Association between low-grade chronic inflammation with adipocytokines and body fat mass in superobese male children

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Abstract

**Background**
Obesity causes adipocytokines dysregulation and enhances the pro-inflammatory response. Low-grade, chronic, inflammation is related to cardiometabolic diseases.

**Objective**
To evaluate the status of low-grade chronic inflammation in pre-pubertal, obese boys and its potential correlation to adipocytokines and body fat mass.

**Methods**
This cross-sectional study included pre-pubertal, male, superobese children as the subjects. We determined obesity status using the CDC 2000 BMI-for-age chart. Body fat percentage was measured using bioelectric impedance analysis (BIA). Fasting blood specimens were collected to evaluate hsCRP, leptin, adiponectin, and TNF-α levels.

**Results**
Eighty subjects were recruited into this study, with a mean age of 6.9 years. Ten subjects (12.5%) had low-grade chronic inflammation (hsCRP level ≥ 1 mg/L). The levels of hsCRP was not correlated with leptin, adiponectin, and TNF-α levels. A weak, but significant correlation was observed between hsCRP level and body fat mass (r = +0.383; P<0.0001). The hsCRP level increased with increasing body fat mass, until it reached its peak at body fat mass of 28 kg. Beyond that point, hsCRP level was stable.

**Conclusion**
Low-grade chronic inflammation begins at a young age in obese children. The hsCRP level has a weak correlation with body fat mass, but no correlations with adipocytokine levels. Prevention and treatment of childhood obesity should be prioritized to prevent further cardiovascular and metabolic diseases.

**Keywords:** hsCRP; adipocytokines; body fat mass; superobese; children

In this century, obesity is one of the most crucial issues in childhood. The prevalence of childhood obesity varies across the world, but it is quite high in Asian countries, including Indonesia. In 2013, the prevalence of overweight-obesity among Indonesian children aged 5-12 years was 18.8%.

Obesity causes impairment of energy homeostasis and dysregulation of lipid and carbohydrate metabolism. An excess of these substances may increase mitochondrial activity, including the electron transport chain. Tissue (especially adipose) becomes relatively hypoxic due to an increase in oxygen demand. Local adipose hypoxia causes dysregulation of adipocytokines, generation of reactive oxygen species, and activation of several kinases that are related to the pro-inflammatory response.

Interaction between immune homeostasis and metabolism has been observed in many studies. Cytokines released from immune cells may affect the ability of adipocytes to regulate carbohydrate and lipid metabolism. Previous studies also found that low-grade chronic inflammation is...
related to cardiometabolic syndrome, including insulin resistance and atherosclerosis.\textsuperscript{8} In addition, adipocytokines (adipokines) released from adipocytes may alter immune systems. Adipocytokines also play an important role in energy homeostasis.\textsuperscript{6,7}

C-reactive protein (CRP), an acute-phase reactant, is produced by the liver. The CRP level may dramatically increase in the presence of significant inflammation. A more sensitive CRP, high-sensitivity CRP (hsCRP), can be used to predict low-grade inflammation caused by medical conditions.\textsuperscript{9}

At present, our knowledge about low-grade chronic inflammation in pre-pubertal obese children is limited. Low-grade chronic inflammation in pre-pubertal children is important because many studies found that the pathogenesis of chronic cardiometabolic disease has developed since a young age. We only focused on male children to rule out the potential effect of sex hormones. In this study, we aimed to determine the status of low-grade chronic inflammation (hsCRP level) in pre-pubertal, obese boys and its potential correlation with adipocytokines and body fat mass.

Methods

This cross-sectional study with pre-pubertal, superobese boys as the subjects, was conducted at Cipto Mangunkusumo Hospital Jakarta, a private hospital at East Jakarta, a private hospital at Bekasi, and a primary school in Central Jakarta. This study was approved by Ethics Committee of the Universitas Indonesia Medical School.

We calculated the minimum required sample size using a formula for numeric-numeric correlation, resulting in a minimum of 38 subjects needed to perform a two-tailed correlation analysis. The inclusion criteria were males, aged 5-9 years, with normal growth and development, and obesity solely caused by excessive food intake, as proven by dietary analysis. The exclusion criteria were acute illness, consumption of medications that affect body weight, obesity caused by endogenous factors (genetic or endocrine disease), and those on a diet program.

Body mass index (BMI) was calculated by dividing body weight (in kilogram) by the square of body height (in meters-squared). Superobese was defined as a BMI > 97\textsuperscript{th} percentile on the CDC 2000 growth chart.\textsuperscript{10} We calculated body fat mass by multiplying body weight by body fat percentage, which was measured by bioelectric impedance analysis (BIA) using a TANITA Inner Scan Body Composition Monitor type BC-545, Japan. Subjects’ blood specimens for quantifying hsCRP and adipocytokine levels were collected after the subjects had fasted for 12 hours. Adipocytokine (leptin, adiponectin, and TNF-\textalpha) levels were examined using ELISA technique. The hsCRP levels were categorized into high (≥1 mg/L) or normal (<1 mg/L).\textsuperscript{11}

Data are presented numerically. The correlations between low-grade chronic inflammation with body fat mass and adipocytokines were analyzed using Pearson’s correlation, with Spearman’s correlation as the alternative. Data analysis was performed using SPSS program version 20.0 (IBM, Chicago, IL, USA).

Results

We recruited a total of 80 pre-pubertal, male children for this study. Mean age of subjects was 6.9 years; and the youngest subject was 5 year old. All subjects were superobese (>97\textsuperscript{th} percentile BMI-for-age CDC chart), with median BMI of 24.35 kg/m\textsuperscript{2}. The subject with the lowest body fat had 22.2\% body fat percentage, which was equal to 6.28 kg of body fat mass (Table 1). Ten subjects (12.5\%) had high hsCRP levels (Table 2).

We did not observed any correlations between hsCRP level with leptin, adiponectin, or TNF-\textalpha level. However, hsCRP level had a weak, but significant positive correlation with body fat mass (\(r = +0.383; P<0.0001\)) (Table 3). A scatter plot of hsCRP and

| Table 1. Demographic and anthropometric characteristics of subjects |
|---------------------------------------------------------------|
| Characteristics | (N=80) |
| Mean (SD) age, years | 6.91 (1.29) |
| Mean body weight (SD), kg | 41.07 (9.77) |
| Mean body height (SD), cm | 126.65 (9.84) |
| Median BMI (range), kg/m\textsuperscript{2} | 24.35 (19.1-39.8) |
| Median body fat percentage (range), % | 33.35 (22.2-63.1) |
| Median body fat mass (range), kg | 14.71 (6.28-42.4) |
body fat mass is shown in Figure 1. The hsCRP level increased along with body fat mass, reaching its peak at body fat mass of 28 kg. Beyond this point, hsCRP level tended to be stable (Figure 1).

![Figure 1. Scatter plot between body fat mass and hsCRP level](image)

**Discussion**

In our study, 12.5% subjects had low-grade chronic inflammation, as defined by high hsCRP level. Low-grade chronic inflammation generates oxidative stress that exacerbates adipocyte malfunction in maintaining metabolic homeostasis. Energy excess is stored in adipocytes, resulting in hypertrophy and hyperplasia of adipocytes. Many studies in the past two decades found that high hsCRP level was significantly associated with cardiovascular and metabolic diseases, including hypertension, coronary arterial disease, peripheral arterial thrombosis, stroke, and metabolic syndrome. It is still unclear whether hsCRP plays a causative role in the pathogenesis or is only a marker of inflammation. However, several studies found that hsCRP has important roles in opsonization, uptake of lipid by macrophages, increased recruitment of monocytes into arterial plaque, dysregulation of endothelial function by disrupting nitric oxide release, and increased expression of plasminogen activator inhibitor-1 (PAI-1) on endothelial surfaces.

Leptin, especially in its free form, plays a major role in regulation of energy balance. Although the concentration of leptin in an obese person is higher than in a normal weight person, it loses its function of controlling food intake. This condition is defined as leptin resistance. We did not find a correlation between hsCRP and leptin levels. It can be explained by the fact that leptin is involved in innate and adaptive immune system maturation, activation, and proliferation. The lack of significance may have been due to genetic, environmental, and lifestyle factors, including sleep duration, which also influences serum adiponectin level.

There was an inverse trend between hsCRP and adiponectin levels, but it was also not significant. The inverse trend is possibly because adiponectin has anti-inflammatory properties by reducing inflammatory cytokines (TNF-α, nuclear factor-κB, vascular cell adhesion molecule-1, E-selectin, and IL-8), inhibiting transformation of macrophage to foam cells, and enhancing production of nitric oxide (NO). Adiponectin plays a protective role in various obesity-related complications. The lack of significance may have been due to genetic, environmental, and lifestyle factors, including sleep duration, which also influences serum adiponectin level.

In this study, TNF-α was not correlated with hsCRP level. The TNF-α is produced by mature adipocytes and stromal-vascular cells, and neutralizes tyrosine kinase, causing insulin resistance, which may be aggravated by inflammation in obesity. Several studies found that hsCRP and TNF-α levels

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**Table 2. High-sensitivity CRP and adipocytokine levels**

| Variables                              | (N=80) |
|----------------------------------------|--------|
| Median hsCRP (range), mg/L             | 0.58 (0.01-2.59) |
| hsCRP, n (%)                           |        |
| Normal (<1 mg/L)                       | 70 (87.5) |
| High (≥1 mg/L)                         | 10 (12.5) |
| Mean leptin (SD), ng/dL                | 20.68 (11.03) |
| Median adiponectin (range), pg/dL      | 5.25 (2.5-35) |
| Median TNF-α (range), µg/dL            | 3,106 (1,737-8,399) |

**Table 3. Correlations between hsCRP with body fat mass, leptin, adiponectin, and TNF-α**

| Variables                              | hsCRP level | r   | P value |
|----------------------------------------|-------------|-----|---------|
| Leptin                                  | 0.185       | 0.101 |
| Adiponectin                             | -0.095      | 0.401 |
| TNF-α                                   | 0.117       | 0.302 |
| Body fat mass                           | 0.383       | <0.0001 |
concurrently increased during various metabolic diseases.\textsuperscript{29,30} However, none of these studies analyzed for correlations between the markers. One study of type 2 diabetes mellitus patients found that hsCRP was not related with the basal value of TNF-\textit{\alpha}.\textsuperscript{31}

We found a positive, weak correlation between hsCRP level and body fat mass. Adipocytes play an important role in causing chronic states of inflammation in obese people. Adipocyte dysfunction in obesity enhances production of reactive oxygen species and pro-inflammatory cytokines.\textsuperscript{32} The positive peak of hsCRP level was reached at a body fat mass of 28 kg. This observation may imply that adipocytes have limitation in producing pro-inflammatory substances in response to metabolic failure.

This study has several limitations. A further study with a larger sample size, controlled diet, and normal weight boys as a control group are needed to evaluate the relationship between the markers mentioned above.

In conclusion, low-grade, chronic inflammatory state (high hsCRP level) in obese children begins at a young age. The level of hsCRP has a weak correlation with body fat mass, but no correlation with adipocytokines. Childhood obesity should be prevented or treated as early as possible to prevent further cardiovascular and metabolic diseases.

Conflict of interest
None declared.

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