

Comparison of inflammation and oxidative stress levels by the severity of obesity in prepubertal children

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Abstract

Background Children with severe obesity have a much more adverse cardiometabolic risk factor profiles at a younger age. Inflammation and oxidative stress associated with childhood obesity may be important in the development of insulin resistance, atherosclerosis, and other comorbid conditions.

Objective To compare levels of high-sensitivity C-reactive protein (hsCRP) and malondialdehyde (MDA) by the severity of obesity in prepubertal children aged 6 to 10 years.

Methods We conducted a cross-sectional study at the Pediatric Nutrition and Metabolic Syndrome Clinic, Sanglah Hospital, Bali, from August to December 2015. Subjects were categorized into three body mass index (BMI) groups, according to the 2000 Centers for Disease Control and Prevention growth chart: overweight (85th-94.9th percentile), obese (95th-98.9th percentile), or severely obese (\geq 99th percentile). Plasma MDA and serum hsCRP were analyzed in blood specimens obtained at enrollment. Data were analyzed by Kruskal-Wallis test, followed by Mann-Whitney U test for post-hoc comparison between groups.

Results Subjects were 20 overweight children, 29 obese children, and 28 severely obese children. Levels of MDA were significantly higher in the severely obese [median 0.25 (IQR 0.1) $\mu\text{mol/L}$] than in obese subjects [median 0.19 (IQR 0.1) $\mu\text{mol/L}$; $P=0.001$], and than in overweight subjects [median 0.16 (IQR 0.1) $\mu\text{mol/L}$; $P<0.0001$]. Also, the severely obese children had significantly higher hsCRP levels compared to obese [median 3.2 (IQR 2.0) mg/L vs. 1.3 (IQR 1.6) mg/L , respectively; $P<0.0001$] and compared to overweight children [median 0.7 (IQR 0.6) mg/L ; $P<0.0001$].

Conclusion Prepubertal children at the \geq 99th percentile for BMI (severely obese) are more likely to have significantly higher hsCRP and MDA compared to those in the obese and overweight groups. [Paediatr Indones. 2017;57:279-84 ; doi: <http://dx.doi.org/10.14238/pi57.6.2017.279-84>].

Keywords: obese; children; MDA; hsCRP

Childhood obesity is increasing at an alarming rate throughout the world. The prevalence of obesity in children rose worldwide by 47.1% between 1980 and 2013.¹ If current trends continue, the number of overweight or obese children globally will increase to 60 million by 2020.² Although once considered a problem only of developed countries, the prevalence of obesity has also risen during the past 30 years in low and middle-income countries. At the same time, low and middle-income countries are still dealing with the prevalent public health issue of undernutrition, a situation often described as the “double burden of malnutrition.” According to a recent report, 12% of children in Indonesia suffer from wasting, while a further 12% are

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overweight. The economic cost of noncommunicable diseases, many of which is diet-related, was estimated to be \$248 billion USD per year.³

Other data further suggested that compared to overweight and obese children and adolescents, youth with severe obesity have a much more adverse cardiometabolic risk factor profiles and are more likely to develop diabetes and cardiovascular diseases at a younger age.^{4,5} Inflammation and oxidative stress associated with childhood obesity appears to be central to the development of insulin resistance and atherosclerosis, and may be important in the pathogenesis of other comorbid conditions.⁶ A previous study documented that obese children with upper quartiles of C-reactive protein (CRP) and oxidative stress markers were more likely to have metabolic syndrome and abnormal lipid profiles, suggesting that inflammation and oxidative stress are interrelated, and might be associated with harmful health effects.⁷

To the best of our knowledge, no previous study has evaluated the levels of inflammatory and oxidative stress markers among overweight, obese, and severely obese prepubescent children. Therefore, the aim of this study was to compare high-sensitivity C-reactive protein (hsCRP) and malondialdehyde (MDA) levels by the severity of obesity in prepubertal children aged 6 to 10 years. We hypothesized that at a very young age, severely obese children have higher hsCRP and MDA levels compared to obese and overweight children.

Methods

We performed a cross-sectional study at the Pediatric Nutrition and Metabolic Syndrome Clinic, Sanglah Hospital, Bali, from August to December 2015. The eligibility criteria for participation in the study were children aged 6-10 years, with BMI at or above the 85th percentile for age and sex on the BMI growth chart from the Centers for Disease Control and Prevention (CDC),⁸ and at prepubertal Tanner stage 1. Children with physical disability, history of acute infection, chronic diseases, chronic medication use, special diet, or whose parents refused to sign the informed consent were excluded from the study. We categorized subjects into three BMI groups of increasing severity: overweight (85th-94.9th percentile), obese (95th-98.9th

percentile), or severely obese ($\geq 99^{\text{th}}$ percentile). The required sample size was determined by a formula of mean difference of two independent groups, with α of 0.05 and power of 80%. Therefore, a minimum of 20 patients per study group was required (total of 60 patients). Subjects were selected by consecutive, nonrandom sampling.

All anthropometric measurements and pubertal developmental staging (Tanner) were made by the same trained general physician and under the supervision of the same pediatrician, using standard protocols. Subjects' weights and heights were measured using a dual-purpose medical weight scale with height stadiometer. The stadiometer was checked for accuracy and the weighing scale was calibrated before the examinations. Height was measured without footwear and recorded to the nearest 0.1 cm from the highest point on the top of the subject's head. Body weight was measured and recorded to the nearest 0.1 kg, again without footwear and wearing lightweight clothing. We calculated the BMI percentile ranks using the CDC Child and Teen BMI Calculator.⁹ Waist circumference (WC) was measured and recorded to the nearest 0.1 cm with a non-elastic tape at a point midway between the lower border of the rib cage and the iliac crest, at the end of normal expiration.

Patients were subjected to blood sampling for evaluation of MDA and hsCRP. The concentration of plasma MDA was measured using the *Bioxytech*® MDA-586 kit (*Oxis International*TM). Serum hsCRP was measured by immunoturbidimetric method (analytic range 0.15-20 mg/L).¹⁰ This study protocol was approved by the Research Ethics Committee of the Udayana University Medical School. Subjects' parents provided written informed consent.

Kruskal-Wallis test, followed by a Mann-Whitney U test for post-hoc comparison, was used to analyze the differences between groups. Data are presented as the mean, standard deviation (SD), median, and interquartile range (IQR). Results with $P < 0.05$ were considered to be statistically significant for all data analyses. Statistical analyses were performed with *SPSS*® version 22.0 software for *Windows*®.

Results

A total of 113 children were screened for inclusion

into this study. Thirty-six children were excluded (34 due to parental refusal and 2 due to acute infection). Therefore, 77 subjects were included in the final analysis (**Figure 1**).

Twenty overweight children (11 boys, 9 girls) with median age 9.5 (IQR 2) years and mean BMI of 21.1 (SD 1.8) kg/m² were compared to 29 obese children (14 boys, 15 girls) with median age 9 (IQR 2) years and mean BMI of 24.3 (SD 2.9) kg/m² as well as to 28 severely obese children (15 boys, 13 girls) with median age 9 (IQR 2) years and mean BMI of 28.2 (SD 2.4) kg/m² (**Table 1**).

Kruskal-Wallis test, followed by post-hoc Mann-Whitney U test, was used to evaluate differences in

levels of MDA and hsCRP across the BMI groups. Kruskal-Wallis test revealed that significant differences existed in total MDA ($P < 0.0001$) and hsCRP levels ($P < 0.0001$) among the three BMI groups. **Figure 2** presents a box plot of MDA values for the three groups of children. Levels of MDA were significantly higher in severely obese [median 0.25 (IQR 0.1) $\mu\text{mol/L}$] than in obese subjects [median 0.19 (IQR 0.1) $\mu\text{mol/L}$; $P = 0.001$], or in overweight subjects [median 0.16 (IQR 0.1) $\mu\text{mol/L}$; $P < 0.0001$].

The serum concentration of hsCRP was also increased with the severity of obesity. The severely obese children had significantly higher hsCRP levels as compared to obese [median 3.2 (IQR 2.0) mg/L

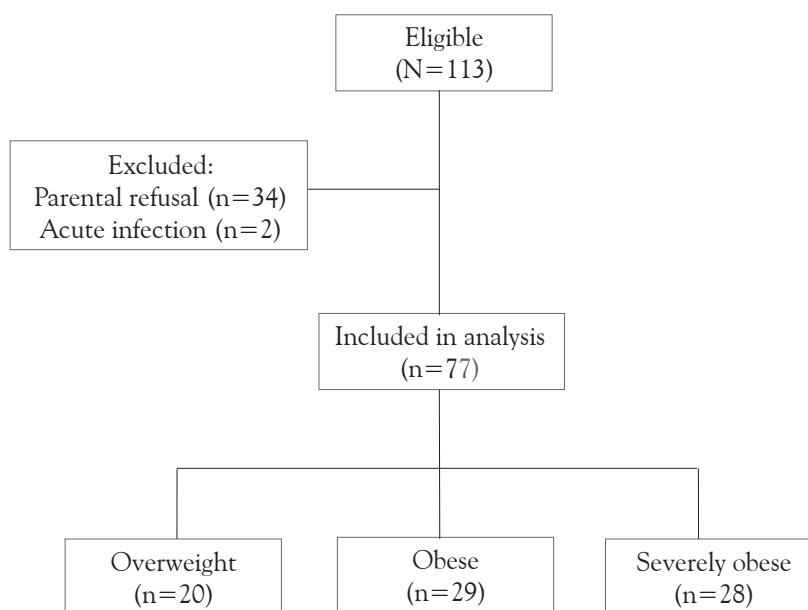


Figure 1. Study enrollment distribution

Table 1. Subjects' characteristics according to BMI category

Characteristics	Overweight (n=20)	Obese (n=29)	Severely obese (n=28)
Median age (IQR), months	9.5 (2)	9 (2)	9 (2)
Gender			
Male, n (%)	11 (55)	14 (48.3)	15 (53.6)
Female, n (%)	9 (45)	15 (51.7)	13 (46.4)
Mean BMI (SD), kg/m ²	21.1 (1.8)	24.3 (2.9)	28.2 (2.4)
Median weight (IQR), kg	38 (11.8)	41.0 (16)	51.5 (13.8)
Mean height (SD), cm	139.2 (10.2)	133.8 (8.9)	137.9 (9.3)
Mean waist circumference (SD), cm	75.7 (6.9)	76.1 (8.3)	82.4 (6.2)

SD: standard deviation; IQR: interquartile range

vs. 1.3 (IQR 1.6) mg/L, respectively; $P < 0.0001$] and compared to overweight children [median 0.7 (IQR 0.6) mg/L; $P < 0.0001$] (Figure 3).

Discussion

Our study provides further confirmation to the close links between obesity, enhanced oxidative stress, and inflammation in prepubescent children. Firstly, levels of MDA as a biomarker for oxidative stress was increased more than 1.5-fold in severely obese compared to overweight children. Secondly, severely obese children had more than a 4-fold increase in hsCRP levels as a proinflammatory marker compared to the overweight group. Some previous studies among youths noted that subjects with more body fat had higher concentrations of inflammatory and oxidative stress markers. In a population-based study, Kelishadi *et al.* reported a significant correlation between CRP and oxidative stress markers (MDA and conjugated diene) in 512 children and adolescents aged 10-18 years with abdominal obesity.⁷ Another study by Oliver *et al.* demonstrated simultaneous elevations in inflammatory and oxidative status in overweight and obese peripubertal children, when compared to healthy controls matched for age, gender, and fitness level. Obese children displayed significantly increased inflammatory cytokine (interleukin-6/IL-6) and systemic levels of lipid peroxidation (F2-isoprostanes). In addition, obese children displayed widespread alterations in their lipid profile, plasma glucose, and insulin compared to the control group.¹¹ Habib *et al.* conducted a case-control study and compared obese children and adolescents aged 5-17 years with healthy control participants matched for age and gender. They also showed significant elevations in proinflammatory adipocytokines (tumour necrosis factor- α and IL-6) and an oxidative stress biomarker (MDA), as well as significant decreases in antioxidant defense mechanisms (glutathione, zinc levels, and superoxide dismutase activity) among obese individuals compared to the control group.¹² Vehapoglu *et al.* recently found that at an early age (2-11 years), obese prepubescent children had concurrently elevated inflammatory (CRP) and decreased antioxidant status compared to underweight and normal-weight controls matched for gender. Furthermore, they observed significantly higher levels of fasting glucose, insulin, total cholesterol, triglycerides, homeostasis model assessment of insulin resistance (HOMA-IR), and homeostasis model assessment of β -cell function (HOMA- β), in obese prepubescent children compared to underweight and

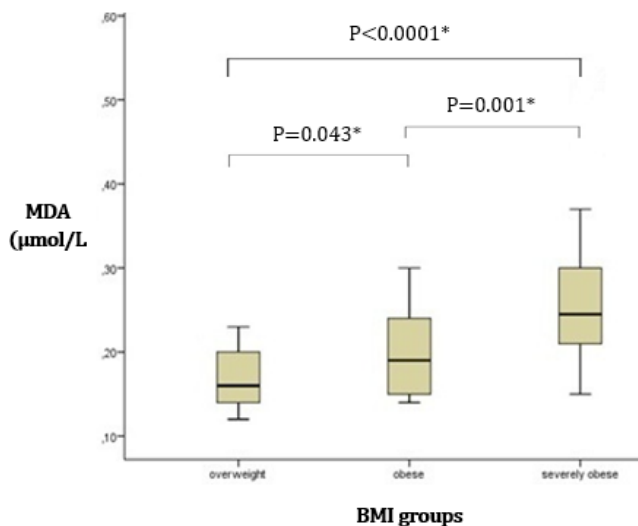


Figure 2. Comparison of MDA level based on BMI groups

*post-hoc analysis (Mann-Whitney U test)

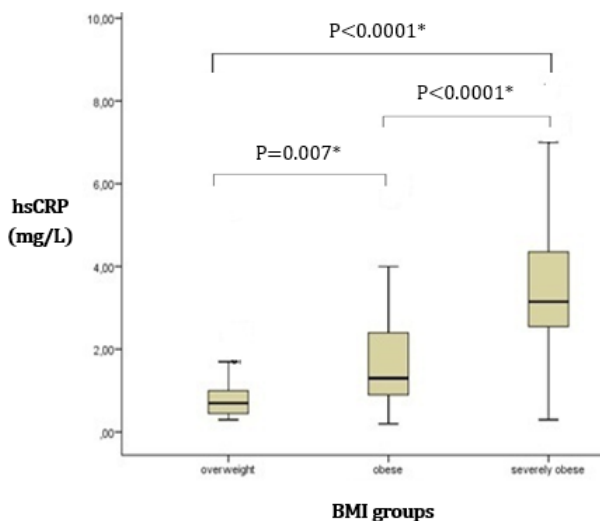


Figure 3. Comparison of hsCRP level based on BMI groups

*post hoc analysis (Mann-Whitney U test)

normal weight children.¹³

Our study applied the 99th percentile cut-off point based on the longitudinal cohort *Bogalusa Heart Study*, that found severely obese children had higher rates of developing severe obesity in adulthood and much more adverse cardiometabolic risk factor profiles. They demonstrated that of those with severe obesity (BMI \geq 99th percentile) at a mean age of 12 years, 100% developed to be adults with BMI \geq 30 kg/m²; 88% developed to have BMI \geq 35 kg/m², and 65% developed to have BMI \geq 40 kg/m². They also compared 6 cardiovascular risk factors (triglycerides, low density lipoprotein, high density lipoprotein, fasting insulin, systolic blood pressure, and diastolic blood pressure) in children based on BMI percentile. The results showed that 39% of children > 95th percentile and 59% of children \geq 99th percentile had \geq 2 risk factors, respectively, which was significantly greater than children in the 85th-95th percentile.¹⁴ Previous studies have shown that CRP has now emerged to be one of the most powerful predictors of inflammatory markers for the evidence of cardiovascular events in adults.^{15,16} There are currently no guidelines associating CRP levels and cardiovascular risk in children, but adults with a CRP > 3 mg/L are considered to have a 1.5 to 2-fold increased risk of cardiovascular disease.¹⁶ In our study, severely obese children had markedly elevated hsCRP values [median 3.2 (IQR 2) mg/L] compared to overweight and obese children. These results suggest that the severely obese children in our study population may be at high risk of future cardiovascular disease according to the adult cut-off point.

Although a chronic inflammatory response is an established fact in obesity, the molecular determinants that trigger this response and maintain it in a sustained state are still poorly understood. Reactive oxygen species (ROS) and free fatty acids have, however, been proposed as potential contributors to this process.¹⁷ Indeed, conditions that lead to increased oxidative stress are also known for their ability to lead to inflammation, in large part through the activation of nuclear factor kappa B (NF- κ B).¹⁸ In turn, activated inflammatory cells release high levels of ROS that potentiate the inflammatory response. Thus, the relationship between oxidative stress and inflammation is more complex than was originally thought, and it is clear that inflammation and

oxidative stress are mutually inclusive and most likely operate by creating a cycle that exacerbates them.¹⁹

Regarding study limitations, we should acknowledge that this study does not show the temporal ordering of the association between oxidative stress, systemic inflammation, and severity of obesity. Further longitudinal investigations are needed to confirm these associations.

In conclusion, prepubertal children at the \geq 99th percentile for BMI in the severely obese category are more likely to have higher hsCRP and MDA. Our data support the concept that at an early age, obesity activates biochemical mechanisms that might be responsible for long-term complications, thereby considerably increasing, if left untreated, the number of years of expected severe morbidity in patients.

Conflict of Interest

None declared.

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