RESEARCH ARTICLE

Comparison of hs-CRP level between low calorie high protein to standard protein diet in obese individuals with weight cycling - a randomised trial [version 1; peer review: 1 not approved]

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V1 First published: 11 Apr 2018, 7:447
<https://doi.org/10.12688/f1000research.13342.1>
Latest published: 11 Apr 2018, 7:447
<https://doi.org/10.12688/f1000research.13342.1>

Abstract

Background: Obesity is associated with various health problems. Low-grade inflammation is a condition found in obese people and contributes to the development of various diseases. The objective of this study was to compare the effects of calorie restriction diet with high-protein (HP) or standard protein (SP) to inflammation marker (hs-CRP) in obese individuals with weight cycling.

Methods: 61 healthy obese men and women (25 – 49 years old) with a history of weight cycling were recruited and were randomly assigned to one of the intervention groups, HP or SP groups. Both groups were suggested to reduce their daily caloric intake by 1000 kcal with regular physical activity for 56 days. Subjects in HP group were given a daily protein intake of 22-30% from total daily caloric intake, while SP group were prescribed 12-20%. Dietary consultation was conducted through daily reminder by phone and weekly counseling. The measurement of hs-CRP level was performed prior to and at the end of the intervention.

Results: 54 subjects completed the program, yet due to several reasons only 32 of them were measured for hsCRP before and after completing the program, 15 from HP group and 17 from SP group. After completing the 56-day diet program, SP group experienced reduction of hs-CRP by -0.446 ± 4.239 , while HP underwent increase by 0.135 ± 2.389 . The mean difference of change in hs-CRP level between the two groups were not statistically significant ($P=0.094$).

Conclusion: Low calorie diet with either HP or SP for 8 weeks significantly reduced body weight ($P<0.001$) and BMI ($P<0.001$) in healthy obese subjects but the difference of change in the hs-CRP level between them were not significant. The protein composition of a

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1. **Denise Mafrá**, Fluminense Federal University, Niterói, Brazil

Any reports and responses or comments on the article can be found at the end of the article.

low calorie diet may not affect the inflammatory state of obese individuals with weight cycling.

Trial registration number: NCT03374150

Keywords

high-protein diet, hs-CRP, obese, weight cycling

This article is included in the **All trials matter** collection.

Associated Research Article

Jutamulia J, Paranoan AN, Prasetya SI et al. » Comparison of body composition changes between low calorie high protein diet to standard protein in obese individuals with weight cycling – a randomised trial, F1000Research 2018, 7:445 (<https://doi.org/10.12688/f1000research.13300.1>)

Prasetya SI, Jutamulia J, Paranoan AN et al. » Comparison of plasma malondialdehyde and glutathione levels between low calorie high protein diet to standard protein in obese individuals with weight cycling – a randomised trial, F1000Research 2018, 7:446 (<https://doi.org/10.12688/f1000research.13227.1>)

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Author roles: **Paranoan AN:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Writing – Original Draft Preparation; **Jutamulia J:** Data Curation, Funding Acquisition, Investigation, Project Administration, Resources; **Prasetya SI:** Formal Analysis, Writing – Original Draft Preparation; **Mudjihartini N:** Conceptualization, Funding Acquisition, Methodology, Resources, Writing – Review & Editing; **Witjaksono F:** Conceptualization, Funding Acquisition, Methodology, Project Administration, Resources, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: Publikasi Internasional Terindeks Untuk Tugas Akhir Mahasiswa (PITTA) grant 2017, Directorate of Research and Community Services (DRPM) Universitas Indonesia.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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How to cite this article: Paranoan AN, Jutamulia J, Prasetya SI et al. **Comparison of hs-CRP level between low calorie high protein to standard protein diet in obese individuals with weight cycling - a randomised trial [version 1; peer review: 1 not approved]** F1000Research 2018, 7:447 <https://doi.org/10.12688/f1000research.13342.1>

First published: 11 Apr 2018, 7:447 <https://doi.org/10.12688/f1000research.13342.1>

Introduction

The global prevalence of obesity according to the World Health Organization (WHO) in 1980 was 5% in the male population and 8% in the female population. In 2014, this increased to 38% in men aged over 18 years and 40% in women. The WHO stated that in the Southeast Asian regions, the prevalence of overweight population varies from 7.6% in male adults in Bangladesh to 53% female adults in Maldives^{1,2}. In Indonesia, a report by RISKESDAS showed that the prevalence of central obesity increased from 18.8% in 2007 to 26.6% in 2013, and the prevalence for males aged >18 years old in the country was 19.7% and females >18 years old was 32.9%. Jakarta province has an obesity prevalence higher than the national rate, 30% for men and 40% for women³.

Obesity is associated with various health problems, mainly hypertension, cardiovascular diseases, dyslipidemia, diabetes, sleep apnea, and osteoarthritis. Obese people are more likely to be disabled in their later years, and many obese-related illnesses cause people in productive years to lose their productivity⁴. Attempts to lose weight by managing dietary intake and increasing physical activity are frequently conducted by many people, but the difficulty in maintaining body weight causes a fluctuation in body weight, called weight cycling^{5,6}. A study by Votruba *et al.* (2002) reported that 16 (57%) of 28 women regained 19% of the body weight they lost after 1 year of follow-up⁷.

Obesity is accompanied by low-grade inflammation, which contributes to the development of cardiac complications⁴. The mild increase in hs-CRP levels, as an indicator of low-grade inflammation, is an independent predictive factor of future cardiovascular diseases⁸. C-reactive protein (CRP) is an abnormal protein that appears in the blood during inflammation. Highly specific (hs-CRP) test can detect low CRP levels (0.2 mg/L) compared to CRP ($N \leq 5$ mg/L)^{4,9}. A previous study by Azadbakht *et al.* showed that energy-restricted HP and SP diet brought about similar hs-CRP reduction in overweight and obese people⁸. Further studies are needed to assess whether in obese individuals with weight cycling history, protein composition could affect inflammation markers. Therefore, the dietary composition that will give the best results for body composition as well as inflammation marker improvement for such population should be investigated. The aim of this study is to evaluate the effects of a low calorie high protein diet compared to a standard protein diet on the level of hs-CRP.

Methods

Study design

This is a part of an open-randomized parallel trial assessing the effect of a weight loss program with low calorie high protein diet conducted through dietary consultation on body composition, oxidative stress, inflammation marker and metabolic syndrome in obese individuals with weight cycling. The main interest of this article is the change of hsCRP levels among the HP and SP group before and after completing this program. There are two intervention groups with the allocation ratio 1:1, namely high protein (HP) group and standard protein

(SP) group. There were no changes made to the methods after the study had been started (original trial protocol can be found in our sister article¹⁰).

Approval from the Health Research Ethical Committee of the Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital has been obtained by the letter number 237/UN2.F1/ETIK/2017. Written informed consent for participation in the trial and publication of patient information was obtained from each patient. A completed CONSORT checklist can be found in [Supplementary File 1](#).

Trial registration number: NCT03374150

Registry name: Clinicaltrials.gov

Date of trial registration: 12/03/2017

Trial link: <https://clinicaltrials.gov/show/NCT03374150>

Participants

This study was conducted at the province's Civil Workers' Health Service Centre of the Special Capital Region of Jakarta (Pusat Pelayanan Kesehatan Pegawai Provinsi DKI Jakarta). Initial screening criteria for subject inclusion was men and women age > 20 years old with body mass index ranged 25 – 35 kg/m². In this study, weight cycling was defined by history of weight loss ≥ 2 kg and regaining weight into or exceeding its initial body weight at least twice in last five years. Weight cycling history was obtained from a questionnaire regarding history of body weight changes (see supplementary materials of our sister article¹⁰). Subjects were excluded if they had diabetes mellitus, history of gastrointestinal tract resection, hormonal disorders, using hormonal contraception, menopause, and having kidney function disruption, which was identified from serum urea and creatinine levels. The recruitment and intervention were conducted in two periods, namely May – July and July – September of 2017.

Intervention

All subjects who meet the inclusion criteria were measured for anthropometric data and were instructed to cease their previous diet program and requested to continue performing their daily physical activity at a regular level. History of previous daily calorie intake was gained from food recall interview 24 hours by asking type, cooking method, and estimation of amount of food consumed using household size, based on food photo books issued by Tim Survey Konsumsi Makanan Individu, Ministry of Health of Indonesia.

Dietary consultation for weight loss program was given for each subject in which all of them were suggested to reduce their daily caloric intake by 1000 kcal from their regular amount with lower limit of 1000 kcal/day. Subjects of HP group were assigned to low calorie-high protein diet with a composition of 22–30% protein, 50–55% carbohydrate, 20–25% fat while SP group were low calorie-balanced composition diet with 12–20% protein, 55–60% carbohydrate, and 20–30% fat. In order to ensure the compliance, follow-up to each subject were conducted by weekly person-to-person encounter with

nutritionist and daily text reminder. Subjects were equipped with diet formula, and logbook. Dietary program was applied for 8 weeks and subjects were instructed to fill logbook everyday to record their food intake. The measurement for hs-CRP level was done pre-intervention and re-measured after each subject has completed the 8 weeks of diet intervention.

Sample size and randomization

A total of 28 participants would be needed in order that the difference between groups could be detected with a two-tailed α of 0.05 and a $(1-\beta)$ of 0.80. Subject allocation into the intervention arms was determined through block randomization method with a block size of two. The group allocation sequence was constructed by the investigator (JJ) by random number generation method in which a computer-generated random number was taken, even number represent an arrangement of HP-SP and vice versa. The allocation sequence for all the participants that will be used throughout the study was generated by the investigator before the study commenced. Envelopes each representing a particular group were arranged according to the allocation sequence and then were orderly numbered. It was given consecutively to each subject who come hence the allocation sequence was blinded from the subject. The investigator assigned the subjects to the treatment group according to the envelope he/she received.

Outcomes and data collection

The primary data of this study is serum hs-CRP level before and after the diet intervention, which was measured using Immunochemmiluminescent device (Immolute 1000 Immunoassay system, Siemens Healthineers, Erlangen, Germany). The secondary outcome was dietary profile comprising mean daily caloric intake and mean daily proportion of macronutrients during the 56-days of diet program. Dietary data was measured with 24 hours food recall, while subject's physical activity was calculated using Global Physical Activity Questionnaire from the WHO. Body weight and BMI measurement were conducted using 8-electrode method of Bio-Electrical Impedance Analysis (BIA; SC-330 Tanita Corp. Tokyo, Japan). No change to the outcome measures was done after the study commenced.

Statistical analysis

The characteristics of subjects were analyzed statistically using independent sample t-test (age and previous calorie intake), Fisher's exact test (gender and weight cycling history), and Mann-Whitney test (weight and Body Mass Index (BMI)). All data except for body weight in HP group given as mean with 95% confidence interval. Thirty-six subjects were included for analysis. Change in body weight and BMI analyzed statistically using independent sample t-test, while intragroup pre- and post- mean difference of body weight and BMI analyzed by Wilcoxon test. The value of measured hs-CRP analyzed

statistically by Mann-Whitney test. Statistical analysis was performed using SPSS for Windows version 20.

Results

The number of participants who were screened, randomised and allocated into treatment, completed the treatment and analysed are provided in [Figure 1](#). There were 54 participants completed the 56-days of diet program but only 15 subjects from SP group and 17 subjects from HP group who completed the treatment and had their hsCRP been measured were analyzed for the primary outcome to the group they were initially assigned. During the course of treatment, no major harms were reported by the participants.

Characteristics of subjects are presented in [Table 1](#). There is no statistically significant difference found in the subjects' characteristics. Meanwhile, dietary profile of the subjects among both groups during the 56-days of dietary programme were presented in the [Table 2](#). Subjects' body weight and BMI before and after the programme as well as the difference are provided in [Table 3](#). The body weight, BMI, as well as the change in the body weight and BMI before and after the diet programme were higher in the SP group than the HP group, although those were not statistically significant. SP and HP groups both showed significant intragroup mean difference in body weight and BMI reduction pre- and post intervention. SP group experienced slightly greater reduction in body weight and BMI compared to HP protein (body weight 0.216 , BMI $p=0.136$). The hs-CRP level before and after completing the intervention are provided in the [Table 4](#). The hs-CRP level before ($P=0.094$) and after ($P=0.063$) the diet programme were higher in the SP group than in the HP group. After completing the 56-day diet programme, SP group experienced reduction of hs-CRP by -0.446 ± 4.239 while HP underwent overall increase by 0.135 ± 2.389 ($p=0.094$).

Discussion

The baseline and post-treatment hs-CRP level of HP group was lower than that of the SP group, although both were insignificant. The change of hs-CRP level of both group were also statistically insignificant. While the mean hs-CRP level of HP group increased, in the SP group it decreased. The insignificant reduction in hs-CRP despite weight loss and the decrease of BMI may because all the subjects were still in the overweight or obese state. Adipokines released by adipocytes or macrophages induced low-grade chronic inflammation in overweight and obese people¹¹; hence the hs-CRP level as the indicator of such condition is maintained.

The result of this study is similar to a study by Due *et al.*, in which SP diet in overweight subjects showed a better reduction of hs-CRP than HP diet¹². The result is also in line with a study by Azadbakht *et al.* on the effects of HP diet to weight,

Figure 1. The flow diagram of the participants.

cardiovascular risk and hs-CRP. Between the HP-low calorie diet (protein, carbohydrate, and fat: 25%, 45%, and 30% respectively) and SP-low calorie diet (protein, carbohydrate and fat : 15%, 55%, and 30%), hs-CRP in the standard group decreased greater by which it reduced $-0.08 \pm 0.11\%$, while in the HP group it only reduced by $-0.04 \pm 0.09\%$ ($p=0.06$)¹³. Another study by Kitabchi *et al.*, which compared various metabolic marker in the HP diet and high

carbohydrate diet for 6 months showed a greater decrease of inflammation marker (CRP) in the group receiving high carbohydrate diet than in the HP group (-2.1 vs. -0.8 mg/L, $p=0.0003$)¹⁴.

Despite the benefit of higher protein diet in increasing satiety, the present study found that SP composition provided larger reduction in body weight and hs-CRP level compared

Table 1. The characteristics of subjects prior to the low calorie-diet, divided according to the intervention with high protein and standard protein. Only subjects who completed the study were included.

Variable	Standard protein group (n=15)	High protein group (n=17)	P value*
Age (years)	33.4 ± 8.6	35.12 ± 8.8	0.598 ^l
Gender (n, %)			
Male	1 (6.7)	3 (17.6)	0.603 ^l
Female	14 (93.3)	14 (82.4)	
Body composition (mean±SD)			
Weight (kg)	76.9 ± 8.55	74.0 ± 12.64	0.496 ^m
Body mass index (kg/m ²)	29.94 ± 2.79	29.16 ± 3.52	0.385 ^m
Frequency of weight cycling history (n, %)			
2–3 times	11 (73.3)	14 (81.7)	0.907 ^p
4–5 times	4 (26.7)	3 (17.7)	
Usual daily caloric intake (mean±SD)	1759.3 ± 345.6	1802.3 ± 452.7	0.767 ^l
Physical activity level			
Very low	3 (20)	0 (0)	0.152
Low	8 (53.3)	11 (64.7)	
Moderate	4 (26.7)	6 (35.3)	

Values are expressed as mean ± SD except ^avalues are medians (min- max)

There were no differences between groups (P>0.05) by ^lIndependent samples t-test,

^mMann-Whitney test, ^pFisher's exact test

BMI = Body Mass Index

Table 2. Subject's dietary profile during 56 days of diet programme.

Variable	Standard protein (n=15)	High protein (n=17)	P-value*
Mean caloric intake (kcal; mean±SD)	964.7 ± 83.33	1032.8 ± 177.27	0.184 ^l
Mean percentage of caloric intake from carbohydrate (%;mean±SD)	50.73 ± 4.23	40.6 ± 5.22	<0.001 ^l
Mean percentage of caloric intake from protein (%;mean±SD)	20.91 ± 2.57	27.74 ± 3.31	<0.001 ^m
Mean percentage of caloric intake from fat (%;mean±SD)	21.05 ± 3.49	24.66 ± 3.73	0.009 ^l
Number of days with diet program compliance* (mean±SD)	20 ± 9	26 ± 12	0.741 ^l

*significance was set at <0.05

^lIndependent samples t-test

^mMann-Whitney test

*Compliance to the daily diet plan of 1000-kcal caloric intake restriction and proportion of caloric intake of 12–20 per cent for the SP group or 22–30 per cent for the HP group

Table 3. Subjects' body weight and body mass index changes.

Group (mean±SD)	Standard group (n=15)	High protein (n=17)	P*
Body weight before intervention	76.9 ± 8.55	74.0 ± 12.64	0.313 ^m
Body weight after intervention	71.8 ± 9.98	70.07 ± 11.97	0.406 ^m
<i>Intragroup pre- and post- mean difference significance</i>	P<0.001	P<0.001	
Body mass index before intervention	29.9 ± 2.79	29.16 ± 3.52	0.496 ⁱ
Body mass index after intervention	27.96 ± 3.42	27.68 ± 3.39	0.815 ⁱ
<i>Intragroup pre- and post- mean difference significance</i>	P<0.001	P<0.001	
Change in body weight	-5.1 ± 2.57	-4.0 ± 2.33	0.216 ⁱ
Change in BMI	-1.97 ± 1.01	-1.47 ± 0.83	0.136 ⁱ

*significance was set at <0.05

ⁱIndependent samples t-test^mMann-Whitney test^pPaired-samples t-test^wWilcoxon test**Table 4. Change in high-sensitive C-reactive protein before and after low calorie diet in high protein and standard protein group.**

hs-CRP	Group of intervention		P-value
	SP group (n=15)	HP group (n=17)	
hs-CRP before intervention	6.08 ± 3.99	3.84 ± 3.75	0.094 ^m
hs-CRP after intervention	5.63 ± 5.65	3.97 ± 4.51	0.063 ^m
<i>Intragroup pre- and post- mean difference significance</i>	0.105 ^w	0.586 ^w	
Change in the hs-CRP	-0.446 ± 4.239	0.135 ± 2.389	0.094 ^m
Mean difference of change in hsCRP (95%CI)	0.582 ((-1.864) - 3.028)		

*significance was set at P<0.05

^mMann-Whitney test^wWilcoxon test

to HP group. The amino acids obtained from protein intake were absorbed then are passed into the liver. The rate of synthesis and degradation of amino acids there is influenced by many factors including immediate food intake and nutritional status¹⁴. Inflammation and physical inactivity in the weight cycling condition which were characterized by accelerated wasting of lean body mass lead to the accelerated depletion of amino acids obtained from the dietary protein intake to counter the amino acids loss during the degradation of protein from body mass¹⁵.

A major limitation of this study is the absence of detailed assessment of the condition of the participants during the course of the intervention, such as the presence of acute illness, the use of drugs that affect the body inflammatory state and so on, which potentially affect the level of hs-CRP. Besides, the dietary consultation failed to maintain the compliance of the participants to the dietary plan to the desired extent. The average number of days in which subjects comply towards

the assigned program is only 20 days in the SP group and 26 in the HP group which may further limit the validity of this study. The majority of participants of this study were office workers which may limit the generalisability of this study.

Conclusions

Although both standard and high protein low-calorie diet for 56 days reduced body weight and BMI significantly in obese people with weight cycling, SP diet of 12–20% of overall daily caloric intake decreased hs-CRP levels, as an inflammation marker, greater than HP diet. However, the mean difference of change in hs-CRP level between the two groups were not statistically significant. This is probably due to the subjects from both groups were still in the obese/overweight state hence the change in the level of inflammation marker were less prominent. A trial with larger participants and/or longer follow-up period are needed to confirm whether protein proportion of dietary programme affects the change in serum hs-CRP level.

Data availability

Dataset 1: Harvard Dataverse. Replication Data for Comparison of low calorie-standard protein or high protein diet on body composition, malondialdehyde and glutathione, and hs-CRP level, <http://dx.doi.org/10.7910/DVN/7H55FP>¹⁶

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

Competing interests

No competing interests were disclosed.

Grant information

Publikasi Internasional Terindeks Untuk Tugas Akhir Mahasiswa (PITTA) grant 2017, Directorate of Research and Community Services (DRPM) Universitas Indonesia.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Supplementary material

Supplementary File 1: CONSORT checklist.

[Click here to access the data.](#)

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<https://doi.org/10.5256/f1000research.14479.r35974>

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Denise Mafrá

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The manuscript is confused and the authors did not explain exactly what the hypothesis is? The conclusion in the abstract shows p values, this is not common and not interesting.

The authors should write more about the role of protein in inflammation.

BMI and weight are not body composition measurements.

The diets were low carb and low fat and what was the exact protein intake expressed in g/kg/d, because 21% of protein is a diet with high protein intake? Normally, CRP values are not parametric data.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

No

Are all the source data underlying the results available to ensure full reproducibility?

No

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Renal nutrition

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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