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Fish oil capsule supplementation in children with obesity reduced c-reactive protein and improved blood pressure

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ABSTRACT

Introduction: Excessive accumulation of body fat in obesity increases morbidities such as hypertension and cardiovascular diseases. This study investigated the effect of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) supplementation on the level of high-sensitivity C-reactive protein (hs-CRP) and blood pressure in children with obesity. **Methods:** Fifty obese children, aged 6-10 years, were randomly assigned to the supplementation group (*n*=25) who received EPA and DHA supplementation or to the placebo group (*n*=25) for eight weeks. The trial was done in a single centre in Denpasar, Bali, Indonesia. Randomisation and allocation to the trial group were done by a computer system. The primary analysis was comparing the blood pressure and hs-CRP level between groups. Analysis of covariance (ANCOVA) and multivariate analysis of covariance (MANCOVA) tests were done to compare the differences between groups, with a *p*-value <0.05 considered as significant. **Results:** A total of 44 children completed the study, 24 (54.5%) were males and 20 (45.5%) were females. Initially, the systolic/diastolic blood pressure and hs-CRP level in the supplementation and placebo groups were 109.5/72.7 mmHg and 3.5 mg/L, 107.9/68.4 mmHg and 2.8 mg/L, respectively. At the end, they were 106.3/67.7 mmHg and 1.7 mg/L, and 108.1/71.8 mmHg and 2.8 mg/L, respectively. Systolic-, diastolic blood pressure and hs-CRP level were decreased by -2.6 mmHg (95% CI: -6.9 to 1.6; *p*=0.220), -7.5 mmHg (95% CI: -12.4 to -2.6; *p*=0.004), and -1.15 mg/L (95% CI: -2.1 to -0.2; *p*=0.022), respectively. **Conclusion:** EPA and DHA supplementation in obese children showed significant decrease in diastolic blood pressure and hs-CRP level.

Keywords: Fatty acid, inflammation, cytokine

INTRODUCTION

Obesity is a global health problem, including in Indonesia. Based on the data from the Basic Health Research (Riset Kesehatan Dasar/Riskesdas) in Indonesia, the prevalence of obesity in children is still high, although it decreased from 12.2% in 2007 to 11.9% in 2010, and further to 8.0% in 2018 (Kemenkes RI, 2018).

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Obesity is associated with increased blood pressure and the development of hypertension. In obese children or adults, their blood pressure is high even in a resting position. Many studies reported that obese individuals showed higher blood pressure than non-obese individuals even though they were still within the range of normal limits (Badeli *et al.*, 2016; Cheung *et al.*, 2017; Zhao *et al.*, 2017).

C-reactive protein (CRP) is a major inflammatory cytokine that functions as a non-specific defense of the immune system against tissue injury or infection. Many studies reported that increased levels of CRP in the blood was strongly associated with the occurrence of cardiovascular diseases (Fonseca & Izar, 2016; Badimon *et al.*, 2018; Zhuang *et al.*, 2019). In obesity, the increasing levels of CRP as a response to increased cytokine secretion in fat tissue has been used as a cardiovascular risk marker (Soeki & Sata, 2016).

Omega-3 fatty acids, especially their active metabolites, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been known to have several beneficial effects on health (Gammone *et al.*, 2019; Sakamoto *et al.*, 2019). A study in young healthy adults reported that blood omega-3 fatty acid levels were inversely associated with blood pressure (Filipovic *et al.*, 2018). Another study reported that omega-3 fatty acid and omega-6 fatty acid modulate blood pressure regulation and vascular function in obese children (Bonafini *et al.*, 2018). However, controversial results were documented. One study in children and adolescents reported that the beneficial effects of high omega-3 fatty acid levels were observed on the blood pressures of thin/normal-weight children, but not in overweight/obese children (Wolters *et al.*, 2016). A meta-analysis reported that increased omega-3 fatty acids had little or no effect on cardiovascular health (Abdelhamid *et al.*, 2018). Based to these evidences, this study investigated the effect of fish oil (containing EPA and DHA) supplementation on C-reactive protein and blood pressure in children with obesity.

MATERIALS AND METHODS

Study design

This study was a randomised, double-blind clinical trial conducted on children aged 6 to 10 years old with obesity. Obesity was classified according to their Body Mass Index (BMI) based on two standard deviation (SD) or above the median of the World Health Organization (WHO) BMI growth reference for age and sex. They were excluded from the study if they were suffering from infections, chronic diseases, neoplasms, and autoimmune diseases, consuming anti-inflammatory medications, and consuming omega-3 fatty acids. Informed consent was obtained from all children who met the study criteria. This study was performed at a single centre in Denpasar, Bali, Indonesia from January 2017 to June 2018. This study was conducted after obtaining ethical approval from the Ethics Committee of Udayana University – Sanglah General Hospital, Bali, Indonesia (No. 92/UN.14.2/Litbang/2016).

Data collection

Data collection included food recall, activity score assessment, anthropometric, blood pressure measurement, and blood samples for hs-CRP examination. Food recall and activity score assessments were obtained by giving a questionnaire to the parents before the intervention. Food recall was obtained from three days of dietary intake and counted for total calories, protein, fat, and omega-3 fatty acid intakes. The physical activity questionnaire was modified from the

Physical Activity Questionnaire-Children (PAQ-C). Anthropometric measurements included weight, height, and BMI (kg/m^2), which was calculated and then plotted onto the WHO BMI growth chart. Blood pressure was measured using a mercury sphygmomanometer and hs-CRP was measured using the particle enhanced immunoturbidimetric assay method. Blood sampling and laboratory tests were carried out by the Prodia® Clinical Laboratory. All data were collected at the beginning and at the end of the study.

Intervention

Children were randomly allocated to the supplementation or placebo groups. Random allocation was done using a computer system. The supplementation group received a fish oil capsule containing 90 mg EPA and 450 mg DHA twice per day, while the placebo group received a similar capsule twice per day containing cellulose flour. Both groups were followed for eight weeks. Investigators and children did not know whether they were in the supplementation or placebo groups before the study ended.

Parents/children met with the investigators for three times during the study period. The first time, they were given a total of 56 capsules (twice per day for 4 weeks). The second time, which was four weeks later, they were again given a total of 56 capsules with similar doses. The third time, at the end of the study, they reported the total capsules consumed.

Parents were asked to supervise and observe their children. The investigators monitored the compliance of children through telephone calls with their parents every week. The parents had to report how many capsules were consumed by their children in a week. They also reported the side effects of the interventions, such as nausea, vomiting, and itching, or other allergic manifestations that occurred. The intervention was stopped if side effects, such as allergic manifestations were found. Children who consumed less than 75% of the capsules were recorded as dropouts and they were not included in the final analysis.

Statistical analysis

The minimal sample size for detecting a difference of 0.17 mg/dL in hs-CRP, 10.8 mmHg in systolic blood pressure, and 6.7 mmHg in diastolic blood pressure between groups, with 80% power, and α=0.05 was 22 samples per group. The Kolmogorov-Smirnov test was performed on each numerical variable for normality. The association between categorical and numerical variables were analysed using chi-square and independent *t*-test. The effects of EPA and DHA supplementation on changes in blood pressure and hs-CRP level were analysed using analysis of covariance (ANCOVA) test. The multivariate analysis of covariance (MANCOVA) test was used to adjust the effects of several variables. The level of significance was *p*-value <0.05 and 95% confidence interval was also calculated. Data analysis was performed using SPSS statistics software version 20.0.

RESULTS

Of the total 92 children who were invited and came to the study centre, 34 children declined participation and 8 children did not meet the inclusion criteria. Fifty children were included in the study and signed the informed consent. They were then randomly allocated to the supplementation and placebo groups. During the study period, six children dropped out because they consumed less than 75% of the capsules. Finally, 44 children completed the study, with 22 children in each group, respectively (Figure 1).

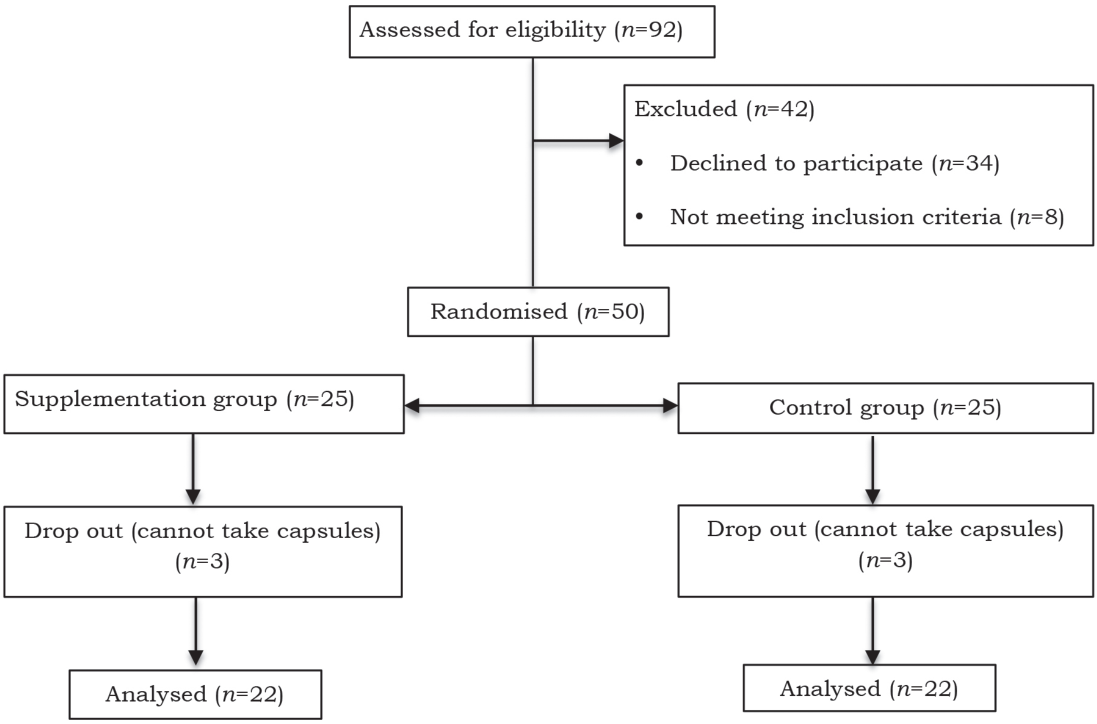

Figure 1. Flow diagram of subjects

Out of the 44 children who completed the study, 24 (54.5%) were males and 20 (45.5%) were females, with a mean age of 9.9±0.9 years and 9.7±0.7 years in the supplementation and placebo groups, respectively. Initially, the mean systolic-/diastolic blood pressure in all children was 108.7/70.5 mmHg and hs-CRP level was 3.2 mg/L, with a minimum and maximum level of 0.3 mg/L and 17.2 mg/L, respectively. Weight, height, BMI, physical activity, and nutrient intakes including calories, protein, fat, and polyunsaturated fatty acid (PUFA) were not statistically different in both groups (Table 1).

Table 2 shows the classification of blood pressure in all children at the beginning of the study, while Table 3 shows the anthropometric data in both groups at the end of the study. Finally, systolic blood pressure between groups was not statistically significant (p=0.220), but diastolic blood pressure and hs-CRP level between groups were statistically significant with p=0.004 and p=0.022, respectively (Table 4).

DISCUSSION

The prevalences of normal, pre-hypertension, and hypertension in this study were 61.4%, 6.8%, and 31.8%, respectively. The prevalence of hypertension in this study was similar to other studies. Badeli and colleagues (2016) reported that the prevalence of hypertension in obese children was 30.1% and they stated that the hypertension rates in obese children were higher than normal-weight children. A systematic review in children and adolescents in Africa reported that the prevalence of hypertension in obese children was 30.8%, which was six times higher than normal-weight children (Noubiap *et al.*, 2017). On the other hand, several studies reported lower results, which varied between 4.5% to 11.5% (Cheung *et al.*, 2017; Diaz & Calandra, 2017). Although the prevalence of hypertension in obese children is low, it is still a worldwide health concern due to its relation to cardiovascular diseases and high mortality rates.

Obesity is a chronic disease that is characterised by the increase of

Table 1. Baseline characteristics of the children

Characteristics	*Group*		*p*
	Supplementation n=22	*Placebo n=22*	
Gender			0.54†
Male, *n*	13	11	
Female, *n*	9	11	
Age (y), mean±*SD*	9.9±0.9	9.7±0.7	0.43‡
Body weight (kg), mean±*SD*	54.3±8.9	51.8±7.7	0.33‡
Height (cm), mean±*SD*	142.9±7.2	142.9±7.2	0.98‡
BMI (kg/m^2), mean±*SD*	26.6±4.9	25.3±2.6	0.27‡
Physical activity score, mean±*SD*	2.5±0.4	2.4±0.5	0.54‡
Calorie intake (kcal), mean±*SD*	2322.0±489.8	2280.4±515.4	0.78‡
Protein intake (g), mean±*SD*	89.3±26.3	91.1±22.9	0.81‡
Fat intake (g), mean±*SD*	65.5±24.6	61.8±20.3	0.59‡
PUFA intake (g), mean±*SD*	23.6±5.9	23.9±5.2	0.84‡

†Chi-square test
‡Independent *t*-test
SD = standard deviation; BMI = body mass index; PUFA = polyunsaturated fatty acid

Table 2. Blood pressure classification of children

Classification	*n*	*%*
Normal	27	61.4
Pre-hypertension	3	6.8
1st degree hypertension	13	29.5
2nd degree hypertension	1	2.3

Table 3. Anthropometric data of both groups at the end of the study

Anthropometric	*Group*		*p*†
	Supplementation	*Placebo*	
Body weight (kg), mean±SD	54.5±8.4	52.7±8.0	0.49
Height (cm), mean±SD	143.7±7.0	143.7±7.3	0.99
BMI (kg/m^2), mean±SD	26.5±4.6	25.4±2.5	0.36

†Independent *t*-test
SD = standard deviation

pro-inflammatory cytokines such as interleukin 1β, interleukin 6, tumour necrosis factor-α, as well as c-reactive protein (Luciardi *et al.*, 2018). This inflammation is associated with the complications of obesity that affect adults as well as children, such as hypertension, insulin resistance, dyslipidaemia, and cardiovascular diseases (Luciardi *et al.*, 2018). In this study, the mean concentration of hs-CRP was 3.2 mg/L, which is between the range of 1.9 mg/L to 3.4 mg/L as reported by other publications (Luciardi *et al.*, 2018; Jain *et al.*, 2017). The levels of hs-CRP should be controlled as low as possible to prevent the complications of obesity in children.

This study showed significantly decreased diastolic blood pressure and hs-CRP level, but not systolic blood pressure in children with obesity after supplementation of fish oil capsules containing EPA and DHA for 8 weeks. The systolic-, diastolic blood pressure, and hs-CRP level decreased from 109.5 mmHg to 106.3 mmHg, 72.7 mmHg to 67.7 mmHg, and 3.9 mg/L to 1.7 mg/L, respectively. Comparing with the placebo group, the decreasing effects of EPA and DHA on systolic-, diastolic blood pressure, and hs-CRP level were -2.6 mmHg, -7.5 mmHg, and -1.15 mg/L, respectively. No study has ever published the effects of omega-3 fatty acid on both blood pressure and hs-CRP level in children with obesity. However, one study did report the effect of prenatal DHA supplementation on blood pressure in obese children. They reported that overweight or obese children whose mothers received DHA supplementation had lower blood pressure (Kerling *et al.*, 2019).

Several variables, such as age, gender, BMI, physical activity, and food intakes have been adjusted in the multivariate analysis of this study. At baseline, the characteristics including

Table 4. Systolic- and diastolic blood pressure and hs-CRP levels in both groups

Variables	*Supplementation*		*Placebo*		*Univariate*†		*Multivariate*‡	
	Before	*After*	*Before*	*After*	*Mean diff. (95% CI)*	*p*	*Mean diff. (95% CI)*	*p*
Systolic (mmHg)	109.5±13.9	106.3±12.6	107.9±11.7	108.1±11.2	-3.0 (-7.4 to 1.4)	0.17	-2.6 (-6.9 to 1.6)	0.22
Diastolic (mmHg)	72.7±9.9	67.7±7.1	68.4±11.0	71.8±11.2	-6.8(-11.1 to -2.4)	<0.01	-7.5 (-12.4 to -0.2)	<0.01
hs-CRP (mg/L)	3.9±3.5	1.7±1.1	3.5±2.8	2.8±1.9	-1.2 (-2.1 to -0.3)	<0.01	-1.15 (-2.1 to -0.2)	0.02

†ANCOVA
‡MANCOVA (adjusted: age, gender, BMI, physical activity, and PUFA intake)

age, gender, body weight, height, BMI, physical activity score, intakes of calories, protein, fat, and PUFA were similar between both groups. Physical activity and healthy diet interventions in children with overweight and obesity are effective in improving BMI and cardiovascular risk score (Larsen *et al.*, 2016). Therefore, physical activity and diet interventions should be promoted as they are closely associated with lowering pro-inflammatory cytokines and BMI in children with obesity.

There are several limitations to this study. Firstly, there was no calculation of sodium in food intake. This is important as it is widely recognised that dietary sodium intake is closely related to blood pressure or hypertension (Grillo *et al.*, 2019). Secondly, the limitation of sample size and duration of the study.

CONCLUSION

The study showed the effectiveness of EPA and DHA supplementation in improving blood pressure and decreasing hs-CRP levels among children with obesity. With that, it may be recommended to give EPA and DHA supplementation to children with obesity to prevent cardiovascular disease-related conditions such as high blood pressure and increased hs-CRP levels.

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Authors' contributions

IGLS, principal investigator, conceptualised and designed the study, prepared the draft of the manuscript and reviewed the manuscript; PDV, led the data collection, advised on the data analysis and interpretation, and reviewed the manuscript; IWBS, led the data collection and reviewed the manuscript.

Conflict of interest

None of the authors have any conflict of interest regarding the publication of this article.

References

Abdelhamid AS, Brown TJ, Brainard JS, Biswas P, Thorpe GC, Moore HJ, Deane KHO, AlAbdulghafoor FK, Summerbell CD, Worthington HV, Song F & Hooper L (2018). Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. *Cochrane Database Syst Rev.* 7(7):CD012345.

Badeli H, Hassankhani A, Naaemi Z, Hosseinzadeh S, Mehrabi S, Pourkarimi M, Hosseini S, Nikoukar PR, Nezamdoust R, Rahimi A & Pourrajabi A (2016). Prevalence of hypertension and obesity-related hypertension in urban school-aged children in Rasht. *IJKD* 10(6):364-368.

Badimon L, Pena E, Arderiu G, Padro T, Slevin M, Vilahur G & Chiva-Blanch G (2018). C-reactive protein in atherothrombosis and angiogenesis. *Frontiers in Immunology* 9:430. Doi:10.3389/fimmu.2018.00430

Bonafini S, Giontella A, Tagetti A, Marcon D, Montagnana M, Benati M, Gaudino R, Cavarzere P, Karber M, Rothe M, Minuz P, Antoniazzi F, Maffeis C, Schunck WH & Fava V (2018). Possible role of CYP450 generated omega-3/omega-6 PUFA metabolites in the modulation of blood pressure and vascular function in obese children. *Nutrients* 10:1689. Doi:10.3390/nu10111689

Cheung EL, Bell CS, Samuel JP, Poffenbarger T, Redwine KM & Samuels JA (2017). Race and obesity in adolescent hypertension. *Pediatrics* 139(5):1-9.

Diaz A & Calandra L (2017). High blood pressure in school children and adolescents in Argentina over the past 25 years: A systematic review of observational studies. *Arch Argent Pediatr* 115(1):5-11.

Filipovic MG, Aeschbacher S, Reiner MF, Stivala S, Gobbato S, Bonetti N, Risch M, Risch L, Camici GG, Luescher TF, Schacky C, Conen D & Beer JH (2018). Whole blood omega-3 fatty acid concentrations are inversely associated with blood pressure in young healthy adults. *J Hypertens* 36(7):1548-1554.

Fonseca FAH & Izar MCO (2016). High-sensitivity c-reactive protein and cardiovascular disease across countries and ethnicities. *Clinics* 71(4):235-242.

Gammone MA, Riccioni G, Parrinello G & D'Orazio N (2019). Omega-3 polyunsaturated fatty acids: benefits and endpoints in sport. *Nutrients* 11:46. Doi:10.3390/nu11010046

Grillo A, Salvi L, Coruzzi P, Salvi P & Parati G (2019). Sodium intake and hypertension. *Nutrients* 11;1970. Doi:10.3390/nu11091970

Jain V, Kumar A, Agarwala A, Vikram N & Ramakrishnan L (2017). Adiponectin, interleukin-6 and high-sensitivity C-reactive protein levels in overweight/obese Indian children. *Indian Pediatrics* 54:848-850.

Kemenkes RI (2018). *Riset Kesehatan Dasar*. Badan Penelitian dan Pengembangan Kesehatan Kementerian Kesehatan RI, Jakarta.

Kerling EH, Hilton JM, Thodosoff JM, Wick J, Colombo J & Carlson SE (2019). Effect of prenatal docosahexaenoic acid supplementation on blood pressure in children with overweight condition or obesity. A secondary analysis of a randomized clinical trial. *JAMA Network Open* 2(2):e190088. Doi:10.1001/jamanetworkopen.2019.0088

Larsen KT, Huang T, Ried-Larsen M, Andersen LB, Heidemann M & Moller NC (2016). A multi-component day-camp weight-loss program is effective in reducing BMI in children after one year: A randomized controlled trial. *Plos One*. Doi:10.1371/journal.pone.0157182

Luciardi MC, Carrizo TR, Diaz EI, Aleman MN, Bazan MC & Abregu AV (2017). Proinflammatory state in obese children. *Rev Chil Pediatr* 89(3):346-351.

Noubiap JJ, Essouma M, Bigna JJ, Jingi AM, Aminde LN & Nansseu JR (2017). Prevalence of elevated blood pressure in children and adolescents in Africa: a systematic review and meta-analysis. *The lancet* 2:e375-e386.

Sakamoto A, Saotome M, Iguchi K & Maekawa Y (2019). Marine-derived omega-3 polyunsaturated fatty acids and hearth failure: current understanding for basic to clinical relevance. *Int J Mol Sci* 20:4025. Doi:10.3390/ijms20164025

Soeki T & Sata M (2016). Inflammatory biomarkers and atherosclerosis. *Int Heart J* 57:134-139.

Wolters M, Pala V, Russo P, Rise P, Moreno LA, Henauw S, Mehlig K, Veldebaum T, Molnar D, Tornaritis M, Galli C, Ahrens W & Bornhorst C (2016). Associations of whole blood n-3 and n-6 polyunsaturated fatty acids with blood pressure in children and adolescents – results from the IDEFICH/I.Family Cohort. *Plos One* 11(11):e0165981. Doi:10.1371/journal.pone.0165981

Zhao Y, Wang L, Xue B & Wang Y (2017). Associations between general and central obesity and hypertension among children: The childhood obesity study in China Mega-Cities. *Scientific Reports* 7:16895. Doi:10.1038/s41598-017-16819-y

Zhuang Q, Shen C, Chen Y, Zhao X, Wei P, Sun J, Ji Y, Chen X & Yang S (2019). Association of high sensitive C-reactive protein with coronary heart disease: a Mendelian randomization study. *BMC Medical Genetic* 20:170. https://doi.org/10.1186/s12881-019-0910-z