

Piperine derived from black pepper increases the plasma levels of coenzyme Q10 following oral supplementation

Vladimir Badmaev, Muhammed Majeed, and Lakshmi Prakash

Sabinsa Corporation, Piscataway, NJ USA

An extract from the fruits of black pepper consisting of a minimum of 98% pure piperine was evaluated in a clinical study using a double-blind design. The relative bioavailability of 90 mg and 120 mg of coenzyme Q10 administered in a single-dose experiment or in separate experiments for 14 and 21 days with placebo or with 5 mg of piperine was determined by comparing measured changes in plasma concentration. The inter-subject variability was minimized by limiting the selection of individuals to healthy adult male volunteers with (presupplementation) fasting coenzyme Q10 values between 0.30 and 0.60 mg/L. The results of the single-dose study and the 14-day study indicate smaller, but not significant, increases in plasma concentrations of coenzyme Q10 in the control group compared with the group receiving coenzyme Q10 with a supplement of piperine. Supplementation of 120 mg coenzyme Q10 with piperine for 21 days produced a statistically significant ($p = 0.0348$) approximately 30% greater area under the plasma curve than was observed during supplementation with coenzyme Q10 plus placebo. It is postulated that the bioenhancing mechanism of piperine to increase plasma levels of supplemental coenzyme Q10 is nonspecific and possibly based on its description in the literature as a thermogenetic (J Nutr Biochem 11:109–113, 2000) © Elsevier Science Inc. 2000. All rights reserved.

Keywords: piper nigrum; black pepper; piperine; bioperine; coenzyme Q10; bioavailability

Introduction

Piperine belongs to a group of compounds known as “vanilloids,” because they are distinguished by the presence of a chemical group based on the structure of vanillin. Piperine is a naturally occurring compound present as the major pungent ingredient (1–9%) in various parts of plants from the family *Piperaceae*.¹ Piperine, an alkaloid (1-peperoyl piperidine), has been previously evaluated for its potential to enhance the serum levels of drugs and nutrients in animals and humans.^{2–9} Compounds studied include drugs such as vasicine,² pyrazinamide,³ rifampicin,⁴ isoniazid,³ propranolol,⁵ theophylline,⁵ and phenytoin,⁶ and nutrients^{8,9}

such as fat soluble beta-carotene, water soluble vitamin B₆, vitamin C, and the mineral selenium in the form of L-selenomethionine. The results of clinical studies of piperine with drugs indicate that piperine administered orally at a single dose of 20 to 50 mg may significantly increase serum drug levels by reducing the clearance of drugs, both naturally derived and synthetic. This effect is primarily due to piperine's ability to inhibit (by a noncompetitive mechanism) the xenobiotic (drug) metabolizing enzymes when administered in a high dose.^{3,4} On the other hand, the increased nutrient absorption in the presence of piperine appears to be independent of the inhibition of the biotransforming enzymes and has been achieved with as little as 5 mg of piperine coadministered with the supplemented nutrient.^{8,9}

The purpose of the this study was to compare the serum response to the biologically important nutritional compound coenzyme Q10, administered to healthy male volunteers with piperine or a placebo under various treatment protocols.

This work was funded by Sabinsa Corporation. Sabinsa is a manufacturer of piperine and coenzyme Q10.

Address correspondence to Dr. Vladimir Badmaev, Sabinsa Corporation, 121 Ethel Road West, Unit 6, Piscataway, NJ USA.

Received August 3, 1999; accepted October 28, 1999

Materials and methods

Subjects

Twelve healthy adult male subjects were enrolled in this study. Prior to selection, the prospective subjects were instructed regarding the study design and its objectives. All individuals were given an opportunity to ask questions regarding the proposed study, and the volunteers gave their written informed consent. The subjects, aged 20 to 47 years, were in good health and were nonsmokers and nondrinkers of alcohol (less than one alcoholic drink per day was allowed). In addition, they had not taken any type of medication, prescription or over-the-counter, or used any nutritional supplements during the 4 months prior to the study. Inclusion in the study was restricted to individuals who had never ingested coenzyme Q10 supplements and who also provided two consecutive (1 week apart) fasting baseline plasma coenzyme Q10 values between 0.30 and 0.60 mg/L.

All subjects stayed on their self-selected diets. They were instructed not to change their eating habits, particularly with regard to fruit or vegetable consumption, during the supplementation period. From day 0 until day 21, the subjects were provided with a standard breakfast that consisted of 8 oz. of whole milk and a large Danish pastry (15 g fat).

The experimental protocol was reviewed and approved by the Institutional Review Board at Our Lady of Mercy Medical Center (Bronx, NY USA). The study was performed at the Biomedical Research Laboratory of Our Lady of Mercy Medical Center (Bronx, NY USA).

Formulations tested

The control group received a GNC (General Nutrition Center) Pro Performance Brand coenzyme Q10 soft-gel capsule (30 mg of coenzyme Q10 per capsule), plus a two-piece, hard-shell placebo capsule provided by Sabinsa Corp. (Lot # RD/BPP/02; Piscataway, NJ USA). They were administered together in a single dose (90 mg of coenzyme Q10), 14 day supplementation (90 mg of coenzyme Q10 per day) and 21 day supplementation (120 mg of coenzyme Q10 per day). The active group received coenzyme Q10 soft-gel capsules and a two-piece, hard-shell capsule containing 5 mg piperine provided by Sabinsa Corp. (Lot # RD/BPC/02). A specific brand of piperine, Bioperine®, was used in the study. Bioperine is composed of a minimum of 98% pure alkaloid piperine extracted from the fruits of black pepper (*Piper nigrum* Linn).^{8,9}

Experimental design

The subjects were randomly assigned into two groups of six subjects each. After assignment, each volunteer arrived at the laboratory between 6:30 and 7:30 AM following an overnight fast on study days -7 and 0. On both occasions, a fasting blood sample was collected from each subject for determination of two baseline plasma coenzyme Q10 measurements. Immediately following the collection of the second baseline blood sample (day 0), each volunteer was started on a particular experimental dosing regimen. The ingestion of the supplement on an empty stomach was fully supervised (in the presence of an appointed supervisor), and 30 minutes later the standardized breakfast was eaten (8 oz. of whole milk and a large Danish pastry). In the single-dose study, fasting blood samples were collected for coenzyme Q10 analysis at intervals of 2, 4, 5, 6, 7, and 8 hours; in the 14-day study, fasting blood samples were collected for plasma coenzyme Q10 analysis on days 2, 4, 7, 10, and 14; and in the 21 day study, fasting blood samples were collected for plasma coenzyme Q10 analysis on days 4, 7, 10, 14, and 21.

Table 1 Hourly changes in coenzyme Q10 plasma values following a single dose administration of 90 mg coenzyme Q10 either with placebo or with 5 mg piperine in 12 volunteers

Time (hr)	Mean increase in plasma coenzyme Q10 levels (mg/L)	
	Q10 + placebo group	Q10 + piperine group
0	0.435 ± 0.145	0.413 ± 0.117
2	0.598 ± 0.138	0.576 ± 0.11
4	0.720 ± 0.111	0.741 ± 0.083
5	0.788 ± 0.091	0.825 ± 0.125
6	0.840 ± 0.106	0.846 ± 0.143
7	0.853 ± 0.208	0.808 ± 0.2
8	0.763 ± 0.119	0.733 ± 0.107

Sample collection and analysis

Blood samples were collected in 7 mL vacutainer tubes that contained ethylenediamine-tetraacetic acid (EDTA) for the anticoagulant and placed in a test tube rack that was protected from heat and light. Plasma was separated by centrifugation (4°C, 15 min, 1,200 × g) within 60 minutes of collection. Immediately following centrifugation, plasma was transferred to 2 mL cryogenic vials and stored at -85°C until analysis. Coenzyme Q10 analyses were completed within 3 weeks of collection by reversed-phase high performance liquid chromatography (HPLC).¹⁰

The area under the plasma concentration curve (AUC) of coenzyme Q10 was calculated as follows:

$$\sum_{i=1}^5 (t_i - t_{i-1}) \left[\frac{(v_i + v_{i-1})}{2} - v_0 \right]$$

where t refers to the day following initiation of supplementation, i refers to the specific sampling period, and v equals the plasma coenzyme Q10 level at the t_i period. The variable v_0 at period one is the arithmetic mean of two baseline measurements.

Statistical data analysis

Analysis of variance and t -tests were used to test the null hypothesis that the means of the baseline coenzyme Q10 values were equal across the two groups, that the mean absolute (and relative) change in plasma coenzyme Q10 values between baseline and each data point during the ingestion period was equal for the two groups, and that the mean area under the plasma coenzyme Q10 curve was equal for the two formulations.¹¹

Results

Hourly changes in coenzyme Q10 plasma values following single-dose administration of 90 mg coenzyme Q10 with placebo or with 5 mg piperine

The single-dose administration of 90 mg coenzyme Q10 with placebo or with piperine indicated a wide variation in the plasma coenzyme Q10 levels in the 12 subjects. The numerically higher net plasma increases (mg/L) were found in the group receiving coenzyme Q10 with piperine rather than in the group receiving coenzyme Q10 with placebo (Table 1). In addition, the Tmax for the coenzyme Q10 and piperine group was attained 1 hour sooner than the Tmax for the control group (6 versus 7 hours; Figure 1). However, the differences between the two dosing groups were not statistically significant.

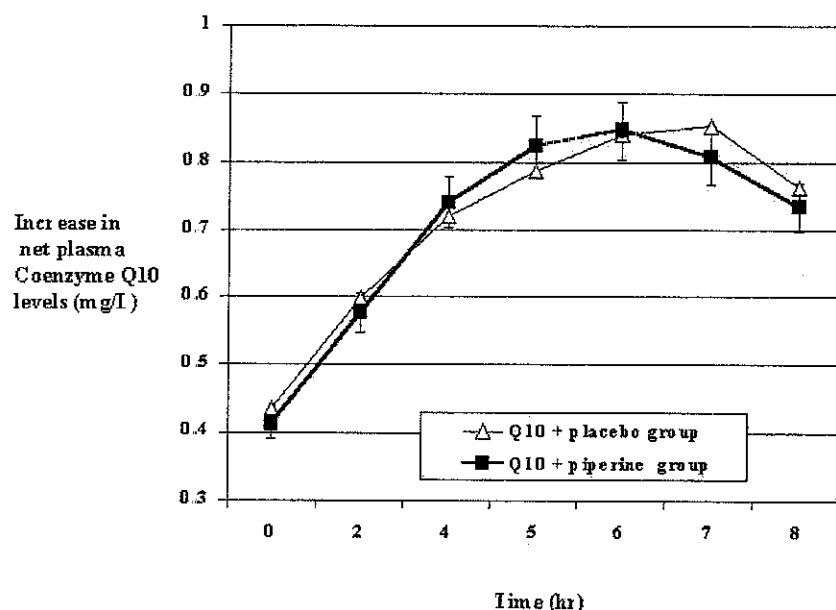


Figure 1 Hourly changes in coenzyme Q10 plasma values following single dose administration of 90 mg coenzyme Q10 either with placebo or with 5 mg piperine in 12 volunteers

Absolute changes in plasma coenzyme Q10 from baseline to day 14

The mean absolute change (change from baseline to post-supplementation value) in plasma coenzyme Q10 values and the mean AUC from baseline to day 14 were numerically greater for the active group (90 mg coenzyme Q10 plus 5 mg piperine per day) than for the placebo group (daily 90 mg coenzyme Q10 with placebo). The mean baseline coenzyme Q10 value was 0.44 ± 0.09 mg/L for the active group and 0.46 ± 0.13 mg/L for the placebo group. The mean absolute change after 14 days of supplementation was 0.21 ± 0.04 mg/L and 0.19 ± 0.07 mg/L, respectively; and the mean AUC was 1.098 ± 0.439 and 1.027 ± 0.441 (mg/L) $\times$ (days), respectively. However, changes in the active group were not significantly different from the values determined for the control group.

Absolute changes in plasma coenzyme Q10 from baseline to day 21

The mean absolute change in plasma coenzyme Q10 values and the mean AUC from baseline to day 21 were significantly greater for the active study than for the placebo study (Table 2).

Coadministration of 5 mg piperine with 120 mg coenzyme Q10 daily resulted in a statistically significant ($p = 0.0369$) absolute increase in plasma levels of coenzyme Q10 of 1.12 mg/L compared with the absolute increase of 0.85 mg/L in the control group (approximately 32% greater increase in active versus control group; Table 2). Corresponding values measured as the area under the plasma coenzyme Q10 curve in the active group receiving piperine were higher and statistically significant ($p = 0.0348$) when compared with the control group (15.38 and 11.81 mg/L; approximately 30% greater increase in active versus control group; Table 2).

Discussion

This study is one in a series of clinical trials conducted on the alkaloid piperine to explore the use of this compound and the mechanism(s) by which it enhances the gastrointestinal absorption of nutrients. The selected nutrient, coenzyme Q10, is different from previously studied vitamins and minerals because the body synthesizes it. The increased plasma response to supplemental coenzyme Q10 is of clinical significance because its deficiency has been found in patients with breast cancer and nonmalignant breast

Table 2 Absolute change in plasma coenzyme Q10 from baseline to day 21 of daily ingestion of 120 mg coenzyme Q10 with placebo or with a concurrent dose of 5 mg piperine in 12 volunteers

Treatment	Mean baseline value $\pm$ SD coenzyme Q10 mg/L	Mean absolute change $\pm$ SD coenzyme Q10 mg/L	Mean area under the curve (AUC) (mg/L) $\times$ (days)
Coenzyme Q10 + placebo	0.50 ± 0.09	$0.85 \pm 0.20^*$	$11.81 \pm 2.66^\dagger$
Coenzyme Q10 + piperine	0.49 ± 0.09	$1.12 \pm 0.20^*$	$15.38 \pm 2.40^\dagger$

* Difference between mean absolute change in placebo and active group statistically significant ($p = 0.0369$)

† Difference between mean absolute change in placebo and active group statistically significant ($p = 0.0348$)

tumors.¹² Low levels of coenzyme Q10 have been found in patients with insulin dependent diabetes mellitus,¹³ patients with severe ischemic heart disease,¹⁴ and endurance trained individuals.¹⁴ Supplementation with coenzyme Q10 has been shown to be beneficial for the treatment of congestive heart failure,¹⁵ dyslipidemia,¹⁶ complications of diabetes,¹⁷ Parkinson's syndrome¹⁸ and as a potential therapy for some forms of male infertility.¹⁹

The normal plasma levels of coenzyme Q10 reported in literature vary. According to one report, it is between 0.36 and 0.8 mg/L for men,²⁰ whereas a range of 0.46 to 0.57 mg/L for men and women is reported by other researchers.²¹ The levels of coenzyme Q10 in different age groups were reported as follows: <30 years, 0.42 ± 0.16 mg/L; 30 to 49 years, 0.48 ± 0.22 mg/L; >50 years, 0.47 ± 0.10 mg/L.²¹ The breakdown of normal coenzyme Q10 values based on gender showed 0.49 ± 0.21 mg/L plasma levels in men and 0.44 ± 0.15 mg/L plasma levels in women. Supplemental coenzyme Q10 is poorly absorbed from the digestive tract and is slowly eliminated from plasma, with a long plasma half-life of 33.19 ± 5.32 hours.²²

The current study tested three dosing regimens of coenzyme Q10 with and without an additional supplement of 5 mg piperine: a single-dose study evaluating the absorption rate of 90 mg coenzyme Q10 within 8 hours, 14-day administration of 90 mg coenzyme Q10 per day, and 21-day administration of 120 mg of coenzyme Q10 per day. The inclusion criterion for participants in this study was a plasma level of coenzyme Q10 of 0.30 to 0.60 mg/L. To further minimize the inter-subject variability, this initial evaluation of the effects of piperine on coenzyme Q10 bioavailability was carried out selectively with healthy, young male volunteers only.

The plasma response to coenzyme Q10 administered in a single dose, as well as in the course of 14-day supplementation, resulted in a wide variation of coenzyme Q10 plasma levels in the control and piperine subjects. The numerically higher net plasma levels of coenzyme Q10 obtained in both these experiments within the piperine groups were not significantly higher than those in the respective control groups. Nevertheless, the results from these experiments suggest that piperine may exert a bioenhancing effect on coenzyme Q10 gastrointestinal absorption.

The third experiment involving the coadministration of 5 mg piperine with 120 mg coenzyme Q10 once daily for 21 days showed significant increase in the plasma levels of coenzyme Q10 in comparison to the control group. The increase in the AUC was 30%, whereas the absolute change in the plasma values was 32%.

Our results suggest that piperine supplementation may result in improved absorption of coenzyme Q10 in healthy subjects. It also appears that a longer period of coadministration of piperine with 120 mg rather than 90 mg of coenzyme Q10 per day may result in significantly better absorption of coenzyme Q10 as measured by its plasma levels.

The mechanism(s) by which piperine increases the absorption of coenzyme Q10 is likely nonspecific and operates directly in the gastrointestinal tract. These mechanisms, based on literature data, may involve increased gastrointestinal blood supply,²³ increased micelle formation,²³ and

epithelial cell wall modification due to the lipophilic nature of the compound.²⁴ The most interesting mode of action of piperine may be due to its postulated thermogenic²⁵ properties and the increase in bioenergetic processes²⁶ in the gastrointestinal epithelium described in our previously published study on its *thermonutrient* activity.⁸

This preliminary report on increased gastrointestinal absorption of the coenzyme Q10 with piperine presents an interesting concept to be further evaluated. The combined supplementation of coenzyme Q10 with piperine may be a practical approach to enhance this nutrient bioavailability, especially in patients diagnosed with low plasma levels of coenzyme Q10 and in elderly individuals with poor gastrointestinal absorption.

References

- Govindarajan, V.S. (1977) Pepper—chemistry, technology, and quality evaluation. *CRC Crit. Rev. Food Sci. Nutr.* 115–250.
- Atal, C.K., Zutshi, U., and Rao, P.G. (1981) Scientific evidence of the role on Ayurvedic herbals on bioavailability of drugs. *J. Ethnopharm.* 4, 229–232.
- Zutshi, U. (1989) A process for the preparation of pharmaceutical combination with enhanced activity for treatment of tuberculosis and leprosy. Indian Patent No. 1232/DEL/89.
- Zutshi, R.K., Singh, R., Zutshi, U., Johri, R.K., and Atal, C.K. (1985) Influence of piperine on rifampicin blood levels in patients of pulmonary tuberculosis. *J. Assoc. Physicians India* 33, 223–224.
- Bano, G., Raina, R.K., Zutshi, U., Bedi, K.L., Johri, R.K., and Sharma, S.C. (1991) The effect of piperine on bioavailability and pharmacokinetics of propranolol and theophylline in healthy volunteers. *Eur. J. Clin. Pharmacol.* 41, 615–617.
- Bano, G., Amala, V., Raina, R.K., Zutshi, U., and Chopra, C.I. (1987) The effect of piperine on pharmacokinetics of phenytoin in healthy volunteers. *Planta Med.* 53, 568–569.
- Atal, C.K., Dubey, R.K., and Singh, J. (1985) Biochemical basis of enhanced drug bioavailability by piperine: Evidence that piperine is a potent inhibitor of drug metabolism. *J. Pharmacol. Exp. Ther.* 232, 258–262.
- Badmaev, V., Majeed, M., and Norkus, E. (1999) Piperine, an alkaloid derived from black pepper increases serum response of beta-carotene during 14-days of oral beta-carotene supplementation. *Nutr. Res.* 19(3), 381–388.
- Majeed, M., Badmaev, V., and Rajendran, R. (1996, 1998) Use of piperine to increase the bioavailability of nutritional compounds. United States Patent No. 5,536,506 and No. 5,744,161.
- Okamoto, T., Fukui, K., Nakamoto, M., Kishi, T., Okishio, I., Yamagami, I., Kanamori, N., Kishi, H., and Hiraoka, E. (1985) High performance liquid chromatography of coenzyme Q-related compounds and its application to biological materials. *J. Chromatography* 342, 35–46.
- STATA (1992) Reference Manual. Computing Resource Center, Los Angeles, CA, USA, Release#3.
- Jolliet, P., Simon, N., Barre, J., Pons, J.Y., Boukef, M., Paniel, B.J., and Tillement, J.P. (1998) Plasma coenzyme Q10 concentrations in breast cancer: Prognosis and therapeutic consequences. *Int. J. Clin. Pharmacol. Ther.* 36(9), 506–509.
- McDonnell, M.G. and Archbold, G.P.R. (1996) Plasma ubiquinol/cholesterol ratios in patients with hyperlipidemia, those with diabetes mellitus and in patients requiring dialysis. *Clin. Chim. Acta* 253, 117–126.
- Karlsson, J., Diamant, B., Edlund, P.O., Lund, B., and Folkers, K. (1992) Plasma ubiquinone, alpha-tocopherol and cholesterol in man. *Int. J. Vitam. Nutr. Res.* 62, 160–164.
- Soja, A.M. and Mortense, S.A. (1997) Treatment of congestive heart failure with coenzyme Q10. *Mol. Aspects Med.* 19(suppl), S159–S168.
- Neuzil, J., Witting, P.K., and Stocker, R. (1997) Alpha-tocopherol hydroquinone is an efficient multifunctional inhibitor of radical-

- initiated oxidation of low-density lipoprotein lipids. *Proc Natl Acad Sci USA* **94**(15), 7885–7890
- 17 Suzuki, S., Hinokio, Y., Ohtomo, M., Hirai, M., Hirai, A., Chiba, M., Kasuga, S., Satch, Y., Akai, H., and Toyota, T. (1998) The effects of coenzyme Q10 treatment on maternally inherited diabetes mellitus and deafness, and mitochondrial DNA 3243 (A to G) mutation *Diabetologia* **41**(5), 584–588
- 18 Schulz, J B., Henshaw, D.R., Matthews, R I., and Beal, M F (1995) Coenzyme Q10 and nicotinamide in neural disorders *Exp Neurol* **135**(2), 279–283
- 19 Lewin, A and Lavin, B (1997) The effect of coenzyme Q10 on sperm motility and function *Mol Aspects Med* **18**(suppl), S213–S219
- 20 Johansen, K., Theorell, H., Karlsson, J., Diamant, B., and Folkers, K (1991) Coenzyme Q10, alpha-tocopherol and free cholesterol in HDL and LDL fractions *Ann Med* **23**(6), 649–656
- 21 Kaplan, P., Sebestianova, N., Turiakova, J., and Kucera, I (1996) Determination of coenzyme Q in human plasma *Physiol Res* **45**, 39–45
- 22 Tomono, Y., Hasegawa, J., Seki, I., Motegi, K., and Morishita, N. (1986) Pharmacokinetic study of deuterium-labeled coenzyme Q10 in man *Int J Clin Pharmacol Ther Toxicol* **24** (10), 536–541
- 23 Annamalai, A R and Manavalan, R (1990) Effect of “Irikatu” and its individual components and piperine on gastrointestinal tracts *Indian Drugs* **27**(12), 595–604
- 24 Johri, R K., Thusu, N., Khajuria, A., and Zutshi, U (1992) Piperine-mediated changes in the permeability of intestinal epithelial cells *Biochem Pharmacol* **43**(7), 1401–1407
- 25 Kawada, I., Sakabe, S.-I., Watanabe, T., Yamamoto, M., and Iwai, K (1988) Some pungent principles of spices cause the adrenal medulla to secrete catecholamine in anesthetized rats *Proc Soc Exp Biol Med* **188**, 229–233
- 26 Reanmongkol, W., Janthasoot W., Wattanatorn, W., Dhumma-Upakorn, P., and Chudapongse, P (1988) Effects of piperine on bioenergetic functions of isolated rat liver mitochondria *Biochem Pharmacol* **37**(4), 753–757