Journal of Basic and Applied Pharmacology
Vol. 3 No. 1 January - June 2023

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Original research article

Effects of carotenoid-rich jelly consumption on changes in blood concentrations of carotenoids and lipid profiles among obese men

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Received 30 May 2023; Received in revised form 9 June 2023
Accepted 11 June 2023; Available online 21 June 2023

ABSTRACT

Excessive fat accumulation leads to the generation of reactive oxygen species, oxidative stress, chronic inflammation, and abnormal lipid signaling pathways that potentially result in metabolic dysregulation. Beta carotene is one of the main provitamin A carotenoids in the diet which has potentially beneficial effects on health. Epidemiological studies have linked high circulating concentrations of beta carotene and metabolic diseases such as obesity, diabetes and cancer. In this pilot randomized controlled trial study, we aimed to determine the effect of carrot jelly consumption on clinical and biochemical parameters related to metabolic syndrome in men with obesity. A group of men with obesity underwent dietary supplementation with carrot jelly rich in beta carotene (N=10) or placebo (N=10) daily for 3 months. The treatment group showed significantly high levels of plasma beta-carotene at 1 month (263.6 ± 1.90 ng/mL) and 3 month (284.6 ± 1.65 ng/mL) after supplementation compared to the baseline (72.4 ± 2.34 ng/mL, with $p<0.05$). After 3 months, the treatment group also showed lower blood pressure; lower plasma low-density lipoprotein cholesterol, triglyceride, high-sensitivity C-reactive protein, and malondialdehyde concentrations; and higher plasma oxygen radical absorbance capacity (all $p<0.05$) compared to baseline. In the follow-up periods, these biochemical markers among the placebo group remained unchanged. Dietary carotenoid supplementation may have beneficial effects in men with obesity and other related metabolic diseases such as dyslipidemia and hypertension.

Keywords: obesity; carotenoid; plasma beta carotene; oxidative stress; high-sensitivity C reactive protein

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1. Introduction

The metabolic syndrome is a significant contributor to mortality in both developed and developing countries, because it comprises insulin resistance, hypertension, and hyperlipidemia; which predispose toward the development of chronic non-communicable diseases (NCDs). A World Health Organization (WHO) report published in 2016 stated that there were 1.9 billion adults aged ≥18 years who were overweight. [1] The percentage of Thai people who are overweight or obese has increased over the last 20 years, as shown by the National Health Surveys (NHS) conducted in 1991, 1997, and 2004. [2] In addition, further increases in the prevalence of obesity in the Thai population occurred, to 33.9% in 2012 and 44.8% in 2018.[3] The factors associated with obesity are advanced age, the presence of chronic metabolic diseases, and lifestyle factors, including smoking, the consumption of >1 cup/week of instant coffee, and high sodium intake.[3] The key pathological changes in obesity are the hyperplasia and hypertrophy of adipocytes, which is associated with adipose tissue dysfunction, leading to systemic oxidative stress, chronic low-grade inflammation, and insulin resistance. These factors exacerbate the dyslipidemia that develops in individuals with obesity.[4]

Obesity is associated with a high plasma concentration of malondialdehyde (MDA), a biomarker of lipid peroxidation. The excessive deposition of intra-abdominal fat (IAF) in individuals with obesity is associated with oxidative stress and insulin resistance, characterized by high concentrations of leptin, 8-iso- prostaglandin F2α, MDA, and fasting insulin; and high HOMA-IR. [5] The plasma high-sensitivity C-reactive protein (hsCRP) concentration is closely associated with abdominal obesity, the metabolic syndrome, cardiovascular disease, and cancer. In addition, a previous study showed a positive correlation between BMI and hsCRP, and an hsCRP concentration of >1.0 mg/L is present in approximately 40% of individuals with BMI ≥ 25 kg/m^2 and in 75% of those with BMI ≥ 30 kg/m^2.[6] In this study, 20% of the male participants with abnormal BMI and blood pressure, and dyslipidemia, had hs-CRP concentrations >1.0 mg/L.[6]

A large number of studies have shown beneficial effects of phytochemicals for the prevention of chronic metabolic diseases. Carotenoid-rich foods are principally fruit and vegetables, including carrots, sweet potatoes, tomatoes, and pumpkins. The phytochemicals derived from these sources have a pro-vitamin A activity, are antioxidants, have anti-inflammatory effects, regulate the immune response and lipid metabolism, and improve intercellular communication. [7] The carotenoids consist of β-carotene, α-carotene, lycopene, lutein, and zeaxanthin, and each has specific characteristics. For example, beta carotene can be converted to vitamin A,[7] similar to lutein and zeaxanthin, which have been shown to reduce the release of cytokines secondary to oxidative stress and proinflammatory gene expression in many ocular diseases.[8] The relationships between carotenoid supplementation and metabolic disease have also been investigated in previous studies. For example, daily carrot juice drinking (470 mL) by patients with dyslipidemia for 3 months caused a significant increase in total antioxidant capacity (0.81 ± 0.04 mM vs. 1.03 ± 0.04 mM) and a reduction in plasma MDA concentration (42 ± 6 μM vs. 18 ± 6 μM) . [9] In addition, 28 days of dietary supplementation with mixed fruit and vegetable juice significantly reduced the concentrations of markers of oxidative stress (serum lipid peroxides and urine 8OHdG) and increased the serum concentrations of beta carotene, lycopene, and alpha tocopherol. [10] These results are consistent with carotenoids and other bioactive compounds derived from fruit and vegetables reducing cellular oxidative damage. However, a large trial showed no beneficial effects of beta carotene (30 mg/day) or vitamin A (25,000 IU of retinol/ day) supplementation for 4 years.[11] Therefore, we aimed to perform a

pilot study to evaluate the effects of carrot jelly with beta carotene on the plasma beta carotene, vitamin, and antioxidant concentrations, and on other clinical and metabolic parameters, in Thai men with obesity.

2. Materials and Methods

2.1 Study design, setting, and participants

A pilot randomized, placebo-controlled study was conducted to evaluate the effects of carotenoid-rich jelly versus those of placebo in men aged 25–40 years, with a BMI ≥ 30 kg/m^2. Individuals with a medical history of cancer, autoimmune disease, thyroid disease, chronic liver or kidney disease, or metabolic syndrome, and who were undergoing medical treatment, were excluded. Using a randomization table created using validated computer software, each participant was randomly assigned to a treatment or a placebo group. Participants in the treatment group consumed one cup of carrot jelly containing beta carotene (10 mg/200 mL) once daily for 3 months, and those in the placebo group consumed similar jelly lacking the beta carotene. During the three months of study, all participants were asked to avoid any supplementation. Every week, research staff called each participant to keep the same dietary pattern for monitoring other confounding factors related to dietary intake. The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of Faculty of Medicine Ramathibodi Hospital, Mahidol University (COA.MURA2021/761). Informed consent was obtained from all the participants in the study.

2.2 Data collection and blood collection

All the participants underwent a comprehensive physical examination and laboratory testing, and structured questionnaires were used to collect information regarding their demographics, medical history, and lifestyle factors. Blood samples were collected at baseline and after 3 months of treatment from an antecubital vein after more than 12 hr of fasting. The fasting blood glucose (FBG) , HbA1c, triglyceride (TG) , total cholesterol (TC) , high-density lipoprotein-cholesterol (HDL-C) , and low-density lipoprotein-cholesterol (LDL-C) concentrations were measured using automated methods (Cobas Mira, Roche Diagnostics, Basel, Switzerland) . The plasma high-sensitivity C-reactive protein (hsCRP) concentration was measured using an immunoturbidimetric method (Roche Diagnostics, Indianapolis, IN, USA).

2.3 Plasma MDA concentration

The plasma MDA concentration was measured by high-performance liquid chromatography (HPLC) using an isocratic method on an Agilent 1200 HPLC system (Agilent Technologies, USA) and a commercial MDA kit (Immundiagnostik AG, Germany). The pH was optimized, then 20 µl of plasma sample were injected into the HPLC apparatus. HPLC separation was performed using an isocratic process at 30℃ on a reversed-phase column over 4 min. The flow rate was 0.8 mL/min. Fluorimetric detection was performed using excitation at 515 nm and emission at 553 nm. The MDA concentrations of the samples were estimated by integrating the peak heights and comparing these with that corresponding to a calibrator solution. The detection limit was 0.15 µmol/L.[12]

2. 4 Plasma vitamin A (retinol) and E concentrations

The plasma vitamin A (retinol) and E concentrations were measured by re-versed-phase HPLC. A commercially available kit was used for sample preparation (Chromsystems Instruments and Chemicals GmbH, Munich, Germany). First, 200 µL plasma, 20 µL internal standard, and 25 µL precipitation reagent I were mixed and vortexed for 30 s. Then, 400 µL precipitation reagent II was added to each vial, which was vortexed again for 30 s, then centrifuged at 9,000 × g for 10 min. Next, 50 µL of each supernatant were injected into the HPLC system. The HPLC parameters were: run time, approximately 9 min; flow rate, 1.5 ml/min; column temperature, ambient

(~25°C) ; gradient, isocratic; wavelength, 325 nm, then after 3.5 min, 295 nm.[13] The coefficient of variation within a run was found to be about 3.5% and from run to run about 5.4%.

2.5 Plasma beta carotene concentration

Plasma beta carotene concentration was determined using an isocratic HPLC system and the samples were prepared using a commercially available kit (Chromsystems Instruments and Chemicals GmbH). First, 100 µl plasma and 50 µl internal standard were mixed briefly, after which 50 µl Precipitation Reagent were added and the mixture was vortexed. Next, 200 µl Extraction Buffer were added and the mixture was vortexed for ~30 s, then centrifuged for 10 min at 16,000 × g. The supernatants (50 µl each) were injected into the HPLC system. The HPLC parameters were: run time, ~10 min, flow rate 1.5–1.8 ml/min; column temperature, ambient (~25°C); gradient, isocratic; wavelength, 453 nm.[14] The coefficient of variation within a run was found to be about 2.4% and from run to run about 3.5%.

2. 6 Plasma oxygen radical absorbance capacity

To measure plasma oxygen radical absorbance capacity (ORAC), the method described by Aebischer et al. was used.[15] First, 100 µL of plasma, 200 µL of ethanol, and 100 µL of water were added to a glass tube and mixed, then 400 µL of hexane was added and the contents were mixed again. After 2 minutes, the mixture was centrifuged for 5 min at 14,000 rpm, and the hexane layer was transferred to an amber tube and dried down under nitrogen. Following this, 400 µL of 0.5 M perchloric acid were added to precipitate the protein, followed by centrifugation for 5 min at 14,000 rpm. Next, 160 µL of supernatant were added to 840 µL of phosphate buffer and mixed, and a 40-µL aliquot of the diluted sample was placed into a well of a 48-well microplate. Four hundred microliters of fluorescein solution were then added to each well, followed by 150 µL of AAPH (17.2 mg/mL, 9.4 µmol/well). The fluorescence intensity of each well was then measured at 485 nm and 520 nm every 2 min for 60 min, and the areas under the curves were calculated. The coefficient of variation within a run was found to be about 3.5% and from run to run about 4.8% . The data are expressed as µmol Trolox equivalents per liter or per gram of sample.[16]

2. 7 Preparation of the carotenoid-rich carrot jelly and its composition

Carrots were extracted with water at a volume ratio of 1:0.5 and then filtered through cheesecloth. The carrot juice obtained was gently heated and stirred regularly, then 5% gelatin was added, followed by 6% sorbitol and 0.025% sucralose. The mixture was stirred constantly, and the temperature was increased to 80°C for 60 sec. Finally, 0.4% citric acid, 0.1% orange flavoring, and 0.01% orange color were added; and the preparation was poured into plastic cups (200 mL, 7.1 × 8.0 cm) and their lids were closed and heat-sealed. The jelly obtained was immediately cooled in an ice bath and then stored at 5°C. The placebo was prepared similarly, except that the carrot juice was replaced by water and a clouding agent was added.

The soluble fiber, insoluble fiber, protein, fat, carbohydrate, moisture, and ash contents of the jelly were determined using standard AOAC methods.[17] The energy content was calculated by adding the amounts contributed by the protein, fat, and carbohydrate. The beta carotene content of the jelly was measured by HPLC, as previously described[18], and this yielded a mean beta carotene content of 10.23 ±2.15 mg/200 mL jelly, as shown in Table 1.

Table 1. Nutritive values of carotenoids-rich carrot jelly and orange flavor jelly (placebo) products.

Products (per cup or 200 mL)	Carotenoids-Rich Carrot Jelly	Orange Flavor Jelly (Placebo)
Soluble fiber (g)	0.5±0.07	<0.05
Insoluble fiber (g)	0.8±0.08	<0.05
Protein (g)	8.6±0.13	8.0±0.28
Fat (g)	0.24±0.03	<0.1
Carbohydrate (g)	15.5±0.42	14.3±0.28
Moisture%	74.6±0.9	77.3±1.1
Ash	0.62±0.01	0.38±0.01
Energy (kcal)	98.6±1.86	90.1±2.78
Beta-carotene (mg)	10.23 ±3.15	-

Dietary assessment: A computer-based nutritional analysis program was used to assess the nutritional status of the participants and to compare the nutrient intakes of the Treatment and Placebo groups. The participants were asked to keep 3-day food records prior to each visit to the clinic to compare with baseline and for further analysis of relationships with other parameters. The participants were asked to record the amounts of all the food items and beverages that they consumed. We used the Thai food composition program INMU-CAL-Nutrients, version 4.0, developed by the Institute of Nutrition, Mahidol University, Thailand 19, for further analysis of dietary composition.

2.8 Statistical analysis

Sample size calculation was performed by Gpower 3.1 software with difference between two independent means (two-groups) with effect size 4.00 (used malondialdehyde level as the study outcome, data from previous study[9]). Basically, sample size in this study was 6 cases/group and a 50% dropout rate was expected during the study. Therefore, the total number of subjects was 9-10 cases/group. Data were analyzed using SPSS Statistics version 24.0 (IBM, Inc., Armonk, NY, USA). We show the mean and SD for continuous data, and percentages (%) for categorical data. Comparisons between the two groups at a single time point were performed using the in-dependent t-test or chi-square test. The relationships between anthropometric, biochemical, and oxidation parameters were evaluated using Pearson's correlation coefficients. Clinical and biochemical data related to the intervention were evaluated using repeated-measures ANOVA. To compare data between baseline and follow-up within each group, we also calculated percentage changes (% change). Differences were considered significant when the p-value was lower than 0.050.

3. Results

3.1 Participant characteristics

The general characteristics, anthropometric parameters, biochemical parameters, and nutrient intakes of the two groups did not differ, as shown in Table 2. The general characteristics, anthropometric parameters, biochemical parameters, and nutrient intakes of the two groups did not differ, as shown in Table 2. The mean BMI for the treatment (33.0 ± 4.6 kg/m^2) and placebo (32.6 ± 3.2 kg/m^2) groups are shown in Table 2. The prevalence of smoking and the frequency of alcohol consumption were significantly higher in the placebo group (80% of smoking and 90% of alcohol consumption of placebo group compared to 30% and 60 % in the treatment group, $p<0.05$). The use of dietary supplements were comparable between the two groups. Compliance with the dietary intervention was assessed by evaluating dietary intake at baseline, after 7 days (by telephone interview), and at the end of the study; and compliance with the supplementation regimen was evaluated through the completion of a questionnaire and the measurement of the plasma beta carotene concentrations of the participants at baseline, and after 1 and 3 months of the intervention.

Table 2. General characteristics and lifestyle factors of the study participants at baseline (data are presented as percentage and mean ± SD).

	Treatment group (N=10)	**Placebo group (N=10)**
Age, years	40.00 ±2.86	39.30± 5.61
Height, cm	173.10± 2.81	169.10± 5.48
Weight, kg	99.83± 15.38	93.54± 8.58
Waist circumference, cm	98.96± 7.97	107.32± 7.81
BMI, kg/m^2	32.97± 4.58	32.62± 3.18
Body metabolic rate, kcal/d	1973± 220	1852± 129
Body fat, %	31.16± 4.42	30.70± 4.12
Body fat mass, kg	31.68± 9.67	28.95± 6.23
Muscle mass, kg	64.64± 5.64	61.25± 3.50
Total body water, %	51.68± 1.69	51.66± 4.23
Bone mass	3.51± 0.28	3.34± 0.19
Visceral fat level	16.5±0 2.46	16.50± 1.58
Systolic blood pressure, mmHg	149.50± 5.26	146.11± 6.35
Diastolic blood pressure, mmHg	92.36± 9.25	91.67± 11.58
Smoking, N (%)[a]		
Never	7 (70%)	2 (20%)
Current	3 (30%)	8 (80%)[a]
Current smoking		
Number of cigarettes /day	5.67 ±0.59	7.40 ±2.51
Time of smoking, year	5.00 ±1.60	14.60 ±5.20[a]
Alcohol consumption, N (%)[a]		
Never	4 (40%)	1 (10%)
Current	6 (60%)	9 (90%)[a]
Frequency of current drinking, N (%)		
1-2 time/week	6 (100%)	5 (55.6%)[a]
Everyday	-	4 (44.4%)
Use of nutritional supplements, N (%)		
No	3 (30%)	2 (20%)
Yes	7 (70%)	8 (80%)

Data are presented as percentages or mean ± SD, calculated by Chi-square test and t-test, respectively. [a] Significant difference from the Treatment group: $p<0.050$.

3.2 Relationships between clinical parameters, oxidative stress, and antioxidants at baseline in the treatment and placebo groups

We first investigated the relationships between antioxidants, oxidative stress, and clinical parameters in the treatment and placebo groups at baseline (Table 3). In the treatment group, there were positive correlations between plasma beta carotene concentration and % body fat, FPG, HbA1C, TC, and TG. For the placebo group, we found significant correlation between plasma beta carotene level and % body fat and TG. Inverse relationships of plasma vitamin E concentration with TC, LDL-C, and TG were found in the treatment group whereas in the placebo group, plasma vitamin E negatively correlated with TC and LDL-C. In addition, TC and LDL-C inversely correlated with plasma ORAC in both groups. The plasma concentration of MDA, a marker of lipid peroxidation, showed significant positive correlations with BMI, WC, % body fat, body fat mass, visceral fat mass, and HbA1c in both groups. In addition, plasma MDA showed positive correlations with LDL-C and TG in the Treatment group

Table 3. Correlation between anthropometric parameters, blood pressure and plasma beta-carotene, vitamin A, vitamin E, MDA and ORAC levels among two group at baseline.

	Treatment group				
	Plasma beta-carotene	**Plasma vitamin A**	**Plasma vitamin E**	**Plasma ORAC**	**Plasma MDA**
BMI, kg/m^2	-0.024	-0.126	-0.234	-0.229	0.685*
Waist circumference, cm	-0.108	-0.150	0.171	-0.208	0.760*
Body fat, %	-0.619*	-0.202	0.269	-0.253	0.766*
Body fat mass, kg	-0.002	-0.072	0.246	-0.326	0.678*
Visceral fat level	0.009	-0.157	0.189	-0.067	0.703*
SBP, mmHg	0.183	-0.292	0.147	-0.323	0.164
DBP, mmHg	0.212	-0.175	-0.145	-0.186	0.076
Glucose, mg/dL	-0.799**	-0.206	0.113	0.124	-0.235
HbA1C, %	-0.451*	-0.169	0.138	-0.624	-0.538*
Total cholesterol, mg/dL	-0.529*	-0.032	-0.767*	-0.662*	0.321
HDL-cholesterol, mg/dL	-0.390	0.203	-0.038	-0.118	0.312
LDL-cholesterol, mg/dL	-0.290	0.179	-0.510*	-0.657*	0.405*
Triglyceride, mg/dL	-0.612**	0.084	-0.816*	-0.648	0.476*
hsCRP, mg/L	0.167	0.277	0.289	0.029	0.311
	Placebo group				
	Plasma beta-carotene	**Plasma vitamin A**	**Plasma vitamin E**	**Plasma ORAC**	**Plasma MDA**
BMI, kg/m^2	0.041	-0.226	-0.228	0.164	0.949**
Waist circumference, cm	-0.123	-0.404	-0.476	-0.431	0.910*
Body fat, %	-0.462*	-0.179	-0.086	0.013	0.893*
Body fat mass, kg	-0.08	-0.335	-0.159	-0.168	0.927**
Visceral fat level	0.092	0.183	-0.397	0.082	0.884*
SBP, mmHg	0.287	0.319	-0.301	0.010	0.358
DBP, mmHg	-0.309	-0.298	-0.308	-0.345	0.038
Glucose, mg/dL	0.249	-0.010	0.231	0.261	0.044
HbA1C, %	0.143	-0.317	0.065	-0.044	0.754*
Total cholesterol, mg/dL	0.258	-0.294	-0.667*	-0.520*	0.268
HDL-cholesterol, mg/dL	0.201	0.198	0.138	0.184	-0.245
LDL-cholesterol, mg/dL	0.109	-0.348	-0.563*	0.535*	-0.021
Triglyceride, mg/dL	0.402*	0.099	0.328	0.033	-0.112
hsCRP, mg/L	-0.366	0.336	0.204	0.276	0.108

*, **Significant correlations, p<0.01and 0.001, respectively analyzed by the Pearson correlation. BMI: body mass index, SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL-c: HDL-cholesterol; LDL-c: LDL- cholesterol; hsCRP: high sensitivity C-reactive protein; ORAC: oxygen radical absorbance capacity; MDA:malondialdehyde.

3. 3 Comparison of the anthropometric parameters, blood pressure, and laboratory data of the two groups at baseline and follow-up

There were no differences in the anthropometric parameters or blood pressure between the treatment and placebo groups (Table 4). The mean SBP and DBP of both groups were in the hypertensive range (149.5 ± 5.3 and 92.4 ± 9.3 mmHg for the treatment group and 146.1 ± 6.4 and 91.7 ± 11.6 mmHg for the placebo group, respectively). Supplementation with carotenoid-rich carrot jelly was associated with significant decreases in SBP and DBP after 1 and 3 months compared to baseline ($p<0.05$). In contrast, the SBP and DBP of the placebo group did not change during the

study. In addition, the body mass, BMI, % body fat, body fat mass, muscle mass, and visceral fat mass of neither of the groups changed during the study period.

There were significant changes in a number of the biochemical parameters during 1 and 3 months of dietary supplementation, particularly in the Treatment group (Table 5). The plasma beta carotene concentration increased by 264% during the first month and was 293% higher after 3 months of treatment. Consistent with this, the plasma vitamin A concentration was 40.0% and 18.3% higher after 1 and 3 months, respectively.

These changes occurred alongside changes in the lipid profile, inflammatory status, and oxidative stress status of the participants. After 3 months of supplementation with jelly rich in carotenoids, the participants had lower FBG concentrations than at baseline (94.10 ± 5.75 vs. 88.20 ± 8.17 mg/dL, $p<0.05$; a change of -6.23%), whereas after 1 month, the Placebo group showed a 4.63% increase. There were also significant effects of the carotenoid jelly supplementation on the plasma LDL-C (-8.3% after 1 month and -11.1% after 3 months), TG (-29.2% after 1 month and -12.8% after 3 months) and HDL-C (+8.8% after 3 months) concentrations in the Treatment group. In addition, the plasma ORAC increased by 17.5% and 20.9% during 1 and 3 months of supplementation, respectively, and the hsCRP and MDA concentrations decreased during the study period.

The mean FPG and HbA1c levels for both groups were mostly within the normal ranges, although 30% and 20% of participants in the Treatment and Placebo groups, respectively, had HbA1c values of 6.0% –6.4%, consistent with prediabetes (Table 5). In addition, the mean plasma LDL-C (144.8 ± 32.9 mg/L for the Treatment group and 159.5 ± 42.7 mg/L for the Placebo group) and TG (197.8±55.5 mg/L for the Treatment group and 161.9 ± 33.9 mg/L for the Placebo group) concentrations of the participants were above their respective reference ranges, and consistent with the presence of the metabolic syndrome. In addition, the mean plasma HDL-C concentration was outside the normal range in the Treatment group. Both the Treatment (2.38 ± 0.37 mg/L) and Placebo (2.91 ± 0.89 mg/L) groups had plasma hsCRP concentrations of >1 mg/dL; concentrations between 1 mg/dL and 3 mg/dL are considered to be associated with a moderate risk of cardiovascular disease.[6] However, there were no significant differences in the plasma concentrations of plasma beta carotene, vitamin A, vitamin E, or ORAC between the Treatment and Placebo groups at baseline.

3.4 Comparison of the energy, macronutrient, micronutrient, and vitamin intakes by the two groups at the various time points

Dietary assessments of the daily energy intake; the energy contributed by carbohydrate, protein and fat; and the sugar, cholesterol, fiber, micronutrient, and vitamin intakes of the participants were made using their 3-day food records (Tables 6 and 7). There were no differences in the nutrient intakes of the Treatment and Placebo groups at any of the time points, except with respect to the beta carotene and sodium intakes of the Treatment group. The daily beta carotene intake of the Treatment group was significantly higher after 1 and 3 months of the study than at baseline, which implies good compliance with the intervention. At baseline, the participants in the Treatment group had significantly higher sodium intake (3,730 ± 852 mg/day) than subsequently (2,553 ± 456 mg/day and 2,456 ± 395 mg/day after 1 and 3 months, respectively).

Table 4. Changes of anthropometric and blood pressure measurements at baseline and follow up.

Anthropometric parameters	Treatment group	%change	Placebo group	%change
Weight, kg				
Baseline	99.83 ±15.38	-	93.81 ±9.06	-
1 month follow up	101.24 ±15.09	1.41±0.49	93.53 ±8.74	-0.30±0.21
3 month follow up	101.18 ±15.89	1.35±0.99	94.38 ±8.98	0.61±0.35
BMI, kg/m^2				
Baseline	32.97 ±4.58	-	32.74 ±3.34	-
1 month follow up	33.17 ±4.52	0.61±0.45	32.33 ±2.88	-1.25±0.47
3 month follow up	33.84 ±5.65	2.64±1.69	32.44 ±2.90	-0.92±0.59
Body fat, %				
Baseline	31.60 ±4.42	-	30.89 ±4.24	-
1 month follow up	31.40 ±4.39	0.63±0.25	30.58 ±2.98	-1.00±0.63
3 month follow up	32.10 ±5.19	1.50±1.07	30.52 ±3.17	-1.20±0.57
Body fat mass, kg				
Baseline	31.68 ±9.67	-	28.77 ±6.58	-
1 month follow up	32.07 ±9.57	1.23±0.34	28.83 ±5.57	0.21±0.18
3 month follow up	31.94 ±11.15	0.82±0.44	29.04 ±5.98	0.94±0.32
Muscle mass, kg				
Baseline	64.64 ±5.64	-	61.66 ±3.44	-
1 month follow up	65.56 ±5.23	1.42±1.02	61.37 ±3.40	-0.47±0.25
3 month follow up	64.52 ±4.75	-0.19± 0.11	61.97 ±3.18	0.50±0.19
Visceral fat level				
Baseline	16.40 ±2.46	-	16.44 ±1.66	-
1 month follow up	16.90 ±2.42	3.05±1.07	16.35 ±1.58	-0.55±0.24
3 month follow up	17.20 ±3.01	4.88±1.95	16.33 ±1.58	-0.67±0.19
SBP, mmHg				
Baseline	149.50 ±5.26	-	146.11 ±6.35	-
1 month follow up	134.60 ±11.43[a]	-7.75±2.32	143.50 ±4.58	-1.80±0.39
3 month follow up	132.47 ±13.04[a]	-11.39±3.24	144.36 ±9.43	-1.21±0.51
DBP, mmHg				
Baseline	92.36 ±9.25	-	91.67 ±11.58	-
1 month follow up	86.49 ±8.83[a]	-7.43±3.58	89.20±8.24	-2.69±1.23
3 month follow up	82.19 ±7.92[a]	-11.01±2.63	94.23 ±11.11	-2.79±0.98

[a] Significant difference from baseline, $p<0.05$. BMI: body mass index, SBP: systolic blood pressure; DBP: diastolic blood pressure.

Table 5. Changes of biochemical parameters among two groups at baseline and follow up.

Biochemical parameters	Treatment group	%change	Placebo group	%change
Glucose, mg/dL				
Baseline	94.10 ±5.75	-	91.33 ±5.59	-
1 month follow up	90.60 ±8.27	-3.72±1.68	95.56 ±2.46[a]	4.63±1.54
3 month follow up	88.20 ±8.17[a]	-6.27±2.54	92.34 ±5.91	1.11±0.57
HbA1C, %				
Baseline	5.71 ±0.48	-	5.66 ±0.18	-
1 month follow up	5.61 ±0.39	-1.75±1.12	5.59 ±0.08	-1.23±0.41
3 month follow up	5.64 ±0.41	-1.23±0.78	5.67 ±0.27	0.17±0.09
Total cholesterol, mg/dL				
Baseline	209.30 ±45.08	-	230.44 ±37.80	-
1 month follow up	214.50 ±36.80	2.39±1.33	235.33 ±35.87	2.12±1.11
3 month follow up	207.90 ±39.62	-0.67±0.51	238.22 ±34.79	3.38±1.58
HDL-cholesterol, mg/dL				
Baseline	38.70 ±7.05	-	44.33 ±9.28	-
1 month follow up	39.30 ±6.49	1.55±1.03	43.00 ±7.15	-3.00±2.01
3 month follow up	42.10 ±7.52[a]	8.79±3.12	45.11 ±9.22	1.76±1.05
LDL-cholesterol, mg/dL				
Baseline	147.80 ±32.86	-	162.11 ±44.39	-
1 month follow up	135.60 ±30.58[a]	-8.25±2.03	170.56 ±33.56	5.21±2.69
3 month follow up	131.40 ±37.40[a]	-11.10±4.65	165.67 ±41.90	2.20±1.23
Triglyceride, mg/dL				
Baseline	160.78 ±55.47	-	162.56 ±54.96	-
1 month follow up	131.56 ±82.72[a]	-29.19±7.03	174.11 ±88.98	7.11±3.33
3 month follow up	140.19 ±67.32[a,b]	-12.79±4.69	178.23 ±96.27	9.64±2.14
hsCRP, mg/L				
Baseline	2.89 ±1.81	-	2.92 ±1.28	-
1 month follow up	2.45 ±0.61[a]	-15.22±5.57	3.63 ±2.10	24.32±4.56
3 month follow up	2.32±0.91[a,b]	-19.72±6.33	3.24 ±1.98	10.96±6.32
Plasma beta-carotene, ng/mL				
Baseline	72.44 ±2.34	-	69.18 ±1.38	-
1 month follow up	263.62 ±1.90[a]	263.91±66.36	72.35 ±1.78	4.58±1.28
3 month follow up	284.56 ±1.65[a,b]	292.83±45.23	73.88 ±0.98	6.79±3.37
Plasma vitamin A, mg/L				
Baseline	0.60 ±0.11	-	0.56± 0.18	-
1 month follow up	0.84 ±0.18[a]	40.0±5.36	0.59±0.29	5.35±2.39
3 month follow up	0.71 ±0.19[a]	18.33±7.68	0.58±0.24	3.57±1.89
Plasma vitamin E, mg/L				
Baseline	13.97 ±3.41	-	13.09 ±2.73	-
1 month follow up	13.08 ±2.91	6.37±4.09	12.77 ±3.74	2.44±1.48
3 month follow up	12.84 ±2.41	8.08±3.25	12.95 ±1.09	1.06±0.69
Plasma MDA, µmol/L				
Baseline	4.03 ±0.23	-	3.93 ±0.28	-
1 month follow up	3.23 ±0.42[a]	-19.87±7.12	4.12 ±0.41	4.83±3.48
3 month follow up	2.23± 0.12[a,b]	-44.67±10.22	4.15±0.39	9.66±3.77
Plasma ORAC, µmol Trolox equivalent /mL				
Baseline	12.45 ±2.01	-	13.21 ±2.99	-
1 month follow up	14.63 ±1.58[a]	17.51±9.13	13.11 ±2.18	-0.76±0.47
3 month follow up	15.05 ±1.29[a]	20.88±4.56	13.13 ±2.29	-0.61±0.35

[a, b] Significant difference from baseline and 1 month follow up, respectively, $p<0.05$. HDL-C: HDL-cholesterol; LDL- C: LDL-cholesterol; hsCRP: high sensitivity C-reactive protein; ORAC: oxygen radical absorbance capacity; MDA: malondialdehyde.

Table 6. Mean of energy and macronutrients intake with distribution from 24-hr dietary record among three groups at 1 month and 3 month follow up.

	Treatment group	**Placebo group**
Energy intake, kcal/day		
Baseline	1623 ±466	1616 ±358
1 month follow up	1375 ±192	1397 ±339
3 month follow up	1380 ±449	1426 ±418
Energy distribution		
Carbohydrate, g		
Baseline	171.00 ±54.35	176.94 ±31.39
1 month follow up	143.93 ±24.18	148.46 ±37.42
3 month follow up	135.71 ±45.80	145.55 ±42.29
% of energy (Carbohydrate)		
Baseline	43.49 ±7.13	45.75 ±6.09
1 month follow up	46.52 ±8.92	45.95 ±5.61
3 month follow up	42.11 ±8.19	42.27 ±6.15
Protein, g		
Baseline	94.78 ±38.90	85.18 ±35.27
1 month follow up	63.25 ±20.80	61.00 ±23.28
3 month follow up	75.66 ±30.61	78.19 ±29.73
% of energy (Protein)		
Baseline	23.15 ±6.03	18.47 ±4.27
1 month follow up	16.70 ±4.01	19.65± 5.59
3 month follow up	20.92 ±4.10	21.19 ±5.69
Protein-animal, g		
Baseline	67.97 ±36.23	58.61 ±36.08
1 month follow up	34.43± 23.48	40.20 ±21.92
3 month follow up	56.82 ±28.48	54.13 ±27.50
Protein-vegetable, g		
Baseline	14.71 ±4.50	10.27 ±2.43
1 month follow up	9.53± 2.75	11.06 ±2.61
3 month follow up	9.49 ±2.29	11.24 ±3.07
Fat, g		
Baseline	65.78 ±24.91	63.11 ±15.85
1 month follow up	53.30 ±13.69	58.79 ±27.53
3 month follow up	60.49 ±11.15	62.35 ±21.39
% of energy (Fat)		
Baseline	33.34 ±6.66	35.76 ±4.65
1 month follow up	36.76± 6.85	34.39 ±4.39
3 month follow up	36.95 ±5.68	36.52 ±6.23
Sugar		
Baseline	26.82 ±18.68	38.35 ±21.93
1 month follow up	25.36 ±17.33	29.36 ±16.96
3 month follow up	27.89 ±20.36	28.44 ±12.98
Saturated fat, g		
Baseline	17.00 ±7.63	26.09 ±14.63
1 month follow up	11.77 ±6.32	12.70 ±6.04
3 month follow up	17.56± 7.38	17.06 ±7.25
Cholesterol, g		
Baseline	274.51 ±166.89	333.86 ±182.37
1 month follow up	241.39 ±111.43	276.12 ±171.68
3 month follow up	271.38 ±166.06	243.69 ±106.53
Fiber, g		
Baseline	6.47 ±2.84	5.29± 1.35
1 month follow up	4.20 ±1.59	5.12 ±2.50
3 month follow up	4.79 ±2.11	5.85 ±2.30

Table 7. Mean of mineral, trace element and vitamin intakes/day among two groups at 1 and 3 month follow up.

		Treatment group			Placebo group	
	Baseline	**FU at 1 month**	**FU at 3 month**	**Baseline**	**FU at 1 month**	**FU at 3 month**
Calcium, mg	560±153	411 ±183	394 ±188	316±190	367 ±225	324 ±197
Phosphorus, mg	808 ±328	596 ±336	714 ±303	686 ±296	564 ±272	670 ±242
Ferrous iron, mg	9.69 ±5.16	7.22 ±1.79	7.45 ±3.07	7.63 ±1.92	8.33 ±4.63	8.53 ±3.18
Ferrous iron - animal, mg	4.60 ±4.36	2.83 ±1.56	3.44 ±1.87	3.37 ±2.08	2.98 ±1.39	4.01 ±2.15
Ferrous iron - plants, mg	3.23±1.49	2.45 ±1.07	3.04 ±2.40	2.34 ±1.09	2.79 ±1.25	2.88 ±1.15
Potassium, mg	1558±584	1101 ±415	1262 ±473	1296.1 ±40.3	1089 ±443	1355 ±460
Sodium, mg	3842 ±461	2553 ±456[a]	2456 ±395[a]	3012±886	2860 ±934	2717 ±1054
Magnesium, mg	59.4±40.05	43.35 ±23.60	30.09 ±25.58	39.98±21.54	36.94 ±29.18	43.01 ±23.06
Selenium, mg	43.34 ±21.54	45.58 ±23.95	46.68 ±23.86	55.58 ±29.67	34.94±8.85	46.29 ±24.56
Zinc, mg	5.32 ±2.40	3.03 ±0.77	3.76 ±0.96	4.02 ±1.45	3.08 ±1.44	3.29 ±1.44
Copper, mg	0.71 ±0.32	0.41 ±0.10	0.76 ±0.45	0.62 ±0.24	0.46 ±0.27	0.57 ±0.26
Vitamin A, REA	256 ±167	323 ±142	356 ±145	266 ±152	230 ±115	241 ±108
Retinol, mg	162±118	150 ±101	102 ±80	165 ±121	138 ±88	167 ±77
Beta-carotene, mg	677 ±375	965 ±111[a]	1020±201[a]	569±290	490 ±193	601±412
Vitamin B1, mg	1.62 ±0.95	1.11 ±0.93	1.86 ±0.93	1.48 ±0.77	1.28 ±0.69	1.63 ±0.99
Vitamin B2, mg	1.31±0.70	0.74 ±0.34	0.88 ±0.37	0.98 ±0.29	0.96 ±0.59	1.04 ±0.50
Vitamin B3, mg	18.21 ±10.55	10.35 ±4.91	16.25 ±8.08	15.49 ±8.13	12.60 ±7.22	13.26 ±6.20
Vitamin B6, mg	0.74 ±0.27	0.53 ±0.35	0.70 ±0.58	0.79 ±0.55	0.53± 0.37	0.62 ±0.43
Vitamin B12, mg	2.44 ±1.47	1.25 ±0.57	1.99 ±0.83	1.87± 1.05	1.34 ±0.61	1.96 ±1.33
Vitamin C, mg	25.14 ±11.95	19.07 ±9.45	16.98 ±8.36	22.99 ±9.93	26.00 ±11.12	17.87 ±11.32
Vitamin E, mg	1.73± 0.94	1.45 ±0.83	0.71 ±0.38	2.14± 0.87	1.66 ±0.91	1.36 ±0.79

[a] significant difference from baseline, $p<0.05$.; Analysis by repeated-measures ANOVA. RAE: retinol activity equivalents.

4. Discussion

We have characterized the effects of carotenoid-rich carrot jelly on the anthropometric parameters, blood pressure, and biochemical parameters in individuals with obesity. Dietary supplementation with carrot jelly caused increases in the plasma beta carotene and vitamin A concentrations of the participants (263.9% and 99.5% for beta carotene, and 40.0% and 18.3% for vitamin A, respectively). These increases were comparable to those achieved in a previous study of the consumption of a carrot juice-based drink for 6 weeks (plasma beta carotene concentration increased by 0.42 ± 0.33 mol/L with a dose of 6 mg/day and by 1.71 ± 0.55 mol/L with a dose of 18 mg/day; slight increase in plasma retinol concentration) [20]. In another study of the effects of 2 weeks of carrot juice supplementation (27.1 mg/day β-carotene and 13.1 mg/day α-carotene) on the immune function of healthy men, there was a rapid increase in the plasma carotenoid concentrations following supplementation, but no significant changes in markers of immune function.[21]

We found that the participants in both groups had hypertension, with mean SBPs and DBPs at baseline of 149.5 ± 15.7 and 88.8 ± 9.3 mmHg, respectively, in the Treatment group and 150.4 ± 12.3 and 92.5 ± 11.2 mmHg, respectively, in the Placebo

group. Weight gain significantly predisposes toward hypertension, especially when combined with excess visceral adiposity. The proposed mechanisms for obesity-induced hypertension include excessive activation of the renin-angiotensin-aldosterone system, greater activity of the sympathetic nervous system, oxidative stress, inflammation, and greater renal tubular sodium reabsorption.[22] In the present study, we found that higher plasma beta carotene and vitamin A concentrations were associated with lower blood pressure at the two follow-up time points, which is similar to the relationship identified between serum antioxidant vitamins and blood pressure in the US population. [23] Thus, carotenoid supple-mentation may reduce blood pressure when this leads to a high plasma concentration of beta carotene. [24] In addition, we have shown that carotenoid administration for 1 or 3 months is associated with an increase in antioxidant capacity, as assessed using plasma ORAC, as well as inverse relationships with the plasma hs-CRP and MDA concentrations. Therefore, the effects of carotenoids on hypertension may be mediated through the suppression of oxidative stress and its consequences, such as ROS-induced cellular damage, lipid peroxidation, and inflammation. A previous study also showed that a high plasma vitamin A concentration induced by similar dietary supplementation reduces SBP and DBP in spontaneously hypertensive rats.[25] The effect of vitamin A on the risk of hypertension has also been evaluated by supplementation with all-trans retinoic acid (ATRA), a biologically active metabolite of vitamin A, which was found to affect the gene and protein expression of angiotensin-converting enzyme 2.[25] Furthermore, the plasma ATRA concentrations of individuals with hypertension were found to be lower than those of healthy controls, which was ascribed to vascular remodeling, leading to lower vascular elasticity, and an impairment in vascular homeostasis, and therefore greater vessel stiffness.[26]

Obesity-induced insulin resistance and type 2 diabetes involve the dysfunction of adipose tissue. A key defect underpinning adipose tissue dysfunction and the associated high circulating free fatty acid (FFA) concentrations, immune cell infiltration, and pro-inflammatory cytokine secretion is abnormal insulin signaling. [27] At baseline, both the Treatment and Placebo groups contained participants with prediabetes, according to their HbA1c values. However, after supplementation with carotenoid jelly for 3 months, the FPG concentrations of the participants in the Treatment group had significantly decreased. In addition, as in a previous study, the total serum concentration of carotenoids negatively correlated with BMI and other indices of glucose metabolism (HOMA-IR and insulin). [28] Hyperglycemia in individuals with obesity is associated with insulin resistance, greater ROS production, greater secretion of proinflammatory cytokines, pancreatic β-cell damage, and lower insulin secretion. These defects have been shown to be ameliorated by beta carotene supplementation, possibly through the suppression of free radical production and the regulation of genes expressing insulin signaling molecules.[29]

The hypertriglyceridemia that characterizes obesity is caused by excessive FFA flux to the liver and hepatic TG accumulation, along with a dysregulation of lipolysis, owing to aberrant expression and function of lipoprotein lipase and cholesteryl ester-transfer protein. Thus, the dyslipidemia of obesity is characterized by high circulating TG and LDL-C concentrations in conjunction with a low circulating HDL-C concentration,[30] which we also identified in the present study. Carotenoid jelly supplementation for 1 or 3 months ameliorated this dyslipidemia by reducing the concentrations of TG and LDL-C and increasing that of HDL-C. Similarly, the daily consumption of 300 g of a frozen vegetable product for 2 weeks increased the plasma lutein and beta carotene concentrations and significantly reduced the

plasma concentrations of TC, LDL-C, and oxidized LDL.[31] In addition, a population-based study of 374 men that evaluated the relationship between dietary carotenoid intake and the risk of the metabolic syndrome showed an in-verse relationship between the intake of beta carotene and plasma TG concentration.[32] Finally, a study of beta carotene supplementation in mice with diet-induced obesity showed lower mRNA expression of macrophage recruitment markers and lower plasma cholesterol and TG concentrations.[33] Taken together, these findings suggest that beta carotene, vitamin A, and pro-vitamin A carotenoids can affect adipose tissue metabolism and dyslipidemia through effects on oxidative stress, inflammation, and gene expression.

A 3-day food record was completed by each participant in the present study prior to their first blood sample being taken and before each of the subsequent examinations to assess their diets and the relationships of dietary components with health outcomes. In fact, the only significant change identified during the study was a decrease in sodium intake in the treatment group between baseline (3,842 ± 461 mg/day) and 1 (2,553 ± 456 mg/day) and 3 (2,456 ± 395 mg/day, $p<0.05$) months of carotenoid supplementation. This finding may at least in part explain the decreases in SBP and DBP identified in the treatment group, and is consistent with previous findings that obesity and high salt intake are associated with high BP.[34] For example, increases in sodium intake, estimated by the measurement of urinary sodium excretion, have also been demonstrated to be associated with significant increases in blood pressure (a 2.58 mm Hg increase in SBP per gram sodium excretion when >5 g per day, and a 1.74 mm Hg increase per gram when it was 3–5 g per day; $p<0.001$).[35] The possible mechanisms underlying this hypertension are alterations in water retention, vascular tone, endothelial function, and the activation of the sympathetic and parasympathetic nervous systems.[36] The decreases in both SBP and DBP in the treatment group in the present study might represent the combined effect of carotenoids and low sodium intake.

Overall, 3 months of dietary supplementation with carotenoid-rich carrot jelly had beneficial effects in the present study, ameliorating the dyslipidemia and reducing the blood pressure of the participants with obesity. However, a meta-analysis of observational studies showed that supplementation with beta carotene increased the risk of cardiovascular mortality,[37] and we previously found no beneficial effects of beta carotene supplementation on the incidence of cardiovascular disease, and potentially negative effects on CVD mortality.[38] The strengths of the present study include the randomized, placebo-controlled experimental design, the comparability of the groups at baseline, the measurement of the circulating plasma beta carotene and vitamin A concentrations, and the assessment of clinical parameters at baseline and during the study. However, there were also some limitations. The sample size was small, and we did not measure the concentrations of other carotenoids, such as cryptoxanthin, lycopene, zeaxanthin, and lutein. In the future, molecular biomarkers should be analyzed to confirm that the carotenoids were responsible for the changes in obesity-related parameters.

5. Conclusion

We performed a pilot randomized controlled study which showed that dietary supplementation with carotenoids can reduce the oxidative stress, inflammation, circulating lipid concentrations, and blood pressure of individuals with obesity. These results suggest that carotenoids improve the health of individuals with obesity and prevent adverse metabolic outcomes. Therefore, a high intake of fruit and vegetables, and especially those with high carotenoid contents, should be recommended.

Acknowledgements

We would like to express our thanks to Dr. Napassorn Peasure, and Miss Anantita Sangsuriyawong, researchers at the Institute of Nutrition, Mahidol University for their assistance in food analysis. We also thank the participants, without whose contribution this study would not have been possible.

Funding

This research project was supported by the Faculty of Medicine Ramathibodi Hospital, Mahidol University (Grant No. RF-63074).

Conflicts of Interest

The authors declare no conflict of interest.

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