Comparison of the Bioavailability of Natural Palm Oil Carotenoids and Synthetic β-Carotene in Humans

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Palm oil carotenoids are a mixture of α- and β-carotenes, which are used as food colorants. They may also be applied as a functional food ingredient because of the provitamin A activity of α- and β-carotenes and their proposed beneficial roles in the prevention of chronic diseases. This paper discusses the results of an incomplete balanced crossover study with 69 healthy adult volunteers to compare palm oil carotenoids with synthetic β-carotene in their efficacies to increase plasma levels of carotenoids. Four days of supplementation with natural palm oil carotenoids (7.6 mg/day of α-carotene, 11.9 mg/day of *all-trans*-β-carotene, 7.5 mg/day of *cis*-β-carotene) or synthetic β-carotene (23.8 mg/day of *all-trans*-β-carotene, 4.4 mg/day of *cis*-β-carotene), added to a mixed meal, resulted in significant increases in plasma levels of the supplied carotenoids as compared to consumption of a low-carotenoid meal (i.e., 7.2-fold increase in α-carotene and 3.5-fold increase in *all-trans*-β-carotene following palm oil carotenoids; 6.9-fold increase in *all-trans* β-carotene following synthetic β-carotene). As the carotenoid content differed between the treatments, the relative plasma responses were calculated per milligram of β-carotene intake. These were similar for the two supplements, suggesting that the presence of α-carotene does not affect the bioavailability of β-carotene from palm oil. It was concluded that 4 days of supplementation with palm oil carotenoids or synthetic β-carotene improves the plasma β-carotene status substantially, whereas α-carotene is additionally delivered by the palm oil supplement.

Keywords: *Palm oil carotenoids; food colorant; α-carotene; β-carotene; bioavailability; humans*

INTRODUCTION

Carotenoids may play a beneficial role in human health beyond their provitamin A function. Several biological activities of carotenoids have been demonstrated in vitro or in vivo, such as scavenging of free radicals, singlet oxygen quenching (Burton and Ingold, 1984; Sies and Stahl, 1995), enhancement of intercellular communication (Zhang, 1991), and immunomodulatory effects (Santos et al., 1996). Among the major carotenoids present in the human body (i.e., α-carotene, β-carotene, lycopene, lutein, zeaxanthin, α-cryptoxanthin, and β-cryptoxanthin), β-carotene has been studied most extensively. Interest in other carotenoids is growing, particularly since several intervention studies showed no protective effect of supplementation with high doses of β-carotene (ATBC Cancer Prevention Study Group, 1994; Hennekens et al., 1996; Omenn et al., 1996). Recent epidemiological studies have indicated beneficial effects of α-carotene (Ziegler et al., 1996), lycopene (Giovannucci et al., 1995), and lutein (Seddon et al., 1994).

In light of the emerging interest in carotenoids other than β-carotene, it is important to increase the knowledge on the bioavailability and metabolism of these carotenoids. Palm oil is a rich source not only of β-carotene but also of α-carotene, and palm oil carotenoids are currently used as food colorants. Ziegler et al. (1996) recently postulated that α-carotene may be more effective in reducing the risk of lung cancer than β-carotene. In line with their observation, in vitro and animal studies have shown that α-carotene is a more potent inhibitor of cancer cell proliferation than is β-carotene (Murakoshi et al., 1992; Levy et al., 1995). Little is known about the bioavailability of α- and β-carotenes from palm oil, and it may well be that the two carotenoids compete for absorption, as has been suggested for lutein and β-carotene (Kostic et al., 1995; Van den Berg and Van Vliet, 1998).

The objective of the present study was to compare the changes in plasma concentrations of α- and β-carotenes following 4 days of consumption of palm oil carotenoids with the changes following consumption of synthetic β-carotene alone.

MATERIALS AND METHODS

Volunteers. A total of 72 apparently healthy volunteers, aged 18−65 years, were enrolled in the study. They did not use dietary supplements (e.g., vitamins, minerals, carotenoids), consume a medically prescribed or weight loss diet, or use excessive amounts of alcohol (i.e., ≤21 glasses/week for females; ≤28 glasses/week for males; ∼10 g of alcohol/glass), and they smoked maximally 15 cigarettes/day. The women were not pregnant or lactating. Volunteers were employees of our laboratory or inhabitants of the Vlaardingen area, and they gave their written informed consent before participation.

Study Design. In an incomplete crossover design of four experimental periods, 72 volunteers received a palm oil carotenoid supplement, a synthetic β-carotene supplement, no supplement (control), or one of four other supplements. The results of the latter treatments are outside the scope of this paper and will be reported separately. All volunteers received

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10.1021/jf981091a CCC: $18.00
Published on Web 03/17/1999

Table 1. Macronutrient and Carotenoid Content of the Experimental Meals[a]

component	type of exptl meal		
	control	palm oil carotenoids[b]	synthetic β-carotene[c]
fat (g)	19.8 (1.3)	21.6 (0.7)	21.8 (1.1)
carbohydrate (g)	82.1 (5.4)	84.5 (2.6)	84.1 (3.1)
protein (g)	32.4 (0.7)	32.4 (1.5)	33.8 (1.7)
fiber (g)	16.0 (1.1)	16.1 (1.1)	15.7 (0.5)
α-carotene (mg)	–[d]	7.6 (0.7)	–
all-trans-β-carotene (mg)	–	11.9 (1.3)	23.8 (3.1)
9-*cis*-β-carotene (mg)	–	5.3 (0.8)	0.48 (0.04)
13-*cis*-β-carotene (mg)	–	2.2 (1.0)	3.9 (0.8)

[a] Values are expressed as mean (SD) per daily serving (n = 3–5). [b] Quest International, Ireland. [c] Hoffmann-La Roche, Switzerland. [d] <0.3 mg/serving. The amounts of lutein, zeaxanthin, and lycopene were each <0.3 mg/serving in all of the meals

the control treatment during one of the experimental periods. The treatments were supplied randomly over the volunteers and in randomized order. The carotenoid supplements were added to a standard hot meal, which was consumed at lunch time on four consecutive days. Fasting plasma levels of carotenoids were assessed at the end of the 4 days. These experimental periods were separated by 10 days of wash out, during which time the volunteers returned to their habitual diet. Volunteers were instructed not to consume any vegetables, fruits, fruit juices, or red sauces (e.g., tomato ketchup, pizza) during the experimental periods. Compliance was assessed by questionnaire, and the experimental meals were consumed under supervision in the laboratory.

Carotenoid Supplements. The palm oil carotenoids (Vegex Natural Carotene, 30% suspension in oil, Quest International, Ireland) or synthetic *all-trans* β-carotene [β-carotene 30% FS (E160a), 30% suspension in oil, Hoffmann-La Roche, Switzerland] were consumed with a standard meal. This was a pasta meal with ham and a white sauce and custard for dessert. The carotenoids were added to the sauce at the end of the preparation, and the sauce remained heated until being served to the volunteers (~80 °C, 5–30 min). Energy and fiber content and macronutrient composition of the meal were similar to that of an average Dutch main meal (Voorlichtingsbureau voor de Voeding, 1993) (Table 1). Carotenoid extraction from the control and supplemented sauces was done according to the method of Hart and Scott (1995). Extracts were appropriately diluted in HPLC solvent A [methanol/acetonitrile/2-propanol (54:44:2, v/v/v)]. Analysis was performed on a 5 μm Suplex pKb 100 column (250 × 4.6 mm, Supelco, Bellefonte, PA), using the following step gradient: 0–10 min, 97% solvent A and 3% water; 10–25 min, 100% solvent A, with a flow rate of 1 mL/min and detection at 450 nm. Peaks were identified spectrophotometrically by diode array detection (model 168, Beckman, Munich) and by coelution with synthetic reference carotenoids. Response factors determined for our HPLC system were used to calculate the carotenoid contents of the sauces. Reference carotenoids were either a gift from Makhteshim Chemical Works (Beer Sheva, Israel) (lycopene) and Hoffmann-La Roche (Basel, Switzerland) (lutein, zeaxanthin) or purchased from Sigma (Deisenhofen, Germany) (α-carotene). All other chemicals were obtained from Merck (Darmstadt, Germany). Results of the analyses are shown in Table 1. The synthetic β-carotene supplement contained small amounts of *cis*-isomers, probably due to isomerization during heating of the sauce. As we intended to supply similar amounts of total carotenoids, the β-carotene content of the palm oil carotenoid supplemented meal was lower than that of the synthetic β-carotene supplemented meal.

Plasma and Serum Analyses. Blood samples, obtained while subjects were fasting, were collected at the end of each experimental period into tubes coated with NaEDTA and tubes containing a serum separator for serum preparation. Plasma and serum were prepared by low-speed centrifugation (1500*g* for 10 min at 4 °C). Plasma was stored at −70 °C under argon for analysis of carotenoids, and serum was stored at −20 °C for analysis of lipids.

Extraction of carotenoids from plasma was performed as described by Wingerath et al. (1995). Dry carotenoid residues from plasma extraction were redissolved in HPLC solvent A [methanol/acetonitrile/2-propanol (54:44:2, v/v/v)], and analysis was performed using the same HPLC system as described above.

Total cholesterol and triacylglycerol concentrations in serum were assessed by using commercially available colorimetric test kits [CHOD-PAP, Boehringer, Mannheim, Germany, and GPO–PAP (Roche, Basel, Switzerland)/GPO-Trinder (Sigma, St. Louis, MO), respectively].

Statistical Evaluation. Analysis of variance with persons as blocks and sex, smoking habits, period, treatment, treatment × sex, and treatment × smoking as factors was used to compare the plasma and serum values found after consumption of the supplemented meals with those found after consumption of the control meal. Significance of the differences was assessed by Dunnett's test ($\alpha = 0.05$). As sex and smoking had no significant effect, these variables were excluded from the ANOVA model. Differences between the two carotenoid supplements were assessed by orthogonal contrasts ($\alpha = 0.05$). Plasma carotenoid concentrations were log-transformed to minimize correlation between mean values and standard errors, and the geometric means are presented with the standard error as percentage of these means.

RESULTS

Three volunteers dropped out of the study before the end of the first treatment period because of lack of time to participate in the trial, and four volunteers were not able to participate in each of the experimental periods for various reasons (e.g., illness, business trip). Two of these latter volunteers did not receive the control treatment. Data of 31 males and 38 females were included in the statistical analyses: $n = 67$ received the control treatment, $n = 31$ received the palm oil carotenoid supplement, and $n = 28$ received the synthetic β-carotene supplement. The average age (SD) of the volunteers was 42 (13) years, and their mean body mass index (SD) was 24.6 (2.3) kg/m^2. Ten of the 69 volunteers were smokers (maximum 15 cigarettes/day).

Carotenoid concentrations in plasma as determined at the end of the experimental periods are shown in Table 2. Unfortunately, the carotenoid supplements induced a carry-over effect in plasma concentrations of α- and β-carotenes. The plasma levels of α- and β-carotenes found in the first and second test periods following consumption of the supplements were therefore excluded from the statistical evaluation (i.e., using a wash-out period of 38 days). For α-carotene, this extended wash-out period was applied only following consumption of the palm oil carotenoid supplement. No carry-over effect was found for lutein, zeaxanthin, and lycopene. The numbers presented in Table 2 are based on the data actually included in the statistical evaluation.

As compared to the control meal, consumption of the carotenoid supplements resulted in significantly increased plasma levels of *all-trans*-β-carotene [mean (95% CI) = 345% (267, 439) for palm oil carotenoids; 686% (539, 867) for synthetic β-carotene] and of 13-*cis*-β-carotene [154% (76.8, 265) for palm oil carotenoids; 265% (149, 536) for synthetic β-carotene), whereas consumption of only the palm oil carotenoid supplemented meal induced a significant increase in plasma concentration of α-carotene [716% (590, 865)]. Plasma concentrations of lutein, zeaxanthin, and lycopene

Table 2. Plasma Carotenoid Concentrations[a] after 4 Days of Consumption of Meals Low in Carotenoids (Control Meal) or Supplemented with Carotenoids

	control		palm oil carotenoids		synthetic β-carotene	
	N	mean (CVM)	*N*	mean (CVM)	*N*	mean (CVM)
α-carotene (μmol/L)	55	0.028 (3.7%)[a]	31	0.23 (5.4%)[b]	24	0.028 (6.0%)[a]
all-trans-β-carotene (μmol/L)	43	0.14 (4.5%)[a]	28	0.60 (5.9%)[b]	24	1.1 (6.6%)[c]
13-*cis*-β-carotene (μmol/L)	39	0.009 (8.6%)[a]	28	0.023 (11%)[b]	24	0.034 (12%)[c]
lutein (μmol/L)	67	0.12 (2.2%)	31	0.13 (3.6%)	28	0.12 (3.8%)
zeaxanthin (μmol/L)	67	0.031 (1.8%)	31	0.033 (2.9%)	28	0.031 (3.1%)
lycopene (μmol/L)	67	0.12 (3.5%)	31	0.13 (5.7%)	28	0.14 (6.1%)

[a] Plasma carotenoid concentrations were log-transformed to minimize correlation between mean values and standard errors and the geometric means are presented with the standard error as percentage of these means (CVM). Means with different superscripts are significantly different ($P < 0.05$). Due to a carry-over effect of the carotenoid supplements, we excluded part of the data on α-carotene and β-carotene (see Results). The values presented are based on the actual data included in the statistical evaluation.

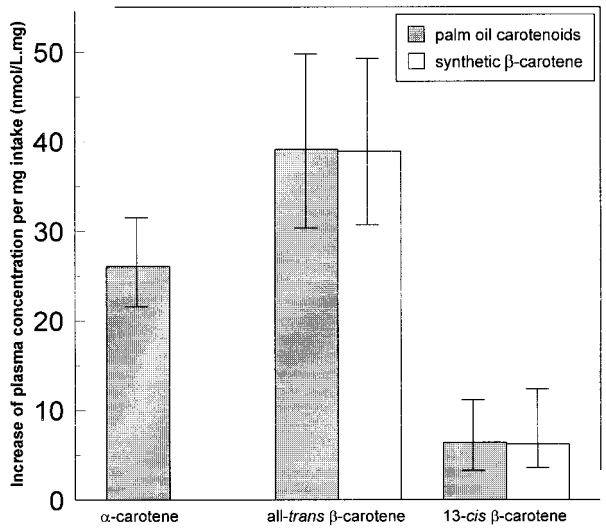

Figure 1. Plasma carotenoid response to 4 days of consumption of palm oil carotenoids or synthetic β-carotene, as compared to a low-carotenoid diet, expressed per milligrams of carotenoid intake (mean, 95% confidence interval).

(Table 2) and serum lipid levels (data not shown) remained unchanged. Data of 9-*cis*-β-carotene are not presented in Table 2 because almost half of the plasma concentrations measured were below the detection level (<0.002 μmol/L). However, after consumption of the palm oil carotenoid supplement, the percentage of volunteers with plasma concentrations of 9-*cis*-β-carotene within the detectable range was significantly larger than after consumption of the control or synthetic β-carotene supplemented meals (84% for the palm oil carotenoids supplemented meal and 55% for the other two meals, $P < 0.005$). None of the treatment effects were significantly different between males and females and smokers and nonsmokers.

Consumption of synthetic β-carotene resulted in a higher plasma level of *all-trans*-β-carotene and 13-*cis*-β-carotene than consumption of palm oil carotenoids (Table 2). On the other hand, consumption of palm oil carotenoids resulted in a 7-fold higher plasma level of α-carotene than consumption of the synthetic β-carotene supplement, which contained no α-carotene at all (Tables 1 and 2). The differences in increases of plasma levels of *all-trans*-β-carotene and 13-*cis*-β-carotene between the palm oil carotenoid and synthetic β-carotene supplements could be explained by the differences in composition of the two supplements. This is illustrated in Figure 1, which shows the increases in plasma concentrations of these carotenoids per milligram of carotenoid intake after consumption of the supplemented meals as compared to those after consumption of the control meal.

DISCUSSION

Four days of supplementation with palm oil carotenoids or synthetic β-carotene, added to a standard hot meal, resulted in significantly increased plasma levels of the supplied carotenoids. The magnitude of the differences between the two supplements in increases of plasma concentrations of *all-trans*-β-carotene and 13-*cis*-β-carotene appeared to be proportional to the differences in level of intake (Figure 1).

No effect was found on plasma levels of lutein, zeaxanthin, and lycopene. The standard meal contained no detectable amounts of these carotenoids (Table 1), and volunteers avoided carotenoid-containing foods during the rest of the experimental days. Hence, it is unlikely that competition for uptake has occurred between these carotenoids and the supplemented carotenoids. In addition, these results suggest that 4 days of supplementation with α- and/or β-carotene does not affect circulating lutein or lycopene levels and tissue uptake or metabolism of these carotenoids.

Our findings of a significantly increased plasma carotenoid status following supplementation with purified α-carotene and/or β-carotene are in line with other studies, and the magnitude of the increases were within the ranges expected (Brown et al., 1989; Micozzi et al., 1992; Rock and Swenseid, 1992; Carughi and Hooper, 1994; Törrönen et al., 1996; Canfield et al., 1997). The relative differences in plasma levels of *all-trans*-β-carotene and 13-*cis*-β-carotene between the two carotenoid supplements were in line with the differences in composition of the supplements (Figure 1). Apparently, at this level of intake a proportional relation exists between intake of these carotene isomers and their plasma response. Other studies also found a proportional association between carotenoid intake and plasma responses after supplementation with 12−90 mg of β-carotene (Dimitrov et al., 1986; Brown et al., 1989; Micozzi et al., 1992). In addition, our findings reveal that *all-trans*- and 13-*cis*-β-carotenes were equally available from the natural palm oil carotenoid supplement and the synthetic β-carotene supplement. Apparently, simultaneous ingestion of α-carotene had no effect on the response of plasma β-carotene levels following supplementation with palm oil carotenoids.

The present data do not allow us to speculate about the effect of β-carotene on the bioavailability of α-carotene because we did not include supplementation with α-carotene only. Although the palm oil carotenoid supplement increased the plasma level of α-carotene significantly, the response per milligram ingested was smaller than that of *all-trans*-β-carotene (Figure 1). In a previous study the plasma response of α-carotene was

more pronounced than that of β-carotene after 4 weeks of supplementation with palm oil carotenoids, resulting in similar final plasma concentrations for α- and β-carotenes (Van het Hof et al., 1998). This difference may be due to the shorter period of supplementation in the present study. The kinetics of the plasma increase may differ between α- and β-carotenes, with a slower rate of increase for α-carotene. As a new steady state may not have been reached in 4 days, the relative difference between α- and β-carotenes may be affected by differences in kinetics between the two carotenoids.

The relative plasma responses were not only different for α- and β-carotenes, they also varied between the isomers of β-carotene. No specific function has been reported for 13-*cis*-β-carotene, whereas 9-*cis*-β-carotene can be converted into 9-*cis*-retinoic acid (Nagao and Olson, 1994; Wang et al., 1994), which is involved in the regulation of gene expression (Heyman et al., 1992; Levin et al., 1992). *all-trans*-β-Carotene is, however, the predominant isomer in plasma, and the present study indicates that this is not entirely due to the fact that *all-trans*-β-carotene is also the major isomer in the diet. As has been reported previously for 9-*cis*-β-carotene (Stahl et al., 1993; Gaziano et al., 1995; Tamai et al., 1995; Ben-Amotz and Levy, 1996; Johnson et al., 1996; Von Laar et al., 1996; Yeum et al., 1996; You et al., 1996), the impact of increased intake of 9-*cis*- and 13-*cis*-β-carotenes on their plasma levels was very low as compared to that of *all-trans*-β-carotene. This may be due to inefficient intestinal uptake or degradation of the *cis*-isomers, more extensive conversion to vitamin A, isomerization to *all-trans*-β-carotene, or rapid uptake by tissue cells. You et al. (1996) found evidence for isomerization of 9-*cis*-β-carotene to *all-trans*-β-carotene. The present study does not support this hypothesis. Figure 1 shows that the increases in plasma level of *all-trans*-β-carotene relative to the intake were similar for both carotenoid supplements. On the basis of the hypothesis of *cis*−*trans*-isomerization, a relatively larger increase would be expected after consumption of the palm oil carotenoid supplemented meal because of the higher content of *cis*-β-carotene isomers. However, the dosage used in this study may have been too large to detect such an effect.

We conclude that 4 days of consumption of a meal supplemented with ∼25 mg/day of either palm oil carotenoids (α- and β-carotenes) or synthetic β-carotene improves the plasma carotenoid status substantially. The presence of α-carotene does not affect the bioavailability of β-carotene from palm oil and may deliver additional benefits.

ACKNOWLEDGMENT

We are grateful to Willy Dubelaar, Bert Dubbelman, Marleen Essenberg, Edward Haddeman, and other colleagues from the Unilever Nutrition Centre for their help in conducting the study and to Wim van Nielen and Sjaak Sies for analysis of the meals and blood samples. We acknowledge Dr. Wilhelm Stahl (University of Düsseldorf, Germany) for helpful discussions, and we thank the volunteers for their interest and participation in the study.

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Received for review October 8, 1998. Revised manuscript received January 22, 1999. Accepted January 27, 1999.

JF981091A