The Galohgor Nutraceutical Cookies Effects on β -Carotene Serum and Oxidative Stress of Postpartum Mothers

Bahriyatul Ma'rifah, Katrin Roosita, and Tiurma Sinaga

Department of Community Nutrition, Faculty of Human Ecology, IPB University, Bogor, West Java 16680, Indonesia

ABSTRACT: The aim of the present study was to analyze the galohgor nutraceutical cookies effects on β -carotene serum levels and oxidative stress of postpartum mothers. To this end, a post-controlled experiment was carried out. Nineteen subjects were recruited to receive 40 g of galohgor cookies (GC) or control cookies (CC) daily for 14 days. Analysis of covariance was applied to assess the effect of the intervention. The results showed that β -carotene serum concentrations were significantly higher in the GC group compared with the CC group ($0.141 \pm 0.094 \mu\text{mol/L}$ vs. $0.106 \pm 0.051 \mu\text{mol/L}$, $P < 0.05$). Meanwhile, malondialdehyde levels of GC group was significantly lower than that of the CC group ($0.82 \pm 0.25 \text{ nmol/L}$ vs. $0.93 \pm 0.27 \text{ nmol/L}$, $P < 0.05$). These results suggest that galohgor nutraceutical may have beneficial effects on improving β -carotene serum levels and oxidative stress of postpartum mothers.

Keywords: β -carotene, galohgor, nutraceutical, oxidative stress, postpartum

INTRODUCTION

The postpartum period is a critical and neglected phase in the lives of mothers, which helps determine the well-being of the mothers and their newborns (World Health Organization, 2013). According to Castillo et al. (2006), onset of lactation is indicative of specific metabolic adaptations that underlie mobilization of lipid and protein reserves. Increasing lipid mobilization to satisfy the increased energy requirement for milk production disrupts several inflammatory and immune functions and promotes production of reactive oxygen species (ROS). Physiologically, excessive concentrations of ROS are counteracted by the natural anti-oxidant system. Whenever this equilibrium is broken, progressive oxidation of other biological substrates including proteins, lipids, RNA, and DNA occurs, which help establish oxidative stress that may contribute to health disorders in lactating mothers (Kumar et al., 2015; Moolchandani and Sareen, 2018). The delivery method and gestational age influence placental oxidative stress and are associated with the oxidative condition of mothers (Hung et al., 2011; Mocatta et al., 2004). A previous study showed that maternal oxidative stress levels (d-ROM levels) gradually decreased over the 3-month postpartum period, however the concentrations remained high (Kuramoto and Kitagawa, 2012; Kuramoto

and Kitagawa, 2017). In recent years, oxidative stress has been shown to be involved in the exacerbation of autoimmune disease in the postpartum state (Kuroda et al., 2010). In the early postpartum stage, lower antioxidant statuses are observed in maternal plasma and there is a positive correlation between antioxidant status in maternal plasma and breast milk (Kuramoto and Kitagawa, 2017).

Oxidative stress can be prevented or reduced through antioxidant phytochemicals present in fruit, vegetables, cereal grains, and medicinal plants. These beneficial effects are due to the antioxidant compounds, such as carotenoid and flavonoids, which may protect key biological sites from oxidative damage (Zhang et al., 2015). Galohgor nutraceutical is a traditional polyherbal made from 56 kinds of plants, containing bioactive compounds, β -carotene, and other nutrients. Our previous study demonstrated galohgor nutraceuticals are beneficial for improving the health status and milk production of Sundanese people in West Java (Roosita et al., 2003; Dahlianti et al., 2005; Roosita et al., 2008). In an animal study, Leatemia (2010) reported that galohgor significantly increases endogenous antioxidant superoxide dismutase (SOD) and reduces malondialdehyde (MDA) plasma. In another study examining type 2 diabetes mellitus patients, nutraceutical galohgor was shown to reduce vis-

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Correspondence to Katrin Roosita, Tel: +62-812-1812-643, E-mail: kroosita2@apps.ipb.ac.id

Author information: Bahriyatul Ma'rifah (Graduate Student), Katrin Roosita (Professor), Tiurma Sinaga (Professor)

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ceral fat and oxidative stress biomarkers (Setyaningsih et al., 2017; Damayati et al., 2018). However, despite various studies that indicate the potential effect of galohgor on diabetes treatment, the effect of galohgor consumption on levels of oxidative stress in postpartum mothers has not been revealed. Thus, the present study aims to analyze the effect of galohgor cookies (GC) on β -carotene serum levels and markers of oxidative stress in postpartum mothers.

MATERIALS AND METHODS

Study design and subjects

This was a post-controlled experimental study conducted in Bogor, West Java, Indonesia. Prospective subjects were identified by involving midwives working at clinics and Primary Health Care Centers. Briefly, the characteristics of the recruited postpartum mothers were as follows: aged 26 and 40 years, had a single pregnancy with a normal delivery, multiparous, apparently healthy, had no medical conditions, or complications during previous pregnancies or deliveries, do not take any medication or regular basis, do not drink or smoke regularly, are able to communicate well, and intended to breastfeed their infants exclusively for at least 6 months. The study protocol was approved by the Research Ethics Committee of IPB University, Indonesia (No.081/IT3.KEPMSM-IPB/SK/2018). All subjects provided written informed consent before participating in the study.

Intervention

A total of 19 eligible subjects were assigned to receive either 40 g GC (n=9) or CC (n=10) daily for 14 days. Galohgor was made according to Roosita et al. (2019) and is already registered on Indonesian patent number IDP000058958. Furthermore, GC were made by CV Nutrasetikal Galohgor (NutriLaktasi, Jakarta, Indonesia) using a cookie formulation that has been acknowledged as a household food production unit. The ingredients for GCs were: galohgor powder, sago flour, margarine, powdered sugar, egg yolks, milk powder, coconut milk, salt, and water. The tools used for cookie production were: mixers, basins, ovens, trays, prints, and scales. CCs was prepared without galohgor powder. 40 g of GCs containing 4 g galohgor powder provided 208 kcal of energy, 3.2 g of protein, 6.8 g of fat, 30.4 g of carbohydrate, 0.0584 mg of β -carotene, 0.33 mg of vitamin C, 1.86 mg of vitamin E, and 1.06 mg of zinc. Meanwhile, 40 g of CCs provided 203 kcal of energy, 2.6 g of protein, 5.9 g of fat, 31.6 g of carbohydrate, 0.0224 mg of β -carotene, 0.76 mg of vitamin C, 1.81 mg of vitamin E, and 1.09 mg of zinc. The compliance of cookie consumption was assessed in follow-up visits.

Subject's characteristic, anthropometric, and dietary assessments

Obstetric and maternal characteristic data were collected from individual medical records and questionnaires. Anthropometric and dietary assessments were carried out following 14 days intervention. Height was measured with a precision of 1 mm using a stature meter and body weight was measured with minimum clothing to the nearest 0.1 kg using a Camry digital weighing scale (Camry Electronic Co. Ltd., Zhongshan, China). Body mass index (BMI, kg/m^2) was calculated to determine the nutritional statuses of the mothers. Mid-upper arm circumferences were measured to the nearest 1 mm using a plastic measurement tape. Dietary intake was assessed by using a 24 h recall method twice for each subject for nonconsecutive days. The food recalls provided information on portion size using household measures and quantities (g). The photographs and detailed descriptions of portion sizes were also provided using a food model to clarify ambiguous food items. Food intake was analyzed using the Nutrisurvey 2007 software package downloaded from www.nutrisurvey.de. The nutrients selected for analysis included β -carotene, vitamin A, vitamin C, vitamin E, and zinc. Calculation of nutrient intake was based on Indonesian Food Composition Database and US Department of Agriculture, National Nutrient Database for Standard Reference, Release 28 (Bhagwat and Haytowitz, 2015). The average nutrient intake was calculated without including the intake of intervention products. To obtain nutrient adequacy ratios (NAR), the nutrient content of each food was taken and divided by the Indonesian recommended dietary allowance of specific nutrients for lactating mothers (0 to 6 months) aged 19 to 49 years.

Blood measurement

Fasting blood samples were collected under standardized conditions between 8 and 9 am. The samples were immediately storage on ice at 4°C and centrifuged at 4,000 rpm for 12 min to separate the blood serum. Serum samples were then stored at -80°C until analysis. β -Carotene serum levels were measured using reverse phase high-performance liquid chromatography (HPLC) according to the method described by Erhardt et al. (2002). For chromatography analysis, 40 μL serum in 0.5 mL vessels were extracted by addition of 100 μL ethanol-butanol (1:1, v/v) containing 5 mg butylated hydroxytoluene/mL as an internal standard. Lipophilic vitamins were extracted by vigorous mixing for 10 s. The mixture was then centrifuged for 5 min at 12,000 rpm and 20 μL of the supernatant fraction was analyzed using HPLC 515 Pump UV-Vis Detector (Waters Corporation, Milford, MA, USA) results were expressed as $\mu\text{mol/L}$ of serum. The thiobarbituric acid (TBA) method was used to assess serum MDA levels as an indicator of oxidative stress.

TBA, trichloroacetic acid (TCA), and 1,1,3,3-tetraethoxy-propane were purchased from Sigma-Aldrich Chemicals, Co. (St. Louis, MO, USA). Serum (400 μ L) was placed into a tube and 200 μ L of 20% TCA was added. The tube was vortexed until the mixture was homogenously combined. After centrifugation at 5,000 rpm for 10 min, the supernatant was moved into a larger tube. Next, 400 μ L of 0.67% TBA was added to the precipitate, and coupling of lipid peroxide with TBA was performed by boiling in a water bath (96~100°C) for 10 min. After cooling in cold water, the absorbance of the supernatant was measured using a spectrophotometer at 530 nm with 1,1,3,3-tetraethoxy-propane as a standard. TBA reactive substances (TBARS) were expressed as nmol/L.

Statistical analysis

Differences in quantitative variables, according to their distribution, were analyzed with using parametric *t*-tests or non-parametric Mann-Whitney U-tests. The potential effect of GCs on the final outcome variables of the 14 days lactation period were assessed using analysis of covariance (ANCOVA) analysis. Values were reported as

mean $\pm$ standard deviation (SD). In all statistical comparisons, differences with $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Subject' characteristics

Subject characteristics including age, parity, gestational age at delivery, delivery method, nutritional status, and vitamin A status did not significantly differ between the two groups (Table 1). The ages of the subjects ranged from 27 to 38 years. All subjects experienced multiparous and normal delivery. The mean gestational age of GC group and CC group at delivery was 38.8 ± 1.5 weeks and 38.6 ± 1.6 weeks, respectively. Most subjects were classified as obese I, using World Health Organization (2000) cut-off reference values. Regarding vitamin A status, the mean of retinol concentrations were considered adequate based on respective cut-off values (≥ 0.70 μ mol/L) (Gibson, 2005).

Dietary intake

Table 2 shows the estimated dietary intake of antioxidants during the intervention period. No significant differences in intake of β -carotene, vitamin A, vitamin C, vitamin E, and zinc were observed between the two treatment groups. The subject's NAR did not significantly differ throughout the intervention. Inadequate nutrient adequacy ratio NARs of vitamin E and zinc were observed in both treatment groups. However, the NARs of vitamin A and vitamin C were adequate for maintaining their roles in human health, particularly in breastfeeding mothers.

Effect of GCs on β -carotene serum concentrations and oxidative stress

As shown on Fig. 1, the mean serum concentration of β -carotene in the GC group was significantly higher than that of the CC group after being adjusted for parity, BMI,

Table 1. Subject characteristics

Variable	GC group (9 subjects)	CC group (10 subjects)	<i>P</i>
Age (years)	32.4 $\pm$ 4.0	30.7 $\pm$ 3.2	0.308
Parity			0.408
Primiparous	0 (0%)	0 (0%)	
Multiparous	9 (100%)	10 (100%)	
Gestational age at delivery (week)	38.8 $\pm$ 1.5	38.6 $\pm$ 1.6	0.808
Type of delivery			1.000
Vaginal	9 (100%)	10 (100%)	
Caesarian	0 (0%)	0 (0%)	
BMI (kg/m ²)	28.2 $\pm$ 4.6	26.1 $\pm$ 3.1	0.254
MUAC (cm)	28.8 $\pm$ 3.0	27.9 $\pm$ 2.1	0.499
Vitamin A status			
Retinol serum (μ mol/L)	1.17 $\pm$ 0.23	1.31 $\pm$ 0.34	0.312

Results are expressed in n (%) or mean $\pm$ SD.

GC, galohgor cookies; CC, cookies without galohgor; BMI, body mass index; MUAC, mid upper arm circumference.

Table 2. Dietary intake of antioxidants during the intervention period

Nutrient	RDA/d	GC group (9 subjects)		CC group (10 subjects)		<i>P</i>
		Amount of intake	NAR (%)	Amount of intake	NAR (%)	
β -Carotene (mg)	ns	9.2 $\pm$ 7.0	ns	8.0 $\pm$ 6.2	ns	0.683
Vitamin A (retinol) (mcg)	ns	168.6 $\pm$ 106.9	ns	386.7 $\pm$ 536.5	ns	0.870
Vitamin A (RAE)	850	1,049.2 $\pm$ 626.5	123.4	935.8 $\pm$ 657.7	110.1	0.683
Vitamin C (mg)	100	108.8 $\pm$ 94.3	108.8	75.9 $\pm$ 94.1	75.9	0.253
Vitamin E (mg)	19	3.2 $\pm$ 1.3	16.8	4.4 $\pm$ 2.3	23.0	0.194
Zinc (mg)	15	7.0 $\pm$ 2.2	46.3	9.7 $\pm$ 3.4	64.6	0.058

Result are presented as mean $\pm$ SD.

RDA, recommended dietary allowance; GC, galohgor cookies; CC, cookies without galohgor; NAR, nutrient adequacy ratio; RAE, retinol activity equivalents.

ns, not shown.

Fig. 1. β -Carotene serum concentration of the intervention groups. Data are expressed as mean $\pm$ SD. Intervention effect was estimated by using adjusted ANCOVA. *Significantly higher than CC group at $P<0.05$. GC, galohgor cookies; CC, cookies without galohgor.

Fig. 2. Oxidative stress condition of the intervention groups. Data are expressed as mean $\pm$ SD. Intervention effect was estimated by using adjusted ANCOVA. *Significantly lower than CC group at $P<0.05$. GC, galohgor cookies; CC, cookies without galohgor; MDA, malondialdehyde.

MDA serum, β -carotene, and zinc intake. The mean MDA serum level in the GC group was significantly lower than that of the CC group after being adjusted for age, parity, BMI, mid upper arm circumference (MUAC), β -carotene serum, retinol serum, vitamin C, vitamin E, and zinc intake. Subjects who consumed GCs showed less oxidative damage, as reflected by MDA serum, at the end of intervention period compared to those in CC group (Fig. 2).

DISCUSSION

Our results show that subjects who received GC experienced higher β -carotene serum concentrations compared with those in the CC (0.141 ± 0.094 $\mu\text{mol/L}$ vs. 0.106 ± 0.051 $\mu\text{mol/L}$). This is in agreement with a previous animal study, during which β -carotene serum concentrations were significantly increased following administration of galohgor powder (Permana, 2011). According to Roosita et al. (2014), β -carotene is the most carotenoid compound present in galohgor nutraceuticals. Consumption of β -carotene-containing foods is assumed to be reflected by serum concentrations of β -carotene (Prasad et al., 2018). Although the effects of lactation on mobilization of carotenoids from tissue stores cannot be ruled out, it is likely that the elevated serum carotenoids in GC groups were attributable to higher β -carotene intake. In our previous *in vitro* study, we showed that higher β -carotene concentrations have a role in differentiation of the mammary gland cell line via altering its antioxidant activity and expression of connexin and β -casein during the lactation period (Roosita et al., 2014).

Many previous studies have reported the responses of supplementation with β -carotene or vegetable sources. Intake of β -carotene from supplements result in larger increases in plasma levels compared to intake from vegetable (Strobel et al., 2007). Gossage et al. (2000) observed

an 8-fold increase in plasma β -carotene concentrations in breastfeeding women after receiving identical amounts of 30 mg/d of β -carotene for 28 days. Canfield et al. (2001) reported elevated levels of β -carotene in serum after 90 mg/d supplementation of breastfeeding mothers with red palm oil.

Our results show that consumption of GCs significantly lowers oxidative stress compared control following 14 days intervention (0.82 ± 0.25 nmol/L vs. 0.93 ± 0.27 nmol/L). These reductions are clinically relevant with respect to natural ways for improving oxidative stress status. It is important to note that the final outcome variables of this study were adjusted for age, parity, BMI, MUAC, β -carotene serum, retinol serum, vitamin C, vitamin E, and zinc intake. Adjusting these covariates provide a more precise effect estimate. Our findings are also similar to the results of other studies. Setyaningsih et al. (2017) indicated that consumption of 2 g/d of galohgor for 38 days improves profiles of oxidative stress markers in patients with poorly controlled type 2 diabetes. In an experimental study, Leatemia (2010) demonstrated that consumption of 0.375 g/kg body weight for 14 days galohgor has protective effects on the oxidant/antioxidant balance of thirty-two of female rats. Our results demonstrate that GCs had antioxidant capabilities and improve the oxidative status of postpartum mothers. It is possible that the higher levels of serum β -carotene in the galohgor treated group may be due to decreased consumption or utilization of free radical detoxification agents, following reduction in MDA serum levels and improvements in β -carotene serum concentrations.

We showed that galohgor has high levels of antioxidant compounds with free radical scavenger actions, such as β -carotene, vitamin C, vitamin E, iron, zinc, copper, and manganese (Roosita et al., 2003; Masrurah, 2004). β -Carotene is the most efficient provitamin A carotenoid due to its unique structure and cleavage efficacy. As an

antioxidant, β -carotene quenches singlet molecular oxygen and scavenges reactive oxygen species, especially peroxyl radicals (Grune et al., 2010).

The synergism of these nutrient compounds may be effective to stimulate the activity of antioxidant enzymes; these appear to be of great importance for controlling the effects of reactive oxygen species (Marcadenti and Assis Coelho, 2015). Zinc is an essential cofactor of the Cu/Zn-SOD enzyme, catalyzes the dismutation of superoxide radicals ($O_2^{\cdot-}$) into the less harmful O_2 and H_2O_2 , which can then be detoxified by catalase and glutathione peroxidase. Zinc also inhibits nicotinamide adenine dinucleotide phosphate oxidases, causing reduced generation of ROS (Gammoh and Rink, 2017). However, after two weeks of treatment, the oxidative stress status was still higher than standard values. Previous studies have reported that oxidative stress is higher during the early postpartum days, and that it remains high 3 months after giving birth. Oxidative stress therefore requires time to return to normal levels (Kuramoto and Kitagawa, 2017).

This study is the first study investigating the effects of nutraceuticals on the oxidative stress balance of postpartum mothers in Indonesia. However, our study has some limitations; the study had a short study duration of only two weeks, a small sample population, use of a fixed dose of galohgor, and followed a post-controlled design only. In conclusion, postpartum mothers in the galohgor group experienced significantly higher β -carotene serum concentrations and lower levels of oxidative stress than those in the control group, which may indicate that galohgor has beneficial effects on the oxidative stress balance in postpartum periods. Further studies with larger sample sizes and longer follow-up periods are encouraged to elaborate the mechanism of GCs on oxidative stress and milk production.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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