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# Comparison of Flow-Mediated Dilatation and Endothelin-1 Levels Between Normal and Obese Adolescents

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SOUHRN

**Kontext:** Endotelová dysfunkce (ED) je jedním z hlavních mechanismů rozvoje různých kardiovaskulárních onemocnění. Endotelová dysfunkce je spojena s obezitou a hypertenzí, což se odráží v nízkých hodnotách průtokem stimulované dilatace tepny (flow-mediated dilatation, FMD) a vysokých hodnotách endotelinu-1 (ET-1) u dospělých osob a starších. Méně se ví o hodnotách uvedených dvou parametrů u adolescentů. Cílem této studie bylo srovnat hodnoty FMD a ET-1 obézních adolescentů s hypertenzí, obézních adolescentů bez hypertenze a u adolescentů s normální tělesnou hmotností.
**Metoda:** Celkem 72 indonéských adolescentů průměrného věku 195 (178–217) měsíců, převážně chlapců (61 %), bylo zařazeno do tří následujících skupin: obezita s hypertenzí (n = 21), obezita bez hypertenze (n = 19) a bez obezity a bez hypertenze (n = 32). Hodnota FMD se měřila na pažní tepně po vyvinutí zvýšeného systolického tlaku na předloktí po dobu pěti minut. Hodnoty ET-1 se měřily metodou ELISA v sendvičovém uspořádání.
**Výsledek:** Medián hodnoty ET-1 byl 42 (2,4–384) pg/ml. Mezi skupinami však nebyly nalezeny žádné významné rozdíly v hodnotách ET-1 (*p* 0,269). I když byly hodnoty FMD podle deskriptivní analýzy vyšší u zdravých adolescentů, nebyly zjištěny žádné statisticky významné rozdíly (*p* 0,159) v hodnotách FMD ve skupinách obezita s hypertenzí (9,1 ± 5 %), obezita bez hypertenze (8,3 ± 3,1 %) a bez obezity a bez hypertenze (10,9 ± 6,2 %). Srovnání hodnot ET-1 mezi skupinami s ED a bez ED (mezní hodnota FMD 7,1 %) nevykázalo žádné rozdíly (*p* 0,77). Navíc hodnoty ET-1 nekorelovaly statisticky významně s hodnotami FMD (*p* 0,66, r –0.053).
**Závěr:** U indonéských adolescentů s obezitou a hypertenzí, obezitou bez hypertenze a s normální tělesnou hmotností a bez hypertenze nebyly zjištěny žádné rozdíly v hodnotách ET-1 a FMD. Korelace mezi ET-1 a FMD není u adolescentů statisticky významná.



ABSTRACT

**Background:** Endothelial dysfunction (ED) is one of the major mechanisms in various cardiovascular diseases. ED is associated with obesity and hypertension, which is reflected by low flow-mediated dilatation (FMD) value and high endothelin-1 (ET-1) level in adults or older. However, their value in adolescents is less known. This study aims to compare the FMD and ET-1 between obesity with hypertension, obesity without hypertension, and normal adolescents.
**Method:** A total of 72 Indonesian adolescents with a mean age of 195 (178–217) months and dominated by males (61%) were classified into the three following groups obesity with hypertension (n = 21), obesity without hypertension (n = 19), and normal (n = 32) adolescents. FMD was measured using the brachial artery by previously applied supra systolic pressure in the forearm in 5 minutes. ET-1 was measured by Sandwich ELISA.
**Results:** The median ET-1 level was 42 (2.4–384) pg/mL. However, there are no significant differences in ET-1 levels between all groups (*p* 0.269). Although FMD values are descriptively higher in normal adolescents, however, there are no statistical differences (*p* 0.159) in FMD values between obese with hypertension (9.1 ± 5%), obese without hypertension (8.3 ± 3.1%), and normal (10.9 ± 6.2%) groups. Comparison of ET-1 levels between groups with ED and without ED (FMD cut-off 7.1%) showed no differences (*p* 0.77) among them. In addition, ET-1 was not significantly correlated with FMD (*p* 0.66, r –0.053).
**Conclusion:** There are no differences in ET-1 and FMD values between obesity with hypertension, obesity without hypertension, and normal Indonesian adolescents. The correlation between ET-1 and FMD is not significant in adolescents.

*Keywords:*
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Endothelin
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## Introduction

People experiencing obesity, along with comorbid conditions such as hypertension[1] and insulin resistance, have an increased risk of developing premature cardiovascular disease due to endothelial dysfunction.[2] Vascular endothelium is the primary regulator in maintaining the body's balance, controlling various processes, including vasoconstriction, vasodilation, thrombogenesis, fibrinolysis, smooth muscle cell proliferation, and cell adhesion. Calcification, stenosis, and atherosclerosis can disrupt these functions, making endothelial dysfunction an early sign of atherosclerosis development and a predictor of future cardiovascular disease.[3]

Flow-mediated dilatation (FMD) assessment provides an independent prognostic value in predicting future cardiovascular events, surpassing even traditional methods. FMD is attributed to its ability to provide an index of nitric oxide (NO) function derived from the endothelium.[4] In the exploration of the dynamics of the cardiovascular system, the interconnection between flow-mediated dilatation (FMD) and endothelin-1 (ET-1) becomes an intriguing focus of research. ET-1 is produced by endothelial cells and plays a key role in regulating vascular tone and overall vascular health.[5] Thus, FMD can be seen as one of the clinical indicators of endothelial dysfunction, while ET-1 can be seen as one of the vasoactive mediators of endothelial dysfunction.[6]

A deeper understanding of the relationship between FMD and ET-1 can provide richer insights into the mechanisms of vascular regulation and its clinical implications in the context of cardiovascular diseases. While some research evaluating ET-1 and FMD in adults or old subjects has been published in the literature, reports on adolescent populations are very scarce. Adolescents have different physiological patterns than adults or the elderly, protecting them from excessive endothelial dysfunction. In addition, identifying endothelial dysfunction in adolescents effectively prevents cardiovascular disease at the very early phase. This study investigated the relationship between FMD and ET-1 between obese adolescents with hypertension, obese adolescents without hypertension, and normal adolescents.

## Subject and methods

### *Subjects*

This study is an analytical cross-sectional study to compare FMD and ET-1 in obese adolescents with hypertension, comparing them with obese adolescents without hypertension, and normal adolescents. This study was conducted in five senior high schools in Surabaya and Sidoarjo districts. Exclusion criteria were dyslipidemia, hormonal therapy usage, smoking, alcohol consumption, and endocrine disorder.

### *Anthropometric measurements*

Weight (kg) was measured using a digital electronic scale (accuracy up to 0.1 kg), and standing height (cm) was measured by a wall-mounted Harpenden stadiometer. Specific BMI centiles for pediatrics were used, and obesity was defined as BMI value in the upper 95th percentile according to CDC growth charts.[7] Subjects sat in a relaxed condition for about 10 minutes before blood pressure measurement.[8] Blood pressure was measured using a standard sphygmomanometer fitting the subject's arm size with at least two measurements to calculate the average value (**Table 1**).[8]

### *Endhotelial-1 and metabolic parameters*

Metabolic parameters evaluated in this study included random blood glucose, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride, and total cholesterol. Metabolic parameters were measured using Auto Chemistry Analyzer TMS 24i Premium. Total cholesterol-HDL ratio was calculated by dividing total cholesterol with HDL concentration. ET-1 con-

**Table 1 – Baseline characteristics**

| Variables | Total (n = 72) | Obesity + HT (n = 21) | Obesity + non-HT (n = 19) | Normal (n = 32) |
|---|---|---|---|---|
| Age (months) | 195 (178–217)* | 197 (178–217) | 197 (185–217) | 195 (179–215) |
| Male (%) | 44 (61%) | 17 (53%) | 14 (74%) | 13 (62%) |
| Systolic blood pressure (mmHg) | 131 ± 16 | 137 ± 12 | 120 ± 12.6 | 114 ± 8 |
| Diastolic blood pressure (mmHg) | 82 ± 11.5 | 87 ± 9.8 | 76 ± 9 | 69 ± 8 |
| Random blood glucose (mg/dL) | 85 ± 7.1 | 86 ± 5.8 | 83 ± 11.3 | 81 ± 4 |
| Total cholesterol (mg/dL) | 175 ± 38 | 178 ± 36.9 | 153 ± 37 | 183 ± 39 |
| HDL (mg/dL) | 45.5 ± 10 | 41.9 ± 7 | 48 ± 8.9 | 57 ± 12 |
| Triglycerides (mg/dL) | 96 (38–247) | 108 (38–247) | 75 (39–133) | 67 (45–134) |
| Total cholesterol – HDL ratio | 4 ± 1.1 | 4.3 ± 0.97 | 3.2 ± 0.9 | 3.3 ± 0.9 |
| LDL (mg/dL) | 112 (54–201) | 123 (66–201) | 98 (54–162) | 105 (87–160) |
| FMD (%) | 9.1 ± 5 | 7.9 ± 3.3 | 10.9 ± 7.6 | 12.1 ± 5.9 |
| ET-1 (pg/mL) | 42 (2.4–384) | 41.2 (2.4–384) | 44 (26–62) | 43 (26–56) |

* Parameters with non-normal distributions
ET-1 – endothelin-1; FMD – flow-mediated dilatation; HDL – high-density lipoprotein; LDL – low-density lipoprotein.

**Table 2 – ANOVA and post-hoc LSD test**

| Variables | Uji-ANOVA | Obesity + HT | Obesity + non-HT |
|---|---|---|---|
| Age (months) | 0.854* | N/S | N/S |
| Systolic blood pressure (mmHg) | < 0.001 | Normal = < 0.001<br>Obesity + non-HT = < 0.001 | Normal = 0.114 |
| Diastolic blood pressure (mmHg) | < 0.001 | Normal = < 0.001<br>Obesity + non-HT = 0.002 | Normal = 0.69 |
| Random blood glucose (mg/dL) | < 0.048 | Normal = 0.019<br>Obesity + non-HT = 0.81 | Normal = 0.06 |
| Total cholesterol (mg/dL) | 0.286 | N/S | N/S |
| HDL (mg/dL) | < 0.001 | Normal = < 0.001<br>Obesity + non-HT = 0.4 | Normal = < 0.001 |
| Triglycerides (mg/dL) | 0.016* | Normal = 0.13<br>Obesity + non-HT = 0.435 | Normal = 0.75 |
| Total cholesterol – HDL ratio | < 0.001 | Normal = < 0.001<br>Obesity + non-HT = 0.794 | Normal = 0.001 |
| LDL (mg/dL) | 0.02* | Normal = 0.16<br>Obesity + non-HT = 0.59 | Normal = 0.57 |
| FMD (%) | 0.149 | N/S | N/S |
| ET-1 (pg/mL) | 0.269* | N/S | N/S |

* Non-parametric test (Kruskal–Wallis test) was used for data with non-normal distribution.
ET-1 – endothelin-1; FMD – flow-mediated dilatation; HDL – high-density lipoprotein; LDL – low-density lipoprotein; N/S – not significant for variables with ANOVA or Kruskal–Wallis *p*-value > 0.05.

**Table 3 – Spearman analysis**

| Variables | Correlation coefficient (r) | *p*-value |
|---|---|---|
| FMD – ET-1 | –0.053 | 0.66 |

centration was measured by the ELISA Rider Bio-rad I-mark with the Sandwich ELISA method. All laboratory parameters were examined on the same day as the blood sampling.

### *Flow-mediated dilation (FMD) measurement*

Flow-mediated dilation was defined as the percentage change of brachial artery diameters from baseline in response to increased flow and calculated accordingly.[5] Baseline measurement of the diameter of the brachial artery was performed in the lower third of the humerus under relaxed conditions. The pneumatic cuff was inflated on the subject to supra systolic pressure for five minutes. The pneumatic cuff was placed in the forearm distal to the ultrasound examination. After five minutes, deflation of the cuff was performed, and the diameter of the brachial artery was measured.[5] Measurement was performed by an experienced cardiologist using Echocardiography GE Healthcare-Ultrasound Vivid IQ V204.

### *Statistical analysis*

Analysis was performed using SPSS Ver. 23. Kolmogorov–Smirnov test was used to evaluate the distribution of variables with continuous data. Continuous data with normal distribution were presented as mean ± SD, while data without normal distribution were presented as median (quartile 1–quartile 3). Categorical data were presented as proportions and analyzed by chi-square analysis.

ANOVA test was conducted on variables with normally distributed data, followed by an LSD post-hoc test if the ANOVA results were significant (**Table 2**). When the distribution was abnormal, the Kruzkall–Wallis non-parametric test was used as an alternative to ANOVA. In addition, we also compared groups with and without endothelial dysfunction according to FMD values. Endothelial dysfunction was defined as FMD ≥ 7.1%, according to a previous study conducted by Maruhashi et al.[9] Correlation tests were performed using the Pearson test on data with normal distribution and the Spearman test without normal distribution. The $p < 0.05$ was considered significant (**Table 3**).

## Results

A total of 72 adolescents were included in this study, which consisted of obese with hypertension (21 subjects), obese without hypertension (19 subjects), and normal (32 subjects). The mean subject age was 195 (178–217) months, and the majority was male (61%). There were no differences in age and gender distribution among the three groups. Systolic and diastolic BP was significantly higher in obese patients with hypertension compared to two other groups. However, there were no differences in systolic and diastolic pressure between obese patients without hypertension and the normal group (systolic BP 120 ± 12.6 vs 114 ± 8 mmHg, *p* 0.11; diastolic BP 76 ± 9 69 ± 8 mmHg, *p* 0.69, respectively for mentioned groups).

**Table 4 – Comparison between two groups based on endothelial dysfunction status**

| Variables | FMD < 7.1% | FMD ≥ 7.1% | *p*-value |
|---|---|---|---|
| Age (months) | 195 (184–217) | 195 (178–217) | 0.792* |
| Male (%) | 18 (69%) | 26 (67%) | 0.62 |
| Systolic blood pressure (mmHg) | 132 ± 17.7 | 123 ± 15.3 | 0.662 |
| Diastolic blood pressure (mmHg) | 83 ± 11.8 | 81 ± 11.6 | 0.529 |
| Random blood glucose (mg/dL) | 85 ± 7.3 | 85 ± 7.1 | 0.799 |
| Total cholesterol (mg/dL) | 177 ± 46.6 | 174 ± 33.4 | 0.746 |
| HDL (mg/dL) | 42.2 ± 7.8 | 47.1 ± 10.8 | 0.037 |
| Triglycerides (mg/dL) | 108 (38–247) | 87 (38–247) | 0.3* |
| Total cholesterol – HDL ratio | 4.3 ± 1.1 | 3.9 ± 1.1 | 0.107 |
| LDL (mg/dL) | 116.5 (54–147) | 110 (54–188) | 0.729* |
| ET-1 (pg/mL) | 43.2 (30.1–384.5) | 42.3 (2.4–103) | 0.77* |

* Non-parametric test (Mann–Whitney) was used for data with non-normal distribution.
ET-1 – endothelin-1; HDL – high-density lipoprotein; LDL – low-density lipoprotein.

### *Metabolic parameters*

Glucose plasma level was higher in obesity with hypertension compared to the normal group ($p < 0.001$), but the value was not statistically significant when compared to obese without hypertension ($p$ 0.81). Although there were no differences in total cholesterol concentration among the three groups (ANOVA $p$-value 0.286), HDL concentration was lower in obesity with hypertension ($p < 0.001$) and without hypertension ($p < 0.001$) compared to the normal group. However, there were no differences in HDL concentration between obese with hypertension and without hypertension (41.9 ± 7 mg/dL vs 48 ± 8.9 mg/dL, $p$ 0.04, respectively). A similar result was observed in the total cholesterol-HDL ratio. In addition, there were no differences in triglyceride and LDL concentration between the three groups.

### *Endothelial dysfunction parameters*

The median ET-1 concentration of the subject was 42 (2.4–384) pg/mL. As comparison, the ET-1 concentration in each group was: obesity with hypertension 41.2 (2.4–384) pg/mL, obesity without hypertension 44 (26–62) pg/mL, and normal 43 (26–56) pg/mL without any significant differences ($p$ 0.269) between them (**Table 4**). There were no differences in FMD ($p$ 0.159) between obesity with hypertension (91 ± 5%), obesity without hypertension (8.3 ± 3.1%) and normal (10.9 ± 6.2%) group. However, we also noted that the value of FMD was descriptively higher (but not statistically) in normal groups than in obesity groups irrespective of the hypertension status. Additional analysis that classifiied the subjects with and without endothelial dysfunction (cut off 7.1%) also showed there were no differences in ET-1 concentration. In addition, there was no correlation between ET-1 concentration and FMD value ($p$ 0.66, r –0.053).

## Discussion

Endothelial dysfunction is a major mechanism underlying various cardiovascular diseases.[5,10] Endothelial dysfunction leads to altered secretion of various vasoactive molecules, including ET-1, ICAM-1, ACE, and IL-6, which are vasoconstrictive and pro-inflammatory.[5] Obesity is associated with increased concentrations of pro-inflammatory and pro-oxidizing molecules that increase the severity of endothelial dysfunction.[11]

Our study showed no significant difference in ET-1 concentration between obese and normal adolescents (regardless of hypertensive status). In comparison, a study conducted by Weil et al.[12] showed higher ET-1 concentrations in overweight and obese groups than in normal groups.[12] However, the participants of Weil's et al. study[12] were older adults with an average age of 55 years.[12] Meanwhile the subjects in our study were adolescents aged 16.5 years. On the other hand, research by Nacci et al.[5] showed higher ET-1 concentrations in obese and overweight children compared to children without obesity.[5] Our study is different from Nacci's et al. study[13] because the subjects in their study were mostly children younger than ten years.

Endothelial dysfunction is associated with increases in ET-1 secretion, where the majority of ET-1 in the circulation is produced by the dysfunctioning vascular endothelium. Endothelial dysfunction can be caused by stress in the form of mechanical stress (e.g., hypertension), chemical stress (hyperglycemia or dyslipidemia), or inducted by pro-inflammatory and pro-oxidation cytokines.[6] The higher percentage of adipose tissue is associated with higher secretion of pro-inflammatory and pro-oxidizing cytokines that cause endothelial dysfunction.[11] The similar concentration of ET-1 in the obese and normal groups in our study indicates a milder degree of endothelial dysfunction in the obese adolescent population compared to obese children or older adults. This could be due to the protective effect of testosterone and estrogen against endothelial dysfunction.[14–16] Testosterone and estrogen have their first peak concentrations at 15 years of age and continue to decline with age.[14,17]

Children younger than ten years, as in the study of Nacci et al.,[13] have low levels of testosterone or estrogen

because they have not yet reached puberty. Likewise, the old adult population in Weil's et al. study[12] has testosterone and estrogen levels that have continued to decline compared to early puberty. In addition, higher steroid hormone receptor sensitivity in early puberty may contribute to the protective effect of endothelial dysfunction in adolescents with obesity.[11] Apart from the age aspect, our study with Nacci's et al.[13] and Weil's et al.[12] differed in race, where their study subjects were Caucasian while our study subjects were Malayan-Mongoloid. On the other hand, metabolic parameters such as glucose and total cholesterol/HDL-c were higher in the obese group compared to normal, and HDL-c was lower in the obese group compared to normal, in accordance with the findings in previous studies.[11–13,18]

FMD is a clinical parameter used to evaluate endothelial dysfunction. Blood vessels with healthy endothelial function will return to their original diameter faster than those with endothelial dysfunction.[5] Theoretically, the laboratory parameter (ET-1) correlates with the clinical parameter (FMD). In the older adult population, the obese group has a significant association between ET-1 and FMD.[19] Our study showed no correlation between ET-1 concentration and FMD. Further analysis by dividing the two groups into clinically endothelial dysfunction and not clinically endothelial dysfunction based on an FMD cut-off of 7.1%[9] showed no significant difference between the two groups, both in obese and normal subjects. FMD is the ability of vessels to re-vascularize after stress to the blood vessels. High basal ET-1 concentrations in endothelial dysfunction are closely related to low FMD values.[19] The absence of differences in ET-1 concentrations between the obese and normal groups in our study illustrates the same basal concentration of ET-1, thus causing the same FMD value in both groups.

## Conclusion

There are no differences in ET-1 concentration and FMD between obese adolescents with hypertension, obese adolescents without hypertension, and normal adolescents. ET-1 concentration was not correlated with FMD values. In addition, ET-1 levels are also not different between adolescents with and without endothelial dysfunction.

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**Conflict of interest**
The authors declare that they have no competing interests.

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**Ethical statement**
This research has been approved by the Ethical Committee of Dr. Soetomo General Hospital with number 271/EC/KEPK/FKUA/2023.

**Informed consent**
Not applicable.

**Availability of data and material**
All data are available in the manuscript

**Authors' contributions**
Meity Ardiana: conceptualization, methodology, investigation, writing – original draft, resources review & editing, supervision: writing and approving final manuscript; Nur Aisiyah Widjaja: conceptualization, methodology, formal analysis, writing – original draft, data curation review & editing, supervision; writing and approving final manuscript; Achmad Faisal Dwi Raharja: acquisition of data, analysis, writing – original draft, project administration, review & editing, writing and approving final manuscript; Achmad Tri Ludfy Avianto: acquisition of data, interpretation for the data, writing – original draft, visualization review & editing, supervision.

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