High-protein Diet and Exercise Improve Inflammatory Marker and Oxidative Stress After Weight Loss Program in Adolescents Obesity A Randomized Control Trial

by Etika Ratna Noer

ORIGINAL ARTICLE

High-protein Diet and Exercise Improve Inflammatory Marker and Oxidative Stress After Weight Loss Program in Adolescents Obesity: A Randomized Control Trial

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ABSTRACT

Introduction: Prolonged low-grade inflammation in obesity is associated with excessive oxidative stress and enhances lipid peroxidation. However, few studies have explored the response of inflammatory markers to the combined high protein diet and exercise (HPE) of weight reduction in the adolescent obese population. The objective of this study was to examine the effects of HPE on high-sensitivity C-reactive protein (hs-CRP) and malondialdehyde (MDA) in the obese adolescent. **Methods:** The study was an 8-week randomized controlled trial in 56 abdominal obese adolescents (F=47, M=9; aged about 19 to 20 years). The subjects were divided into four groups; high protein and exercise (HPE, n=15), high protein diet (HP, n=15), exercise (E, n=13), and control (C, n=13). The meal diet consisted of 55% carbohydrate, 25%protein and 20%fat. The exercise conducted by circuit training. Body compositions (weight and fat mass) were measured by Bio Impedance Analyzer. hs-CRP and MDA were measured using *enzyme-linked immunosorbent assay (ELISA)*. **Results:** The change percentage of hs-CRP and MDA were decreased in HPE group by (-23.6±2.9%; -30.9±7.2%), exercise group by (-9.0±4.9%; -9.6±3.2%), HP group by (4.8±2%; -7.3±6.3%) and in the control group (-5±3%) respectively. There was a significant reduced in delta hs-CRP and MDA among four groups (p=0.0001). The change of hs-CRP and MDA were correlated positively with the change in BW and waist circumference (p<0.01). **Conclusion:** High protein diet and exercise has decreased hs-CRP and MDA levels after weight loss among obese adolescents.

Keywords: hs-CRP, MDA, High Protein Diet, Exercise, Obesity

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INTRODUCTION

Obesity, characterized by a chronic energy imbalance, has become a major problem in developed and developing countries. Obesity is regarded as a disease in the last century according to the World Health Organisation (1). Based on Indonesian Basic National Health Research, obesity prevalence in young people increases tremendously from 18, 8 to 31% for 10 years (2). Obesogenic environment, defined as the ubiquitous and accessible palatable energy-dense food, contributes essentially to the emerging obesity (3). Excessive energy intake and physical inactivity result in adipocyte hypertrophy followed by hyperplasia which in turn triggers low-grade chronic inflammation. Prolonged low-grade inflammation is associated with excessive oxidative stress and enhancement of lipid peroxidation (4).

Obese individuals tend to have elevated levels of the inflammatory biomarker high-sensitivity C-reactive protein (hs-CRP) and malondialdehyde (MDA). The hs-CRP is an inflammatory indicator as well as a risk of cardiovascular disease and metabolic syndrome which becomes one of the indicators in obesity (5). The systemic inflammatory indicator showed by hs-CRP is substantially sensitive, where its elevation through overexpression of inflammatory mediators reveals a pathological response (6). The management of obesity through weight loss programs can decrease the hs-CRP level.

Another consequence of obesity is oxidative stress, which is considered as the main mechanism of obesity-related complications. Numerous mechanisms have been proposed to explain the oxidative stress state in obesity, in which the homeostasis disturbance of redox resulted from lipid peroxidation seems to be the crucial one. Malondialdehyde, the last product of lipid peroxidation, represents a significant biomarker for oxidative stress (6,7). Oxidative stress leads to cellular damage followed by an overproduction of pro-inflammatory cytokines stimulating hs-CRP secretion in the liver.

Obesity management has been encouraged in many ways, such as dietary modification. A previous study in Indonesia showed that low-calorie high-protein diet successfully reduced the body weight and body mass index (BMI), fat mass, fat mass percentage, and muscle mass in adult obesity (8). Previous research revealed that weight loss caused a significant reduction in inflammatory cytokines (9)(10)(11) and oxidative stress (12). The positive effect of low-calorie diet and exercise on obesity encouraged us to evaluate the effects of a hypocaloric high-protein diet and exercise on the level of hs-CRP and MDA.

MATERIALS AND METHODS

Diet protocol

A high-protein diet (55% carbohydrate, 25% protein, 20% fat of the total energy value) was administered to all participants. Diet was given as much as 1200 kcal day-1. The protein sources came mainly from fish and tempeh. We chose both protein sources because they are considered to have high arginine content, compared to other proteins. Arginine is a potent satiety agent. Both protein sources are also abundantly available in Indonesia. The adherence to the diet meals was measured by 24-h records conducted by a daily logbook. Diet was provided 5 days/week (working days) for 7 weeks.

Exercise protocol

Subjects were given a circuit training consisting of aerobic and resistance training 5 times/week. Each session consisted of 45 minutes and was supervised by a professional trainer. The subjects were monitored using a heart rate monitor (Pollar HR-7, Polland) that was attached at the chest and must reach a 75% maximum heart rate. Each exercise session was held in the exercise laboratory and monitored by the researcher.

Study design and participants

This study was conducted from May to September 2018. This experimental study was approved by the local ethics committee of the Faculty of Medicine Diponegoro University-Dr. Kariadi Semarang Hospital (427/EC/FK-UNDIP/VII/2018). All participants (n=60) agreed to join and signed the informed consent. Of all sixty subjects recruited, 56 subjects completed the study and four were lost followed-up. The inclusion criteria in this study were BMI >25 kg m-2, aged 19 to 20 years non-diabetic, and no comorbid conditions. The subjects were divided into four groups: HPE (n=15), a group which received high protein plus exercise program; HP (n=15), a group which received high-protein diet; E (n=13), a group which received exercise program and C (n=13), a control group. The control group (C) received a hypocaloric, high-protein dietary advice, and was recommended to do exercise 30 minutes daily. The interventions were given for 8 weeks. Exercise and meal diet were prescribed by a physical activity trainer and professional nutritionist respectively.

Anthropometric and laboratory data collection

Bodyweight (barefoot and light clothes) and percent body fat were monitored once a week using bio impedance analyzer (Tanita, DC 360, Japan). The height was measured to the nearest 0.1 cm using stadiometer (SECA 213, Japan). BMI was stated by body weight divided by the square of height (kg/m2).

Overnight-fasting (12hr) blood samples were withdrawn from the vein in the arm and collected in EDTA tubes. Blood samples were centrifuged at 3000 × g for 10 minutes at 4˚C to obtain serum and stored at -80˚C until analyzed. The serum concentration of MDA and hs-CRP were measured by an enzyme-linked immunosorbent assay kits (Elabscience), using the ELISA technique (ELX 808; Bio-Tek Instruments, USA). Blood samples were withdrawn twice, before and at the end of the study. Serum samples were collected by qualified staff and were measured in the Central Laboratory of Diponegoro National Hospital, Semarang.

Statistical analysis

All results were expressed as mean ± standard deviation (SD). The Kolmogorov Smirnov test was used to verify the normal distribution of data. Independent t-test and Mann-Whitney was used to identify differences between groups. Correlation analysis (Pearson or Spearman correlation coefficient as appropriate) was performed between changes in inflammatory marker and oxidative stress from baseline to 8 weeks. The statistical tests were 2-sided and considered as significant at $p<0.05$ and a confidence interval of 95%. Data analyses were conducted using SPSS version 21.0 for windows (IBM, USA).

RESULTS

A total of 56 subjects completed in our weight loss program for 8 weeks. The baseline characteristics of the subjects are shown in Table I. All parameters at baseline were homogenous between four groups (P>0.05). The proportion of obese females was higher than that of males. All participants were considered abdominal obesity with waist circumference >80 cm for females and >90 cm for males.

The reduction in total body weight was achieved by 3% in HPE and HP groups. Nevertheless, the bodyweight of the E group appeared stable. Our treatments successfully resulted in decreasing hs-CRP and MDA levels, and the percentage of change was determined (Table. II). In general, the improvement of the variables was achieved by the combination of diet and exercise treatment followed by exercise only and diet only per se. After 8 weeks of study, hs-CRP and MDA levels of treatment groups significantly decreased ($p<0.05$). In line with anthropometric indices, the highest reduction of hs-CRP and MDA levels occurred in the HPE group followed by E, HP, and control group respectively (Fig. 1 & 2). Table. III indicates that there was a positive correlation between change of body weight, waist circumference with hs-CRP, and MDA levels.

Table I : The baseline characteristic of subjects

Variable	HPE	HP	E	C
Subjects (n)	15	15	13	13
Age (y)	19.3	18.9	19.2	19.5
BW (kg)	79.7±14.7[b]	69.7±7.5[b]	75.8±13.59	78.7±13
WC (cm)	87.2 ±10.06	85.06±3.84	89.48±8.44	94.15±9.31
BMI (kg/m²)	31±4[b]	29±3[b]	29±3	30±2
MDA (ng/mL)	243±151.7	201.4± 95.6	219.7± 161.1	175.7±68.9
hs-CRP (mg/mL)	3004±1215	1700±1167	2821± 959	2579±501

HPE, High protein and exercise; HP, High protein; E, Exercise; C, Control; BW, Body weight; WC, waist circumference; BMI, Body mass index; MDA, Malondyaldehide; hs-CRP, High sensitive C reactive protein

Values are expressed as mean ± standard deviation (SD).

all groups are homogenous ($p>0.005$)

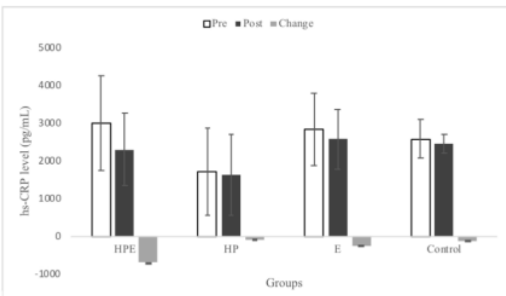

Fig. 1 : Change of hs-CRP levels during interventions.

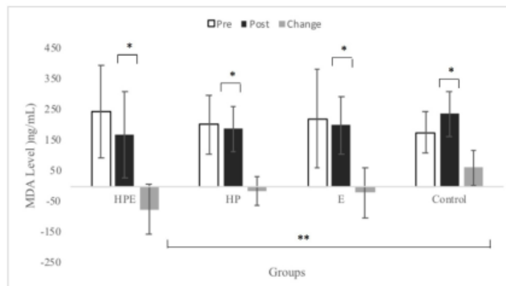

Fig. 2 : Change of Malondialdehyde levels during intervention.

Table II : Change in anthropometry, inflammatory and oxidative stress parameters

Variable	ΔHPE (n=15)		ΔHP (n=15)		ΔE (n=15)		ΔC (n=15)		p
	Absolute change	Relative change (%)	Absolute change	Relative change (%)	Absolute change	Relative change (%)	Absolute change	Relative change (%)	
BW (kg)	-2.3±1.9	-2,9±2.3	-1.9±2.2	-2.6±2.8	0.14±1.4	0.2±1,8	1.8±1.26	2.2±1.6	0.0001[a]
BMI (kg/m²)	-0.9±0.7	-2.9±2.3	-0.8±0.9	-2.6±2.8	0.04±0.5	0.1±1,9	0.6±0.47	2.2±1.6	0.0001[a]
PBF (%)	-2.53±2.3	-6.8±2.3	-0.75±1.4	-2.1±1.1	-1.2±3.4	-3.0±1,3	-0.6±1.27	-1.6±1.3	0.045[a]
WC (cm)	-3.3±1.7	-3.8±2.5	-1.94±3.5	-2.3±4.6	-0.45±1.3	-0.5±1,4	0.9±0.55	1.1±0.6	0.0001[b]
hs-CRP (mg/mL)	-709±11.5	-23.6±2.9	-82±11.2	-4.8±2	-254±9.1	-9±4.9	-13±4.6	-5±3	0.001[a]
MDA (ng/mL)	-75.2±8.1	-30.9±7.2	-14.7±4.6	-7.3±6.3	-21±12.5	-9.6±3.2	59.9±5.8	34.1±12.3	0.001[a]

HPE, High protein and exercise; HP, High protein; E, Exercise; C, Control; BW, Body weight; BMI, Body mass index;
PBF, Percent Body Fat; WC, waist circumference; hs-CRP, high-sensitive C-reactive protein; MDA, Malondyaldehide
Values are expressed as mean ± standard deviation (SD)
[a] Kruskall wallis test, Significant at level $p<0.05$
[b] Anova test, Significant at level $p<0.05$

Table III : Correlation between change of anthropometry and inflammatory and oxidative stress

	BW (kg)	WC (cm)
hs-CRP	r= 0.473*	r= 0.439*
MDA	r=0.366*	r = 0.358*

BW, Body weight; WC, waist circumference; BMI, Body mass index; MDA, Malondyaldehide; hs-CRP, High sensitive C reactive protein.
*Spearman test is significant at the 0.01 level (2-tailed)

DISCUSSION

The purpose of this study was to examine the 8-weeks effects of high protein diet, exercise, and the combination of them in obesity. Inflammation and oxidative stress occurred in obesity due to the presence of adipose tissue which is the main source of inflammation (13,14).

This study highlighted the combination of treatment showed the best improvement towards inflammatory marker and oxidative stress in obesity by reducing hs-CRP and MDA levels. The result was in line with a study by Azadbakht et al. and Hruby et al. on the effects of diet and exercise on oxidative stress (15,16). The possible explanation is the improvement of the inflammatory process through the adipocytes size reduction and pro-inflammatory marker discharge (10).

Interestingly, the benefit of high-protein diet and exercise resulting in a larger reduction in hs-CRP and MDA levels compared to a single treatment. The mechanism of protein in reducing the hs-CRP level warrants further studies Our hypothesis of protein in improving hs-CRP is the loss of adipose tissue through weight loss. A high-protein diet in weight loss program gave a higher satiety effect which potentially helps the adherence for dieters promoting weight loss (17). Exercise is well established for obesity improvement. The modalities of exercise seem to have beneficial effects on hs-CRP and MDA alleviation. The combination of aerobic and resistance training can attenuate inflammatory and oxidative levels in the weight loss program(19). This study is matched with the previous study that the greater effect of low grade-inflammation reduction through abdominal obesity improvement could be achieved by a combined exercise(20). The primary limitations of this study are the presence of under-reporting diet records, which potentially affected the treatment, also the length of study which was only 8 weeks. Nevertheless, we directly provided a meal diet from our laboratory which was strictly controlled.

CONCLUSION

Weight loss can improve inflammation marker and oxidative stress. A high-protein diet and combined-exercise give a beneficial effect on decreasing MDA and Hs-CRP.

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