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Effects of Red Palm Oil on Serum Lipids and Plasma Carotenoids Level in Chinese Male Adults

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Objective Effects of red palm oil on major plasma carotenoids, tocopherol, retinol and serum lipids were evaluated when used in Chinese diet. **Methods** Red palm oil group (RPO) composed of 20 male subjects(aged 18-32) and soybean oil group (SBO) composed of 22 male subjects (aged 18-32). Dietary fat provided about 28% of total calories, and the test oil accounted for about 60% of total dietary fat. In the 3 weeks of pretest period, diets were prepared with soybean oil, and then in the next 6 weeks subjects in each group consumed the diet prepared by test oil. **Results** Plasma α-carotene, β-carotene and lycopene concentration of RPO group significantly increased at the time of interim (21 days) and of the end (42 days) ($P<0.05$), and α-tocopherol concentration significantly increased at the time of the end (42 days) in this study. Though Chinese plasma retinol level was relatively low when compared with that of Westerners, red palm oil diet showed no significant effect on adult Chinese plasma retinol level. Serum concentration of total cholesterol, triglyceride, high density lipoprotein cholesterol, apolipoprotein AI and apolipoprotein B of all subjects showed no significant changes in RPO group during the study. **Conclusions** The data in our study suggest that red palm oil is a good source of carotenoids and vitamin E when used in Chinese diet preparation, and it can significantly increase plasma concentration of α-carotene, β-carotene, lycopene and α-tocopherol.

Key words: Red palm oil; Retinal; α-carotene; β-carotene; α-tocopherol; Serum cholesterol; Serum triglyeride; Apolipoprotein AI; Apolipoprotein B

INTRODUCTION

Red palm oil (RPO), derived from the mesocarp of the oil palm (Elaeis guineensis), has been consumed for many centuries in some African countries. Although categorized as a saturated fat, it can play a dual role in providing provitamin A and fulfilling the energy needs in developing countries. Red palm oil contains about 15-300 times as many retinol equivalents as carrots, leafy green vegetables and tomatoes, which are considered as rich sources of provitamin A. It is these carotenoids that give an orange-red color to crude palm oil. As a great improvement in oil processing has been achieved, the newly developed process can remove most free fatty acids and odoriferous materials while retains more than 80% of carotenoids and most of vitamin E contents which are originally present in crude palm oil[1]. The edible grade of unrefined RPO is one of the richest natural sources of

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β-carotene and other carotenoids as well as vitamin E (Table 1). The edible red palm oil has recently been introduced to China.

TABLE 1

Carotenoid and Vitamine E Contants in Red Palm Oil

Red Palm Oil	mg/L
β-carotene	258.3
α-carotene	201.6
γ-carotene	2.7
δ-carotene	3.3
ζ-carotene	7.1
Lycopene	8.2
Phytoene	10.9
Zeacarotene	1.0
Vitamine E (tocopherol, tocotrienols)	468.0

In recent years, an interest is being focused on the nutritional aspects of carotenoids, especially on the better understanding of carotenoid metabolism and the bioavailability of carotenoids from natural foods. Among vegetable oils, red palm oil contains the highest known concentration of agriculturally derived carotenoids[2]. Reverse-phase high-performance liquid chromatography (HPLC) analysis has shown that it also contains many kinds of carotenoids such as α-and β-carotene, phytofluene, δ-, ζ-, γ- carotene, β-zeacarotene, α-zeacarotene and lycopene[3], which make edible red palm oil an ideal natural source of carotenoids.

Chinese diet usually contains an inadequate amount of retinol equivalent (RE). Data from the 1992 national nutrition survey showed that the average total intake of RE was about 61% of the Chinese RDA[4] and data from the total diet study also indicated that the average intake of RE was even less than 200 μg per capita per day (% RDA) in some areas[5]. In China, serum vitamin A level was reported to be lower than 20 μg/dL in 20%-40% of rural preschool children and serum beta-carotene concentration of the Chinese appeared to be significantly lower than that observed in western countries and the concentrations of some other carotenoids, like lycopene were even lower than the detection limit in sera of the Chinese[6, 7].

About one million tons of RBD palm oil are imported in China each year. Our previous study indicated that RBD palm oil had nonhypercholesterolaemic effects and even lowering effects on serum cholesterol when compared with lard and peanut oil[8]. Since red palm oil is completely a new type of edible oil for the Chinese, its color, nutrition value and safety are unfamiliar to the Chinese people. The purpose of this study was to study the effects of red palm oil on serum lipids and plasma carotenoinds level in the Chinese subjects.

SUBJECTS AND METHODS

Subjects

After volunteers with hypercholesterolemia, hypertension, liver and kidney disorders, and those who were habitual smokers and drinkers were excluded, 42 male soldiers aged 18-32 years, were recruited as voluntary subjects. Among them, 20 subjects from one army company were assigned to consume the red palm oil diet and the other 22 in another army

company were assigned to consume soybean oil diet. All the subjects lived in the same barracks and carried out the same drills. No one took any medication or nutrient supplements during the test period. The general information on the subjects is showed in Table 2.

TABLE 2

Characteristics of Study Subjects at Baseline

Items	Red Palm Oil Group	Soybean Oil Group
No. of Subjects	20	22
Age (years)	18 – 32	18 - 29
Body Height (m)	1.72±0.05	1.70±0.04
Body Weight (kg)	63.7±6.8	63.0±3.9
BMI (kg/m^2)	21.4±1.9	21.9±1.6

Diets

The basic experimental diet was composed of rice, wheat flour, lean pork, egg, bean curd and some local green vegetables. The menu was developed according to the preferences of the subjects and daily meals were prepared by cooks of the company under the direction of a nutritionist to meet the experimental requirements. Red palm oil was provided by the Malaysia Palm Oil Research Counsel and soybean oil was purchased from the local edible oil company. According to the routine dietary regime, average nutrient intakes were calculated based on the Chinese Food Composition Table. The main nutrient intakes of the test diets including the intakes of main carotenoids are shown in Table 3.

TABLE 3

Average Daily Nutrients Intake of Subjects From the Red Palm Oil Diet and the Soybean Oil Diet

Test Diet	RPO	SBO
Energy (MJ)	10.9	11.5
Protein (g)	68.3	71.0
Fat (g)	71.2	70.3
Carbohydrate (g)	418.9	430.1
Cholesterol (mg)	134	132
Vitamin A (μg)	50.8	51.9
Vitamin E (mg)	28.3	10.6
β-carotene (μg)	8356.6	855
α-carotene (μg)	5646.2	-
Lycopene (μg)	228.9	-

Biochemical Analyses

Blood samples were collected on day 0, 21 and 42 after 12 h fasting and centrifuged at 3 000 rpm for 15 min. One part of the serum samples was stored at -20℃ for serum lipids analysis and the other part was put into liquid nitrogen and transported to the central laboratory and then stored at -70℃ for serum carotenoids analysis.

Serum total cholesterol (TC), triglyceride (TG), apolipoprotein AI and apolipoprotein B were measured by using Chinese Zhong Sheng High-Tech Bioengineering Company

enzymatic kits. High density lipoprotein cholesterol (HDL-C) was assayed using the enzymatic kits after precipitatation with phosphotungstic acid and magnesium chloride.

Plasma retinol, β-carotene, α-carotene, lycopene and α-tocopherol were assayed by the HPLC method[9]. The HLPC system consists of Waters 490 multiwavelength detector, Waters millipore model 510 pump and with a model 730 data module, Waters model 710B WISP injector, Dubon Zorbax ODS, 250×4.6 mm, 5-um C18 reverse column.

Chemicals Standards of α-tocopherol, β-carotene, α-carotene and lycopene were obtained from the Sigma Chemical Company. Standards of trans-retinol and trans-retinol acetate were the gifts from F. Hoffmann La-Roche, Switzerland. HPLC-grade acetonitrile, G.R. methanol, ethanol and A.R. chloroform, hexane were purchased from Beijing Industrial Chemical Plant. All solvents were distilled, filtered and degassed prior to their use.

Sample Preparation for HPLC The fat soluble compounds were extracted from human serum samples using the procedures described by Miller. K. W[9]. In brief, an internal standard, retinol acetate in a 200 μL of ethanol was added to 200 μL plasma and mixed. Then 460 μL hexane was added and mixed in a vortex. The hexane phase (300 μL) was removed and dried under N_2, and the residue was dissolved in 100 μL ethanol. A 40 μL sample was injected into the HPLC and the column was eluted isocrytically with methanol: acetonitrile:chloroform (25:60:15) at a flow rate of 1.2 mL/min.

Statistical Analysis

The data were analyzed with the SPSS/PC statistics program (V4.0, SPSS, Chicago, IL). The differences between the two test groups were assessed with the Student *t* test (two-tailed). In all cases, $P<0.05$ was considered statistically significant and data are presented in the text and tables as $\bar{x}\pm s$.

RESULTS

Serum lipid The effects of the two tested oils on serum lipids are shown in Table 4. The average serum TC, TG, HDL-C, ApoAI and ApoB concentrations of the two groups at baseline were basically the same. Although red palm oil contained a larger amount of tocotrienol, it did not cause any significant effect on serum lipids when compared with the baseline level of the SBO group. The results of lipids measurement showed that the blood lipids levels were the same between the SBO and RPO groups.

TABLE 4

Effects of RPO and SBO on Serum Lipids ($\bar{x}\pm s$)

Serum Lipids	SBO			RPO		
	d 0	d 21	d 42	d 0	d 21	d 42
TC (mmol/L)	3.49±0.57	3.55±0.56	3.54±0.56	3.52±0.52	3.67±0.47	3.62±0.59
TG (mmol/L)	0.80±0.40	0.83±0.35	0.84±0.35	0.81±0.32	0.85±0.31	0.86±0.33
HDL-C (mmol/L)	1.17±0.19	1.17±0.15	1.21±0.15	1.16±0.20	1.12±0.18	1.18±0.18
Apo AI (g/L)	1.37±0.15	1.37±0.22	1.39±0.20	1.42±0.15	1.35±0.19	1.39±0.15
Apo B (g/L)	0.77±0.08	0.78±0.07	0.76±0.11	0.78±0.05	0.78±0.08	0.78±0.09

Plasma retinol, α-tocopherol and carotenoids Plasma retinol concentration was related to dietary β-carotene concentration. Although there was a large amount of β-carotene in red palm oil, subjects' plasma retinol did not show any increment (Table 5). Plasma α-tocopherol increased significantly after 42 days supplementation (Table 5). However, the isomer of vitamin E, especially the various kinds of tocotrienols which have been proved to have cholesterol-lowering effect, were not measured because of the limitation of equipment. Compared with the entry level of the SBO group, serum β-carotene, α-carotene and lycopene increased significantly after 21 days, and a more significant increase was observed at the end of the study (42 days).

TABLE 5

Plasma Retinol, α-tocopherol and Carotenoids Concentration of Subjects ($\bar{x} \pm s$)

	n	Retinol	α-tocopherol	β-carotene	α-carotene	Lycopene
SBO		(μmol/L)	(mmol/L)	(mmol/L)	(mmol/L)	(μmol/L)
d 0	22	1.27 ± 0.31^{a}	18.47 ± 4.13^{a}	0.20 ± 0.12^{a}	0.032 ± 0.033^{a}	0.013 ± 0.014^{a}
d 21	22	1.36 ± 0.31^{a}	18.81 ± 3.69^{a}	0.22 ± 0.14^{a}	0.033 ± 0.037^{a}	0.015 ± 0.013^{a}
d 42	22	1.30 ± 0.23^{a}	18.54 ± 2.95^{a}	0.22 ± 0.16^{a}	0.033 ± 0.036^{a}	0.014 ± 0.014^{a}
RPO						
d 0	20	1.35 ± 0.30^{a}	17.64 ± 4.12^{a}	0.21 ± 0.11^{a}	0.027 ± 0.032^{a}	0.014 ± 0.019^{a}
d 21	20	1.49 ± 0.23^{a}	$19.49 \pm 5.56^{a,b}$	0.68 ± 0.24^{b}	0.110 ± 0.070^{b}	0.042 ± 0.035^{b}
d 42	20	1.48 ± 0.29^{a}	21.67 ± 4.77^{b}	1.16 ± 0.50^{c}	0.160 ± 0.120^{c}	0.061 ± 0.040^{c}

Note. Values within a column with different superscripts letter a,b,c were significantly different ($P<0.05$).

DISCUSSION

We designed this study with a defined diet to control the absorption and utilization of red palm oil carotenoids. The diet in our study had about 24%-25% of the energy from fat, 10%-11% from protein, and 64%-65% from carbohydrate. Furthermore, the treatments were strictly monitored throughout the study to ensure good compliance.

Our previous study showed that RBD palm oil had nonhypercholesterolaemic effect on serum lipids when compared with soybean oil and hypocholesterolaemic effect on serum lipids when compared with lard and groundnut oil. In this study, soybean oil, a kind of polyunsaturated and widely used edible oil in China, was selected for control diet preparation. Though, compared with RBD palm oil, red palm oil had a large amount of tocotrienol which was proved to have the effect of inhibiting cholesterol synthesis limited enzyme-HMG-CoA and lowering the serum cholesterol and apoB concentration of mild hypercholesterolemia adults in a tocotrienol rich capsule supplementation study[10], the concentration of serum TC, TG, HDL-C, apoAI and apoB of the subjects who consumed red palm oil diet showed no obvious changes in this study. The reason for this might be that the soybean oil was also a kind of polyunsaturated oil and though the concentration of tocotrienol was abundant in red palm oil when compared with other kinds of edible oil, the concentration was much less than that in tocotrienol rich capsule. Besides, the original lipids level of the subjects was normal and the intervention period was not long enough (42 days), so the effect of red palm oil on serum lipid might be very limited.

Some studies showed that high dose of supplementation of β-carotene could increase serum retinol level in children, and Rukmini concluded that the significant increase of serum

retinol as well as liver retinol store appeared when red palm oil was used to prepare Indian school children's snacks[11]. In this study, compared with soybean oil, red palm oil did not show any effect on serum retinol concentration, although the serum retinol level of the Chinese was low when compared with that of westerners. This is in accordance with some previous studies that β-carotene supplement did not bring any changes on serum or plasma retinol in adults in spite of the dramatic increases in plasma β-carotene concentration[12]. Our data again confirmed that circulating retinol did not appear to be affected by β-carotene supplementation from natural foods in subjects with normal baseline concentrations.

A few reports in the literature have suggested that pharmacological dose of β-carotene may adversely affect vitamin E concentrations in blood or tissues, but the studies were fairly few[13]. The suggestion of an adverse effect of supplemental β-carotene on plasma vitamin E concentrations, first published in 1992, prompted further investigations of interactions between β-carotene and vitamin E. Nierenberg *et al.* analyzed the data from more than 500 patients enrolled in a US polyprevention trial and reported that blood vitamin E concentrations did not change after 9 months of supplementation with 25 mg β-carotene/d (2% increase relative to baseline measurements)[14]. Different from pure β-carotene supplement in those previous studies, red palm oil increased both β-carotene intake and vitamin E intake, so in this study, serum α-tocopherol in RPO group showed a significant increase compared with that in SBO group. Because of the limited equipment condition, we only measured the concentration of α-tocopherol without any other isomers of vitamin E, especially tocotrienol which has been proved to have the inhibitory effect of HMG-CoA.

Some studies of carotenoid bioavailability have shown that 2 carotenoids administered concurrently can negatively affect the absorption of each other[15], while others showed positive effects. For example, Wahlqvist *et al.* reported that supplementation with 20 mg β-carotene/d for 24 months elevated both plasma α-carotene concentrations (211% in men and 166% in women) and lycopene concentrations in women (n=224)[16]. In this study, subjects in RPO group consumed many kinds of carotenoids per day rather than pure β-carotene, thus resulting in an increase of plasma α-carotene, β-carotene, lycopene concentration concurrently. Plasma lutein and canthaxanthin concentrations were not measured in this study, so the effect of red palm oil on lutein and canthaxanthin was unconfirmed. However, the results still suggested that compared with soybean oil, red palm oil could provide much more dietary carotenoids and natural food might have more advantages over pure carotene capsule.

Carrots provide a good source of both α- and β-carotene. Some other nature foods such as tomato, potato and broccoli are also good sources of lycopene, α-carotene, β-carotene and other carotenoids. But in a study, participants proved that it would be difficult to physically consume 272 g of carrots or 300 g broccoli in diets per day. So getting much more carotenoids from nature food is not an easy thing. Although the proportion of vegetables, tomato, potato and carrot is high in Chinese diet, inhabitants' absolute intake per day is still quite low, especially in those undeveloped districts. So the plasma carotenoid and retinol level in the Chinese is very low compared with that in westerners. The carotenoids in red palm oil can be easily absorbed and in our study, there is no difficulty for subjects to accept this kind of new edible oil. Thus, red palm oil may provide another natural source of carotenoids for the Chinese.

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