The Effect of Vitamin E Supplementation on Lipid Profiles and Adiponectin Levels in Obese Adolescents: A Randomized Controlled Trial

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ABSTRAK

Latar belakang: *inflamasi kronik derajat rendah pada obesitas menyebabkan abnormalitas lipid dan resistensi insulin. Vitamin E mempunyai efek antioksidan dan dapat meningkatkan sensitivitas insulin dengan perantara adiponektin. Penelitian ini bertujuan untuk menilai efek suplementasi vitamin E terhadap profil lipid dan kadar adiponektin pada remaja dengan obesitas.* ***Metode:*** *penelitian ini merupakan uji klinis acak tersamar ganda. Remaja obesitas berusia 14-18 tahun tanpa riwayat konsumsi obat-obatan antiobesitas atau antioksidan diikutsertakan dalam penelitian ini. Mereka dibagi menjadi dua kelompok menggunakan metode randomisasi: kelompok vitamin E dan plasebo. Dosis vitamin E yang digunakan adalah 400 IU/hari. Intervensi diberikan selama 2 bulan. Profil lipid dan kadar adiponektin diukur sebelum dan setelah pemberian intervensi. Hasil utama dianalisis menggunakan prinsip "per-protocol analysis". Analisis statistik menggunakan uji t independen, dan uji Mann-Whitney U sebagai alternatifnya.* ***Hasil:*** *sebanyak 66 subyek menyelesaikan penelitian, terdiri dari 34 subyek pada kelompok vitamin E dan 32 subyek pada kelompok plasebo. Profil lipid dan kadar adiponektin setelah intervensi selama 2 bulan tidak berbeda bermakna antara kedua kelompok. Perubahan parameter-parameter tersebut dari nilai dasar juga tidak berbeda bermakna antara kedua kelompok dan tidak konsisten dari satu subyek ke subyek lainnya.* ***Kesimpulan:*** *pada remaja dengan obesitas, suplementasi vitamin E dengan dosis 400 IU selama 2 bulan tidak berpengaruh secara signifikan terhadap profil lipid dan kadar adiponektin*

Kata kunci: *vitamin E, profil lipid, adiponektin, obesitas, uji klinis tersamar ganda.*

ABSTRACT

Background: *low-grade chronic inflammation in obese individuals contributes to the development of lipid abnormality and insulin resistance. Vitamin E has antioxidant and insulin-sensitizing properties, mediated by adiponectin. In this study, we aimed to evaluate the effect of vitamin E supplementation on lipid profiles and adiponectin levels in obese adolescents.* ***Methods:*** *this was a randomized, double-blind, controlled study. Obese adolescents aged 14-18 years, with no history of taking anti-obesity or antioxidant drugs, were recruited and randomized into two groups: vitamin E and placebo. The dose of vitamin E was 400 IU/day. Intervention was administered for two months. Lipid profiles and adiponectin levels were measured at baseline and after intervention. Primary outcomes were analyzed using the per-protocol analysis principle. Statistical analysis was performed using the independent t-test or the Mann-Whitney U test.* ***Results:*** *a total of 66 subjects completed the intervention study, 34 in the vitamin E group and 32 in the placebo group. Lipid profiles and adiponectin levels at 2 months after intervention did not differ significantly between the two groups. Changes from the baseline*

levels were also not significantly different between the two groups and were inconsistent from one subject to another. ***Conclusion:*** *in obese adolescents, vitamin E supplementation of 400 IU/day for 2 months does not significantly affect lipid profiles and adiponectin levels.*

Keywords: *vitamin E, lipid profiles, adiponectin, obesity, randomized controlled trial.*

INTRODUCTION

Childhood obesity has become a critical issue in Indonesia. In line with the nation's evolving economic situation, the prevalence of obesity is rising. On the other hand, malnutrition is also a persistent problem. This condition leads to what is called the "double burden of malnutrition".[1,2] Obesity is characterized by the presence of low-grade chronic inflammation that contributes to the development of metabolic syndrome and cardiovascular diseases later on.[3] Chronic inflammation leads to an increased production of reactive oxygen species (ROS) with ensuing damage to various organs.[4] Obese adolescents are more likely to develop insulin resistance in adulthood compared to normal weight adolescents.[3]

Low-grade chronic inflammation in obese individuals is related to a reduction in their adiponectin levels. Adiponectin, also known as ACRP30 or AdipoQ, is an adipocytokine that is primarily secreted by adipose tissue.[5] Increased adiponectin levels elicit a cascade that eventually results in an increased glucose uptake into cells and decreased gluconeogenesis.[6-7] Additionally, adiponectin also plays an important role in increasing fatty acid oxidation and in lowering lipid peroxidation due to oxidative stress.[8]

Several studies have revealed multiple benefits of vitamin E in healthy individuals. Vitamin E is well-known as a potent lipid-soluble antioxidant involved in the prevention of lipid peroxidation within cell membranes. A previous study found that vitamin E supplementation of 400 IU/day was able to improve endothelial function in children.[9]

Vitamin E supplementation may increase adiponectin levels through various mechanisms. Vitamin E has anti-inflammatory properties, which may help in neutralizing low-grade chronic inflammation in obese individuals. Suppression of pro-inflammatory cytokines may lead to an increased production of adiponectin. Previous studies have found that vitamin E is similar in structure to ligands which bind to the peroxisome proliferator-activated receptor-γ (PPAR-γ). Thus, vitamin E administration activates the PPAR-γ-responsive promoter region in the adiponectin gene, resulting in increased expression of adiponectin.[10-12]

To date, there has been a paucity of data on the beneficial effects of vitamin E in obese children. Therefore, in this study, we aimed to evaluate the effect of vitamin E supplementation on the lipid profiles and adiponectin levels in obese adolescents.

METHODS

This was a randomized, double-blind, controlled study. The study was conducted at high schools in Central Jakarta from September 2017 to February 2018. We chose this location due to its proximity to the Cipto Mangunkusumo Hospital. We included participants from twelve different high schools in this study.

Study Participants

Subject size was estimated using the formula for the mean difference between two independent populations, with type I error = 0.05 and power = 80 %. Pooled variance was calculated from previous studies.[13,14] Mean changes of 0.8 μg/mL for adiponectin, 20 mg/dL for total cholesterol, 10 mg/dL for LDL cholesterol, 5 mg/dL for HDL cholesterol, and 30 mg/dL for triglycerides were assumed as clinically significant. Based on the calculations, a minimum number of 31 subjects in each group was needed for this study. Obese adolescents aged 14-18 years were enrolled in this study. According to the Indonesian Guideline for Pediatric Nutrition Care, the term "obese" is defined as a body mass index (BMI) > 95th percentile in the CDC 2000 curve.[15] Subjects were excluded if they consumed anti-obesity or antioxidant drugs.

Randomization

Subjects were allocated into vitamin E and placebo groups using permutated block randomization with a size of four. An independent person who was not part of the study team placed either code A or code B into an envelope, which was then given to each subject. Code A was for placebo and code B was for vitamin E supplementation. The code list was concealed until the end of study. All study teams, subjects, and other related parties were blinded to the treatment allocation.

Intervention

The Indonesian Pediatric Society Guideline was basis of care for all subjects. Each subject received a capsule containing either vitamin E 400 IU or a placebo, which was consumed once daily. The placebo was made by the Cipto Mangunkusumo Hospital's pharmacy unit and was identical in flavor and packaging to the vitamin E capsule. The subjects received intervention for two months.

To minimize the number of dropout subjects and to increase their compliance, we built a system (peer groups) using social media so that the subjects could remind each other to consume the capsules every day. We also distributed the capsules in two divided visits: at baseline and at one month of intervention. At the one-month and end-of-intervention visits, we asked the subjects to bring all the remaining capsules to assess their compliance.

Nutritional Consultation and Other Documentation

All selected subjects received a consultation from a nutritionist experienced in treating obese adolescents. Energy intake per day for each subject was determined using 24-h dietary recall. Each subject received information about daily energy requirements and the best dietary plan for treating obesity.

We documented the frequency of exercise per week but did not evaluate the type of exercise and the number of calories burned. We recorded every drug that was consumed by the subjects before and during the intervention.

Outcome Measurements

Subjects were required to fast for 12 hours before blood samples were collected for the measurement of lipid profiles and adiponectin levels. The lipid profile included triglycerides, total cholesterol (total-C), LDL cholesterol (LDL-C), and HDL cholesterol (HDL-C). Adiponectin levels were quantified using the Human Adiponectin ELISA Kit for Total and Multimers (Daiichi Pure Chemicals Co, Ltd.).

Statistical Analysis

Numeric data is presented in mean (SD) for normal data distribution, otherwise the data is presented in median (min-max). Outcome results were analyzed by the per-protocol analysis principle. Statistical analysis between groups was performed using the independent t-test, or the Mann-Whitney U test. We used the software SPSS ver 20.0 (IBM, Chicago, IL, USA) to analyze the data.

Ethical Statement

This study has been approved by the Ethical Committee of the Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, with Ethical Approval Number 530/UN2.F1/ETIK/2017.

RESULTS

We measured the body weights and the heights of 2,856 adolescents from twelve high schools around Jakarta. Based on their BMI values, only 148 adolescents were classified as obese. For this eligible study population, we provided information regarding obesity, performed anthropometry examination, and distributed informed consent and assent forms for the subjects and their parents. A total of 79 adolescents was excluded from this study for various reasons, including: an unwillingness to participate, denial of permission by their parents, and obesity criteria not being met. The remaining 69 adolescents were recruited as study subjects. We collected blood samples, provided them with nutritional counseling, and performed physical and anthropometric examinations. They were then randomized into two groups; 35 subjects were assigned to the vitamin E group and the

rest were assigned to the placebo group. In the vitamin E group, one subject withdrew from the study; in the placebo group, one subject was lost to the follow-up and one subject withdrew (**Figure 1**).

The median age of the 69 randomized subjects was 16.42 years, with the youngest and the oldest subjects aged 14 and 18 years, respectively. Mean BMI of all subjects was 33.76 (3.41) kg/m^2. Subjects in both the vitamin E and the placebo groups had similar baseline demographic characteristics, including age, body weight, body height, BMI, and waist circumference (**Table 1**).

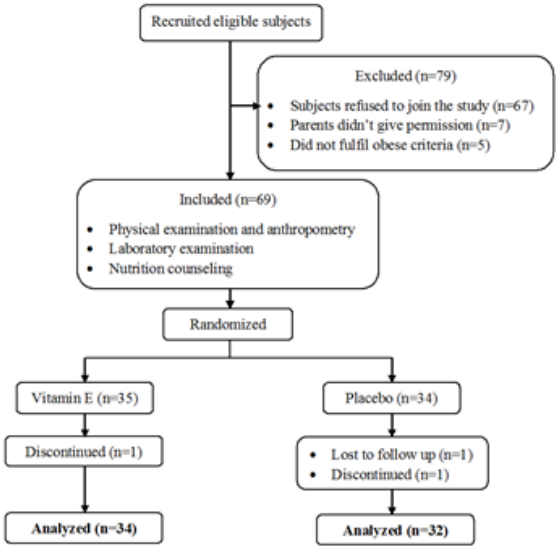

Figure 1. Flow diagram of randomized controlled study

After 2 months of intervention, lipid profile parameters in both vitamin E and placebo groups were not significantly different. A similar result was also observed in the adiponectin levels.

Table 1. Demographic characteristics

Characteristics	Mean (SD)	
	Vitamin E (n=35)	Placebo (n=34)
Age, years	16.12 (0.94)	16.84 (0.88)
Gender, n (%)		
- Male	21 (60)	22 (64.7)
- Female	14 (40)	12 (35.3)
Body weight, kg	92.32 (8.88)	93.18 (15.9)
Body height, cm	165.27 (7.03)	165.91 (8.59)
BMI, kg/m^2	33.82 (2.81)	33.7 (3.97)
Waist circumference, cm	104.06 (9.5)	105.32 (11.45)

Despite a higher value observed in the vitamin E group compared to the placebo group, the difference was not significant (**Table 2**).

Compared to the baseline, the changes in the lipid profiles and adiponectin levels after 2 months of intervention did not differ significantly between the groups. The changes were also inconsistent from one subject to another in both groups (**Table 3**).

DISCUSSION

In this study, subjects in the two groups were comparable because they had similar baseline demographic characteristics, and they were all assigned by a double-blind randomization procedure. All subjects had a waist circumference ≥ 80, which indicates that a large amount of fat was distributed in their intra-abdominal organs.[16,17] Compared to subcutaneous fat, abdominal fat is more metabolically active and resistant to insulin. Visceral adipose tissue is known to actively produce free fatty acids (FFA) and adipocytokines, hence obese children with a high waist circumference are more at risk of

Table 2. Lipid profiles and adiponectin levels at baseline and after 2 months

Variables	Baseline		After 2 months		P value
	Vitamin E (n=35)	Placebo (n=34)	Vitamin E (n=34)	Placebo (n=32)	
Triglycerides[a], mg/dL	115.5 (55-269)	117.5 (63-464)	112.5 (82-453)	132 (57-392)	0.426
Total-C[b], mg/dL	169 (29.05)	163.53 (30.73)	171.38 (29.61)	165.99 (28.57)	0.453
LDL-C[b], mg/dL	113.35 (26.13)	104.81 (29.49)	117.62 (28.4)	107.86 (27.32)	0.161
HDL-C[b], mg/dL	43.18 (7.64)	45.19 (10.35)	41.94 (7.06)	44.56 (9.87)	0.217
Adiponectin[a], µg/mL	3.16 (2.14-5.83)	3.38 (1.05-8.9)	4.18 (2.39-8.17)	4.13 (1.08-18.27)	0.423

[a] median (min-max), [b] mean (SD)

Table 3. Lipid profiles and adiponectin levels changes from baseline

Variables	Post-Pre		p value
	Vitamin E (n=34)	Placebo (n=32)	
Triglycerides[a], mg/dL	8 (-111 - 184)	21 (-71 - 250)	0.323
Total-C[b], mg/dL	2.38 (13.63)	2.43 (17.37)	0.989
LDL-C[b], mg/dL	4.26 (14.36)	3.06 (15.06)	0.741
HDL-C[a], mg/dL	-1 (-9 - 6)	0 (-12 - 15)	0.557
Adiponectin[b], µg/mL	1.33 (1.42)	1.18 (2.16)	0.742

[a] median (min-max), [b] mean (SD)

developing metabolic syndrome.[16-18]

Current studies support the role of vitamin E in controlling lipid profiles owing to its antioxidant property. Compared to other plasma lipids, LDL is more easily oxidized by free radicals, forming oxidized LDL (oxLDL). This oxLDL has a high affinity for the macrophage scavenger receptor, resulting in fatty streak formation. HDL, on the other hand, plays a crucial role in buffering oxLDL by directly protecting the vascular wall and removing lipid hydroperoxide (LOOH) from oxLDL.[19]

In this study, we found that after 2 months of treatment, subjects in the vitamin E group had lower triglyceride and HDL levels but higher total-C and LDL-C levels compared to subjects in the placebo group. However, the differences between the two groups were not significant. Similarly, changes in the lipid profiles before and after intervention in the placebo and the vitamin E groups did not differ significantly. Our result supports previous studies.[20-22] In apparently healthy adults, vitamin E administered in an escalating dose up to 400 mg/day (equal to 600 IU/day) for 21 days did not show a significant difference in lipid profiles pre- and post-intervention.[20] A study of diabetes type 2 subjects showed that the administration of 400 IU of vitamin E twice a day for 3 months did not significantly change plasma lipid levels.[21] Another study in hemodialysis and peritoneal dialysis patients also reported similar results.[22]

The exact mechanism of adiponectin production is still unclear. Despite being secreted by many cells in the body, it is predominantly produced by adipocytes. Adiponectin is known to play an important role in energy homeostasis by improving insulin sensitivity, increasing fatty acid oxidation, and decreasing gluconeogenesis.[23,24] It also shows antifibrotic properties by downregulating the expression of aldehyde oxidase and tissue growth factors.[24-26]

In this study, we found that the adiponectin levels between the placebo and vitamin E groups at the end of the study were inconsistent and were not significant. The change in adiponectin levels before and after intervention was also not significantly different between the two groups. This inconsistent result may imply that the severity of chronic inflammation among obese individuals varies from one individual to another.

A study involving overweight adults similarly found that vitamin E supplementation at 800 IU/day for 6 months did not change adiponectin levels.13 One possible reason is a defect in adiponectin production. Vitamin E activates the promoter region of the adiponectin gene.[10-12] However, obesity causes DNA methylation of this gene, resulting in the suppression of adiponectin expression.2[7,28] Therefore, the expectation that vitamin E supplementation will increase the adiponectin levels higher than placebo is not realized. Obesity also lowers adiponectin receptor (AdipoR1) expression, leading to a state of adiponectin resistance.[28,29]

A previous, double-blind, randomized controlled trial (RCT) study reported that vitamin E supplementation at a dosage of 400 IU/day for 6 weeks was sufficient to improve endothelial function in children.[9] However, our results did not support this finding as we found that supplementation with vitamin E at 400 IU/day for a longer duration did not change the adiponectin levels significantly. Mohammadi-Sartang M. et al.[30] conducted a meta-analysis study to evaluate the role of vitamin E supplementation

(with or without other supplements) on plasma adiponectin levels. Their sub-group analysis results showed that a significant increase in adiponectin levels was found only in those trials that used a dose of vitamin E ≥ 400 mg/day (with or without other supplements) and had a duration of intervention of ≥ 6 months.[30]

Although the difference between the vitamin E and placebo groups was not significant in this study, the overall variable changes favored the vitamin E group. Compared to the placebo group, subjects in the vitamin E group had a smaller increase in triglyceride levels and a larger increase in adiponectin levels.

In clinical practice, the findings from this study imply that vitamin E supplementation may be beneficial for obese children. A higher dosage and longer duration of supplementation must be considered to produce more consistent effects. The results from this study may be used as a basis for further RCT studies. Studies using various dosages and durations of vitamin E supplementation and measuring low-grade chronic inflammation markers are necessary to comprehend the beneficial effects of vitamin E supplementation on adiponectin in obese children.

This study has several strengths and weaknesses. This was the first study in this field with adolescents as subjects. It was a randomized, double-blind, controlled study with similar baseline characteristics between the groups. The drawbacks of this study were the absence of controlled diets and activities.

CONCLUSION

Vitamin E supplementation of 400 IU/day for 2 months does not significantly affect lipid profiles and adiponectin levels in obese adolescents. Further studies are necessary to comprehend the beneficial effects of vitamin E in obese adolescents.

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