

PIPERINE, AN ALKALOID DERIVED FROM BLACK PEPPER INCREASES SERUM RESPONSE OF BETA-CAROTENE DURING 14-DAYS OF ORAL BETA-CAROTENE SUPPLEMENTATION.

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ABSTRACT

The effectiveness of an extract from the fruit of black pepper, consisting of a minimum of 98.0% pure alkaloid piperine, was evaluated for its ability to improve serum response of beta-carotene during oral supplementation using a double-blind, crossover study design. Subjects were randomly selected to ingest a daily beta-carotene dose (15 mg) either with 5 mg of piperine or placebo during each of two 14-day supplementation periods. Inter-subject variability in pre-supplementation serum beta-carotene levels was minimized by limiting the selection of volunteers to healthy, adult males with fasting serum beta-carotene values $<20 \mu\text{g/dL}$. The results indicate that significantly greater increases ($P<0.0001$) in serum beta-carotene occurred during supplementation with beta-carotene plus piperine ($49.8 \pm 9.6 \mu\text{g/dL}$ vs. $30.9 \pm 5.4 \mu\text{g/dL}$) compared to beta-carotene plus placebo. Supplementation with beta-carotene plus piperine for 14-days produced a 60% greater increase in area under the serum beta-carotene curve (AUC) than was observed during supplementation with beta-carotene plus placebo. We suggest that the serum response during oral beta-carotene supplementation is improved through the non-specific, thermogenic property(s) of piperine, described in this paper as thermonutrient in action.

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KEY WORDS: Piperine, Bioperine, Beta-carotene, Bioavailability

INTRODUCTION

Piperine belongs to the chemical family of cinnamamides, naturally occurring compounds present as the major pungent ingredient, 1 to 9%, in various parts of plants from the family Piperaceae (1). Piperine, an alkaloid (1-peperoyl piperidine), has been previously evaluated for its ability to increase the serum response and lengthen the serum half-life of drugs in both experimental animals and human volunteers (2,3,4,5,6,7). These studies suggest that orally administered piperine may significantly increase serum levels, and lengthen serum half-lives of chemically diverse group of naturally occurring and synthetic drugs, i.e. vasicine (2), pyrazinamide (3), rifampicin (4), isoniazid (3), propranolol (5), theophylline (5) and phenytoin (6).

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The present study was conducted to evaluate the potential of piperine to enhance the serum response of a fat-soluble nutrient, the pro-vitamin beta-carotene. A specific brand of piperine was used in the current study, Bioperine®, which is composed of a minimum 98% pure alkaloid piperine extracted from the fruits of black pepper (*Piper nigrum* Linn) (8).

MATERIALS AND METHODS

Subjects

Adult male volunteers were selected for this study following a screening procedure to identify individuals with serum beta-carotene levels <20 µg/dL following an overnight fast. Twelve volunteers (22-43 years) were enrolled. Subjects were in good health, non-smokers and consumed <1 ounce of 80⁰ ethanol-equivalents per day. They also were not taking any type of medication, prescription or over-the-counter, and had not used any nutritional supplements during the four months prior to the study. Informed consent for participation was obtained from all volunteers following internal review board approval. All subjects remained on their self-selected diets throughout both 14-day testing phases. They were instructed not to change their eating habits, particularly with regard to fruit or vegetable consumption during the supplementation period.

Formulations tested

During both supplementation phases, a commercially available beta-carotene product (soft-gel capsule, GNC, General Nutrition Center), consisting of 15 mg of beta-carotene as a 30% suspension in vegetable oil, was used. Beta-carotene capsules for both testing phases were from the same GNC manufacturing lot (#45185LV) and all had the same expiration date. In addition, two-piece, hard-shell capsules that contained 5 mg of 98% pure alkaloid piperine, as commercially available Bioperine®, (lot# RD/PC/02) or placebo (lot# RD/PC.01) were provided by Sabinsa Corporation.

Testing Phase Design

During the first study phase, volunteers were randomly assigned into two groups of six subjects. They arrived between 6:30AM and 8:00AM, after an overnight fast on study day 1. Blood samples were collected to determine baseline or pre-supplementation serum beta-carotene values. Within 15 minutes, the daily supplements (beta-carotene capsule plus placebo or piperine capsule) were provided. Volunteers then consumed a standard snack consisting of 8 ounces of whole milk and a cheese danish pastry (15 g fat). Coffee and tea also were available to all volunteers. This procedure, supplementation followed by snack, was repeated daily for 14 days. In addition on days 2, 4, 7, 10 and 14, fasting blood samples were obtained prior to supplementation for serum beta-carotene analysis. After completion of the 14-days of beta-carotene supplementation, volunteers returned to their normal daily routine for three months. During this time, they resumed their normal daily eating habits and were instructed not use any form of nutritional supplementation. After

three months, fasting blood samples confirmed that the serum beta-carotene levels of volunteers had returned to $< 20 \mu\text{g/dL}$. At that time, the volunteers repeated the cross-over portion of the study during which they ingested the same daily beta-carotene supplement plus the placebo or piperine capsule that they did not ingest during the first 14-day trial.

Sample Collection and Analysis

Blood samples were collected in 7 mL vacutainers that did not contain anticoagulant. Serum was separated by centrifugation (4°C , 10 min, $1200 \times g$) within 60 minutes of collection. After centrifugation, serum for vitamins A, E and beta-carotene analyses was transferred to 2 mL cryogenic vials and stored at -85°C until thawed for analysis. Serum for vitamin C was acidified and deproteinized by addition (1:1, v/v) to 10% m-phosphoric acid prior to storage at -85°C . Vitamin and beta-carotene analyses were completed within 2 weeks of completion of each testing phase by published methodology (9,10).

Statistical analysis

Paired t-tests were used to test the hypothesis that the mean baseline serum beta-carotene values were equal, that the mean absolute (and relative) change in serum beta-carotene values between baseline and day-14 were equal, and that the mean serum beta-carotene AUC were equal for the two supplementation groups (beta-carotene + piperine vs. beta-carotene + placebo). Paired t-tests also were used to test the hypothesis that the mean serum vitamin A, vitamin E and vitamin C values were unaffected by either beta-carotene supplementation trial (11).

RESULTS

Baseline (pre-supplementation) serum beta-carotene levels

The mean baseline beta-carotene values were calculated from the average of two baseline blood samples during both (Phase I and Phase II) 14-day supplementation periods. The mean baseline beta-carotene values were not to differ significantly at the start of each supplementation period (16.3 ± 2.6 vs. $15.9 \pm 3.2 \mu\text{g/dL}$) (Table 1).

Absolute and relative change in serum beta-carotene levels from baseline to day 14

The mean absolute change in serum beta-carotene values from day 1 to day 14 was statistically greater after supplementation with beta-carotene plus piperine compared to beta-carotene plus placebo ($P < 0.0001$) (Table 1). The relative change in serum beta-carotene values was defined as the absolute change from baseline to day 14 divided by the baseline beta-carotene value. The mean relative change in serum beta-carotene values were significantly greater ($p < 0.0001$) after supplementation with beta-carotene plus piperine (3.1) as compared to supplementation with piperine plus placebo (1.89).

Table 1
Serum beta-carotene levels before and after 14 day supplementation*

Treatment	Baseline beta-carotene µg/dL	Day 14 beta-carotene µg/dL	Change beta-carotene µg/dL	AUC (µg/dL)x(time in days)
Beta-carotene + placebo	16.3 ± 2.6	47.2 ± 6.4	30.9 ± 5.4	272±47.6
Beta-carotene + piperine	16.0 ± 3.1	65.8 ± 9.7	49.8 ± 9.6	435±74.2

*Comparisons (baseline vs. day 14, placebo vs. piperine at day 14, placebo vs. piperine change at day 14) indicated statistically significant differences $p < 0.0001$.

Area under the serum beta-carotene curve from baseline to day 14

The mean area under the serum beta-carotene curve (AUC) differed significantly ($p < 0.0001$) between the volunteers when they received beta-carotene plus piperine compared to when they received beta-carotene plus placebo. A 60% increase in serum beta-carotene AUC was observed when beta-carotene plus piperine was used as compared to piperine plus placebo receiving group (Table 1).

Serum vitamin A, E and C levels at baseline and at day 14 of the supplementation

The baseline and day 14 mean values of vitamin A (retinol), vitamin E (alpha-tocopherol) and vitamin C (ascorbic acid) were compared during both beta-carotene supplementation phases. The results indicate that there were no significant changes from baseline to day 14 for vitamin A, vitamin E and vitamin C levels with either beta-carotene supplementation regimen (beta-carotene + piperine or beta-carotene + placebo) (Table 2) and (Table 3).

Table 2
Serum vitamin A levels at the baseline and at day 14 *

Treatment	Baseline	Day 14 retinol µg/dL	Change
Beta-carotene + placebo	66.0 ± 11.5	65.1 ± 10.2	-0.9 ± 2.4
Beta-carotene + piperine	65.2 ± 11.9	65.0 ± 10.6	-0.2 ± 2.1

*Comparison (baseline vs. day 14, placebo vs. piperine at day 14, placebo vs. piperine change at day 14) indicated no statistically significant differences.

Table 3
Serum vitamin C and E levels at the baseline and at day 14 *

Treatment	Baseline		Day 14	
	vitamin C μg/dL	vitamin E μg/dL	vitamin C μg/dL	vitamin E μg/dL
Beta-carotene + placebo	0.62 ± 0.13	0.80 ± 0.18	0.64 ± 0.10	0.84 ± 0.15
Beta-carotene + piperine	0.66 ± 0.12	0.83 ± 0.16	0.65 ± 0.11	0.84 ± 0.16

*Comparison (baseline vs. day 14, placebo vs. piperine at day 14, placebo vs. piperine change at day 14) indicated no statistically significant differences.

DISCUSSION

The current study addresses an important problem of nutrient bioavailability. It has been estimated that the improved nutritional status of the U.S. society maybe one of the important reasons why the age adjusted death rate showed a drop of nearly 50% from 1930 to 1980 as well as the marked increase in life expectancy at birth (12). Additional health benefits may be expected from the improved nutrient bioavailability.

The results of our study suggest that piperine supplementation may offer a simple and effective nutritional intervention to enhance the serum levels of beta-carotene in healthy subjects. This study is one of several clinical evaluations involving piperine in the form of Bioperine® with nutrients including: water soluble vitamin B6, vitamin C, selenium in the form of L-selenomethionine, and coenzyme Q10 (unpublished data, 1996). All these studies involving piperine supplementation with nutrients show its significant effectiveness in increasing the serum levels of the nutrients studied.

The 5 mg/person/day dose of piperine together with 15 mg of beta-carotene administered orally in the course of 14 day supplementation, resulted in a 60% increase in serum beta-carotene levels as compared to beta-carotene administered with placebo. In addition, the levels of retinol remained unchanged during the course of the beta-carotene plus piperine trial, suggesting that the 5 mg dose of piperine had no effect on the metabolic pathways that convert beta-carotene to retinol. This is important because retinol is produced by the metabolic conversion of beta-carotene, predominantly in the gastrointestinal epithelium which is also the proposed site of piperine action. Additional findings following 14 days of supplementation with beta-carotene plus piperine indicate

that serum vitamin E and vitamin C levels (not supplemented nutrients) were not affected suggesting that the improved serum response of one nutrient by piperine did not alter the normal serum status of other micronutrients. The present findings suggest that piperine may act through a selective uptake mechanism in the intestinal mucosa and that for piperine to be effective, a nutrient-piperine interaction must occur in the gut. When this specific interaction does not occur, there is no serum nutrient response due to piperine supplementation.

The diversified nature of the earlier mentioned drugs and nutrients whose serum response was improved by piperine suggest that piperine has a broad and nonspecific ability to improve circulating levels of drugs and nutrients. However, it is possible that there may be important distinctions between piperine's effect on drugs and piperine's effect on nutrients. Drugs, as examples of xenobiotics, are subject to biotransformation primarily in the epithelial cells of the gastrointestinal tract and the liver. The primary result of this biotransformation is the elimination of the xenobiotic from the body. As such, biotransformation plays a major protective role to prevent high serum levels of a drug. Most nutrients, on the other hand, enter the general metabolism and are essential chemical building blocks or biochemical co-factors which are not subject to the biotransformation process. Thus, a major obstacle to improved nutrient status for nutrients is the ability or the efficiency to cross the gastrointestinal barrier, rather than a concern about premature elimination from the circulation by biotransformation processes. Our findings suggest that piperine improves gastrointestinal uptake of beta-carotene leading to increased serum status and probably tissue status.

The piperine dose (5 mg) used in this study was calculated as far below an average dose that was reported to affect the biotransformation of drugs. Earlier studies dealing with drug bioavailability suggested that piperine was effective as a non-competitive inhibitor of xenobiotic biotransforming enzymes when administered in oral doses of 20-50mg/subject/day (3,4) and that the duration of inhibition was dose-dependent and that it lasted for only a few hours (7).

In summary, we believe that piperine increases the serum response of beta-carotene by non-specific mechanisms which operate directly on the gastrointestinal tract and the liver. These mechanisms may involve increased micelle formation (13), hyperemia (13), epithelial cell wall modification due to the lipophilic nature of piperine (14), or an increase in the bioenergetic processes of the gastrointestinal epithelium due to the thermogenic properties of piperine (15). These thermogenic properties may be of particular importance in explaining how such a small amount of piperine (5 mg) can afford such a profound effect on serum nutrient levels. It is possible that when piperine is ingested, it has a localized thermogenic effect on epithelial cells which increase the uptake of nutrients from the gut. This action would be of short duration, and could explain the enhanced serum response of beta-carotene reported here. It should also be noted that the proposed thermogenic action of piperine may not be shared by structurally similar compounds. For example, capsaicin (a pungent compound in *Capsicum annum*) which is structurally similar to piperine is well known for its thermogenic properties. Nevertheless, capsaicin does not appear to have the

ability to affect the serum response of nutrients (16,17). It is also noteworthy that the effect of piperine and capsaicin on bioenergetic processes is different. While piperine increases the ATPase activity (15), capsaicin on the contrary inhibits its activity (18). In view of these findings we propose that piperine ingested in relatively small amounts will act as a *thermonutrient*, which by virtue of a local thermogenic action on the epithelial cells would increase the rate of absorption of supplemented nutrient(s).

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REFERENCES

1. Govindarajan VS. Pepper-Chemistry, Technology, and Quality Evaluation. CRC Critical Reviews in Food and Science and Nutrition 1977: 115-250.
2. Atal CK, Zutshi U, Rao PG. Scientific evidence of the role on Ayurvedic herbals on bioavailability of drugs. J Ethnopharm 1981; 4: 229-232.
3. Zutshi U. A process for the preparation of pharmaceutical combination with enhanced activity for treatment of tuberculosis and leprosy. Indian Patent 1989; No. 1232/DEL/89.
4. Zutshi RK, Singh R, Zutshi U, Johri RK, Atal CK. Influence Of Piperine On Rifampicin Blood Levels In Patients Of Pulmonary Tuberculosis. JAPI 1985; 33: 223-224.
5. Bano G, Raina RK, Zutshi U, Bedi KL, Johri RK, Sharma SC. The effect of piperine on bioavailability and pharmacokinetics of propranolol and theophylline in healthy volunteers. Eur J Clin Pharmacol 1991; 41: 615-617.
6. Bano G, Amala V, Raina RK, Zutshi U, Chopra CL. The Effect of Piperine on Pharmacokinetics of Phenytoin in Healthy Volunteers. Planta medica 1987; 53: 568-569.
7. Atal CK, Dubey, RK, Singh, J. Biochemical basis of enhanced drug bioavailability by piperine: Evidence that piperine is a potent inhibitor of drug metabolism. J Pharmacol Exp Ther 1985; 232: 258-262.
8. Majeed M, Badmaev V, Rajendran R. Use of piperine to increase the bioavailability of nutritional compounds. United States Patent 1996 No. 5,536,506.
9. Laboratory Procedures used by the Clinical Chemistry Division, Centers for Disease Control, for the 2nd Health and Nutrition Survey (NHanes II) 1976-1980, USDHHS, Public Health Services, IV. Analytical Methods, Vitamin C, Atlanta, GA, 1979; pp. 17-19.

10. Craft NE, Brown ED, Smith JC. Effects of storage & handling procedures on concentrations of individual carotenoids, retinol, and tocopherol in plasma, Clin.Chem. 1988,34:44-48.
11. Zar JH. Biostatistical Analysis, 2nd edition, Prentice-Hall, Inc., Englewood Cliffs, NJ, 1984.
12. Mendeloff, AI. Diet and colorectal cancer. Am J Clin Nutr 1988; 48:780-781.
13. Annamalai AR, Manavalan R. Effect of "Trikatu" and its individual components and piperine on gastrointestinal tracts: Trikatu - a bioavailable enhancer. Ind Drugs 1990; 27(12): 595-604.
14. Johri RK, Thusu N, Khajuria A, Zutshi U. Piperine-mediated changes in the permeability of intestinal epithelial cells. Biochemical Pharmacology 1992; 43(7): 1401-1407.
15. Reanmongkol W, Janthasoot W, Wattanatorn W, Dhumma-Upakorn P, Chudapongse P. Effects Of Piperine On Bioenergetic Functions Of Isolated Rat Liver Mitochondria. Biochem Pharmacol 1988; 37(4):753.
16. Sambaiah K, Srinivasan MR, Satyanarayana MN, Chandrasekhara N. Influence of capsaicin on the absorption of amino acids and fat in rats. J Food Sci Technology 1984; 21:155.
17. Monsereenusorn Y, Glinsukon, T. Inhibitory effect of capsaicin on glucose absorption in vitro. Food Cosmet Toxicol 1978: 16.
18. Chundapongse P, Janthasoot W. Mechanism of the inhibitory action of capsaicin on energy metabolism by rat liver mitochondria. Biochem Pharmacol 1976; 30:735.

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