Original Article

Tumour necrosis factor-α and risk of cardiovascular disease among overfat Indonesian adolescents

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Background: Overfatness (overweight and obesity) is associated with an increased risk of cardiovascular disease, including elevated blood pressure, dyslipidaemia, and insulin resistance. Chronic inflammation may play a role in mediating these associations. **Objective:** To investigate the association between plasma tumour necrosis factor-α and risk factors for cardiovascular disease among overweight and obese adolescents. **Methods and Study Design:** This study was an observational analysis with a cross-sectional design for high school students in Yogyakarta, Indonesia. One hundred and fifteen overweight and obese adolescents (mean age 16.8 years; 48.3% female) were involved in the study. Overfatness was specified by body mass index z-scores. Anthropometric measurements, blood pressure, lipid profiles, and fasting glucose were obtained. Fasting plasma insulin and plasma tumour necrosis factor-α were quantified using enzyme-linked immunosorbent assay. Insulin resistance was represented as the homeostatic model assessment value. Data were analysed using SPSS for Windows, version 23. **Results:** Plasma tumour necrosis factor-α was significantly associated with total cholesterol (p=0.046) and diastolic blood pressure (p=0.018) among the overweight and obese adolescents. Results from path analyses showed that there were indirect effects of z-score BMI on systolic and diastolic blood pressures, HDL and fasting plasma glucose mediated by plasma tumour necrosis factor-α concentrations. Meanwhile, there were indirect effects of waist circumference on systolic and diastolic blood pressure by age and height percentile and HDL. There was no significant association between plasma tumour necrosis factor-α and insulin resistance. **Conclusion:** The study showed that a proinflammatory marker, plasma tumour necrosis factor-α, is associated with blood pressure, HDL and fasting plasma glucose in overweight and obese adolescents. This indicates that inflammation in overweight and obesity may play a role in increasing the risk of cardiovascular disease.

Key Words: tumour necrosis factor-α, overfatness, adolescents, blood pressures, lipid profile, insulin resistance

INTRODUCTION

Obesity is a major health problem in the twenty-first century. Based on data from the Indonesian National Health Research Survey (RISKESDAS) 2013,[1] the prevalence of obesity in adolescents aged 16–18 years in Indonesia rose from 1.4% in 2007 to 7.3% in 2013. The Special Region of Yogyakarta has one of the highest prevalences of obesity among adolescents. In early 2014, an obesity research group at the Universitas Gadjah Mada found that the prevalence of obesity is higher, amounting to 10.68% in a screening of more than 4000 adolescent high school students in the city of Yogyakarta.[2] This indicates an approximately 7% increase in obesity prevalence since 2007.

Overfatness (overweight and obesity) is a known risk factor for degenerative diseases in adulthood such as diabetes, CVD, and cancer.[3] In a previous study (2007), we found that more than 50% of obese adolescents in Yogyakarta have insulin resistance and hypertension. Furthermore, we recently reported that obese adolescents have significantly higher blood pressure (BP), triglycerides, cholesterol, LDL, and insulin resistance (homeostatic model assessment, HOMA-IR), as well as low HDL.[2]

Our previous studies have confirmed that overfatness in adolescents could lead to early-onset CVD by affecting its risk factors. The percentage of body fat among the Asian population is higher compared with that among the Caucasian population at the same body mass index.[4] Therefore, Asians have higher risks for various diseases associated with increased body fat, and closer attention is required to prevent an increase in the incidence of various degenerative diseases and cancer later in life.

Overfatness leads to chronic inflammation that is characterized by an increase in proinflammatory cytokines such as tumour necrosis factor-α (TNF-α) and interleukin-

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6 (IL6) in the fatty tissues and blood plasma.[5] In addition to inflammation in fat tissues, mononuclear leukocytes in adults who are obese are also in the proinflammatory state, characterized by increased activation of nuclear factor ƙB and increased expression of proinflammatory genes.[6] High concentrations of inflammatory mediators are also associated with insulin resistance[7] and cardiovascular risk. Among healthy adults, concentrations of plasma proinflammatory cytokines are positively correlated with BP[8] even after body mass index is controlled for;[9] as such, the TNF-α concentration is a strong predictor of hypertension. In addition, TNF-α has been associated with decreases in lipoprotein lipase (LPL) activity and LPL mRNA expression.[10] A positive association of TNF-α with body mass index was previously reported in adolescents.[11] However, data on the association of TNF-α with BP and dyslipidaemia remain limited. Therefore, the objective of this study was to investigate the association between the plasma concentration of TNF-α and CVD risk factors in overweight and obese adolescents (Figure 1).

MATERIALS AND METHODS

Study participants

The study was cross-sectional and consisted of 115 overweight and obese high school students in Yogyakarta, Indonesia, who were aged between 16 and 18 years and apparently healthy according to a medical check-up. Gender was almost equally distributed (51.7% vs 48.3%, respectively). For people aged <19 years, obesity is defined as BMI ≥+2 standard deviations (SDs) (World Health Organization [WHO], 2005), and overweight is defined as BMI between +1 SD and +2 SD (WHO, 2007). BMIs were considered z-scores. The study participants were cluster-sampled from high schools in Yogyakarta, Indonesia. Informed consent was obtained from both the participants and their parents. Those with known metabolic diseases such as diabetes mellitus, renal impairment, or congenital heart diseases were excluded. The study obtained ethical clearance from the Medical and Health Research Ethics Committee, Faculty of Medicine, Universitas Gadjah Mada–Dr. Sardjito General Hospital, Yogyakarta, Indonesia (KE/FK/1383/EC/9th December 2016).

Methods

Anthropometric and BP measurements

These measurements were conducted as previously described.[2] Anthropometric measurements included body weight, height, and waist circumference. Systolic and diastolic BP were measured twice and the average for each was used.

Blood collection

Blood samples were withdrawn from participants who had previously been screened and found to be overfat.[2] Blood was drawn by a phlebotomist and kept in a cool icebox before being centrifuged to isolate plasma. On the same day (within an hour after drawing), plasma was transferred and kept in a deep freezer at −80°C prior to analysis.

Lipid profiles and glucose and insulin concentrations

Lipid profiles (triglycerides, cholesterol, LDL, and HDL) were quantified using DiaSys. Insulin concentrations were quantified using ELISA. Glucose concentrations were quantified using the glucose oxidase–phenylamine phenazone method. HOMA-IR was calculated by multiplying fasting plasma glucose and insulin and dividing it by a constant: HOMA-IR = [fasting insulin (μIU/mL) × fasting glucose (mg/dL)/405].[12] The participants were considered to have insulin resistance when their HOMA-IR index exceeded 3.2.[13]

Plasma TNF-α

Plasma TNF-α concentrations were quantified using ELISA (Abcam Simple Step, cat# ab181421, Abcam, MA, USA). Standard curves were plotted for every plate, as well as a control high and control low, which were analysed in duplicate. Male and female adolescent plasma was analysed in the same plate to minimize experimental bias.

Data analysis

Linear regression analysis was performed to investigate the association between TNF-α concentrations (predictor) and CVD risk factors (outcomes) such as elevated BP, abnormal lipid profiles, and insulin resistance. To further investigate the direct association of overfatness (z-score BMI and waist circumference) on cardiovascular disease (CVD) risk factors (elevated BP, dyslipidaemia, and insulin resistance), and indirect association of overfatness on CVD risk factors mediated by TNF-α, path analyses were performed. A diagram representing path analyses is shown in Figure 2. Two models of linear regression were performed to obtain standardized β values. Model 1 includes z-score BMI and waist circumference (independent variables) as predictors of TNF-α (a dependent variable). Model 2 includes z-score BMI, waist circumference and TNF-α (independent variables) in predicting CVD risk factors (measures of blood pressures, lipid profiles, fasting plasma glucose, fasting plasma insulin and HOMA-IR). Standardized β values from these analyses were then used to calculate the indirect effect of z-score BMI and waist circumference on CVD risk factors

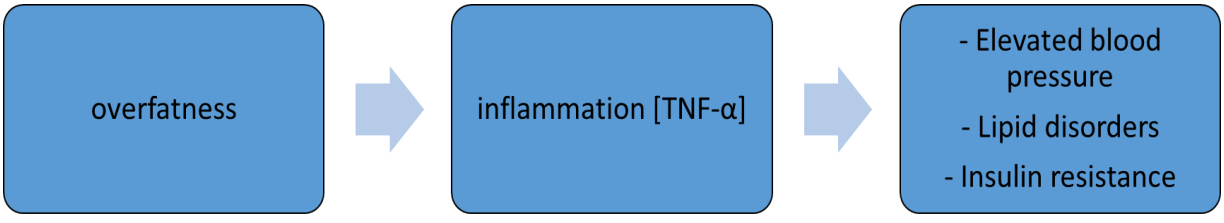

Figure 1. Putative association between overfatness, inflammation, and risk factors for cardiovascular disease.

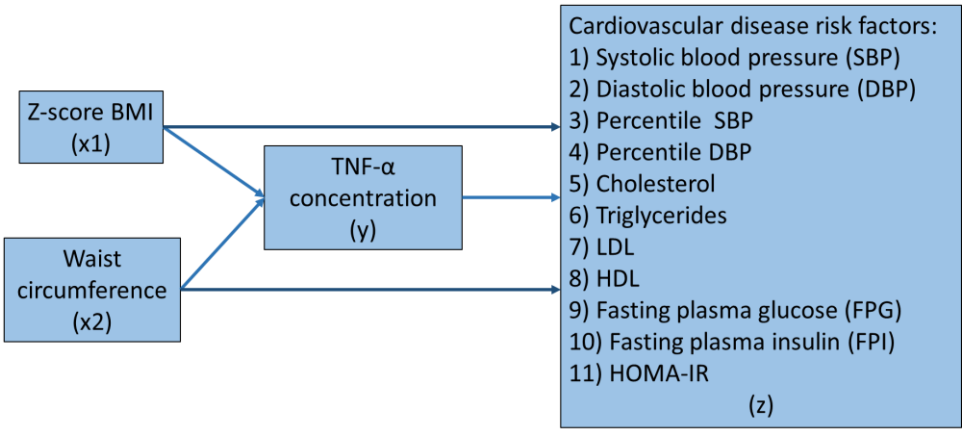

Figure 2. Diagram representation of path analyses performed

Table 1. Metabolic characteristics of participants

Characteristics	Average (standard deviation)	Min-Max
Age, years	16.8 (0.619)	18.0-16.8
BMI z-score	3.51 (1.20)	1.00-6.70
WC, cm	94.2 (11.1)	69.0-120
SBP, mmHg	120 (11.7)	95.0-150
Percentile SBP	68.6 (28.0)	4.00-100
DPB, mmHg	81.9 (9.44)	55.0-100
Percentile DBP	85.8 (16.1)	18.0-100
Cholesterol, mg/dL	178 (37.3)	113-344
Triglycerides, mg/dL	116 (64.1)	40.0-312
LDL, mg/dL	101 (38.2)	14.0-245
HDL, mg/dL	45.5 (9.78)	21.0-70.0
Fasting glucose, mg/dL	4.65 (0.559)	3.77-6.16
Fasting insulin, µmol/mL	2.14 (9.34)	6.61-55.5
HOMA-IR	4.36 (1.98)	1.32-10.6
TNF-α (pg/mL)	77.7 (26.8)	9.63-189

SBP: systolic blood pressure; DBP: diastolic blood pressure; HOMA-IR: homeostatic model assessment–insulin resistance; TNF-α: tumour necrosis factor-alpha.

Table 2. Simple linear regression analyses of TNF-α and risk factors for cardiovascular disease

Outcomes	Standardized β	95% CI	Adjusted R^2	*p*
SBP	0.140	-0.016-0.148	0.013	0.133
Percentile SBP	0.163	-0.017-0.382	0.020	0.080
DBP	0.219	0.015-0.148	0.041	0.018*
Percentile DBP	0.142	-0.024-0.382	0.012	0.128
Cholesterol	0.185	0.001-0.517	0.025	0.046*
Triglycerides	0.035	-0.367-0.542	-0.008	0.711
LDL	0.171	-0.011-0.508	0.022	0.067
HDL	0.090	-0.032-0.107	0.001	0.336
Fasting glucose	0.005	-0.072-0.074	-0.009	0.955
Fasting insulin	-0.001	-0.071-0.067	-0.009	0.991
HOMA-IR	-0.018	-0.016-0.013	-0.008	0.851

SBP: systolic blood pressure; DBP: diastolic blood pressure; HOMA-IR: homeostatic model assessment–insulin resistance.
*Significant at $p<0.05$.
TNF-α: tumour necrosis factor-alpha; predictor variable. In multilinear analysis, all predictors of plasma TNF-α were placed in the same model.

mediated by TNF-α. Data were analysed using SPSS for Windows, version 23 (SPSS Inc., Chicago, IL, USA).

RESULTS

As shown in Table 1, although BP was normal, cholesterol, triglycerides, and HDL were borderline according to cut-offs for children at 10–19 years of age (170–199 mg/dL, 90–129 mg/dL, and 40–45 mg/dL, respectively).[14] LDL concentrations were normal. Furthermore, insulin resistance was prominent, with levels well above normal (>3.2).[15]

As shown in Table 2, plasma TNF-α was significantly associated with cholesterol (p=0.046) and diastolic BP (p=0.018) among the overweight and obese adolescents. In addition, although not significant, there were positive associations between TNF-α and percentile systolic BP

Table 3. Summary path analysis (standardized β coefficients) on the effect of overfatness (z-score BMI) mediated by TNF-α on cardiovascular disease risk factors

	Direct effect	Indirect effect
Direct effect of z-score BMI on TNF-α	-0.048	
SBP		
TNF-α	0.129	(0.129)×(-0.133) = -0.0172‡
z-score BMI	-0.133	
DBP		
TNF-α	0.218	(0.218) × (-0.023) = -0.00501‡
z-score BMI	-0.023	
Percentile SBP		
TNF-α	0.177	(0.177) × (0.112) = 0.0199†
z-score BMI	0.112	
Percentile DBP		
TNF-α	0.154	(0.154) × (0.071) = 0.0109†
z-score BMI	0.071	
Cholesterol		
TNF-α	0.167	(0.167) × (0.106) =0.0177†
z-score BMI	0.106	
Triglycerides		
TNF-α	0.020	(0.020) × (0.179) = 0.00358†
z-score BMI	0.179	
LDL		
TNF-α	0.165	(0.165) × (0.175) = 0.0289†
z-score BMI	0.175	
HDL		
TNF-α	0.117	(0.117× (-0.025) = -0.00293‡
z-score BMI	-0.025	
Fasting glucose		
TNF-α	-0.005	(-0.005) × (-0.069) = 0.000345‡
z-score BMI	-0.069	
Fasting insulin		
TNF-α	-0.026	(-0.026) × (0.303) = -0.00788†
z-score BMI	0.303	
HOMA-IR		
TNF-α	-0.043	(-0.043) ×0.281) = -0.0121†
z-score BMI	0.281	

SBP: systolic blood pressure; DBP: diastolic blood pressure; TNF-α: tumor necrosis factor-alpha; HOMA-IR: homeostatic model assessment-insulin resistance.
†Denotes when direct effect of overfatness on CVD risk factors is bigger than the indirect effect of overfatness on CVD risk factors mediated by TNF-α.
‡Denotes when indirect effect of overfatness on CVD risk factors mediated by TNF-α is bigger than the direct effect of overfatness on CVD risk factors.

and LDL (p=0.080 and p=0.067, respectively). There was no significant association between plasma TNF-α and insulin resistance (p>0.05).

Path analysis in Table 3 and 4 showed the role of TNF-α in mediating the association between overfatness (z-score BMI and waist circumference) on CVD risk factors. As shown in Table 3, TNF-α mediated the effect of overfatness represented by z-score BMI, on cardiovascular disease (CVD) risk factors, in particular: systolic BP, diastolic BP, HDL and fasting plasma glucose. Meanwhile as shown in Table 4, TNF-α mediated the effect of overfatness represented by waist circumference (WC), on cardiovascular disease (CVD) risk factors, in particular: percentile systolic BP, percentile diastolic BP and HDL.

DISCUSSION

Obesity is a risk factor for CVD, as it has been associated with dyslipidaemia (elevated triglyceride, cholesterol, and LDL, and low HDL concentrations), elevated BP, and insulin resistance. Studies have also shown that obesity is often accompanied by increased proinflammatory markers, suggesting chronic inflammation. Kern et al confirmed that in obesity, the expression of the proinflammatory markers IL6 and TNF-α increased in adipocytes.[5] In our study, a significant association was observed between TNF-α and cholesterol but not between TNF-α and triglycerides, LDL, or HDL. Increased TNF-α has been associated with dyslipidaemia through inhibition of LPL, which stimulates lipolysis in adipose tissues.[16] This could then increase the concentrations of circulating nonesterified fatty acid, which could lead to insulin resistance.[17] Despite the strong association between TNF-α and cholesterol, we did not find that TNF-α mediates insulin resistance. We found no significant association between TNF-α and fasting plasma glucose, insulin, or HOMA-IR. Using a similar dataset, we also found no association between IL17 and insulin resistance.[18] This disagrees with other studies, such as Hotamisligil et al, where a strong positive correlation was observed between mRNA expression levels of TNF-α in adipose tissue and hyperinsulinaemia, which is a proxy for insulin resistance in adolesecents.[19] This may be because the circulating TNF-α is not sufficiently reflective of or sensitive to TNF-α expression at the tissue level.

Table 4. Summary path analysis (standardized β coefficients) on the effect of overfatness (waist circumference-WC) mediated by TNF-α on cardiovascular disease risk factors

	Direct effect	Indirect effect
Direct effect of WC on TNF-α	0.132	
SBP		
TNF-α	0.129	(0.129) × (0.251) = 0.0324†
WC	0.251	
DBP		
TNF-α	0.218	(0.218) × (0.061) = 0.0133†
WC	0.061	
Percentile SBP		
TNF-α	0.177	(0.177) × (-0.131) = -0.0232‡
WC	-0.131	
Percentile DBP		
TNF-α	0.154	(0.154) × (-0.181) = -0.0279‡
WC	-0.181	
Cholesterol		
TNF-α	0.167	(0.167) × (0.127) = 0.0212†
WC	0.127	
Triglycerides		
TNF-α	0.020	(0.020) × (0.087) = 0.00174†
WC	0.087	
LDL		
TNF-α	0.165	(0.165) × (0.041) = 0.00677†
WC	0.041	
HDL		
TNF-α	0.117	(0.117) × (-0.167) = -0.0195‡
WC	-0.167	
Fasting glucose		
TNF-α	-0.005	(-0.005) × (0.113) = -0.000565†
WC	0.113	
Fasting insulin		
TNF-α	-0.026	(-0.026) × (0.079) = -0.00205†
WC	0.079	
HOMA-IR		
TNF-α	-0.043	(-0.043) × (0.095) = -0.00409†
WC	0.095	

SBP: systolic blood pressure; DBP: diastolic blood pressure; TNF-α: tumor necrosis factor-alpha; HOMA-IR: homeostatic model assessment-insulin resistance.
†Denotes when direct effect of overfatness on CVD risk factors is bigger than the indirect effect of overfatness on CVD risk factors mediated by TNF-α.
‡Denotes when indirect effect of overfatness on CVD risk factors mediated by TNF-α is bigger than the direct effect of overfatness on CVD risk factors.

Another association that was investigated was the association between TNF-α and BP. We discovered a significant and positive association between TNF-α and diastolic BP and a positive association between TNF-α and percentile systolic BP, suggesting a potential role of TNF-α as a mediator that underlies the role of BP as a risk factor for CVD. One of the pathways that connects the concentration of TNF-α in plasma and BP is a signal by TNF-α that increases the expression of angiotensin receptor,[20] which in turn increases BP through aldosterone secretion. In children and adolescents, hypertension is often clinically assessed using percentile BP, unlike in adults. However, understanding the association between TNF-α concentrations and systolic and diastolic BP in millimetres of mercury is important for identifying how much BP rises with the increase in TNF-α concentration. Data from this study showed that every 1 pg/mL increase in TNF-α concentration is significantly associated with a 0.22 mmHg increase in diastolic BP. Furthermore, although not significant, a 1 pg/mL increase in TNF-α concentration is associated with a 0.14 mmHg increase in systolic BP.

The association of TNF-α and BP remained significant even after adjustment for measures of fatness (z-score BMI and waist circumference) and other risk factors of CVD (elevated BP, dyslipidaemia, and insulin resistance) (Table 3 and Table 4). Using path analysis, it was clear that TNF-α mediates the effect of overfatness (z-score BMI and waist circumference) on BP and HDL. There was a modest mediation by TNF-α in the association of z-score BMI and fasting plasma glucose. The similar association was not seen with measure of fatness as waist circumference. This suggests that the role of proinflammatory cytokines in contributing to risk factors for CVD is highly related to their role in BP regulation. There are many mechanisms by which obesity-related chronic inflammation, marked by elevated proinflammatory cytokines, can lead to elevated BP. Elevated inflammatory cytokines such as TNF-α can lead to increased nitric oxide and endothelin, which in turn could increase vasoconstriction and BP. In addition, an epigenetic mechanism has been suggested to play a role in mediating the proinflammatory cytokines in BP maintenance. Methylation in

the promoter region of TNF-α in leucocytes could influence TNF-α concentrations in plasma or serum, which in turn could affect BP.

The methylation status in the promoter region of various genes is modified in certain environmental conditions such as obesity and was reported by one study to be associated with age and gender,[21] although another study found no association with gender.[22] Monocyte maturation and high glucose levels have also been shown to increase histone acetylation TNF-α loci associated with increased expression of TNF-α.[23] In addition, the level of CpG methylation in the promoter region of TNF-α was positively associated with PUFA intake.[24] Chronic inflammation such as that from obesity can trigger its own epigenetic status with changes in the methylation status of CpG, which then triggers a persistent inflammatory response. Methylation in the promoter region is known to inhibit the attachment of transcription factors that inhibit gene expression. However, a decrease in gene expression is not always associated with high levels of DNA methylation in the promoter region of the gene.[25] The level of DNA methylation, especially in peripheral blood cells, can be evaluated as a risk and therapeutic marker, especially for conditions associated with increased leukocyte activity, such as obesity. Methylation of CpG in the promoter area of *TNF-α* gene obtained from peripheral blood and subcutaneous fat has been reported to be associated with good response to therapy in calorie restriction on the adult subjects[26-29] so these tests can also be developed as a marker of therapeutic efficacy. DNA samples from a peripheral blood draw should be used, because this form of sample collection is relatively easy compared with fat tissue biopsy. Therefore, future studies on epigenetic mechanisms such as DNA methylation and other mechanisms including miRNA regulation are necessary for examining the underlying mechanism by which proinflammatory markers contribute to risk factors for CVD.

Conclusions

This study shows that there is a significant association between plasma TNF-α and diastolic blood pressure and cholesterol. After further analysis by taking measures of overfatness represented by z-score BMI and waist circumference into account, plasma TNF-α was shown to mediate the association between overfatness and systolic and diastolic blood pressure, HDL and fasting plasma glucose in overfat Indonesian adolescents. No significant associations were found between TNF-α and the profiles of other lipids and insulin resistance among overweight and obese adolescents, suggesting that the role of chronic inflammation in mediating cardiovascular disease risk factors warrants further study.

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AUTHOR DISCLOSURES

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