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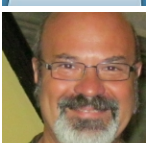
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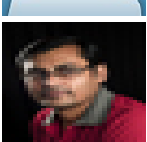
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## Abstract

**Aim:** Antioxidants, such as vitamin C and vitamin E, is widely used as supplements. The aim of this study is to analyze the profile of blood glucose, serum insulin, and HOMA in active teenagers after vitamin C and vitamin E supplementation.

**Methods:** Subjects (14-16 y.o) consisted of 12 boys and 5 girls, divided into 3 groups: control (4 boys, 2 girls), 'moderate dose' of vitamin C and vitamin E combination group (5 boys, 1 girls), and 'high dose' of vitamin C and vitamin E combination group (3 boys, 2 girls). The treatment was given for 5 days. Vitamin C and vitamin E for 'moderate dose' was 500mg; 200IU, and for 'high dose' was 1000mg; 400IU. Fasting Blood Glucose (FBG) and 1 hour BG (1hr\_BG), fasting serum insulin (FSI) and 1 hour SI (1hr\_SI) was collected after treatment. We also calculated the HOMA-IR and HOMA- $\beta$ .

**Result:** There was no significant difference on FBG, 1hr\_BG, FSI, 1hr\_SI, HOMA-IR, and HOMA- $\beta$  ( $p \geq 0.05$ ). However, mean FBG and 1hr\_BG tended to be higher on the treatment groups. The control group had the lowest HOMA-IR and the highest HOMA- $\beta$ .

**Conclusions:** We suggest that the supplementation of vitamin C and vitamin E in active teenagers is not essential on glucose homeostasis.

**Keywords:** vitamin C; vitamin E, blood glucose, insulin, HOMA, teenager.

## 1. Introduction

Antioxidants, such as vitamin C and vitamin E, widely used as one of the supplements to maintain or improve immunity. On medical application, external antioxidants required only for certain circumstances, because in normal physiological conditions, the body itself is able to make antioxidants (endogen). Specific requirement of exogenous antioxidants are needed as in illness where there is an inflammatory process. However, it has evolved during this perception in the community that when the body's physical activity increases, it needs additional supplement including antioxidants to prevent illness.

High physical activity, such as athletes performing aerobic or anaerobic activity is known to induce more free radicals [1]. Athletes consume antioxidant supplements as an effort to increase endurance and performance. Some study suggested that high free radicals trigger a disruption in insulin secretion, thus increasing the risk of diabetes mellitus, because it lead to cells damage [2]. However, in other studies mentioned that free radicals can actually stimulate insulin secretion [3,4].

Several studies indicated some advantageous of antioxidant. It can improve the performance of athletes after 5 days of vitamin C and E consumption [5]. Another study related to blood glucose in diabetics mellitus, indicates antioxidants can help decrease blood glucose level [6]. While other studies related to the provision of antioxidants lead to an increase in blood glucose level on diabetes and cancer patient [7,8].

The results of previous studies provided contradictory information related to antioxidant administration on glucose metabolism,

including in normal people and diabetes. Therefore, this study is conducted to determine the effect of vitamin C and vitamin E on glucose, insulin level, and insulin resistance-secretion models with HOMA (Homeostatic Model Assessment), focused on normal people who are physically active.

## 2. Methods

This study had obtained ethical approval from the Medical Research Ethics Committee of the Faculty of Medicine, Universitas Airlangga. Seventeen teenagers aged 14-18 years were (12 boys and 5 girls) divided into 3 groups, control group (4 boys, 2 girls), 'moderate dose' (5 boys, 1 girl), and groups of 'high dose' (3 boys, 2 girls).

The subjectss were performing routine activities as junior and senior high school students in the morning until noon, and in the afternoon they still doing regular physical exercise, swimming and diving 6 times per week.

The combination of vitamins C and E was administered for 5 days before data measurement. Medium dose was vitamin C 500mg and vitamin E 200IU, and high dose was vitamin C 1000mg and vitamin E 400IU [5]. On the 6th day, fasting blood glucose (FBG) and fasting serum insulin level (FSI) data was taken. Then, the subjectss were given 200gram glucose solution dissolved in 200mL water and followed by anerobic acute physical exercise, 100m surface diving [5].

After 1hour glucose administration, 1-h postprandial blood glucose (1hr\_BG) and 1-hour post-prandial insulin level (1hr\_SI) were obtained. Blood glucose level was measured by GOD-PAP

method which is one of the photometric enzymatic methods using supernatant samples from whole blood or serum. Serum insulin level was measured by Elisa method, using serum sample. Further detailed for method procedure of this study can be seen in Fig. 1.

Insulin resistance and secretion model with HOMA was calculated by the following formula: HOMA for insulin resistance or HOMA-IR = (FBG x FSI): 22.5, and HOMA for insulin secretion or HOMA- $\beta$  = (20 x FSI):( FBG-3.5). FBG in mmol/L; FSI in  $\mu$ U/mL.

Data for age, fasting blood glucose (FBG), 1-hour post-prandial blood glucose (1hr\_BG), glucose change (BG\_diff), fasting serum insulin (FSI), 1 hour post-prandial insulin serum (1hr\_SI), insulin serum alteration (SI\_diff), and insulin resistance-secretion with HOMA were analyzed with Anova (if the distribution of data was normal) or Kruskal wallis (if the distribution of data was not normal).

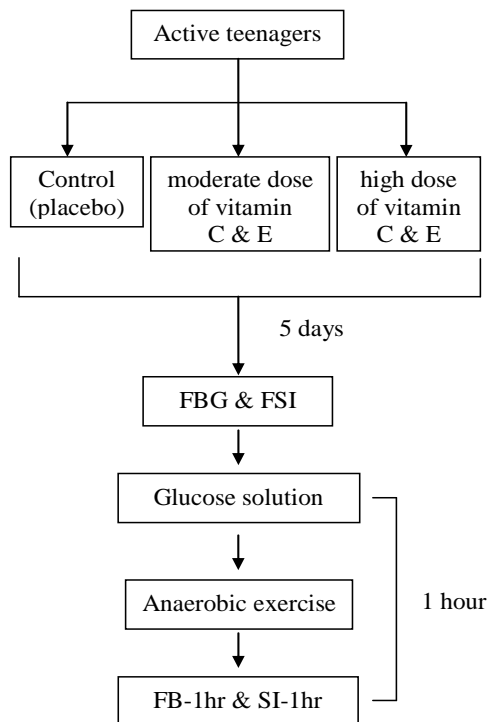


Fig. 1: Method Procedure of the study

### 3. Result and Discussion

#### 3.1. Characteristics of Subjects

The average age of the subjects in each group indicated not significantly difference ( $p \geq 0.05$ ), can be seen in Fig. 2. It told that the subjects's age was homogeneous with a mean of 15-16 years.

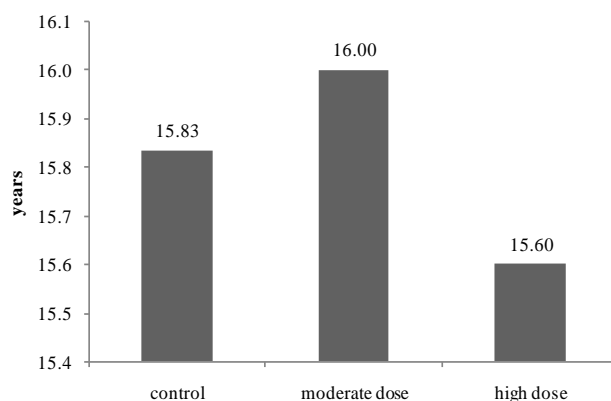


Fig. 2: Average age of subjects in each group

Subjects weight of each group can be seen in Fig. 3 and table 1. It appeared that the mean body weight had not significant difference ( $p \geq 0.05$ ). Therefore, in terms of body weight was considered homogeneous. However, the changes of weight was not analyzed because of the short duration (5 days) of these supplement.

Insulin is a hormone that affecting the metabolism, by inhibits fat burning and increase the synthesis of free fatty acids, therefore it can increase body weight [9].

The results of this study were not in line with higher insulin level in the 'high dose' group who had lower body weight. This might be due to a 5-day effect of vitamin C and vitamin E supplementation had not provided significant changes in serum insulin level yet.

Studies was conducted in overweight and non-overweight young adults found that HOMA-IR was higher in overweight subjects, and might be decreased by antioxidant administration for 8 weeks [10]. In this study antioxidant given for 5 days, so the effects of acute and chronic was not visible.

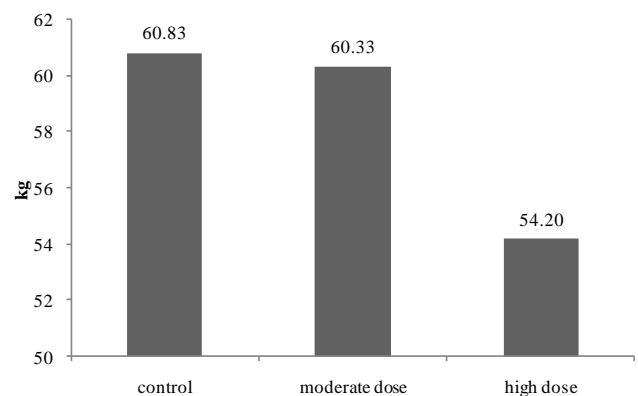


Fig. 3: Average of body weight in each group

#### 3.2. Effects of Antioxidant Supplementation on Glucose Level

Fasting blood glucose level, 1 hour post-prandial, and changes in blood glucose level can be seen in Fig. 4.

Fasting blood glucose (FGB) level showed no significantly difference ( $p \geq 0.05$ ). It told that vitamin C and vitamin E did not give significant effect. However, mean FBG was higher on treatment group. It was in line with the 1 hour post-prandial blood glucose level (1hr\_BG), although with a non-significant difference ( $p \geq 0.05$ ).

It suggested that supplementation of vitamin C and vitamin E began to affect glucose homeostasis which increase blood glucose level, either in fasting state or through glucose released in the blood by physiological effect of physical exercise.

Blood glucose level are controlled hormonally through absorption in the gastrointestinal tract, glycogenolysis, gluconeogenesis, glucose transport in cells, and urinary glucose excretion, in which insulin plays a major role in glucose homeostasis.[11]

The previous study stated that vitamin C supplementation affected blood glucose level. It is known that one form of vitamin C metabolism is dehydroascorbic acid is known has a resemble with glucose structure and also using glucose transporter (GLUT) in order to get into the cells. Several glucose transporter has a higher affinity for dehydroascorbic acid than for glucose [12].

Meanwhile, different results were shown in vitamin C supplementation for 6 weeks in type 2 diabetic patients, which decreased fasting blood glucose. It might that vitamin C as an antioxidant reduced insulin resistance by endothelial function improvement and lowering oxidative stress. Four weeks of vitamin E also improved insulin-mediated glucose disposal in hypertensive patients. [13, 14]. Nevertheless, recently, vitamin E supplementation did not show sufficient evidence to support

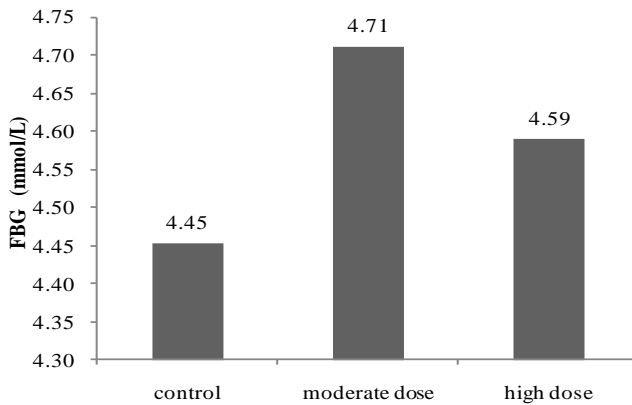
potential beneficial effects in both glucose and insulin homeostasis in subjects with type 2 diabetics. [15]

Changes in blood glucose level (BG\_diff) also indicated no significant differences. However, the average increase in blood glucose level were highest on 'high-dose' group, followed by 'moderate dose', and the lowest was on the control group.

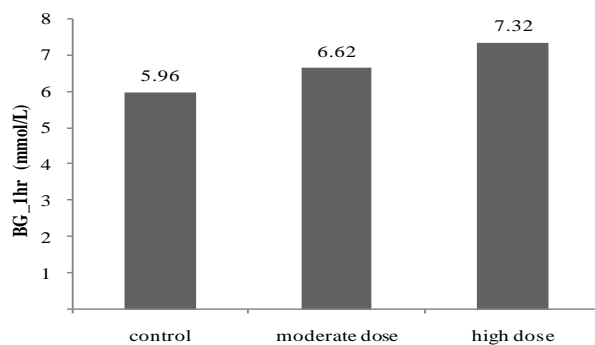
During physical exercise, free radicals are also formed. Free radicals are known to activate AMPK (AMP-activated protein kinase) [16, 17]. Activation of AMPK has been known to be one of the mechanisms that result in increase glucose uptake by skeletal muscle [18].

The vigorous physical exercise trigger excessive free radical formation. Regular exercise (training) can reduce the formation of free radicals as well as antioxidants can neutralize the free radicals [19]. It is known that the contraction through the increasing of calcium ions can lead to generate free radicals ( $H_2O_2$ ) which induce GLUT4 translocation and further effect is glucose uptake enhancement [20].

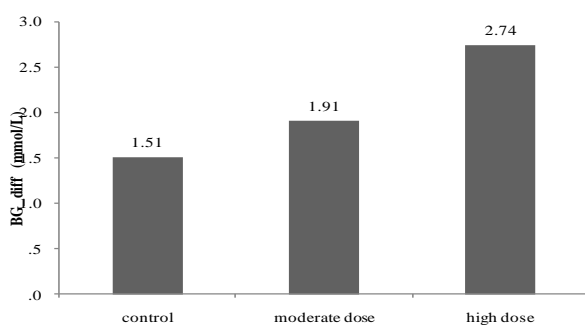
Physical exercise also activates sympathetic nervous system. The sympathetic nervous system simultaneously stimulates epinephrine release from the medulla of the adrenal gland which then increases blood glucose by stimulating glycogenolysis in the liver and skeletal muscle, inhibiting insulin secretion, and increasing glucagon secretion. It explains the increase of glucose after physical exercise. [11]



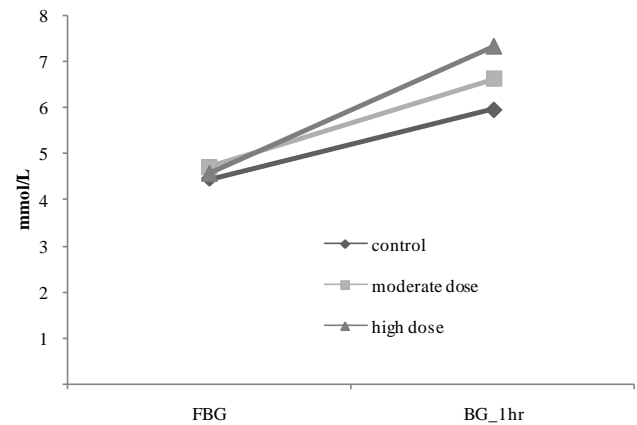
(a)



(b)



(c)



(d)

**Fig. 4:** Average of blood glucose level in each group A: Fasting blood glucose (FBG), B: Blood glucose of 1 hour post-prandially (1hr\_BG), C: Blood glucose difference between 1hr\_BG and FBG, D: time based of blood glucose level changing. No significantly difference among groups

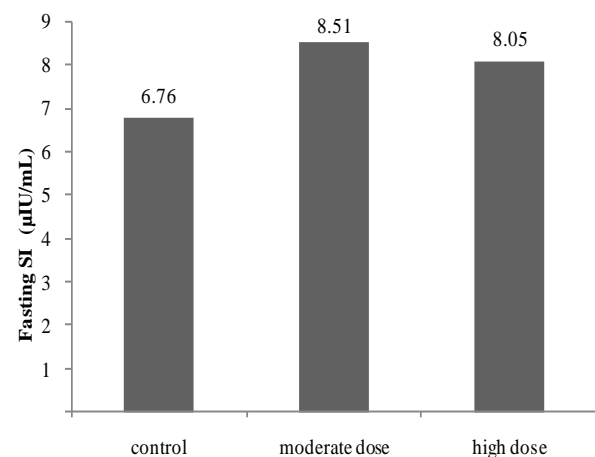
### 3.3. Effects of Antioxidant Supplementation on Insulin Level

Serum insulin level presented no significantly difference ( $p \geq 0.05$ ), both at fasting (FSI) and 1 hour post-prandially (1hr\_SI). Based on the average increase in serum insulin level change (SI\_diff), the 'high dose' group had the greatest increase. While the 'moderate dose' group had an almost equal improvement. After glucose loading, elevated blood glucose level of the 'moderate dose' group was not as high as the 'high dose' group. These results were in line with studies conducted on the group with metabolic syndrome compared with the normal group of subjects [21].

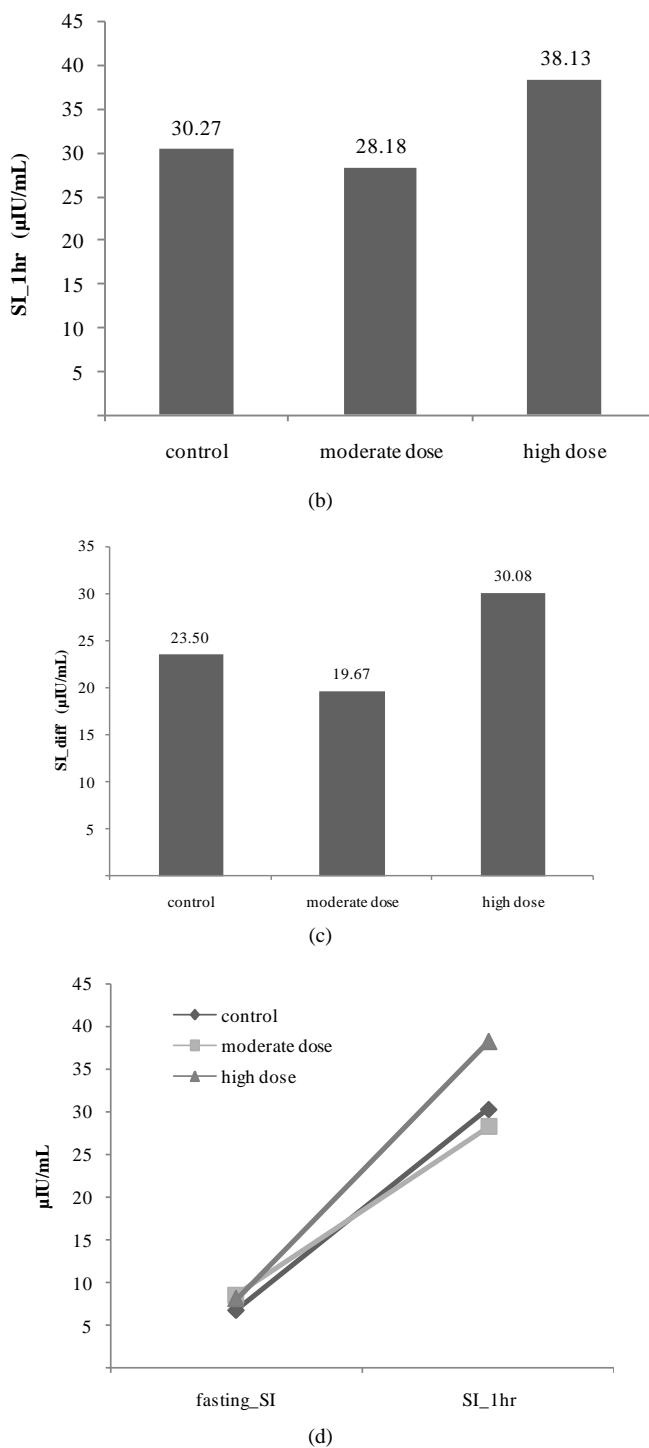
Other studies using ALA (alpha lipoic acid) antioxidants, omega 3 fatty acids, and vitamin E revealed an improvement on insulin sensitivity in type 2 diabetic patients [22]. When the insulin level increased, it lead to increase glucose uptake by the cells, thus blood glucose level became lower.

However, in this study the different result was occurred, greater blood glucose level and also greater serum insulin level in the 'high dose' group. It could be because the high dose of vitamin C and vitamin E as antioxidants triggered a shift in the balance of antioxidants and free radicals. The excess of antioxidants could lead antioxidants to become free radicals. Excessive free radicals would impair blood glucose uptake by cells [17].

In the other study using short term of vitamin E in obese mice, it showed that supplementation of vitamin E produced metabolic deregulation characterized by hyperglycemia which were typical features of insulin resistance, as confirmed by the increased HOMA-IR values [23].



(a)



**Fig. 5:** Average of serum insulin (SI) level in each group A: Fasting serum insuli (Fasting SI), B: Serum insulin of 1 hour post-prandially (1hr\_SI), C: Blood Serum insulin of blood glucose level, D: time based of serum insulin level changing.

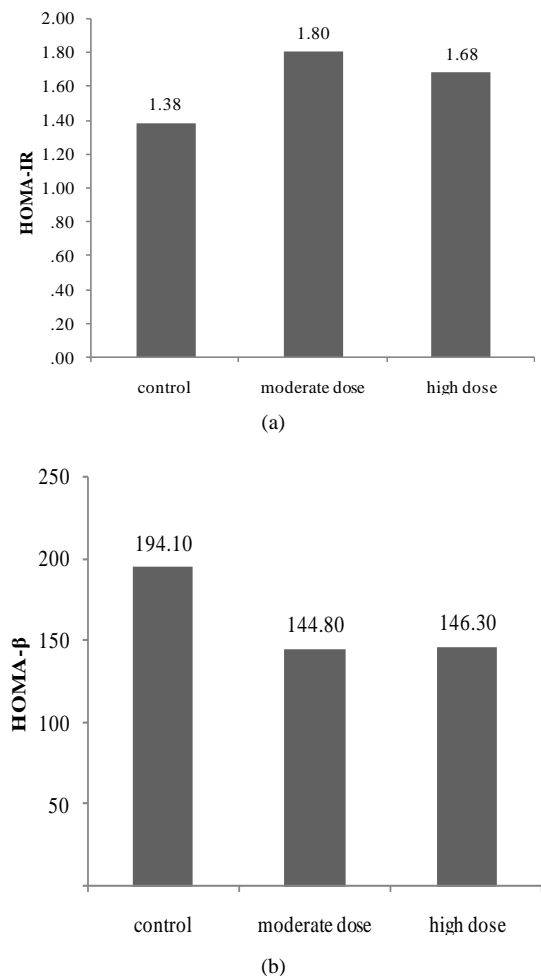
### 3.4. HOMA

Homeostatic model assessment (HOMA) is an approach to predict insulin resistance and insulin secretion. HOMA is expected to reflect the balance of glucose production by hepar and insulin secretion by beta cells during basal homeostatic conditions [24]. The result of insulin resistance or HOMA-IR on a subjects that was an active normal teenagers should be in normal range of 1.7-2.0. An indicator of insulin resistance, HOMA-IR is more than 2.0, whereas in diabetic patients the HOMA-IR is more than 4.00 [25]. Nevertheless, in this study, the calculation of HOMA-IR was analyzed due to analyze of the effect of vitamin C and E.

Beside, HOMA-IR, to predict whether beta cells are still good secreting insulin can be used model of HOMA-β. Since the subjects was normal active teenagers, HOMA-β was also in normal range.

The results of HOMA-IR and HOMA-β calculations showed no significantly difference ( $p \geq 0.05$ ). However, the control group had the lowest HOMA-IR and the highest HOMA-β. This could be interpreted that the antioxidant supplements in the form of vitamin C and vitamin E had a tendency to affect beta cells and beta cell receptors.

In studies conducted on athletic athletes who had high HOMA-IR (> 2.5), when given a high carbohydrate diet there was increasing level of insulin [26]. In our study, the HOMA-IR did not exceed 2.



**Fig. 6:** Average of model of insulin resistance and secretion in each group A: HOMA-IR, B: HOMA-B

In the 'moderate dose' group there was an interesting phenomenon where in FBG had the highest level compared to other groups. FSI level compared with the 'high dose' group was almost the same. The higher HOMA-IR calculations in the 'moderate dose' group was because HOMA calculations was based on fasting conditions. However, the increase of 1hr\_BG was not high. We suspected that moderate vitamin C and vitamin E supplementation increased the sensitivity of the insulin receptor to glucose and improved GLUT4 translocation in the skeletal muscle, so that blood glucose level decreased. Further research are needed to uncover the underlying mechanism.

Changes in blood glucose level in the 'high dose' group, which had greater increase along with a tendency of elevated serum insulin level. It indicated a potential antioxidant supplementation of vitamin C and vitamin E might trigger glucose metabolism. Although the results in this study was not significant, many other studies had demonstrated the same pattern as this research.

**Table 1:** The effect of vitamin C and vitamin E combination on body weight, blood glucose level, and serum insulin level. Data were presented as mean±SD

|                     | control       | moderate dose | high dose     | p    |
|---------------------|---------------|---------------|---------------|------|
| body weight (kg)    | 60.83 ± 5.46  | 60.33 ± 7.12  | 54.2 ± 12.76  | .406 |
| FBG (mmol/L)        | 4.45 ± 0.60   | 4.71 ± 0.32   | 4.59 ± 0.34   | .613 |
| BG_1hr (mmol/L)     | 5.96 ± 1.72   | 6.62 ± 2.20   | 7.32 ± 2.14   | .554 |
| BG-diff (mmol/L)    | 1.51 ± 2.0    | 1.91 ± 2.2    | 2.74 ± 2.0    | .390 |
| Fasting_SI (μIU/mL) | 6.76 ± 4.03   | 8.51 ± 7.47   | 8.05 ± 5.32   | .723 |
| SI_1hr (μIU/mL)     | 30.27 ± 16.43 | 28.18 ± 24.65 | 38.13 ± 37.04 | .815 |
| SI_diff (μIU/mL)    | 23.50 ± 19.08 | 19.67 ± 24.97 | 30.08 ± 37.54 | .726 |

## 4. Conclusions

Based on our study, we conclude that vitamin C and vitamin E supplementation have no effect on glucose level, serum insulin level, and insulin resistance-secretion models with HOMA. However, there is a tendency that moderate dose of vitamin C and vitamin E supplementation increase fasting glucose level, but can improve post-prandial glucose level. While the combination of high dose vitamin C and vitamin E, it tends that post-prandial blood glucose increase, but it is equalized by increasing insulin level. Therefore, it suggests that, on glucose homeostasis, the supplementation of vitamin C and vitamin E in active adolescents is not essential.

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