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Plasma Total Antioxidant Capacity (TAC) in Obese Malaysian Subjects

Lim SH, Fan SH & Say YH*

Department of Biomedical Science, Faculty of Science, Universiti Tunku Abdul Rahman (UTAR) Perak Campus, 31900 Kampar, Perak, Malaysia

ABSTRACT

Introduction: There is a pressing need to better understand the complex biochemical pathways that lead to the pathogenesis of obesity. Increased oxidative stress and decreased antioxidant capacity have been identified to be associated with obesity. Therefore, the objectives of this study were to determine the plasma total antioxidant capacity (TAC) levels of Malaysian subjects and to evaluate its potential association with obesity and related anthropometric measurements. **Methods:** Plasma TAC of 362 multi-ethnic Malaysian subjects from the Kampar Health Clinic (138 males, 224 females; 124 ethnic Malays, 152 Chinese, 86 Indians; 192 non-obese, 170 obese) was measured using Trolox equivalent antioxidant capacity (TEAC) 96-well plate assay. **Results:** Plasma TAC was significantly lower in obese subjects ($M \pm SE = 292 \pm 10.4$ μmol/L) compared to non-obese subjects (397 ± 8.58 μmol/L), whereas it was significantly higher in males and those in the 21-30 age group. Those with salty food preference and practising a strict vegetarian diet also had significantly higher plasma TAC. However, no association was found for other dietary habits (coffee intake) and lifestyle factors (physical activity, smoking). Plasma TAC was also significantly negatively correlated with diastolic blood pressure, waist and hip circumferences, weight, body mass index, total body fat, % subcutaneous fat, visceral fat level, resting metabolism and % skeletal muscle. **Conclusion:** Plasma TAC was found to be associated with obesity, strict vegetarian practice, salty food preference and all obesity anthropometric indicators, except systolic blood pressure and pulse rate. Obese people have decreased plasma TAC indicating a compromised systemic antioxidant defence and increased oxidative stress.

Keywords: Plasma total antioxidant capacity, Trolox equivalent antioxidant potential, oxidative stress, obesity, Malaysia

INTRODUCTION

The prevalence of obesity, a multifactorial disease caused by an interaction of genetic factors with lifestyle and environmental factors, is rapidly increasing worldwide. The 2006 Third Malaysian National Health and Morbidity Survey (NHMS III) found the prevalence of overweight to have increased to 29.1% and that of obesity to 14.0% compared to the 1996 NHMS II at 16.6% and 4.0%, respectively (Institute of Public Health Malaysia, 2006). There is a pressing need to better understand the complex biochemical

* Correspondence author: Say YH; Email: *sayyh@utar.edu.my*

pathways that lead to the pathogenesis of obesity and the associated complications that arise from it, such as metabolic syndrome. In the last few years, antioxidant defence system and systemic oxidative stress have been identified to have a significant impact on the pathophysiology of obesity and metabolic syndrome (Furukawa *et al*., 2004).

Antioxidants are defined as compounds that significantly decrease or delay oxidation of macromolecules such as lipid, protein, nucleic acid and carbohydrate by preventing the consequences of chemical reactions involving free radicals (Halliwell & Gutteridge, 1995). Oxidative stress is established when there is an imbalance of the antioxidant defence system and free radicals production which compromises cell functions leading to cell death by apoptosis and necrosis, and contributes to the development of a wide variety of diseases including atherosclerosis, hypertension, diabetes mellitus, cancer and obesity (Young & Woodside, 2001). Oxidative stress is induced in obesity (Dandona *et al*., 2001; Furukawa *et al*., 2004). Oxidative damage of amino acids and protein as well as lipid peroxidation and reactive oxygen species (ROS) are greater in obese subjects than those in normal subjects (Dandona *et al*., 2001). Furthermore, human, *in vitro* cell culture and *in vivo* mice studies revealed that systemic oxidative stress is increased in obesity, which involves fat-derived hormone adipocytokines (Furukawa *et al*., 2004). Blood total antioxidant capacity (TAC) of obese individuals was also found to be significantly lower than in non-obese individuals (Amirkhizi *et al*., 2010; Chrysohoou *et al*., 2007).

Plasma TAC is used as a biomarker for non-enzymatic antioxidant status and oxidative stress (Miller *et al*., 1993). Plasma TAC assay provides an integrated parameter that takes into consideration the cumulative action of all the antioxidants present in plasma, rather than just the simple sums of measurable antioxidants (Serafini & Del Rio, 2004). Therefore, the objectives of this study were to measure the fasting plasma TAC levels of subjects from a Malaysian clinic cohort by using the Trolox equivalent antioxidant capacity / potential (TEAC) assay, to determine the potential relationship between the plasma TAC and obesity, and to evaluate correlation of plasma TAC with anthropometric measurements, blood pressure, dietary habits and lifestyle factors.

METHODS

Subjects

Three hundred and sixty-two subjects were recruited by convenience sampling from April to December, 2010 at the Kampar Health Clinic (*Klinik Kesihatan Kampar*) in the state of Perak, Malaysia. Exclusion criterion of the subjects was having acute respiratory infection, cold or flu, chronic viral infection, or having undergone any major surgery recently preceding the sample collection. This study was registered under the National Medical Research Registry of Malaysia (NMRR-09-826-4266) and the protocol was approved by the Medical Research and Ethics Committee, Ministry of Health, Malaysia. An informed consent form was signed by all the respondents in this study and the blood samples were taken in accordance with the World Medical Association (WMA) Declaration of Helsinki (as revised in Seoul, 2008).

Questionnaire, anthropometric and blood pressure measurements

A questionnaire-interview session was conducted by the field team to gather demographic data, which provided information on age, gender and self-indentified ethnicity. Systolic and diastolic blood pressure (SBP; DBP) and pulse rates of the subjects were measured using the HEM-712C blood pressure monitor (Omron,

Japan) and duplicate readings were obtained after the subjects were in resting condition for at least 10 minutes. Besides height, waist and hip circumferences were determined using a measuring tape to the nearest 0.1 cm. Waist-Hip Ratio (WHR) was calculated by dividing the waist circumference (WC) by hip circumference (HC). The HBF-362 Karada scan bio-impedance scale (Omron, Japan) was used to analyse anthropometric measurements namely weight, body mass index (BMI), total body fat (TBF), subcutaneous fat (SF), visceral fat (VFL), resting metabolism (RM) and subcutaneous muscle (SM). The BMI cut-off point for obesity for this study was 27 kg / m^2 (Deurenberg-Yap *et al*., 2000). Dietary habits like salty food preference (intake of at least a serving or dish of high sodium foods such as salted fish or salted vegetables once a week), strict vegetarian practice (defined as diet where meat, poultry or seafood, excluding egg and milk products are absent), habitual coffee intake (at least a cup per day) and lifestyle factors such as habitual exercise (physical activity of $\geq$3 times per week for at least 30 minutes per session) and current smoking status (at least a stick of cigarette per day) were also surveyed. Subjects responded with a 'yes / no' choice.

Plasma TAC assay using the 96-well microplate TEAC method

About 5 ml of overnight fasting blood samples were collected by medical practitioners into EDTA anticoagulant tubes and plasma was separated. Plasma TAC was assessed using the Trolox equivalent antioxidant potential (TEAP) 96-well microplate method (Kambayashi *et al*., 2009), where TAC was assessed using lag time by antioxidants against the myoglobin-induced oxidation of 2,2'-azino-di(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) with hydrogen peroxide (H_2O_2), and expressed as Trolox equivalent. A serial dilution of plasma which ranged from 1: 2 to 1:100 was performed; lag time was linear with the dilution of 1: 25 and was therefore used in subsequent assays. Ninety microlitres of 10 mM phosphate-buffered saline, 50 μl of myoglobin solution, 20 μl of 3mM ABTS solution and 20 μl of diluted plasma or Trolox solution were added into the wells of 96- well microplate, mixed by vibration and maintained at 25°C for 3 min. Reaction was started by the addition of H_2O_2 (20 µl) and followed at 600 nm with the microplate reader for 5 min, 25°C. The Trolox standard curve was obtained by plotting the average absorbance against Trolox concentration standards of 0, 5, 10, 15, 20 and 25 μM (R^2 = 0.986). Plasma TAC of test sample was then calculated using the equation obtained from the linear regression of the standard curve (Miller & Rice-Evans, 1997). The equation was as follows: Plasma TAC level (µM Trolox equivalent) = [$y(A_{600})$-Intercept] / slope × dilution factor, where $y(A_{600})$ was the average absorbance of the test sample at 600 nm, intercept is the intercept of *y* axis by the standard curve, and slope is the slope of the standard curve. The intra- and inter-assay coefficients of plasma TAC variation did not exceed 3%.

Statistical analysis

The data obtained were analysed using Statistical Package for Social Sciences (SPSS) version 17.0 (SPSS Inc., Chicago, IL). Frequencies and percentages for demographics, dietary habits and lifestyle factors were determined using descriptive statistics. The normality of the sample distribution was examined using the Kolmogorov-Smirnov test. The significant differences of means of anthropometric measurements, blood pressure and plasma TAC between BMI groups, gender, ethnicities, dietary habits and lifestyle factors were compared using the Mann-Whitney *U* test or Kruskal-Wallis test. Additionally, correlations of plasma TAC with anthropometric measurements and blood pressure were determined by Spearman's rho correlation analysis. A *p*-

value of < 0.05 was considered as statistically significant.

RESULTS

Sample characteristics, blood pressures and anthropometric measurements

As shown in Table 1, 362 subjects who ranged in age from 21 to 80 years ($M \pm SD$ = 53.8 ± 12.6 years) were recruited into this study. Non-obese and obese subjects were almost equally distributed, whereas females (61.9%) outnumbered males (38.1%). Ethnic Chinese formed the majority of the subjects, followed by Malays and Indians. All anthropometric measurements between non-obese and obese groups were significantly different except for pulse rate and WHR, where the means tended to be higher in obese subjects compared to non-obese, while the opposite was true for SM (Table 1). Age-wise, obese subjects were significantly younger than non-obese subjects (52.3 ± 10.9 *vs.* 55.2 ± 13.7; $p = 0.03$ by Mann-Whitney U test); males were significantly older than females (55.4 ± 13.3 *vs.* 52.8 ± 12.0; $p = 0.03$ by Mann-Whitney U test) and age was significantly different among Malays, Chinese and Indians (52.7 ± 12.6, 56.6 ± 13.5, 50.6 ± 9.4, respectively; $p < 0.001$ by Kruskal-Wallis test).

Plasma TAC and its association and correlations with demographics, anthropometrics, blood pressure, dietary habits and lifestyle factors

The association of plasma TAC with demographics such as gender, ethnicity and obesity status is shown in Figure 1. Plasma TAC was significantly higher in males as compared to females and also in non-obese compared to obese, but was not significantly different between ethnic groups and age groups. Nevertheless, Chinese subjects had the highest plasma TAC followed by Indians and Malays; in terms of age, the age groups of 21-30 and 71-80 had the highest plasma TAC levels. By age, plasma TAC seemed to show an increasing trend as the age group increased, albeit starting from age group 31-40 onwards. When stratified according to gender and ethnicity, the obese group still had significantly lower plasma TAC compared to the non-obese, whereas the difference in plasma TAC was not significant between genders and ethnic groups within the non-obese and obese groups, respectively (Figure 1).

Meanwhile, dietary habits such as salty food preference and strict vegetarian practice were associated with plasma TAC, where those who preferred salty food and practising vegetarians seemed to have significantly higher plasma TAC levels (Figure 2). When stratified according to obesity status, this association still holds true among non-obese and obese for salty food preference, but was lost among non-obese and obese for strict vegetarian practice. Other dietary habits and lifestyle factors like habitual exercise / physical activity and current smoking status did not seem to be associated with plasma TAC.

Table 2 illustrates the correlation of plasma TAC with anthropometric measurements and blood pressures. SBP and pulse rate did not have significant correlations with plasma TAC. All other variables like DBP, WC, weight, BMI, TBF, SF, VFL and RM had significant negative correlations with plasma TAC, while WHR and SM had significant positive correlations with plasma TAC. Among all the variables, BMI showed the strongest correlation with plasma TAC, indicating that the increase in BMI is a strong predictor for the decrease in plasma TAC level.

DISCUSSION

In assessing the association between plasma TAC with obesity in the multi-ethnic Kampar health clinic cohort, it was found that an increase in anthropometric indices of obesity like WC, BMI, TBF, SF, VFL and

Table 1. Demographic characteristics, blood pressure and anthropometric measurements of subjects

Variables	*Non-obese* *N = 192 (53.0)*		*Obese* *N = 170 (47.0)*	*Total* *N = 362 (100)*
Gender				
Male	82 (42.7)		56 (32.9)	138 (38.1)
Female	110 (57.3)		114 (67.1)	224 (61.9)
Age group				
21-30	14 (7.3)		3 (1.8)	17 (4.7)
31-40	12 (6.2)		22 (12.9)	34 (9.4)
41-50	37 (19.3)		48 (28.2)	85 (23.5)
51-60	54 (28.1)		58 (34.1)	112 (30.9)
61-70	50 (26.0)		32 (18.8)	82 (22.7)
71-80	25 (13.0)		7 (4.1)	32 (8.8)
Ethnicity				
Malay	48 (25.0)		76 (44.7)	124 (34.3)
Chinese	101 (52.6)		51 (30.0)	152 (42.0)
Indian	43 (22.4)		43 (25.3)	86 (23.8)
Anthropometric measurements				
SBP (mmHg)	138 ± 24.0		143 ± 20.0	140 ± 22.2
p		0.008		
DBP (mmHg)	79.5 ± 10.9		84.9 ± 11.3	82.0 ± 11.4
p		<0.001		
Pulse rate (bpm)	73.6 ± 13.7		73.8 ± 12.4	73.7 ± 13.0
p		0.716		
WC (cm)	84.7 ± 10.0		98.6 ± 10.0	91.2 ± 12.1
p		<0.001		
WHR	0.89 ± 0.09		0.90 ± 0.07	
p		0.182		
BMI(kg/m^2)	23.8 ± 2.30		31.6 ± 3.90	27.5 ± 5.0
p		<0.001		
TBF(%)	31.2 ± 6.63		37.6 ± 5.14	34.2 ± 6.8
p		<0.001		
SF(%)	24.4 ± 6.46		32.5 ± 7.73	28.2 ± 8.2
p		<0.001		
VFL(%)	8.55 ± 3.46		17.5 ± 6.87	12.8 ± 7.0
p		<0.001		
RM(kcal)	1336 ± 202		1560 ± 230	1442 ± 243
p		<0.001		
SM(%)	25.4 ± 4.04		23.4 ± 3.29	24.5 ± 3.8
p		<0.001		

Values in parenthesis are percentages of the total variable in the same column.
SBP: systolic blood pressure; DBP: diastolic blood pressure; WC: waist circumference; WHR: waist-to-hip ratio; BMI: body mass index; TBF: total body fat; SF: subcutaneous fat; VFL: visceral fat level; RM: resting metabolism; SM: skeletal muscle.
Values for blood pressure and anthropometric measurements are mean ± standard deviation; *p* values by Mann-Whitney *U* test.

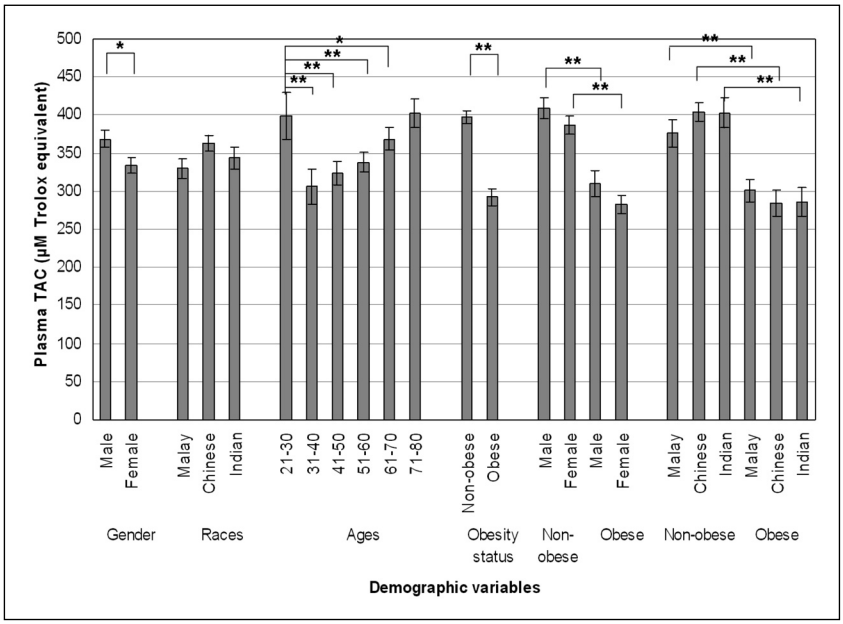

Figure 1. Means of plasma TAC by gender, ethnicity, age groups and obesity status. The non-obese and obese groups are also further stratified into gender and ethnicity. Means were compared using the Mann-Whitney *U* test or Kruskal-Wallis test. Error bars are standard errors of means (SEM) and significance is indicated by * for $p<0.05$ and ** for $p<0.01$.

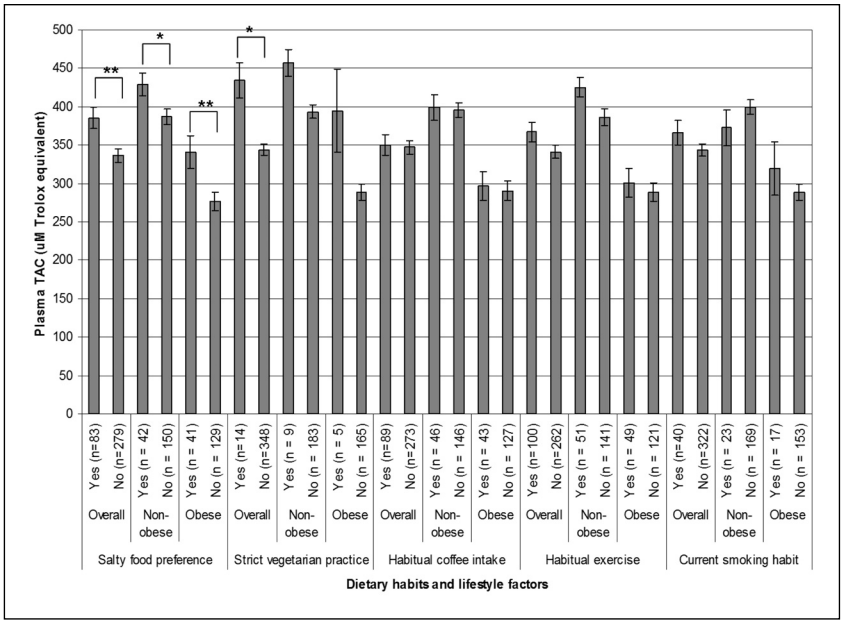

Figure 2. Means of plasma TAC by dietary habits and lifestyle factors. For each factor, subjects were stratified according to overall, non-obese and obese. Number of subjects for practitioners and non-practitioners is given in parenthesis. Means were compared using the Mann-Whitney *U* test. Error bars are standard errors of means (SEM) and significance is indicated by * for $p<0.05$ and ** for $p<0.01$.

Table 2. Correlations of blood pressure and anthropometric measurements with plasma TAC

Anthropometric measurements and blood pressure	*Plasma TAC*	
	r	*p*
SBP (mmHg)	-0.060*	0.256
DBP (mmHg)	-0.176**	0.001
Pulse rate (bpm)	0.020	0.698
WC (cm)	-0.204**	<0.001
WHR	0.105*	0.045
Weight (kg)	-0.279**	<0.001
BMI(kg / m^2)	-0.339**	<0.001
TBF (%)	-0.233**	<0.001
SF (%)	-0.260**	<0.001
VFL (%)	-0.280**	<0.001
RM(kcal)	-0.162**	0.002
SM (%)	0.143**	0.006

SBP: systolic blood pressure; DBP: diastolic blood pressure; WC: waist circumference; WHR: waist-to-hip ratio; BMI: body mass index; TBF: total body fat; SF: subcutaneous fat; VFL: visceral fat level; RM: resting metabolism; SM: skeletal muscle.
r and *p* values by Spearman's rho correlation; *correlation significant at the 0.05 level (2-tailed); **correlation significant at the 0.01 level (2-tailed).

RM correlated with decreased plasma TAC. These findings are consistent with studies in Greece (Chrysohoou *et al*., 2007) and Iran (Amirkhizi *et al*., 2010). The latter also reported significant negative correlation with WHR and a lower correlation coefficient for BMI (-0.27 *vs*. -0.34), suggesting that BMI rather than WHR is a better predictor of plasma TAC among the Malaysian subjects in this study.

Although plasma TAC was significantly higher in non-obese compared to obese subjects in each ethnic group studied, there was no significant difference between the groups when classifed into non-obese and obese groups separately. The difference in plasma TAC between non-obese and obese groups has not been previously reported among Malaysians. In a study among type 2 diabetic patients in Malaysia, markers of oxidative stress, including antioxidant enzymes and non-enzymatic antioxidant (FRAP), were more apparent in Indian patients compared with Malay and Chinese patients (Kuppusamy, Indran & Rokiah, 2005).

Our finding of higher plasma TAC levels in males than in females is in accord with the results among Japanese subjects (Kambayashi *et al*., 2009), but not among Greek subjects (Chrysohoou *et al*., 2007). Among the females, the non-obese had a significantly higher plasma TAC compared to obese subjects, which is consistent with an Iranian study (Amirkhizi *et al*., 2010). The means of plasma TAC in males and females in this study were both relatively lower than in the Japanese subjects (Kambayashi *et al*., 2009) but relatively higher than in Greek subjects (Chrysohoou *et al*., 2007). Plasma TAC level may be influenced by sex, age, lifestyle factors and dietary habits (Anderson *et al*., 2000; Kambayashi *et al*., 2009). An *in vivo* study on female rats has shown that plasma TAC is affected by estrogen levels in the body (Lam *et al*., 2006). By age, using the 21-30 age group as reference, all the older age groups (except 71-80 years) had significantly lower plasma TAC, consistent with a previous study which showed that elderly subjects (45-92 years) had a lower antioxidant enzyme level and

higher oxidative stress markers compared to young subjects (18-26 years) (Tanabe *et al*., 2006). As age is also a factor that can influence plasma TAC level, it could be a confounding factor when comparing plasma TAC levels across all the variables. The reduced plasma TAC in obese and female subjects could have been higher, as obese and female subjects were significantly younger than their counterparts. Nevertheless, the mean age of the obese and non-obese subjects still fell within the age group of 51-60 years, and therefore we predict that the effect of age would not have a drastic effect on plasma TAC, as observed between age groups 21-30 and 51-60.

Plasma TAC concentration was associated with strict vegetarian practice in this study, consistent with the study of Szeto, Kwok & Benzie (2004) which found that plasma antioxidant status was higher in long-term vegetarians compared to non-vegetarians. High intake of vegetables and fruits in vegetarians contributes to the exogenous enzymatic and non-enzymatic components of the antioxidant defence system, preventing free radical formation in the body (Lampe, 1999). Plasma TAC level was also found to be significantly higher among subjects with a preference for salty food. Excess intake of sodium enhances NADPH oxidase and oxidation activity of ROS, leading to increased oxidative stress (Kitiyakara *et al*., 2003). It is not known why subjects who had a salty food dietary habit had significantly higher plasma TAC, even when stratified according to obesity status. The term 'salty food preference' is defined for purposes of the study as 'intake of at least a serving or dish of high sodium foods such as salted fish or salted vegetables once a week' may not be sufficiently specific. Perhaps, other ingredients used in the preparation of salted fish or salted vegetables might have influenced or contributed to the increase in plasma TAC (Li & Hsieh, 2004).

According to Pellegrini *et al*. (2003), intake of coffee showed a statistically significant increase in the plasma TAC after one hour which gradually decreased over time. They suggest that coffee intake affected plasma TAC due to the presence of phenolic compounds in coffee, which may act as free radical scavengers. However, plasma TAC was not associated with coffee intake in this study, based on long term habitual coffee intake without taking into consideration its type and frequency.

The association between plasma TAC with lifestyle factors such as habitual exercise and current smoking status was not associated with plasma TAC. This is inconsistent with other studies that suggest that antioxidant capacity may rise and oxidative stress (ROS levels) may decrease in moderate physical activity training (Evelo *et al*., 1992). Moreover, other studies have shown that plasma TAC and concentrations of blood and body tissue antioxidants are significantly lower in smokers as compared to non-smokers (Duthie *et al*., 1993; Petruzzelli *et al*., 1997). Inconsistencies in the findings of this study compared to previous ones could be due to the fact that the number of subjects who did not practise dietary habits and lifestyle factors were two-fold more than those who practised, thereby limiting the efficacy of the statistical analysis of results.

The SBP had no significant correlation with plasma TAC whereas DBP had a significant negative correlation with plasma TAC. The results are comparable with the findings of Amirkhizi *et al*. (2010), who proposed that elevated blood pressure levels could lead to excessive production of oxidative stress, reduction of TAC and an increase in cardiovascular risk.

According to Furukawa *et al*. (2004), NADPH oxidase-induced free radicals such as ROS increased in cultured adipose tissue exposed to elevated levels of fatty acids, while antioxidant enzyme levels declined

in obese mice. Besides, oxidative stress from accumulated fat in obesity causes dysregulated production of adipocytokines, which participate in the pathogenesis of obesity-associated metabolic syndrome like the development of thrombosis and insulin resistance (Furukawa *et al.*, 2004). Furthermore, the study of Vincent & Taylor (2006) stated that oxidative stress in obesity increases the risk of diseases such as diabetes, hypertension and heart disease because of oxidant conditions.

Oxidative stress levels elevated in human obesity are changeable with various lifestyle and dietary modifications (Vincent & Taylor, 2006), supplementation of antioxidants (Vincent & Taylor, 2006) and surgical interventions (Melissas *et al.*, 2006). Therefore, this study proposes the potential use of plasma TAC as a biomarker for obesity, which could be routinely performed for the diagnosis and prognosis of obesity and metabolic syndrome. Henceforth, antioxidant supplementation could be prescribed in order to compensate for the reduced antioxidant defence system in obese patients. However, without further long term clinical studies, one could not necessarily translate increases in plasma TAC into a potential decreased risk of chronic diseases related to obesity such as diabetes and cardiovascular diseases.

CONCLUSION

Obesity was shown to be associated with decreased plasma TAC levels, indicating compromised systemic antioxidant defence and increased oxidative stress.

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