Effects of Vitamin E Supplementation to Metabolic Markers on Diet-Induced Obesity in Mice

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Abstract

Introduction: Obesity creates health problems by increasing the risks of chronic diseases such as type 2 diabetes and cardiovascular disorders. Obesity leads to insulin resistance, higher blood glucose and cholesterol levels. Adipose tissues synthesize adiponectin which acts as anti-inflammatory, antidiabetic, and anti-atherogenic agent. Meanwhile, vitamin E is an antioxidant that acts as an anti-inflammation.

Aim: The purpose of this study was to analyze the effects of vitamin E supplementation to metabolic markers on diet-induced obesity in mice.

Materials and methods: Twenty-four mice (Mus musculus, L) aged four weeks were divided into six groups which were fed different diets and given vitamin E in different dosages or methods. The period of treatment was 18 weeks. The mice body weights were measured every week; blood sugar and cholesterol levels were measured every six weeks, and the adiponectin level measurement was done at week 18.

Results: A repeated measures ANOVA showed that body weight and cholesterol level within groups were not significantly different [F(15, 54)=1.417, 0.173 and F(10, 36)=1.391, 0.224 respectively]. The glucose levels were found to be significantly different [F(7.646, 27.526)=2.625, 0.030]. There was no significant difference in the adiponectin levels.

Conclusions: Vitamin E supplementation could not prevent the increase of body weight, the elevation of blood sugar and cholesterol levels, and also could not increase adiponectin level.

Keywords
adiponectin, cholesterol, glucose, obesity, vitamin E

INTRODUCTION

The worldwide obesity prevalence is continuously on the increase - more than 600 million people were estimated to be obese in 2014 which means that the global prevalence of obesity has more than doubled since 1980. The prevalence of obesity is high not only in high-income and developed countries but also in moderate and low-income countries, especially in the urban areas. For example, in Africa, the number of children with obesity has nearly doubled from 5.4 million in 1990 to 10.6 million in 2014; in 2014, almost half of the children under 5 years of age in Asia were...
categorized as obese. Meanwhile, the prevalence of overweight and obese adults in Indonesia in 2013 was 13.5% and 15.4%, respectively. But the proportion of obesity in Indonesia was reduced to 8% in 2018 compared to 11.9% in 2013, though it is still high.

Obesity has been recognized as the leading factor in the pathophysiology of type 2 diabetes mellitus (DM2), insulin resistance, dyslipidemia, hypertension, and atherosclerosis. The oversecretion of free fatty acids by adipose tissues in obesity induces oxidative stress to the endoplasmic reticulum in adipose or non-adipose tissues and prevents the clearance of triacylglycerol serum. These free fatty acids cause lipotoxicity and insulin receptor dysfunction. Moreover, the great amount of adipose cells secrete many cytokines that cause vascular dysfunction such as atherosclerosis.

On the other hand, adipose tissues secrete bioactive molecules called adipokines or adipocytokines like leptin, adiponectin, resistin, tumour necrosis factor, interleukin-6, chemokine (C-C motif) ligand 2, interleukin-10 and transforming growth factor. Adiponectin is known as an anti-inflammatory and anti-atherogenic adipokine that has insulin sensitizing effect. Based on its molecular weight, there are two types of adiponectin in circulation: low molecular weight (LMW) and high molecular weight (HMW). The HMW adiponectin is the main and most active adiponectin that induces metabolic effect to the peripheral tissue. The main metabolic effect of adiponectin is to improve the insulin sensitivity, decrease glucose secretion by the liver, increase glucose uptake and adipogenesis. Adiponectin also suppresses the migration of monocyte or macrophage and its transformation to foam cells in the vascular wall.

The high cost that must be paid to cure diseases caused by obesity motivates researchers to find treatment or prevention of the diseases. Meanwhile, vitamin E (α-tocopherol) acts as an anti-inflammation agent. Vitamin E supplementation to DM2 patients reduces interleukin (IL)-1β, IL-6, tumour necrosis factor alpha (TNF-α), PAI-1, and C reactive protein (CRP) serum level. Studies on animals and humans have shown that vitamin E supplementation halts low-density lipoprotein (LDL) peroxidation and prevents the oxidative stress related to DM2. Then, administration of vitamin D3 and vitamin E into mice that are fed high fat diet induces a decrease in the IL-6 production on its epidermis adipose tissues. Also, vitamin E supplementation can reduce lipid storage in liver and improve insulin sensitivity.

**AIM**

The aim of this study was to analyze the effects of vitamin E supplementation to the metabolic markers such as body weight, blood glucose, cholesterol and adiponectin level on diet-induced obesity in mice.

**MATERIALS AND METHODS**

The Ethics Committee at the Faculty of Medicine in the University of Northern Sumatra approved all procedures of animal treatment in this study. Twenty-four male mice (Mus musculus, L) aged four weeks were obtained from the Animal Laboratory, in the Faculty of Biology at the University of Northern Sumatra. All mice were housed under conditions of constant temperature, humidity, and standard dark-light cycles of 12 hours with ad libitum access to food and water. After adaptation for one week, all mice were fed with standard diet with additional high fat milk except the control group, which was given only the standard diet. The high fat milk contained 6 g of fat, 5 g of protein, and 21 g of total carbohydrate. Two spoons of milk (30 g) were poured into 300 ml of water and filled a pot from which the mice could drink ad libitum every day. The mice were divided into six groups: group 1 was the control group which received a standard diet; group 2 (the positive control group) was fed a standard diet plus additional high fat; groups 3 and 4 were fed a standard diet plus high fat diet and also supplemented with vitamin E orally in different dosages of 0.4 mg/day and 0.8 mg/day, respectively (HFE 0.4, HFE 0.8). Groups 5 and 6 were given a standard diet plus high fat and supplemented with peroral vitamin E when entering the fifth week with different dosages of 0.4 mg/day and 0.8 mg/day, respectively. The fifth week of study was chosen because another study had found that high-fat diet mice became obese at five weeks. The mice were caged in groups. The period of treatment was 18 weeks.

Vitamin E was administered forcibly by oral gavage in a dose of 0.4 mg daily. This specific dosage was used based on the findings of a study that demonstrated that supplementing the high-fat diet of mice with α-tocopherol (0.8 g/kg of diet) reduced the production of proinflammatory cytokines.

**Data collection**

Body weight was measured every week with digital weight measurement (Krisbow). The blood glucose and total cholesterol were measured every six weeks using Easy Touch Point of Care Tests as per manufacturer’s instructions. Blood samples were taken from tail without fasting.

The blood glucose and cholesterol level were measured to determine metabolic parameters that indicate the signs of metabolic syndromes. After mice were sacrificed, the HMW adiponectin serum level was measured at the end of treatment (18 weeks) using reagent ab108785--Adiponectin Mouse ELISA Kit and absorbance was read with wavelength 450 nm. To get the effect of anti-inflammation that was caused by vitamin E, the adiponectin level was measured at the end of treatment. Blood samples were taken from aorta after mice were sacrificed.

**Statistical analysis**

All data are expressed as means ± standard deviation (SD).
The data distribution was analyzed using the Shapiro-Wilk test of normality, otherwise data transformation were conducted. The difference in the parameters such as body weight, blood glucose and cholesterol levels within groups during study period was analyzed using repeated measure analysis of variance (ANOVA).

Meanwhile the difference of all parameters including adiponectin level between groups was analyzed using one-way ANOVA, if data were normally distributed, otherwise using Kruskal-Wallis test.

The p values less than 0.05 were considered statistically significant.

RESULTS

Effect of vitamin E supplementation on body weight

Repeated-measure ANOVA with sphericity assumption analysis showed that the body weight within groups were not significantly different between time points [F(15, 54)=1.417, 0.173] (p=0.355, Wilks’ lambda multivariate test).

The significantly different body weight between groups was found at 6 and 18 weeks of study with the p=0.012 and p=0.041, respectively (Table 1).

Table 1. The difference of body weight within groups (g)

<table>
<thead>
<tr>
<th>Group</th>
<th>1 week</th>
<th>6 weeks</th>
<th>12 weeks</th>
<th>18 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>29±1.826</td>
<td>39.25±2.22</td>
<td>34.75±2.36</td>
<td>44.5±1.3</td>
</tr>
<tr>
<td>HF</td>
<td>25.25±2.986</td>
<td>34.75±0.96</td>
<td>33.25±6.29</td>
<td>44±1.41</td>
</tr>
<tr>
<td>HFE 0.4</td>
<td>28.75±1.258</td>
<td>34.75±2.5</td>
<td>35.75±2.5</td>
<td>40±4.76</td>
</tr>
<tr>
<td>HFE 0.8</td>
<td>26.50±4.435</td>
<td>30.25±2.75</td>
<td>32.50±5.32</td>
<td>38.5±8.43</td>
</tr>
<tr>
<td>HFE 0.4 at 5 weeks</td>
<td>26.75±3.594</td>
<td>35±4.24</td>
<td>31.75±2.22</td>
<td>34.75±2.36</td>
</tr>
<tr>
<td>HFE 0.8 at 5 weeks</td>
<td>27±1.414</td>
<td>35.25±3.2</td>
<td>35.25±5.56</td>
<td>36.5±4.93</td>
</tr>
<tr>
<td>p value</td>
<td>0.441</td>
<td>0.012*</td>
<td>0.745</td>
<td>0.041*</td>
</tr>
</tbody>
</table>

Data presented as mean±SD, one-way ANOVA test, *p value<0.05; post hoc Bonferroni with p value <0.05 at 6 weeks: control vs. HFE 0.8 at 5 weeks

Effect of vitamin E supplementation on blood glucose level

Repeated measures ANOVA with the Greenhouse-Geisser correction showed that the glucose levels within groups were significantly different between time points [F(7.646, 27.526)=2.625, 0.030] with p=0.003 as assessed by the Wilks’ Lambda multivariate test. The blood glucose levels at 6 weeks compared to that at 18 weeks, and at 12 weeks compared to that at 18 weeks were significantly different (p<0.001).

We found significant differences in the blood glucose levels between groups at 6 and 18 weeks of study (p<0.001 and p<0.017, respectively). At 6 weeks, the highest blood glucose level was found in the control group (171±19.75 mg/dl), and the lowest blood glucose level - in the HFE 0.8 group (93±8.29). The control group showed again the highest blood glucose level at 18 weeks (180.75±28.92 mg/dl) while the lowest blood glucose level was found in the HFE 0.4 group at 5 weeks (113±13.09 mg/dl) (Table 2).

Table 2. Differences of blood glucose levels between groups (mg/dl)

<table>
<thead>
<tr>
<th>Group</th>
<th>6 weeks</th>
<th>12 weeks</th>
<th>18 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>171±19.75</td>
<td>142.5±16.36</td>
<td>180.75±28.92</td>
</tr>
<tr>
<td>HF</td>
<td>131.25±22.43</td>
<td>102±24.25</td>
<td>140±20.2</td>
</tr>
<tr>
<td>HFE 0.4</td>
<td>156.25±9.98</td>
<td>147.5±20.09</td>
<td>132.75±10.81</td>
</tr>
<tr>
<td>HFE 0.8</td>
<td>93±8.29</td>
<td>129.5±43.31</td>
<td>125±12.94</td>
</tr>
<tr>
<td>HFE 0.4 at 5 weeks</td>
<td>120.75±34.33</td>
<td>113.25±25.79</td>
<td>113±13.09</td>
</tr>
<tr>
<td>HFE 0.8 at 5 weeks</td>
<td>158.75±15.69</td>
<td>153.5±56.03</td>
<td>137±38.26</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.001*</td>
<td>0.261</td>
<td>0.017*</td>
</tr>
</tbody>
</table>

Mean ± SD; one-way ANOVA test; *p<0.05; Post hoc Bonferroni analysis, p<0.05 at 6 weeks: control vs. HFE 0.8, control vs. HFE 0.4 at 5 weeks, HFE 0.4 vs. HFE 0.8, HFE 0.8 vs. HFE 0.8 at 5 weeks, and at 18 weeks: control vs. HFE 0.4 at 5 weeks.
The effect of vitamin E supplementation on cholesterol level

Repeated-measures ANOVA with sphericity assumption analysis showed that cholesterol levels between groups were not significantly different between time points \([F(10, 36) = 1.391, 0.224]\) \((p = 0.126, \text{ multivariate Wilks' Lambda test})\).

There was no significant difference in the cholesterol levels between the groups \((p > 0.05)\). At 18 weeks, the highest cholesterol level was found in the control group \((195.75 \pm 50.96 \text{ mg/dl})\) and the lowest level was found in HFE 0.4 group \((139 \pm 26.5 \text{ mg/dl})\) \(\text{(Table 3)}\).

Effect of vitamin E supplementation on adiponectin level

There were no significant differences in the adiponectin levels between the groups \((p = 0.431)\) \(\text{(Table 4)}\). The highest level of adiponectin was found in the HFE 0.4 group \((5216.22 \pm 3530.77 \text{ ng/ml})\) and the lowest level - in the HF group \((2618.81 \pm 753.89 \text{ ng/ml})\) \(\text{(Table 4)}\).

Table 3. Differences of cholesterol level between groups (mg/dl)

<table>
<thead>
<tr>
<th>Group</th>
<th>6 weeks</th>
<th>12 weeks</th>
<th>18 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>201.5\pm50.15</td>
<td>151.25\pm22.5</td>
<td>195.75\pm50.96</td>
</tr>
<tr>
<td>HF</td>
<td>150\pm28.32</td>
<td>162.5\pm19.77</td>
<td>174.75\pm43.33</td>
</tr>
<tr>
<td>HFE 0.4</td>
<td>145.25\pm14.68</td>
<td>146.25\pm24.24</td>
<td>139\pm26.5</td>
</tr>
<tr>
<td>HFE 0.8</td>
<td>161.75\pm19.8</td>
<td>169.5\pm26.94</td>
<td>159.5\pm29.2</td>
</tr>
<tr>
<td>HFE 0.4 at 5 weeks</td>
<td>138\pm15.17</td>
<td>136.25\pm33.2</td>
<td>170\pm30.5</td>
</tr>
<tr>
<td>HFE 0.8 at 5 weeks</td>
<td>148.25\pm41.75</td>
<td>162.75\pm13.23</td>
<td>158.25\pm39.475</td>
</tr>
</tbody>
</table>

\(p\) value 0.134 0.416 0.435

Kruskal-Wallis test

Table 4. Differences in the adiponectin level (ng/ml) between groups at 18 weeks

<table>
<thead>
<tr>
<th>Group</th>
<th>Adiponectin (ng/ml)</th>
<th>(p) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2938.06\pm724.79</td>
<td>0.431</td>
</tr>
<tr>
<td>HF</td>
<td>2618.81\pm753.89</td>
<td></td>
</tr>
<tr>
<td>HFE 0.4</td>
<td>5216.22\pm3530.77</td>
<td></td>
</tr>
<tr>
<td>HFE 0.8</td>
<td>3442.72\pm707.04</td>
<td></td>
</tr>
<tr>
<td>HFE 0.4 at 5 weeks</td>
<td>4962.25\pm3418.87</td>
<td></td>
</tr>
<tr>
<td>HFE 0.8 at 5 weeks</td>
<td>3988.88\pm2323.78</td>
<td></td>
</tr>
</tbody>
</table>

Kruskal-Wallis test

DISCUSSION

The effect of vitamin E supplementation on body weight

This study found that the control group had the highest body weight at 6 and 18 weeks of study. These results were in line with the characteristics of the control group having the highest body weight at baseline. However, all groups increased their body weights at 6 weeks. Previous studies have found that the increased body weight of mice with diet-induced obesity can be noticed after two weeks of consuming high-fat diet, but becomes apparent after four weeks, the mice exhibiting 20-30% increase in their body weight.10 Mice fed high-fat diet for four weeks have been demonstrated to have significantly higher body weight than the mice receiving normal diet11,12. But this was not seen in this study, because the mice were not grouped based on their initial weight that have aimed to avoid the influence of early weight on subsequent weight gain, glucose and cholesterol levels.13

Other theory stated there are two types of responses on rodents when they are exposed to high fat diet. The first response is called diet-induced obesity (DIO) rodents; this response occurs in the C57BL/6 mouse when exposed to high-fat diet with increasing its body weight. The second response is called dietary resistant (DR) strains, which occurs in wild rodents without an increase in their body weight. However, other studies using outbred Sprague-Dawley rats found the diversity of responses when exposed to a high-fat high-palatability diet, some had DIO and others had DR responses. This diversity may be underlain by brain physiology, especially the expression of certain neuropeptides that may be linked to the dietary intake. Other aspects that may also underlie the diversity are the genetics factor (via QTL mapping), and the role of taste and olfactory systems in food intake.10 Another possibility that may contribute to the different results of this study is the source of fat diet that was obtained from 30 g milk powder that was put into 300 ml of water. Previous study that also used goat milk
with 20.0 ml/kg body weight as fat diet by forced fed Dawley juvenile rats (5 to 6 weeks old) did not show significant difference of body weight compared to others.\textsuperscript{14}

So, the highest body weight found in the control group at 6 and 18 weeks was because of the highest baseline body weight. The lowest body weight found in the HFE 0.8 group at 6 weeks was also because this group had the lowest body weight at baseline, and the HFE 0.4 group at 5 weeks had the lowest body weight at 18 weeks because it showed the lowest body weight in the previous week.

**The effect of vitamin E supplementation on blood glucose level**

Many studies have found a correlation between the higher body weight and type 2 diabetes.\textsuperscript{13} The present study found the higher body weight, the higher blood glucose could be and if the body weight is lower, the blood glucose is also lower.

A previous study has shown that mice on high-fat diet for four weeks showed no significant increase of their blood glucose levels. But after eight weeks of high-fat diet, all metabolic parameters like blood glucose and cholesterol level were significantly increased. Also, mice that received docosahexaenoic acid (coarabin) for two and four weeks had no significant changes in their blood glucose level.\textsuperscript{12}

**The effect of vitamin E supplementation on cholesterol level**

This study showed that there were no significant differences in the blood cholesterol levels between groups. This result is in contrast with another study that found that vitamin E supplementation reduced significantly the circulating triglycerides in mice with diet-induced obesity.\textsuperscript{9}

**The effect of vitamin E supplementation on adiponectin level**

Adiponectin is an anti-inflammatory adipokine secreted by adipose tissue to reduce the inflammatory effect caused by obesity. Adiponectin is one of the adipokines that has its expression, secretion by adipose tissue, and the serum level decreased in obese compared to eutrophic individuals. The functions of adiponectin are to increase insulin sensitivity, fatty acid oxidation, glucose uptake by adipose tissue, adipogenesis, glucose metabolism, free fatty acid oxidation by muscle and decrease free fatty acid uptake and glucose secretion by liver.\textsuperscript{5} Adiponectin expression is induced by \(\gamma\)-tocopherol in mice that are force fed 4 mg of \(\gamma\)-tocopherol daily for 4 days.\textsuperscript{15} The present study showed that there are no significant differences in the adiponectin serum levels between groups. Previous studies have shown that 39 overweight subjects that received 800 IU/day of natural vitamin E for 3 months and an increase dose of 1200 IU/day for further 3 months also showed no significant changes of plasma adiponectin compared to 41 placebo controls.\textsuperscript{16} But another research by Campos et al. shows that vitamin E promotes the adiponectin expression.\textsuperscript{17} Meanwhile, a systematic review and meta-analysis research by Sartang et al. stated that the influence of vitamin E on the circulating adiponectin levels was found to be dependent on the duration of supplementation.\textsuperscript{18} The study on mice that were supplemented with vitamin E in their high-fat diet (2.000 IU/kg) showed no reduction of the systemic oxidative stress, hyperlipidemia and obesity after 12 weeks of treatment.\textsuperscript{19}

**CONCLUSIONS**

We found that the increase of body weight of mice could be achieved after 6 weeks of treatment, and the magnitude was dependent on the body weight at baseline. The increase of blood glucose was in line with the increased body weight. However, this study could not conclude that vitamin E supplementation prevents the increase of the metabolic parameters or improves the adiponectin level.

**Acknowledgments**

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Влияние добавок витамина Е на метаболические маркеры при ожирении, вызванное дietsой у мышей

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Резюме

Введение: Ожирение создаёт проблемы для здоровья, увеличивая риск хронических заболеваний, таких как диабет 2 типа и сердечно-сосудистые заболевания. Ожирение приводит к инсулинорезистентности, повышению уровня сахара и холестерина в крови. Жировая ткань синтезирует адипонектин, который является противовоспалительным, антидиабетическим и антиатрогенным агентом. Кроме того, витамин Е – антиоксидант, обладающий противовоспалительным действием.

Цель: Целью этого исследования было проанализировать влияние добавок витамина Е на метаболические маркеры при ожирении, вызванном пищей у мышей.

Материалы и методы: Двадцать четыре мыши (Mus musculus, L) в возрасте четырёх недель были разделены на шесть групп на разные четыре диеты и получали витамин Е в разных дозах или методами. Срок лечения составил 18 недель. У мышей измеряли еженедельно: массу тела и уровни холестерина. Уровни сахара и холестерина в крови измеряли каждые шесть недель, и уровень адипонектина измеряли на 18-ой неделе.

Результаты: Множественные измерения ANOVA показали, что масса тела в группах существенно не различалась [F (15, 54)=1.417, 0.173 и F (10, 36)=1.391, 0.224, соответственно]. Уровни сахара и холестерина в крови измеряли каждые шесть недель, а уровень адипонектина измеряли на 18-ой неделе.

Заключение: Добавка витамина Е не может предотвратить увеличение веса, высокий уровень сахара в крови и холестерина, но также не может повышить уровень адипонектина.

Ключевые слова
адипонектин, холестерин, глюкоза, ожирение, витамин Е