Salivary adiponectin concentration in healthy adult males in relation to anthropometric measures and fat distribution

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Objectives. Body fat content, fat distribution, and adiponectin level are important variables in the development of obesity related complications. Anthropometric indices may provide an economic and faster method in measuring the risk for complications through their predictive effect of fat distribution and adiponectin concentration. We aimed to determine, which of the waist circumference (WC), waist-to-hip ratio (WHR), and body mass index (BMI) may be the best predictor for the total fat percentage (WF), visceral fat level (VF), and subcutaneous whole-body fat (SCWBF). We aimed also to investigate the potential use of the anthropometric measures and fat distribution as predictors for the salivary adiponectin level in the healthy adult males.

Subjects. A total of 88 adult males aged between 18–25 years with a wide range of BMI were studied. Anthropometric indices were measured using standardized methods and salivary adiponectin level was assessed using an enzyme-linked immunosorbent assay.

Results. In path analysis of the Structural Equation Model (SEM) using IBM@SPSS AMOS, version22, BMI and WC, but not WHR, were strong predictors for WF and SCWBF (p<0.05). BMI but not WC was a strong predictor for VF (p<0.001). WF was strong predictor for SCWBF (p<0.001), but not for VF. BMI, WC, WHR, WF, VF, and SCWBF were poor predictors of the salivary adiponectin level.

Conclusion. BMI is the best predictor for the total body fat and fat distribution. However, WHR seems to be of a little value and the salivary adiponectin level independent of BMI and body fat in healthy adult Malay males.

Key words: adiponectin, body mass index, waist-to-hip ratio, visceral fat, subcutaneous fat, structural equation modeling

The obesity has become a major worldwide health problem that is increasing in prevalence in both developed and developing countries. The dramatic increase in the prevalence of obesity has been reported to be associated with the increase in the prevalence of chronic diseases. The excessive fat deposition in obese individuals has been established to be correlated with the development of metabolic and cardio- vascular complications (Chew et al. 2014). The health consequences associated with obesity differ depending on the place where the adipose tissue is stored; for instance, subcutaneously versus internally (visceral) (Pou et al. 2007; Ducluzeau et al. 2010; Wildman et al. 2011). Although in not all obese individuals of type 2 diabetes or cardiovascular diseases will eventually develop, abdominal adiposity, especially visceral fat,

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has been found to be a risk for the developing of the metabolic syndrome, insulin resistance, type 2 diabetes mellitus, and cardiovascular diseases (McLaughlin et al. 2011; Neeland et al. 2013).

In addition to the regional fat distribution, as being accountable for variations in the development of obesity-related complications, adiponectin (an adipokine released from the adipose tissue) has been reported to be also involved in the pathogenesis of obesity and insulin resistance (Gao et al. 2012). Results of some studies have indicated a positive correlation between the circulating adiponectin levels and the insulin sensitivity (Goropashnaya et al. 2009; Gao et al. 2012; Vega and Grundy 2013). Lower plasma adiponectin concentrations have been detected in obese patients and patients with insulin resistance when compared to the healthy non-obese individuals. Therefore, low adiponectin levels have been reported to be associated with an increased risk of the development of type 2 diabetes (Vega and Grundy 2013).

Accurate measurement of the compartmental fat distribution using imaging techniques, as magnetic resonance imaging (MRI) and computed tomography (CT) (Lee et al. 2015), are relatively expensive and complex. The adiponectin level assay which uses standardized method is also expensive, especially in the clinical risk assessments among large groups of population and large-scale studies. Simple clinical anthropometric measures, such as BMI, WC, and WHR, can be good alternative methods to assess the risk of developing obesity-related complications in the case that they are proven to be good predictors for fat and compartmental fat distribution; visceral versus subcutaneous, as well as adiponectin level.

There are conflicting findings regarding the possible influence of BMI and body fat on the level of adiponectin. Some studies have reported a significant association between the adiponectin level with BMI and the fat distribution (Staiger et al. 2003; Goropashnaya et al. 2009). However, a lack of association of adiponectin with BMI has been also reported by others (Kuo and Halpern 2011). These have become an important reason to carry out this study by using a statistical tool to elucidate this predicament.

The saliva has recently become an attractive diagnostic tool that can be used as an alternative tool for the measure of blood biomarkers because of its non-invasive sampling method and the good correlation between the protein levels of saliva and plasma (Desai and Mathews 2014). Assays are now available to analyze various parameters in saliva including the measurement of the salivary adiponectin. Many studies have reported a successful measurement of adiponec-tin in the saliva of healthy individuals and diabetic patients (Toda et al. 2007; Thanakun et al. 2013).

Various Asian populations have been reported to have high percentage of body fat even with low BMI (Wang et al. 1994) suggesting that even with low or normal BMI, there is still a risk of the cardiovascular diseases and metabolic syndrome development. To rule out other confounding race-related effects, this study hereby focuses only on the Malay population. To the best of our knowledge, no previous studies have analyzed the possible predictive effect of anthropometric measures, visceral fat, and subcutaneous fat on the salivary adiponectin level in the healthy adult Malay males.

The present study is aimed to determine the relative contribution of WC, WHR, and BMI in predicting the total body fat and its distribution (visceral and subcutaneous fat) and the potential use of these anthropometric measures (WC, WHR, and BMI) and fat distribution as salivary adiponectin level predictors in the healthy adult males via path analysis of a structural equation modeling that is constructed and statistically tested for fitness with the saturated perfect model using IBM@SPSSAMOS, version22.

Materials and Methods

Ethical clearance and study population. The study design and consent forms were approved by Research Management Committee of MAHSA University, Kuala Lumpur, Malaysia. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. A total of 88 healthy adult Malay males aged between 18–25 years old participated in the study. All the participants were fully informed about the objectives of the study before completing their written consent forms. Subjects with history of diabetes or cardiac diseases were excluded from the study.

Anthropometric measurements. The height of each subject was measured to the nearest 0.1 cm using the stadiometer. Body weight, BMI, whole fat %, visceral fat level, and whole body subcutaneous fat were measured by Omron Karada Scan HBF-362 Bio-electrical impedance. This bioimpedance method of measuring fats has been proven to correlate well with body fat analyzed by Dual-energy X-ray absorptiom-etry (DEXA) (Fakhrawi et al. 2009; Sluyter et al. 2010) and even by CT (Lee et al. 2015). Each subject was asked to stand barefooted and upright in the middle

of the sensor platform and hold the fat analyzer with his extended arms. Waist and hip circumferences of the subjects were measured to the nearest 0.1 cm using measuring tape on standing. Waist circumference was measured by placing the measuring tape placed around the waist at midpoint between the lower costal border (10th rib) and the iliac crest at the end of normal expiration. Hip circumference was measured at the maximum circumference over the buttocks. Then the WHR was calculated (Cameron et al. 2012).

Saliva collection. Non-stimulated saliva of an average of 5 ml was collected using the drooling technique from each subject. All subjects were advised not to eat or drink for at least one hour before saliva collection. They were given proper instructions to ensure collection of suitable and uncontaminated saliva samples and were advised to rinse their mouths with water thoroughly before saliva collection. Over duration of three weeks, saliva samples were collected into test tubes between 9:00 a.m. and 12:00 noon, and kept in ice then centrifuged for 15 min at 1000 rpm at 4 °C. The supernatant was then divided into aliquots of 1 ml in Eppendorf tubes and stored at –80 °C till analysis (Thanakun et al. 2013).

Assay for the salivary adiponectin level. The saliva samples were brought to room temperature and adiponectin was measured by enzyme-linked immunosorbent assay (ELISA). The commercial ELISA kit for human adiponectin (Abnova KA3019) was used. The assay was conducted according to the manufacturer's instructions. Saliva samples were analyzed without dilution. The standards and the samples were measured each in duplicates.

Statistical analysis. Data were analyzed using a structural equation modeling in IBM@SPSS AMOS version22 with path analysis (Hoyle 1995; Bardenheier et al. 2013; Lee et al. 2016). A model of 7 observed variables (BMI, WC, WHR, WF, VF, SCWBF, and adiponectin) was structured where BMI, WC and WHR represented independent variables for predicting WF, VF, SCWBF, whereas the total fat percentage was an independent variable for predicting VF and SCWBF. BMI, WC, WHR, WF, VF, SCWBF were independent variables for predicting salivary adiponectin level. This structural equation modeling is advantageous over the traditional regression analysis because of its ability to test the direct and indirect effects of multiple independent variables on dependent variables simultaneously, as well as the associations amongst the independent variables (Kline 2010). A direct effect is represented as an arrow emerging from an independent variable and pointing to an outcome (dependent variable) whereas an indirect effect is represented by the presence of a mediating variable in between the independent and dependent variables. The mediator has an arrow pointing to it from an independent variable and another arrow pointing on to yet another dependent variable. A two-headed arrow indicates the correlation between two variables. The hypothesized model was then assessed for its statistical fit to the data using standardized criteria including chi-square, degree of freedom, probability level, root mean residual (RMR), goodness of fit index (GFI), normed fit index (NFI), incremental fit index (IFI), Tucker-Lewis Index (TLI), comparative fit index (CFI) and the root mean square error of approximation (RMSEA) (Hoyle 1995; Schumacker and Lomax 2004). Modification indices were used to assess specific paths for best fit model. Lastly, model fit statistics, correlations between BMI, WC and WHR as independent variables, squared regression analysis and the standardized regression weights estimates (direct, indirect and total) of anthropometric measurements, total fat and fat distribution on the salivary adiponectin level were reported. All paths with $p<0.05$ are considered significant.

Results

Characteristics of the participants. Table 1 shows the means of the anthropometric characteristics, VF level, SCWBF, WF, and the salivary adiponectin level of the participants. In average, the subjects were young adults with wide range of BMI, mainly overweight and pre-obese with high WF according to the World Health Organization (WHO) classification (WHO 2004). However, their average VF level is found to be of 8.59 that is equivalent to 80.59 cm², which is lower than 100 cm²; the VF threshold for increased risk (Nicklas et al. 2003; Pickhardt et al. 2012) as well as the mean of their WC is considered normal for this age group (Lean et al. 1995).

Fitness of the final structural equation model. Table 2 shows the fit measures of the final structural equation model shown in Figure 1. The fit measures indicate that our model is not different from the saturated model or the perfect model that fit our data. This indicates that the model has correctly described the relations between the measured variables. However, researcher can still modify the model to fit different data of different population until a better fit model is obtained.

Results of path analysis of the model.

BMI, WC and WHR as predictors of WF, VF, and SCWBF. The analysis revealed a strong positive correlation between the three anthropometric measure-

Table 1
Anthropometric characteristics, visceral fat, subcutaneous whole-body fat and salivary adiponectin levels of the 88 subjects

Parameter	Mean±SD
Age (year)	20.64±2.01
Height (cm)	168.82±5.51
Weight (kg)	71.80±20.1
BMI (kg/m²)	24.92±6.55
WC (cm)	85.40±16.46
HC (cm)	100.24±12.48
WHR	0.85±0.07
WF (% from body weight)	20.10±8.11
VF (*)	8.59±6.71
SCWB (%)	14.39±6.15
Salivary adiponectin (ng/ml)	17.24±5.02

Data are presented as mean ± SD.
Abbreviations: BMI – body mass index; WC – waist circumference; HC – hip circumference; WHR – waist-to-hip ratio; WF – total fat percentage; VF – visceral fat level; SCWB – subcutaneous whole-body fat. *Visceral fat levels are relative and not absolute values. Visceral fat area (0 - approx. 300 cm²) are distributed over 30-unit levels; one-unit level = 10 cm².

Table 2
Model fit measurements

Model	Default model
Chi-square	0.853
Degree of freedom	2
Probability level	0.653
RMR	0.005
GFI	1.00
NFI	0.999
IFI	1.001
TLI	1.009
CFI	1.00
RMSEA	0.000

Abbreviations: RMR – Root Mean Residual, zero represents a perfect fit; GFI – Goodness of Fit Index, values that approach 1 indicate acceptable fit; NFI – Normed Fit Index, 1 is ideal; IFI – Incremental Fit Index, values that exceed .90 are regarded as acceptable; TLI – Tucker-Lewis Index, values over 0.90 or over 0.95 are considered acceptable; CFI – Comparative Fit Index exceeds 0.93; RMSEA – Root Mean Square Error of Approximation, a zero value indicates perfect model fit.

ments (BMI, WC and WHR; $p<0.05$), the highest correlation was seen between BMI and WC ($r=0.96$). WC and BMI but not WHR were strong predictors for WF ($p<0.05$), only the BMI was a strong predictor for

Table 3
Squared multiple correlations for the predictors of total body fat, visceral fat, subcutaneous whole-body fat and salivary adiponectin level

Parameter	R²	Lower	Upper	p-value
WF	0.831*	0.731	0.886	0.004
SCWBF	0.987*	0.970	1.000	0.001
VF	0.992*	0.987	0.995	0.003
Salivary adiponectin	0.043	–	0.066	0.467

WF – total body fat (%); SCWBF – subcutaneous whole-body fat; VF – visceral fat. *$p<0.05$

VF (when BMI goes up by 1 standard deviation, VF goes up by 0.93 standard deviations), BMI and WC were good predictors for SCWBF ($p<0.05$). For WF, as a predictor for SCWBF versus VF, it seemed to be a strong predictor for SCWBF but not VF ($p<0.001$ and 0.74, respectively) (when WF goes up by 1 standard deviation, SCWBF goes up by 0.763 standard deviations) (Figure 1). It is estimated that the predictors of WF could explain 83.1% of its variance, the predictors of SCWB could explain 98.7% of its variance and the predictors of VF could explain 99.2% of its variance as indicated by the squared multiple correlations estimates (R^2) (Table 3).

The direct, indirect and total predictive effects of the anthropometric and body fat mass measurements on the salivary adiponectin level. The analysis showed that the anthropometric measurements were poor predictors for the salivary adiponectin level directly, indirectly (mediated by their effects on WF, VF, and SCWBF) and total effects of both direct and indirect (Table 4). It is estimated that the predictors for the salivary adiponectin level could explain only 4.3% of its variance. In other words, the error variance of adiponectin is approximately 95.7% of the variance of adiponectin itself (Table 3).

Discussion

The correlation and regression analysis suggest that in healthy young adult Malay males, who are on average overweight-to-obese, WC and BMI, but not WHR, are good predictors for WF. WC and BMI were not significantly different in predicting the SCWBF. Many studies have investigated the association of the anthropometric measures with body fat percentage and its distribution (Goulding et al. 1998; Steinberger et al. 2005; Suchanek et al. 2012; Ejtahed et al. 2014). Suchanek et al. (2012) have reported

Table 4
Standardized regression estimates of direct, indirect and total effect of body mass index, waist circumference, waist-to-hip ratio, total body fat, subcutaneous whole-body fat, and visceral fat on salivary adiponectin level

	WHR	WC	BMI	WF	SCWB	VF
Standardized total effects						
WF	0.014	0.483	0.426*	–	–	–
SCWB	0.010	0.483*	0.461*	0.763*	–	–
VF	0.000	0.061	0.937*	0.017	–	–
Salivary adiponectin	–0.063	–0.170	0.108	0.062	–0.348	1.861
Standardized direct effects						
WF	0.014	0.483	0.426*	–	–	–
SCWB	–	0.114*	0.136*	0.763*	–	–
VF	–	0.052	0.930*	0.017	–	–
Salivary adiponectin	–0.064	–0.258	–1.602	0.296	–0.348	1.861
Standardized indirect effects						
WF	–	–	–	–	–	–
SCWB	0.010	0.368	0.325*	–	–	–
VF	–	0.008	0.007	–	–	–
Salivary adiponectin	0.001	0.088	1.710	–0.234	–	–

Abbreviations: BMI – body mass index; WC – waist circumference; WHR – waist-to-hip ratio; WF – total body fat (%); VF – visceral fat; SCWBF – subcutaneous whole-body fat. *p<0.05
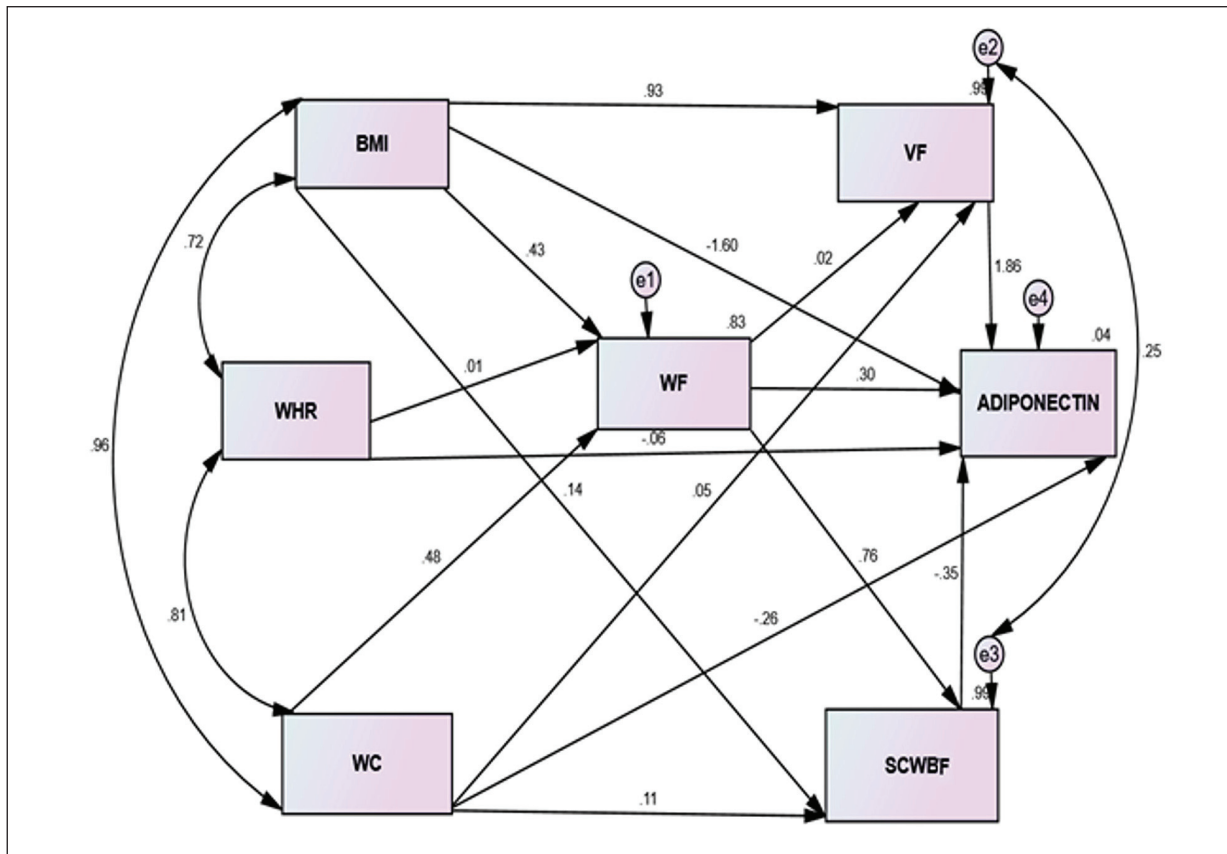
Figure 1. The final Structural Equation Model (SEM) with standardized estimates.

a poor predictive effect of WHR on total body fat in Caucasians and similar findings have been reported among Iranian adolescents by Ejtahed et al. (2014). The poor effect of WHR in predicting WF found is supported by studies on the French-Canadian males and females with an age range from 18–72 years (Rankinen et al. 1999). Similarly, a study being done on weight loss evaluation in obese males and females have reported that WHR is not appropriate for evaluating the changes in the visceral fat depot (van der Kooy et al. 1993).

The body mass index rather than WC predicted VF. This poor predictive effect of WC for VF is contradictory to the findings of Rankinen et al. (1999) about WC being the best predictor for VF. This can be explained by the difference in the race used as body fat distribution is affected by race.

The waist circumference reflects not only the intraabdominal but also the abdominal subcutaneous fat, concluding that in Malay adult males who are overweight, the fat is rather accumulated subcutaneously than viscerally. This is evidenced in our study by the strong positive predictive effect of the total fat percentage on the subcutaneous body but not the visceral fat. These findings strengthen the results of a similar study done on healthy adults (Chen et al. 2014). However, they were different from a study done on children and reported a significant association of WF with both the visceral and the subcutaneous fat (Saelens et al. 2007). This difference can be explained by the different age groups used.

The analysis of the structural equation model revealed that parameters such as BMI, WC, WHR, WF, VF, and SCWBF, each or in combination, are not suitable to use as predictors for the salivary level of adiponectin in our study population as evidenced by the insignificant direct, indirect, and total effects of each of these anthropometrics on the adiponectin (as shown in Figure 1 and Table 4) and the low R^2 of their total effect on adiponectin (shown in Table 3). This is consistent with the conclusion drawn from a meta-analysis study of Kuo and Halpern (2011). They have performed analysis of several obesity studies and plasma adiponectin levels in healthy adults and rejected a hypothesis that obesity may independently affect plasma adiponectin. The insignificant negative effect of WHR, SCWBF, and WC in predicting adiponectin concentration (shown in Table 3) is in agreement with the study being done to investigate the relationship of serum adiponectin concentrations with body fat distribution which re-

ported a negative correlation between WHR as well as WC and adiponectin (Staiger et al. 2003). Our findings indicate that BMI, WF, VF, and SCWBF are poor predictors for the considering of the salivary adiponectin concentrations in the healthy adult males. This supports the conclusions drawn from other studies on the tight physiological regulation of adiponectin concentration that is not essentially affected by just an expansion of the body fat. This has been evidenced in some obese individuals who remained healthy due to their higher capacity to hold storage fat because of genetic or environmental factors (Virtue and Vidal-Puig 2010). It has also been observed that adiponectin resistance had developed earlier than insulin resistance following a high fat diet in mice (Mullen et al. 2009) reflecting the regulation of adiponectin by feedback mechanism. Hypoadiponectinemia has been found to be associated with the obesity related pathological conditions such as metabolic syndrome (Liu and Liu 2009) that is considered as a condition of tissue inflammation (Gustafson et al. 2007). In addition to the presence of high plasma glucose, fat intake, and dyslipidemia with metabolic syndrome, are all known to inhibit adiponectin secretion in vitro (Oller do Nascimento et al. 2009; Sun et al. 2009; Lapointe et al. 2011).

Conclusions

Our study declassified the interrelations of anthropometric indices in tandem with the levels of the salivary adiponectin as detected in a group of subjects with a wide range of BMI. In the healthy young adult Malay males, BMI and WC, but not WHR, showed up as strong predictors for the changes in WF and SCWBF. BMI, but not WC, can be used as a predictor of VF. However, the salivary adiponectin level is independent on BMI and body fat.

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