Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 Jul 18; 10(B):1767-1773.
https://doi.org/10.3889/oamjms.2022.9302
eISSN: 1857-9655
**Category:** *B - Clinical Sciences*
***Section:*** *Cardiology*





# The Benefits of High-Resistant Starch and Beta-Carotene Snack in Ameliorating Atherogenic Index and Inflammation in Obesity

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**Edited by:** Igor Spiroski
**Citation:** Sunarti S, Rubi DS, Pramana AAC, Huriyati E, Santoso U. The Benefits of High-Resistant Starch and Beta-Carotene Snack in Ameliorating Atherogenic Index and Inflammation in Obesity. Open-Access Maced J Med Sci. 2022 Jul 18; 10(B):1767-1773. https://doi.org/10.3889/oamjms.2022.9302
**Keywords:** Atherogenic index; Beta-Carotene; Inflammation; Obesity; Resistant starch; Oxidized low-density lipoprotein
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**Received:** 08-Mar-2022
**Revised:** 08-May-2022
**Accepted:** 08-Jun-2022

**Funding:** This work was supported by the Ministry of Research and Technology Republic of Indonesia.
**Competing Interest:** The authors have declared that no competing interest exists


## Abstract

**BACKGROUND:** In obesity, lipid abnormalities may be related to the higher risk of cardiovascular diseases associated with increased oxidation of lipids and inflammation. Resistant starch and beta-carotene reduce atherosclerosis risk related to low-grade inflammation and oxidative stress in obesity.

**AIM:** This study aimed to evaluate the benefits of a snack containing high-resistant starch and beta-carotene in improving the atherogenic index of plasma (AIP) and inflammation in obesity.

**METHODS:** This study used a single-blinded and randomized controlled design. Fifty subjects received 42 g of snacks per day for 6 weeks, either tested snacks or standard snacks. Anthropometry, body composition, lipid profile, tumor necrosis factor-alpha (TNF-α), and oxidized low-density lipoprotein (ox-LDL) were measured before and after intervention.

**RESULTS:** The snack containing high-resistant starch and beta-carotene significantly decreased LDL, AIP, and TNF-α ($p < 0.05$). Positive correlations were found between AIP and triglycerides in both snacks ($p < 0.05$), LDL or TNF-α in the standard snack ($p < 0.05$), and TNF-α and ox-LDL in both snacks ($p < 0.05$). A negative correlation was found between AIP and HDL in both snacks ($p < 0.05$).

**CONCLUSIONS:** A snack containing high-resistant starch and beta-carotene reduced AIP and inflammation by preventing LDL oxidation.

## Introduction

In obesity, lipid abnormalities may be related to the higher risk of cardiovascular diseases associated with increased oxidation of lipids and inflammation. Metabolic phenotypes in obesity that is estimated to be related to cardiovascular risk factors are total cholesterol/high-density lipoprotein (HDL), low-density lipoprotein (LDL)/HDL, and triglycerides (TGs)/HDL indices. The most common causes of cardiovascular diseases are atherosclerosis and/or hypertension. Obese individuals with dysmetabolism have higher atherogenic risk than those whose condition is dysmetabolic non-obese [1]. There are three formulas that can be used to calculate the atherogenic indices: (1) Cardio risk ratio (CRR) = CH/HDL [2]; (2) atherogenic coefficient (AC) = (CH–HDL)/HDL [3]; and atherogenic index of plasma (AIP) = log (TG/HDL) [4]. The AIP indicates plasma atherogenicity which is also a significant independent predictor of cardiovascular diseases [5].

According to Klop *et al*., abnormality of lipids may be associated with a gradient of pro-inflammatory factors that directly affect the endothelium [6]. Adipose tissue contains various cell types that are involved in the regulation of metabolism, storage of energy, and the function of neuroendocrine and immune responses, including adaptive and innate. Therefore, adipose tissue is an immune organ at the cross-roads between immunity and metabolism [7]. In obesity, excess adipose tissue causes an abnormality of synthesis and secretion of adipokines and pro-inflammatory cytokines [8], such as tumor necrosis factor-alpha (TNF-α). TNF-α is a multipotent cytokine that, with other inflammatory cytokines, stimulates the activation of the nuclear factor-kappa B (NF-κB) pathway and induces oxidative stress in adipose tissue [9]. Therefore, chronic low-grade inflammation with permanently increased oxidative stress is a prevalent condition in obesity, and the process of oxidative stress and pro-inflammatory conditions are closely related. Activation of immune cells generates free radicals and induces inflammation. Excessive adipose tissue produces pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), that cause oxidative stress. TNF-α binds with specific receptors promoting NF-κB signaling and induces reactive oxygen species (ROS) that promote oxidative stress [10]. According to Viuda-Martos

*et al*., lower serum carotenoid concentrations, including beta-carotene, are cardiovascular risk factors in obesity [11]. Beta-carotene can scavenge ROS such as singlet oxygen, superoxide anion ($\bullet O_2^-$), hydroxyl (•OH), peroxyl (RCOO•), and nitric oxide (NO) [12], and also can inhibit TNF-$\alpha$, and other inflammatory genes' expression in macrophages and animals induced by lipopolysaccharides [13]. Therefore, this study used beta-carotene as a component of the snack to prevent or reduce lipid oxidation and inflammation that are commonly found in obesity.

In addition, resistant starch has been shown to improve lipid profile. Fuentes-Zaragoza *et al*. reported that resistant starch from beans of Adzuki and Tebou could decrease circulating cholesterol through increased hepatic SR-B1 (scavenger receptor class B1) and cholesterol 7alpha-hydroxylase mRNA levels [14]. Resistant starch is a fraction of starch that can be classified as a type of dietary fiber, which is resistant to digestion in the small intestines [15]. In addition, it has multiple non-nutritive health effects that can prevent and reduce the risk of atherosclerosis and cardiovascular disease [16]. Our previous study showed that high fiber and beta-carotene from sweet potatoes and pumpkin inhibit sterol regulatory binding protein 1c (SREBP-1c) in the liver of hypertriglyceridemic rats [17]. Therefore, this study aimed to evaluate the benefits of a snack containing high-resistant starch and beta-carotene in improving atherogenic index and inflammation in individuals obese.

## Materials and Methods

### *Participants*

This study uses a single-blinded and randomized controlled design that involved either men or women (body mass index (BMI) ≥ 25 kg/m$^2$) between the ages of 25 and 56 years old which were voluntarily and randomly divided as control and treatment groups (n = 55). The subjects' exclusion criteria were as follows: Pregnancy, smoking, and using antidiabetic or weight loss treatments. The study was conducted according to the established guidelines of the Helsinki Declaration, and the ethical clearance was approved by the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia. All subjects gave written informed consent before participating in the study.

### *Dietary compliance*

Participants consumed 42 g of snacks per day, either tested snacks or standard snacks, for 6 weeks. The tested snack was made of arrowroot, pumpkin, sweet potato, and cassava, whereas the standard snack was made of wheat flour and cassava.

During the study, the participants were suggested to maintain their usual diet and living habits. The composition of both snacks is listed in Table 1.

**Table 1: Nutrient composition of the snacks per 100 g**

| | Standard snacks (100 g) | Test snacks (100 g) |
|---|---|---|
| Carbohydrate (%) | 72 | 71.7 |
| Protein (%) | 1.2 | 4.1 |
| Lipid (%) | 20.2 | 16.8 |
| Water (%) | 4.8 | 5.4 |
| Ash (%) | 1.7 | 2.1 |
| Beta-carotene (mg) | 2.11 | 3.52 |
| Soluble dietary fiber (%) | 0.2 | 0.8 |
| Insoluble dietary fiber (%) | 11.7 | 8.7 |
| Resistant starch (%) | 3.6 | 9.5 |

### *Anthropometrics*

Anthropometry and body compositions were measured before and after the intervention. The anthropometry data included body weight and height used to measure BMI. The body compositions were body age, body fat and visceral fat percentage, and resting metabolism, with subcutaneous fat percentage (whole body) and subcutaneous muscle percentage (whole body) measured by KARADA Scan (Omron, HBF-375). Height was measured with a wall-mounted stadiometer while the participants were barefoot.

### *Biochemical analyses of fasting blood samples*

After fasting 8–10 h overnight, 5 mL of whole blood were drawn from the antecubital vein and added into an EDTA tube. The tube was left for 15 min at room temperature, then centrifuged at 3000 rpm for 15 min to obtain the plasma. The plasma was divided into three tubes for analysis of lipid profile, TNF-$\alpha$, and ox-LDL levels. Lipid levels were analyzed by Cobas C111 Analyzer (Roche). Plasma TNF-$\alpha$ and ox-LDL levels were determined by enzyme-linked immunosorbent assay (ELISA) methods (FineTest, Wuhan, China). The atherogenic index was calculated using a formula: Log triglyceride/high-density lipoprotein (TG/HDL) [18].

### *Statistical analyses*

The data were presented as mean ± standard deviation (SD). If the data had a normal distribution, they were analyzed by paired sample t-test, independent sample t-test, and Pearson correlation test. The data were analyzed by Wilcoxon, Mann–Whitney, and Spearman correlation tests if the data had no normal distribution. $p < 0.05$ was considered statistically significant. Independent sample t-tests were used to determine the effect of the intervention between each group. Paired t-tests were used to determine the difference before and after the intervention in the

groups, and Pearson tests were used to determine the correlation between variables.

## Results

The results in Table 2 show that neither the consumption of standard snacks nor test snacks (high-resistant starch and beta-carotene snacks) for 6 weeks statistically significantly influenced the value of anthropometry and body composition of obesity subjects ($p > 0.05$).

Lipid profile analysis indicated that the test snacks with high-resistant starch and beta-carotene only significantly decreased the LDL levels by 9.8%. The delta of LDL before and after consuming it was also significantly different from the delta of standard snacks. Although not statistically significant, the test snacks could reduce triglyceride levels by 5.9%, which may lead to a decrease of AIP by 9.3%, which was statistically significant after the test snack consumption ($p < 0.05$). In addition, based on the independent sample t-test, we found that the mean difference in AIP value before and after snacks consumption between subjects who consumed standard snacks and tested snacks was significantly different ($p < 0.05$) (Figure 1).

Based on the Pearson correlation test, AIP value was positive, strong and significantly correlated with triglyceride levels (in the standard snacks $r=0.81$, $p < 0.05$; and in the test snack $r=0.76$; $p < 0.05$), and it was negatively correlated with HDL levels (in the standard snacks, $r = –0.53$; $p < 0.05$ and in the test snack, $r = –0.62$; $p < 0.05$) (Figure 2).

According to the Pearson correlation test, the AIP value and ox-LDL levels were positive, weak, and no significant correlation in both the standard snack group ($r = 0.44$; $p > 0.05$) and the test snack group ($r=0.43$; $p > 0.05$). However, AIP value and LDL levels had a positive and moderate correlation ($r=0.52$; $p<0.05$) in the standard snack group and a negative, very weak, and not significant correlation ($r = –0.16$; $p > 0.05$) in the test snack group (Figure 3).

The subjects' TNF-$\alpha$ before and after consumption standard and tested snack are presented in Figure 4. According to these results, we observed consumption of the test and standard snacks for 6 weeks might decrease the level of TNF-$\alpha$. Both standard snacks and test snacks could reduce TNF-$\alpha$ levels, but the reduction of TNF-$\alpha$ was higher in the test snack group (22.3%) than those in the standard snack group (9.05%) (Figure 4).

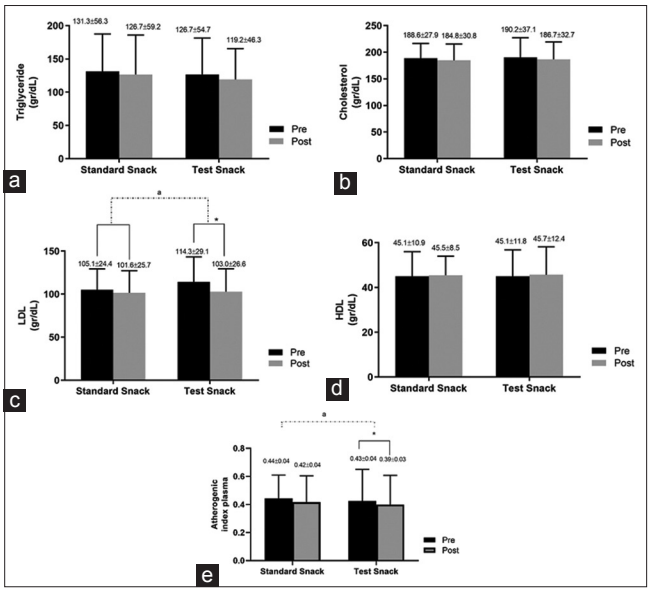


*Figure 1: The subjects' lipid profile and AIP before and after snack consumption. Data were analyzed using paired sample t-test and presented as mean ± standard deviation (SD). The symbol * indicates significantly different ($p < 0.05$) before and after test snack consumption, and [a] indicates the significantly different ($p < 0.05$) mean difference between the standard snacks and test snacks groups according to independent sample t-test*

According to the Pearson correlation test, there was a positive and strong correlation between TNF-$\alpha$ levels and ox-LDL in the standard snack group ($r=0.95$; $p < 0.05$) and in the test snack group ($r = 0.90$; $p < 0.05$) (I). The TNF-$\alpha$ levels also had a positive but weak correlation with AIP in the standard snack group ($r=0.46$; $p < 0.05$) and in the test snack group ($r = 0.39$; $p > 0.05$) (II) (Figure 5).

## Discussion

This present study found the consumption of high-resistant starch and beta-carotene snacks did not change the subjects' anthropometry and body

**Table 2: Changes in subjects' anthropometry and body composition before and after intervention**

| Characteristics | Standard snack | | | | Test snack | | | | p-value[#] |
|---|---|---|---|---|---|---|---|---|---|
| | Pre-intervention | Post-intervention | Δ | p-value* | Pre-intervention | Post-intervention | Δ | p-value* | |
| Body weight (kg) | 76.3 ± 15.7 | 76.7 ± 15.5 | 0.45 ± 1.4 | 0.154 | 78.6 ± 18.6 | 78.9 ± 18.4 | 0.37 ± 1.2 | 0.109 | 0.83 |
| BMI (kg/m$^2$) | 30.4 ± 4.7 | 30.7 ± 4.7 | 0.26 ± 0.7 | 0.095 | 30.7 ± 5.0 | 30.8 ± 5.0 | 0.17 ± 0.5 | 0.082 | 0.61 |
| Body age (years) | 54.3 ± 9.5 | 54.7 ± 9.1 | 0.45 ± 1.8 | 0.149 | 52.9 ± 10.3 | 53.1 ± 10.1 | -0.37 ± 1.6 | 0.257 | 0.46 |
| Resting metabolism (calorie) | 1527.3 ± 270.7 | 1531.3 ± 268.6 | 4.0 ± 16.7 | 0.149 | 1612.3 ± 343.5 | 1616.6 ± 340.6 | 4.23 ± 20.8 | 0.270 | 0.955 |
| Body fat (%) | 35.6 ± 5.6 | 35.9 ± 5.4 | 0.27 ± 0.8 | 0.154 | 34.6 ± 5.2 | 34.7 ± 5.1 | 0.13 ± 0.9 | 0.459 | 0.576 |
| Visceral fat (%) | 13.3 ± 6.2 | 13.5 ± 6.0 | 0.25 ± 0.7 | 0.135 | 14.4 ± 6.6 | 14.6 ± 6.6 | 0.2 ± 0.7 | 0.095 | 0.937 |
| Subcutaneous fat whole body (%) | 31.5 ± 7.5 | 31.6 ± 7.5 | 0.14 ± 0.6 | 0.331 | 29.3 ± 7.4 | 29.4 ± 7.5 | 0.1 ± 0.7 | 0.457 | 0.807 |
| Subcutaneous muscle whole body (%) | 24.0 ± 3.4 | 23.7 ± 3.3 | -0.25 ± 0.57 | 0.065 | 25.2 ± 3.5 | 25.2 ± 3.4 | 0.30 ± 0.50 | 0.747 | 0.158 |

BMI: Body mass index. The value presented as mean ± standard deviation (SD). p value* indicates $p > 0.05$ according to paired sample t-test. p-value[#] indicates a significant difference from mean between each group ($p > 0.05$) according to the independent sample t-test.

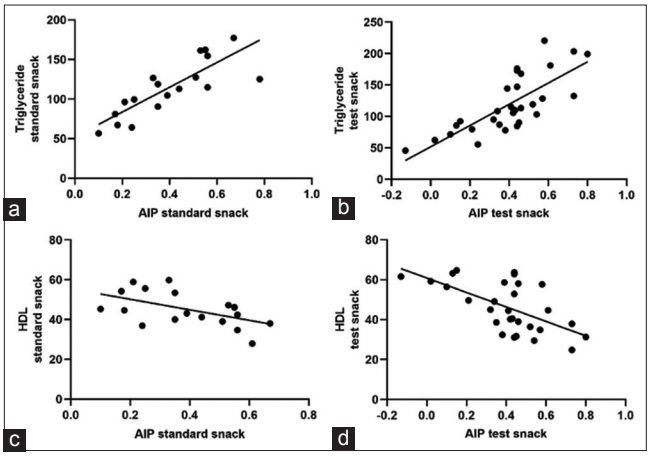


*Figure 2: Correlation between AIP and triglyceride levels in the standard and test snack groups (a and b). Correlation between AIP and HDL levels in the standard and test snack groups (c and d)*

composition (Table 1), triglyceride, cholesterol, nor HDL levels, but it significantly lowered the levels of LDL and AIP (Figure 1). Decreased LDL levels could be the effects of high soluble fiber and resistant starch in the test snacks. Each 100 g of the standard snack contained 0.2% soluble fiber and 3.6% resistant starch, whereas the test snacks contained 0.8% soluble fiber and 9.5% resistant starch. In humans, fiber and resistant starch are resistant to intestinal digestive enzymes [19], and the potential benefits of resistant starch for health are similar to the benefits of soluble fiber related to the improved function of the digestive tract, microbiome, circulating cholesterol, and glycemic index of food [14]. In the gastrointestinal tract, the fiber and resistant starch can trap cholesterol and bile which are then excreted through feces, leading to decreased absorption of the cholesterol and bile, thereby stimulating the liver to synthesize bile acid using circulating cholesterol as a precursor. LDL is a major transporter of cholesterol in the circulation, so increased cholesterol uptake by the liver leads to decreased circulating LDL levels. In addition, resistant starch from Adzuki and Tebou beans were reported to decrease circulating cholesterol through increased hepatic SR-B1 (scavenger receptor class B1) and cholesterol 7alpha-hydroxylase mRNA levels [14].

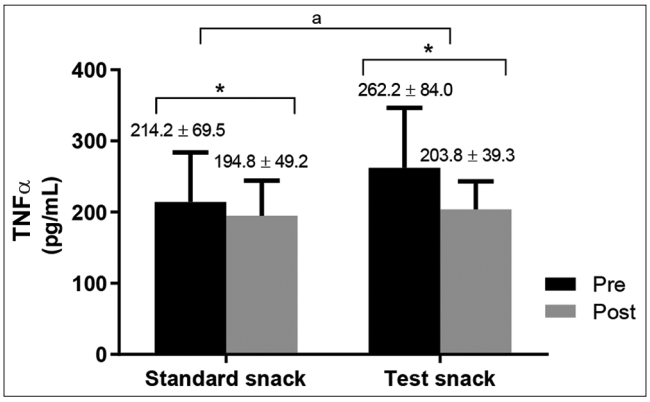


*Figure 4: The levels of TNF-α before and after the intervention of snacks. Data were analyzed using paired sample t-test and presented as mean ± standard deviation (SD). The symbol * indicates significantly different ($p < 0.05$) before and after test snack consumption and [a] indicates the significantly different ($p < 0.05$) mean difference between the standard snack and the test snack groups according to the independent sample t-test*

In this study, although the high-resistant starch and beta-carotene in the snack did not significantly decrease triglyceride levels nor increase HDL, it gave the benefit by reducing the AIP, which means it can reduce the risk of cardiovascular diseases, especially atherosclerosis. According to Lafta [5], atherosclerosis and/or hypertension are the most common causes of cardiovascular diseases related to obesity. The previous study has reported that AIP is positively and strongly associated with obesity and can be used as a direct biological target for obesity prevention and treatment. Furthermore, AIP is usually used as an indicator of dyslipidemia and related diseases and consists of triglycerides and LDL [20]. According to Lafta [5], as an AIP which indicates plasma atherogenicity, the ratio of triglycerides to HDL is shown as a good predictive value for future cardiovascular events and a significant independent predictor of cardiovascular diseases. In this study, the AIP was calculated using the formula: Log (TG/HDL) [18], and the AIP value

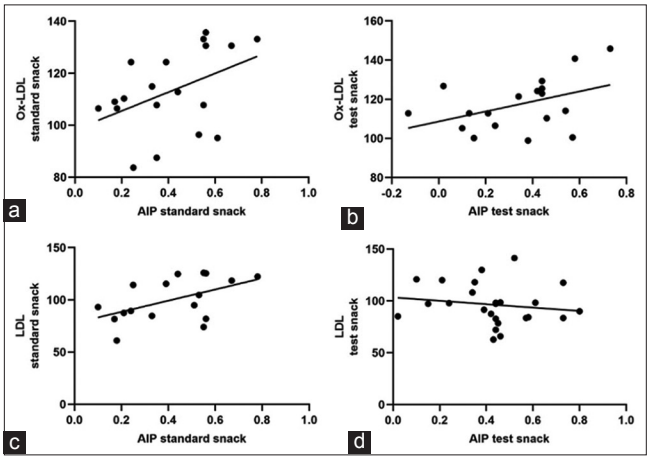


*Figure 3: Correlation between AIP and ox-LDL levels in the standard and test snack groups (a and b). Correlation between AIP and LDL levels in the standard and test snack groups (c and d)*

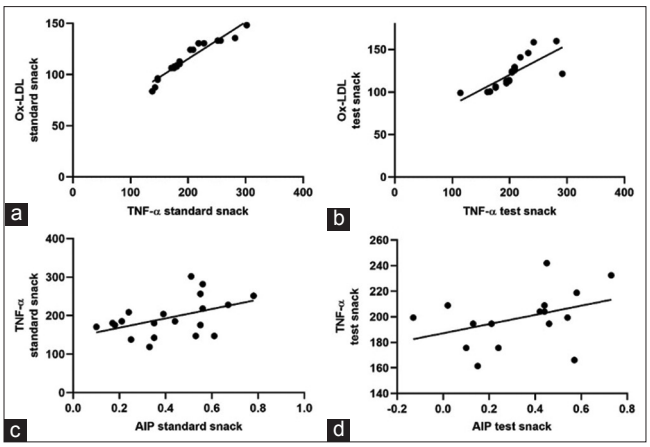


*Figure 5: Correlation between TNF-α and ox-LDL levels in the standard and test snack groups (a and b). Correlation between AIP and TNF-α levels in the standard and test snack groups (c and d)*

was also strongly positively correlated with triglyceride levels and moderately negatively correlated with HDL levels (Figure 2). In obesity, high triglyceride levels are caused by an increase of free fatty acid (FFA) in the liver. Once accumulated, it increases synthesis of very low-density lipoprotein (VLDL). High circulating triglyceride levels are also caused by decreased mRNA expression of lipoprotein lipase (LPL) in adipose tissue and LPL activity in skeletal muscles and competition for lipolysis between VLDL and chylomicrons, which impair lipolysis of VLDL. In addition, hypertriglyceridemia stimulates an increased exchange of cholesterol esters and triglycerides between VLDL and HDL and LDL by cholesteryl ester-transfer-protein, which decreases HDL levels and triglyceride content in LDL. Therefore, in obesity, there are elevated triglyceride levels and a decreasing of the HDL level that can be used to predict the atherogenesis risk. Besides that, hepatic lipase removes triglycerides and phospholipids from LDL to form triglyceride-depleted small dense LDL [6].

LDL is a risk factor for atherogenesis when it undergoes oxidation. Ox-LDL stimulates the development of atherosclerotic plaque and destabilization. Figure 3, in the present study, indicates a positive correlation between ox-LDL levels and AIP, although it was weak and not significant. Ox-LDL has a pro-atherogenic role through several mechanisms: (1) Chemotactic and proliferating actions on monocytes/macrophages, stimulating their transformation into foam cells; (2) stimulation of smooth muscle cells (SMCs) recruitment and proliferation in the tunica intima; and (3) eliciting endothelial cells, SMCs, and macrophages apoptosis with ensuing necrotic core development [21]. On the other hand, this study found a significant, positive correlation between LDL and AIP values only in subjects consuming the standard snacks ($p<0.05$), but not in those who consumed the high-resistant starch and beta-carotene snacks. The different correlations of both of the snacks may be related to the high content of beta-carotene in the test snacks. The high beta-carotene in the test snacks can prevent the negative effects of LDL by inhibiting the oxidation of LDL. Beta-carotene is a major carotenoid in vegetables and fruits with antioxidant effects [22]. The antioxidant activity of beta-carotene is related to its ability to scavenges ROS such as singlet oxygen, superoxide anion ($\bullet O_2^-$), hydroxyl (•OH), peroxyl (RCOO•), and nitric oxide (NO) [12]. Many studies in animals reported the benefit of antioxidants in preventing oxidative stress and atherosclerosis. Cohort studies demonstrate an association between ox-LDL and cardiovascular events. Some studies showed antioxidants including carotenoids (alpha- and beta-carotene, lutein, lycopene, zeaxanthin, and β-cryptoxanthin) could prevent atherosclerosis. According to D'Odorico *et al*., the levels of alpha- and beta-carotene plasma were inversely associated with the prevalence of atherosclerosis in the carotid and femoral arteries [23]. Consumption of beta-carotene rich containing foods has been highly recommended to get a sufficient antioxidant intake and lower the risk of chronic diseases [24]. The potential of the preventive benefit of beta-carotene could be reached by the plasma containing 0.4 μmol/L beta-carotene that can be achieved by consumption of 2–4 mg/d beta-carotene [25]. In this study, the content of beta-carotene per 100 g of the standard snack is 2.11 mg, and in the test snack, it is 3.52 mg. The subjects consumed 42 g of snacks per day, so they get beta-carotene 0.89 mg from the standard snacks or 1.48 mg from the test snacks per day.

One characteristic of obesity is excessive fat mass produced adipose tissue that leads to chronic low-grade inflammation. Inflammatory mainly is caused by infiltrated macrophages producing pro-inflammatory cytokines such as TNF-α, which disturbs the function of adipocytes. In obesity, hypoxia condition contributes to dysfunction and oxygenation changes in adipose tissue that affects the metabolic homeostasis [26]. According to Goossens *et al*., dysfunction of adipose tissue contributes to chronically low-grade inflammation, and hypoxia may stimulate inflammation [27]. Many studies showed, in rodent models of obesity, that oxygen partial pressure at adipose tissue is reduced [28]. Our study indicates a significant reduction of TNF-α as a biomarker of inflammation by 22.3% in subjects consuming the test snacks and 9.05% in those who consumed the standard snacks (Figure 4). According to Kawata *et al*., beta-carotene can trap free radicals only at a low oxygen partial pressure [12].

Therefore, our results may be related to the role of beta-carotene in the prevention of LDL oxidation, such as in Figure 5 that shows a positive and strong correlation between ox-LDL and TNF-α levels (standard snack; $r = 0.95$; $p < 0.05$ and test snack; $r = 0.90$; $p < 0.05$). These results are in line with Santiago-Fernández *et al*. that showed in both non-obese and obese subjects, a significant elevation of TNF-α due to ox-LDL which was dependent on dose [29]; however, secretion of TNF-α was higher in adipocytes from obese than non-obese subjects. Mounien *et al*. reported that adipose tissue is the main storage site for carotenoids, and obese individuals were reported as having low circulating carotenoids [30]. The carotenoids supplementation can modulate immune responses involving stimulating macrophages activity and cytokine production. In addition, a positive correlation between TNF-α and AIP value was found significantly only in the subjects consuming the standard snacks ($p < 0.05$) and not in the test snacks ($p > 0.05$). TNF-α is a key regulator response of inflammatory that is related to atherosclerosis. One study found that the anti-TNF-α therapy inhibits Western diet-induced atherosclerosis in hyperlipidemic mice [31]. Bai *et al*. reported that beta-carotene inhibited TNF-α, and other inflammatory gene expressions in macrophages and animals induced by lipopolysaccharides [13].

In addition to the small number of participants and short duration, another limitation of this study

was the use of the SQ-FFQ assessment of dietary consumption based on the subject's memory. The 6-week dietary recall may result in inaccuracy when determining the amount of fiber consumed. A longer, more controlled prospective study with a larger sample population is needed to confirm our findings.

## Conclusions

The snacks containing high-resistant starch and beta-carotene reduced AIP and inflammation by preventing LDL oxidation. Consuming the tested snacks significantly decreased LDL, AIP, and TNF-$\alpha$ ($p < 0.05$). There were positive correlations between AIP and triglycerides in both snacks ($p < 0.05$), LDL or TNF-$\alpha$ in the standard snacks ($p < 0.05$), and TNF-$\alpha$ and ox-LDL in both snacks ($p < 0.05$). High-resistant starch in the test snacks may increase excretion of cholesterol and bile that stimulate the liver synthesis of bile acid from circulating cholesterol and lead to decreasing LDL levels as a major transporter of cholesterol in circulation. High beta-carotene content in the test snacks can prevent LDL's adverse effects through inhibiting LDL oxidation in stimulating AIP and inflammation.

## Acknowledgments

We would like to thank Yunina Ratnasari, Fatma Syukrina, and Fadlillah Zahra Murti.

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