Original Paper

Absorption, by Humans, of β-Carotene from Fortified Soybean Oil Added to Rice: Effect of Heat Treatment

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Objective: This study was carried out to evaluate the absorption of β-carotene in humans when rice is prepared with refined cooking soybean oil fortified with β-carotene and to assess the effect of heat treatment on its bioavailability.

Methods: Sixteen healthy adults subjects participated in two experimental trials. Studies were carried out during two experimental periods of 11 days with a 12-day interval between them. Beta carotene was added to the soybean cooking oil and rice was cooked with it or it was added to the rice after cooking. Experimental diets included these two kinds of rice during the first day and fasting blood samples were collected on different days. All of the test diets were low in carotenoids. Plasma carotenoids were measured by HPLC method. β-carotene absorption was calculated through postabsorptive peak rise in plasma β-carotene and the total area under the absorption curve was determined by the trapezoidal method for the 11-day period.

Results: Absorption of carotene from heated or unheated fortified soybean oil were similar. Peak plasma carotene rise was different in men and women, $p < 0.05$ (0.66 ± 0.097 vs. 1.04 ± 0.117 μmol/l, respectively). Plasma α-carotene and retinol showed no variation.

Conclusions: Results demonstrate that β-carotene added to soybean oil used in the preparation of rice is absorbed, heated or not, and could be a practical source of provitamin A. Developing countries looking for strategies to increase vitamin A intake could use fortification of vegetable oils with synthetic β-carotene as a simple method.

INTRODUCTION

In many parts of the less industrialized world, vitamin A deficiency is a problem that affects nutritional status and health. In several countries, provitamin A carotenoids are the primary, if not the only, source of vitamin A [1–4]. In humans, carotenoids and especially β-carotene of vegetables are an important source of vitamin A. Of 600 carotenoids from natural sources that have been characterized, fewer than 10% serve as precursors of vitamin A. β-carotene, the most nutritionally active carotenoid, forms 15% to 30% of the total serum carotenoid [5]. On the other hand, prevention of vitamin A deficiency through food fortification is a well recognized approach to solve nutritional problems, mainly in developing nations [6]. Some regions of Brazil present higher indices of vitamin A deficiency, such as semi-arid regions of Paraíba [7] and the Jequitinhonha Valley State of Minas Gerais [8].

In Brazil and in other countries, it is important to select a suitable vehicle for fortification or enrichment with vitamin A. Vegetable oil utilization, particularly soybean oil, has increased rapidly in Brazil, especially among low socioeconomic groups. Vegetable oil appears to be a suitable carrier for fortification with vitamin A or beta-carotene. It is a daily fat source, high in energy, polyunsaturated fatty acids, and naturally occurring antioxidant vitamin E [9]. Furthermore, processing of vegetable oil and technology of fortification with vitamin A or β-carotene are simple. Vitamin A and β-carotene are soluble in oils. Previous studies in our laboratory have shown refined soybean oil to be a good vehicle for vitamin A fortification. They have also demonstrated the possibility of using retinyl palmitate

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fortified soybean refined oil as a carrier of vitamin A since it remains stable during cooking and storage procedures [6]. In rat bioassay, vitamin A palmitate fortified soybean oil retains 100% of this biological value heated at 100°C for about 20 minutes [10]. In humans, similar experiments showed that vitamin A, when added to soybean oil, is well absorbed even after heat treatment during cooking [11]. Other studies showed lower retention of β-carotene after cooking [12]. Manorama and Rumini showed β-carotene retention of 88% in palm crude oil when prepared with others foods [13].

The objective of this study was to investigate, in humans, the absorption of synthetic β-carotene from fortified soybean oil, heat or unheated. The procedure utilized rice, one of the most popular foods eaten in Brazil, to demonstrate this absorption.

MATERIALS AND METHODS

Subjects and Experimental Design

Sixteen healthy adults subjects (10 women and six men), health workers and university students from the University of São Paulo Medical School of Ribeirão Preto, Brazil, participated in two experimental trials. Each subject served as his own control, eating their basic local diet, controlled. The two studies were carried over an 11-day period. An interval of 12 days was included between them. Informed consent was obtained from subjects participating in this study and the project was approved by the Human Experimental Committee of the University of São Paulo Medical School, Ribeirão Preto, Brazil.

Fortification of Oil with β-Carotene

Refined cooking soybean oil, industrialized and consumed in Brazil, was fortified with β-carotene. The amount of β-carotene added was 45 mg per g of refined soybean oil. The form of β-carotene used for fortification was all-trans-β-carotene (Hoffman-La Roche, Basel, Switzerland), available in a 30% oil-miscible concentration. β-carotene was added to soybean oil and carefully mixed for 15 to 20 minutes. It was used the same day. The meals on the first day of each trial consisted of cooked rice (150 g), red kidney beans (100 g) and cooked beef (100 g). The fortified soybean oil was added to the rice preparation so that each portion of cooked rice contained 45 mg of β-carotene during the first trial. In the second trial all foods were cooked as usual with refined unfortified soybean oil, except that a 1 g of fortified soybean oil containing 45 mg of β-carotene was added to the cooked rice; β-carotene was not exposed to heat treatment during cooking. The supplementation with β-carotene was applied only on the first day of each 11-day trial. During this 11-day period each subject ate their own diet low in carotenoids. This included coffee, milk, sugar and bread for breakfast and rice, beans, meat, salt, vegetables and desert for lunch and dinner, all poor sources of carotenoids as suggested and controlled by the dietitian. Participants were carefully informed about the purpose of this study.

Collection of Blood and Analysis of β-Carotene

During each trial, five fasting blood samples were collected from each subject. Heparinized fasting blood samples (10 ml) were collected from each subject at 1, 2, 3, 7 and 11 days of each experiment period. Plasma was separated by centrifuging at 1000 $\times$ g for 10 minutes at 4°C. Plasma was removed and stored at -20°C until analyzed. Blood and plasma samples were protected from light at all times. The plasma α-carotene and β-carotene and retinol were determined by the HPLC technique of Arnaud et al [22].

Calculations and Statistical Analysis

The absorption of β-carotene from fortified soybean oil was evaluated by measuring the postabsorptive peak rise in plasma β-carotene and the total area under the absorption curve by the trapezoidal method of the 11-day periods, representing change in plasma β-carotene from first and last day of study. Peak rise was calculated by subtracting the baseline value from the highest value. The data were statistically analyzed by one way analysis of variance and the comparisons were made between group values for significance, $p < 0.05$ using Tukey's test [21].

RESULTS

The fortification of the soybean oil was carried out in our laboratory and did not effect the taste or acceptance of the rice. The color changed to yellowish but was well accepted by all participants. Plasma overnight fasting sample showed normal values of retinol, α-carotene and β-carotene. Table 1 shows the area under curve and peak rise of postprandial absorption of β-carotene in women and men separately, when they received their food with soybean fortified oil, heated or unheated. Retinol and α-carotene values on the 1st, 2nd, 3rd, 7th and 11th days of the study, in heated or unheated soy oil, are presented in Table 2. Plasma levels of β-carotene in different days of the enriched oil added during cooking or after cooking is shown in Fig. 1.

DISCUSSION

Results of the present study showed adequate plasma levels of retinol, α-carotene and β-carotene in our volunteers, as expected in healthy individuals. It was also shown that the intake of synthetic β-carotene fortified soybean oil used in the rice preparation significantly increased plasma β-carotene in the following days, when oil is heated or not. Our findings demonstrate that fortification of soybean oil with synthetic

Table 1. Effect of Heat Treatment During Rice Cooking with β-Carotene Fortified Soybean Oil and Its Impact on Postprandial Absorption of β-Carotene in Human Subjects. Data as X ± SEM.

	Area under curve (μmol/h/L)		Peak rise (μmol/L)	
	Cooked	Uncooked	Cooked	Uncooked
Men (n = 6)	1.69 ± 0.260	1.33 ± 0.232	0.66 ± 0.097	0.49 ± 0.062
Women (n = 10)	2.25 ± 0.358	2.86 ± 0.584	1.04 ± 0.117*	1.14 ± 0.238
Total (n = 16)	2.04 ± 0.248	2.27 ± 0.415	0.90 ± 0.091	0.90 ± 0.168

* Peak rise in cooked trial is significantly different when compared men and women's by student's "t" test, at $p < 0.05$.

Table 2. Plasma Concentrations of Retinol, α-Carotene and β-Carotene after Rice Intake with β-Carotene Fortified Soybean Oil Added During or After Cooking. Data as X ± SEM.

1st day	2nd day	3rd day	7th day	11th day
Retinol (μmol/L)				
Cooked				
1.67 ± 0.49[a]	1.65 ± 0.47[a]	1.71 ± 0.44[a]	1.72 ± 0.49[a]	1.67 ± 0.44[a]
Uncooked				
1.70 ± 0.33[a]	1.75 ± 0.37[a]	1.73 ± 0.34[a]	1.74 ± 0.35[a]	1.65 ± 0.31[a]
α-Carotene (μmol/L)				
Cooked				
0.29 ± 0.31[a]	0.27 ± 0.24[a]	0.26 ± 0.20[a]	0.26 ± 0.19[a]	0.18 ± 0.15[a]
Uncooked				
0.28 ± 0.20[a]	0.35 ± 0.26[a]	0.31 ± 0.20[a]	0.35 ± 0.25[a]	0.31 ± 0.21[a]
β-Carotene (μmol/L)				
Cooked				
0.78 ± 0.57[a]	1.41 ± 0.76[ab]	1.54 ± 0.78[b]	1.42 ± 0.81[ab]	1.03 ± 0.67[ab]
Uncooked				
0.67 ± 0.38[a]	1.35 ± 0.70[b]	1.42 ± 0.82[b]	1.25 ± 0.57[ab]	1.11 ± 0.54[ab]

Values followed by different superscript letters in same line were significantly different by Tukey's test ($p < 0.05$).

β-carotene is a feasible and practical option to carry β-carotene to humans. In Brazil, especially in the southern region, the basic daily diet of the low economic population is a combination of rice and beans eaten in large amounts. These foods are usually boiled. Frying is much less frequently used.

Although green edible plants and fruits are a natural source of β-carotene, their utilization in diets of several developing countries may be unrealistic due to cultural bias [17]. In addition, as plasma β-carotene increases more with purified β-carotene compared to natural sources, enrichment of daily foods with synthetic β-carotene may be more effective than the use of natural sources in humans [14]. Probably, inhibitory effects of dietary fiber could reduce the bioavailability of β-carotene [15]. It has been shown that intake of cooked carrots does not change plasma β-carotene. Cooking may lead to isomerization of plant carotenoids, and isomers are not handled equally in the intestines [16]. On the other hand, fat increases β-carotene absorption [18]. The source of fat also influences the absorption of carotenoids, for example, corn oil was shown to enhance hepatic accumulation of β-carotene and canthaxanthin in rats [19] and the same must be true with soy oil.

The fortification of soybean oil with carotenoids and vitamin A is an easy technological process that less industrialized countries are able to do and vegetable oils are largely consumed in a large number of countries. The color of oil changes with the addition of β-carotene, but the taste does not.

Our data showed women with significantly higher values of β-carotene than men. Quantification of the area under curve and specially postabsorptive peak rise they were higher in females. Serum concentrations of provitamin A carotenoids, mainly β-carotene, is show in the literature to vary according to sex and season [20]. Analysis of our data without sex differentiation may not reflect the overall β-carotene status. For example in the uncooked group, the area under curve and peak rise in women is two times higher than values presented by men. Plasma concentration of β-carotene increased in the groups receiving cooked and uncooked fortified soybean oil. Higher absorption peaks were found on the 3rd and 11th days, with similar basal values. The peak rise and area under curve in two trials showed no differences among the groups receiving fortified soybean oil added during cooking or after cooking ($p < 0.05$). Others parameters analyzed, plasma α-carotene and retinol showed no variation and no sex differences.

CONCLUSION

In conclusion, our data showed β-carotene to be well absorbed from rice cooked in carotene-fortified soybean oil, and heat treatment did not influence absorption. Therefore, preparing rice with fortified soybean cooking oil could be a good

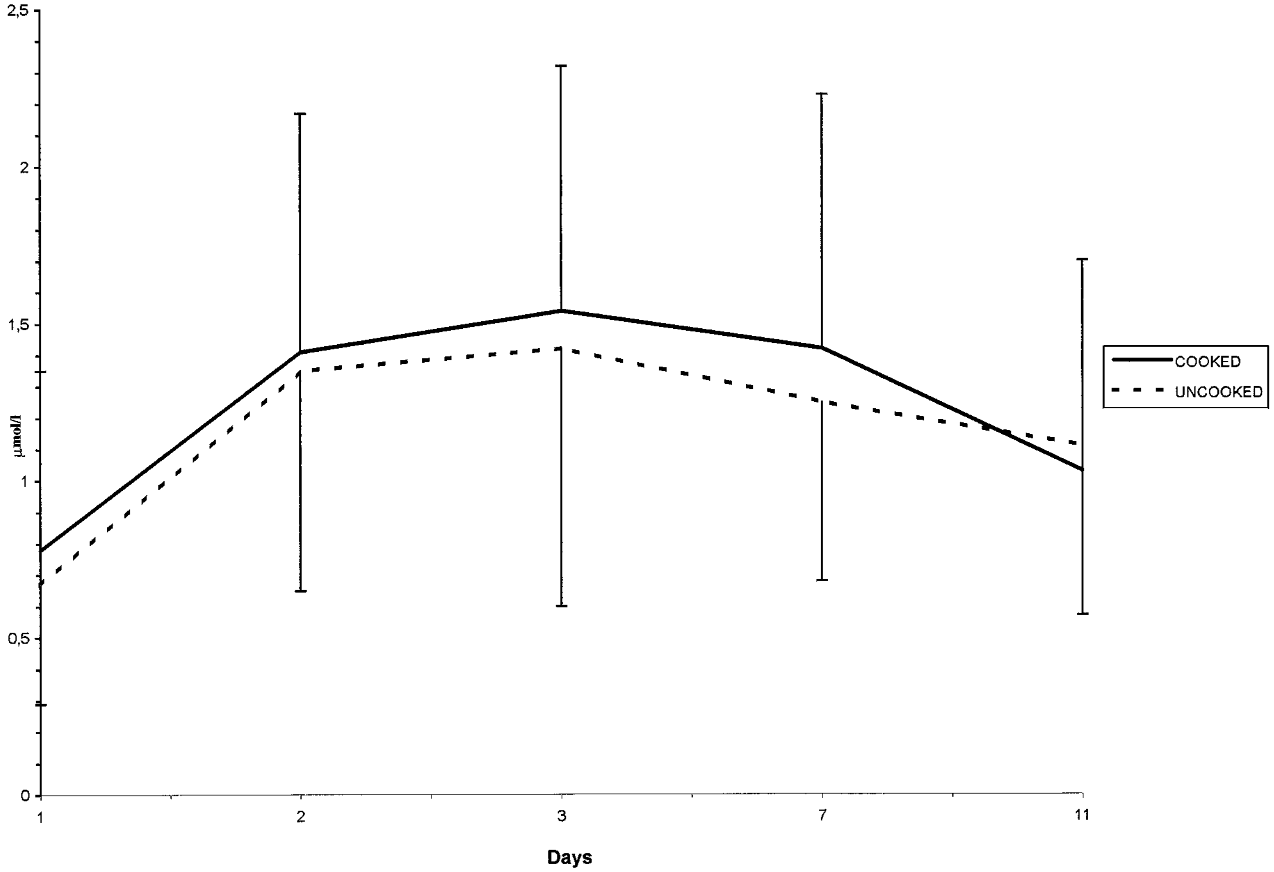

Fig. 1. Plasma fasting levels of β-carotene after rice intake with carotene fortified soybean oil, added during or after cooking. Data as X ± SEM.

alternative way to supply β-carotene to the population in Brazil.

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