In the present study, the PCL assay was performed according to measures the total
291 antioxidant capacity of all antioxidants present in serum. In this study, we showed that
292 overweight asymptomatic, i.e. apparently healthy, individuals have higher serum levels of TAC
293 than normal weight controls. The most likely explanation for our observations is that body
294 responded to increased oxidative stress accompanying overweight/obesity by increasing TAC.
295 Moreover, this phenomenon was evident in both men and women.

296 In the present study TAC tended to be higher with various metabolic risk factors,
297 especially overweight/obesity parameters, inflammation and increased serum levels of Cys.
298 TAC was significantly positively related with increased weight, BMI, waist circumference, BF
299 and was significantly higher in healthy overweight subjects. Obesity has shown to increase
300 oxidative stress by increased LDL oxidation and malondialdehyde (MDA), and these markers
301 of oxidative stress have found to be related with BMI and waist to hip ratio (Mohn et al. 2007).
302 In addition, growing evidence indicates that the mitochondria of white adipose tissue,
303 particularly of obese persons, are the main site of ROS generation, accompanied by decreased
304 expression of antioxidative enzymes (Roberts et al. 2006). However, TAC measured with
305 various methods has not seemed to decrease in overweight and obese people (Kim et al. 2000;
306 Molnar et al. 2004; Bae 2006). Similar to the findings from the present study, TAC was
307 significantly higher in overweight adult males (Kim et al. 2000) and tended to be, but not
308 significantly, higher in obese children (Molnar et al. 2004), as compared to controls. In addition,
309 TAC was found to be significantly positively correlated with weight and BMI in 158 adults
310 with and without hypertension (Kim et al. 2006). These findings, including the results from the
311 present study, consistently suggest that weight gain may increase serum or plasma TAC.

312 Emerging evidence shows that metabolically benign overweight individuals have a
313 greater subclinical cardiovascular disease burden than those of normal weight (Khan et al.

2011), and also that cardiovascular diseases are correlated with oxidative stress-generating conditions (Schnabel and Blankenberg 2007; Lear et al. 2012). In previous studies, obesity has often been accompanied with increased serum glucose, TAG and total cholesterol (Ahn et al. 2005). In conjunction with obesity, serum glucose and insulin significantly positively correlated with TAC in male subjects, while serum TAG significantly positively correlated with TAC in female subjects. However, the findings in the association between TAC and increased glucose, TAG and/or total cholesterol in various metabolic conditions have shown to vary (Kural et al. 2003; Kural et al. 2004). Based on these finding, it is possible to justify that in the present study, the highest serum TAC was associated with higher TAG circulating levels. In addition, obesity is also associated with a chronic state of low-grade inflammation, which can further drive oxidative stress (Wisse 2004; Sun et al. 2012). Indeed, in the present study positive association between CRP and TAC were found, considering the increase in serum TAC as a compensatory system with the purpose of restoring homeostatic balance.

We observed substantially higher levels of Cys in overweight asymptomatic population. High plasma Cys showed to be linked to obesity – Cys might even cause obesity and have an insulin-like action on adipocytes (Elshorbagy et al. 2012). Total Cys showed to have strong positive correlation with fat mass and to be a stronger predictor of fat mass than serum lipids such as TAG, HDL, and total cholesterol (Elshorbagy et al. 2008). Serum Cys imbalances therefore correlate with markers of metabolic dysfunction. We found significant positive correlations between serum Cys and HOMA-IR, CRP, TNF-α, WC and a body shape index in recent work (Mohorko et al. 2015), and here we presented significant positive correlation between Cys and TAC. Cys is involved in body's antioxidant defense as one of the amino acids involved in synthesis of glutathione (GSH), together with glutamate and glycine (Wu et al. 2004).

In physiological conditions, a great part of TAC appears to be due to the endogenous component activity (Crews et al. 2001); however, many studies report the ability of the dietary intake to modulate antioxidant status after the acute consumption of antioxidant rich foods (Hassimotto et al. 2008; Potter et al. 2011). In our study, both caloric intake and nutrient intake and foods habits were quite similar between normal and overweight participants; however, normal weight subjects consumed more vegetables and less meat products per day than overweight subjects and at the same time had lower TAC. It is possible that overweight subjects responded through an inflammatory reaction to high fat meat diet, accompanied by an increased production of the endogenous antioxidants. Indeed, between normal and overweight groups were significant differences in meat and meat products intake.

Moreover, there are some other confounders influencing serum levels of TAC. Increased physical activity is known to be negatively correlated with the MetS (Larsson et al. 2012), and a better FI level means a better antioxidant protective mechanism (Atalay and Sen 1999). Physical activity has an important role to increase the TAC in healthy human blood after a single bout of exercise maximal or submaximal. This reinforcement of the antioxidant system due to training may be explained by the fact that exercise stimulates the expression of the genes (mainly NF-κB) involved in the regulation of the antioxidant enzymes in redox sensitive signal transduction pathways (Berzosa et al. 2011). However, in the present study normal weight subjects with lower TAC, were more physically active and had higher FI. Similar was shown in the study of Gawron-Skarbek et al. (2015), who found that the serum total antioxidant capacity is inversely correlated with fitness characteristics in men with coronary heart disease. The authors concluded that uric acid, the main determinant of serum TAC, may be partially responsible for those associations (Gawron-skarbek et al. 2015). Therefore, the increased TAC levels in the overweight subjects of our study might be related with the uric acid level, especially since a higher consumption of meat and meat products were found in this group.

In the current study, TAC was significantly positively associated with obesity measures, as well as number of metabolic risk factors, although these conditions have known to be high in oxidative stress. Therefore, it seems that TAC appears to be affected by changes in individual antioxidant parameters which might be altered by various metabolic conditions. These antioxidant parameters affecting TAC values could be increased not only by body's increased antioxidant status, but by body's compensatory mechanism to counteract increased oxidative stress (Ames et al. 1985). Indeed, in obese subjects, higher levels of RO metabolites were found (Vassalle et al. 2013). It means that overweight/obesity may stimulate TAC and high values of antioxidant potential among overweight/obese individuals may be explained by secondary response to intensified oxidative stress characterizing subjects with higher amount of adipose tissue (Keaney et al. 2003). Adipose tissue in general possesses relatively high levels of antioxidant defensive enzymes for managing high ROS production (Yang et al. 2008). Therefore, in early stages of metabolic disorders, but already in the presence of risk factors (overweight/obesity, elevated blood pressure, lipids,…), the antioxidant defence system may respond to sustained oxidative stress by increasing its activity, but by the advanced stage the balance between generation of free radicals and antioxidant defence is probably impaired as a result of decreased antioxidant levels or activity. Studies have reported low levels of antioxidant enzymes in obese individuals (Sharma and Sharma 2007; Waggiallah and Alzohairy 2011).

The present findings are concordant with prior reports on obese humans and animal models which found that in the early stages of obesity development there may be an initial elevation in antioxidant enzymes to counteract oxidative stress (Woo et al. 2012; Gawron-Skarbek et al. 2014).

There are several limitations to our study that should be mentioned. It included relatively small numbers of participants, 48 were normal weight and 48 were overweight and obese (obese and overweight subjects were merged in one group, because BMI of our participants was

between 26 and 31). However, we are aware that these participants could be metabolically different. Moreover, our study is a cross-sectional study and warrants confirmation through a future prospective study. Since there are no individual antioxidant data available in the present study, we may not be able to strongly assume the factors directly affecting TAC in subjects with and without metabolic risk factors. However, it is worthy to note that positive associations between TAC and increased metabolic risk factors, especially obesity related parameters, were consistently found in the present study and a few previous studies. This implies that increased TAC may not always represent one's healthier condition or condition with a low oxidative stress, suggesting that TAC may not be used as a sole indicator of oxidative stress marker. Further, it is necessary to clarify the unknown factors affecting TAC with relation to various metabolic conditions.

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Disclosure statement

The authors have no conflicts of interest to report.

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609 **TABLES**

610

611 **Table 1.** Anthropometric and biochemical parameters of the normal weight and overweight

612 group [1]

	Normal weight group		**Overweight group**	
Gender	M	F	M	F
Number of participants	16	32	16	32
General characteristics				
Age (years)	34.3±6.2	37.7±6.1	37.9±6.3	39.3±6.0
BMI (kg/m^2)	23.1±2.2	21.4±2.0	29.3±2.9***	29.5±2.7***
WC (cm)	84±7	72±5	100±6***	91±7***
Weight (kg)	75.9±9.4	60.1±6.1	95.6±8.2***	80.9±9.3***
Body fat (%)	15±5	24±5	24±3***	39±3***
SBP (mmHg)	131±14	118±14	139±19	121±15
DBP (mmHg)	74±16	69±10	79±10	74±5*
RMR (kcal/day)	1605±355	1387±244	1908±240**	1453±295
Biochemical parameters				
Fasting glucose (mmol/L)	5.2±0.5	5.0±0.4	5.6±0.3*	5.0±0.4
Fasting insulin (unit)	5.4±1.4	5.5±1.8	10.8±5.7***	8.4±3.2***
HOMA-IR	1.3±0.4	1.2±0.5	2.7±1.5***	1.9±0.8***
TAG (mmol/L)	1.1±0.8	0.9±0.3	1.7±0.8*	1.2±0.7**
Total cholesterol (mmol/L)	4.9±1.0	5.0±0.8	5.7±1.3	5.5±1.1
HDL-cholesterol (mmol/L)	1.4±0.3	1.6±0.3	1.2±0.2*	1.4±0.3*
LDL-cholesterol (mmol/L)	3.1±0.9	3.1±0.6	3.8±1.2	3.5±0.9*
CRP (mg/L)	1.1±1.1	0.7±0.7	1.8±1.5	3.6±3.4***

TNF-α (pg/L)	2.0±1.6	2.0±1.6	5.5±3.3**	5.1±3.4***
IL-6 (pg/L)	2.6±1.2	2.8±0.8	3.2±0.9	3.3±0.9
Cysteine (µmol/L)	1.33±1.21	0.31±0.28	5.40±5.21***	3.94±3.64***
Lifestyle parameters				
MetS (n, %)[2]	0 (0)	0 (0)	7 (43.8)***	4 (12.5)***
FI	101±13	111±15	82±18*	83±15***
Physical activity (min/week)	214±211	168±117	89±79*	122±82*
Current smokers (n, %)	1 (6.3)	5 (15.6)	1 (6.3)	3 (9.4)
Dietary parameters – nutrient intakes				
Proteins (g/day)	88±25	73±25	110±39	77±28
Carbohydrates (g/day)	279±80	227±69	245±93	247±102
Dietary fiber (g/day)	23±8	22±11	20±12	24±16
Fatty acids (g/day)	92±26	71±26	94±30	70±23
Saturated FAs (g/day)	29±10	22±9	33±14	23±9
Monounsaturated FAs (g/day)	26±14	20±9	24±12	20±8
Polyunsaturated FAs (g/day)	13±6	10±4	13±6	10±5
Vitamin C (mg/day)	121±90	145±70	67±38*	116±60
Folic acid (µg/L)	5.92±2.54	6.70±2.89	5.71±1.64	7.32±2.62

Note: [1]All values are mean ± SD. M, male; F, female; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; RMR, resting metabolic rate; HOMA-IR, homeostasis model assessment for insulin resistance; HDL, high density lipoproteins; LDL, low density lipoproteins; CRP, reactive protein C; TNF, tumor necrosis factor; IL, interleukin; FI, physical fitness; FA, fatty acid; TAG, triacylglycerols.

[2]MetS, metabolic syndrome. Components of the MetS are hypertriglyceredimia (≥ 1.7 mmol/L), HDL levels (< 1mmol/L in men and < 1.3 mmol/L in women), large waist circumference (≥ 94

620 cm in men and ≥ 80 cm in women), elevated blood pressure (systolic ≥ 130 mmHg and/or
621 diastolic ≥ 85 mmHg) and elevated plasma glucose (≥ 5.6 mmol/L). Subject, identified as MetS,
622 has 3 or more components of the MetS.
623 Comparisons of physical, biochemical, nutritional, and other characteristics between two
624 groups (for males and females separately) were completed using one-factor analysis of the
625 variance. A chi square (χ^2) was used to investigate whether distributions of categorical variables
626 differ from one another (MetS (%), smokers (%)). The mean difference is significant at the 0.05
627 level; ***$P < 0.001$, ** $P < 0.01$, * $P < 0.05$
628
629

630 **Table 2.** Energy value of food group of the normal weight and overweight participants [1]

	Normal weight group		**Overweight group**	
gender	M	F	M	F
Number of participants	16	32	16	32
Milk and milk products (kcal/day)	319±171	233±110	269±141	225±139
Vegetable (kcal/day)	75±62	46±41	**33±22***	53±45
Fruit (kcal/day)	127±121	143±111	76±72	137±75
Starchy food (kcal/day)	722±203	528±155	658±258	617±323
Meat and meat products (kcal/day)	349±145	280±135	**571±277***	**364±147***
Fat and fatty food (kcal/day)	529±207	396±163	518±233	403±172
Sugars (kcal/day)	199±136	161±125	165±147	175±206

631 **Note:** [1]All values are mean ± SD. M, male; F, female.

632 Comparison of nutritional characteristics between two groups (for males and females
633 separately) were completed with using one-factor analysis of the variance. *$P < 0.05$

634

635

636

637 **Table 3.** Pearson correlation coefficients of total antioxidant capacity measure to age, selected
638 anthropometric, biochemical, and blood pressure characteristic in men and women

	Men	**Women**
Variable	**TAC**	
	r (*P*)	**r (*P*)**
Age (years)	0.296 (0.204)	0.066 (0.653)
Body mass (kg)	0.359 (0.101)	**0.438 (<0.001)**
BMI ($kg \cdot m^{-2}$)	**0.627 (0.003)**	**0.463 (0.012)**
WC (cm)	**0.444 (0.050)**	**0.335 (0.024)**
BF (%)	**0.564 (0.006)**	**0.419 (0.016)**
SBP (mmHg)	0.090 (0.706)	0.116 (0.698)
DBP (mmHg)	0.155 (0.513)	0.187 (0.376)
FI	-0.237 (0.360)	-0.112 (0.372)
Glucose (mmol/L)	**0.454 (0.044)**	0.153 (0.120)
Cholesterol (mmol/L)	0.258 (0.271)	0.048 (0.744)
HDL (mmol/L)	-0.139 (0.558)	-0.143 (0.326)
LDL (mmol/L)	0.216 (0.359)	0.132 (0.320)
TAG (mmol/L)	0.387 (0.075)	**0.280 (0.026)**
CRP (mg/L)	0.397 (0.067)	**0.258 (0.041)**
Insulin (µU/mL)	**0.529 (0.011)**	0.202 (0.116)
HOMA-IR	**0.545 (0.009)**	0.172 (0.182)
Cysteine (µmol/L)	**0.489 (0.029)**	0.122 (0.345)

639 **Note:** BMI, body mass index; WC, waist circumference; BF, body fat; SBP, systolic blood
640 pressure; DBP, diastolic blood pressure; FI, fitness index; HDL, high density lipoprotein; LDL,

641 low density lipoprotein; TAG, triacylglycerols; CRP, C-reactive protein; HOMA-IR,
642 homeostatic model assessment of insulin resistance.
643 The statistically significant association is at $P < 0.05$ (bold)
644

645 **Table 4.** Partial correlation coefficients of total antioxidant capacity measure to selected
646 anthropometric and biochemical parameters after adjustment for age, physical activity, total
647 energy intake, smoking, and common cardiometabolic risk factors (blood pressure, glucose,
648 LDL/HDL-cholesterol, TAG) in men and women

	Men	**Women**
Variable	**TAC (after adjustments)**	
	r (*P*)	**r (*P*)**
BMI (kg·m^{-2})	**0.453 (0.033)**	**0.416 (0.002)**
BF (%)	**0.344 (0.046)**	**0.321 (0.017)**
CRP (mg/L)	0.298 (0.082)	**0.249 (0.051)**
Cysteine (µmol/L)	**0.530 (0.032)**	0.147 (0.287)

649 **Note:** BMI, body mass index; WC, waist circumference; CRP, C-reactive protein.
650 The statistically significant association is at $P < 0.05$ (bold)
651
652

653 **FIGURE LEGENDS**

654

655 **Fig. 1** Total antioxidant capacity in serum, expressed as nanomoles of ascorbic acids per μL of
656 serum, in the normal weight group in comparison to the overweight participants, in both men
657 and women was analyzed by an independent-sample t-test; a *P* value of less than 0.05 was
658 considered statistically significant.

659

660 **Fig. 2** Pearson's correlation analyses were performed to detect correlations between total
661 antioxidant capacity in serum, expressed as nanomoles of ascorbic acids per μL of serum and
662 selected anthropometric characteristics (body mass index (A) and body fat (B)) in women (1;
663 green lines and green spots) and men (0; blue lines and blue spots)

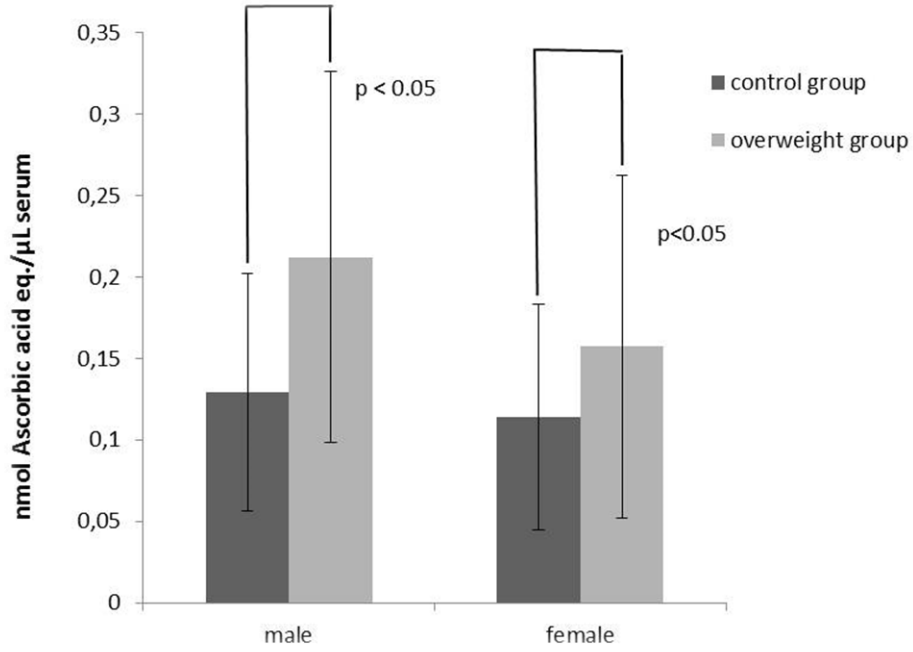
664

665 **FIGURES**

666

667 >> **Fig. 1**

668



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673 >> **Fig. 2 (A, B)**

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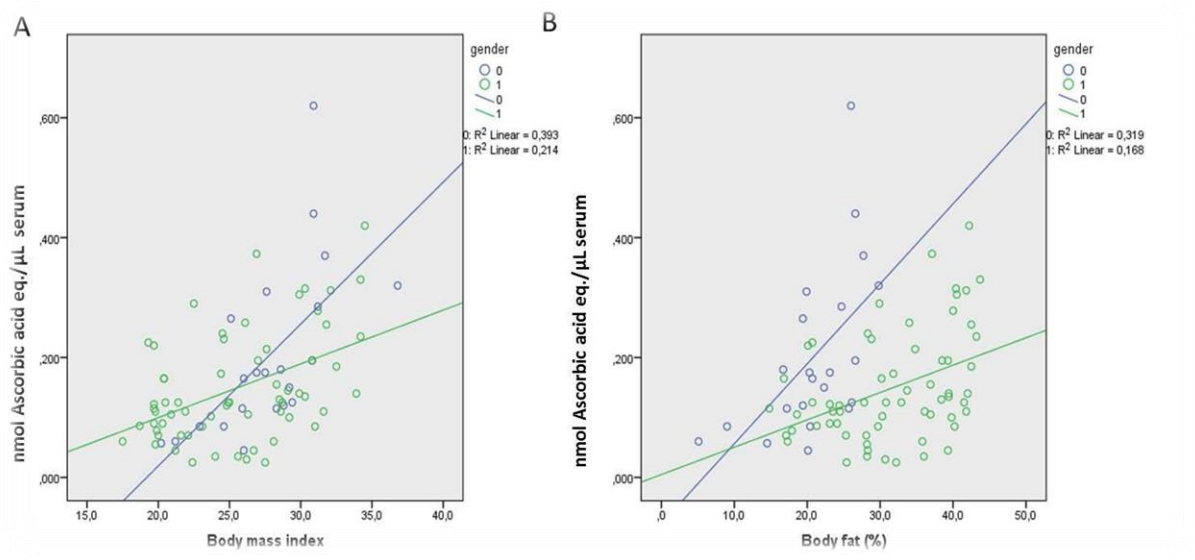

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