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Note

Comparison of Uptake between PureSorb-Q^{TM}40 and Regular Hydrophobic Coenzyme Q_{10} in Rats and Humans after Single Oral Intake

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Summary Coenzyme Q_{10} (CoQ_{10}) is a lipid-soluble antioxidant and essential component of the mitochondrial electron transfer system in the body, and is in wide use as a functional food material and cosmetic raw material. However, as CoQ_{10} is extremely lipid-soluble, absorption by the body is not easy. In general, people use soft-gel capsules in which CoQ_{10} is suspended in oil, and take these capsules with food. PureSorb-Q^{TM}40 (P40) was developed to improve CoQ_{10} processability and absorption when taken without food, and the present study compared the effects of food on absorption between P40 and conventional lipid-soluble CoQ_{10} in rats and humans. The results of a rat study showed higher uptake when P40 was administered in the fasting state or with food compared to lipid-soluble CoQ_{10}. The results of a human study showed that uptake was favorable when P40 was administered in the fasting state, and even when administered postprandially, a significant difference was noted in uptake rate up to 6 h after intake and uptake volume up to 8 h after intake when compared to lipid-soluble CoQ_{10}. These results show that any CoQ_{10} product using P40 can be quickly and reliably absorbed by the body regardless of dosage form or intake time.

Key Words coenzyme Q_{10}, water-soluble, PureSorb-Q^{TM}40, uptake

Coenzyme Q_{10} (CoQ_{10}) is a lipid-soluble substance that plays important roles in the body as an essential factor in the mitochondrial electron transfer system. This biofactor is directly involved in the energy production system of the body (*1, 2*). CoQ_{10} was first chemically synthesized in 1958 (*3*), and has been used since 1974 as an effective agent for improving symptoms associated with congestive heart failure. At present, CoQ_{10} has been closely examined as a functional food material and cosmetic raw material in and outside of Japan, and because of its excellent safety record, CoQ_{10} is beginning to gain wide use (*4–6*). CoQ_{10} is biosynthesized not only by mitochondria, but also by the microsome-Golgi system, and thus does not fit the definition of a vitamin (*7*). In the tissues of many animals, including humans, CoQ_{10} exists mostly as reduced CoQ_{10}, not oxidized CoQ_{10}. Therefore, CoQ_{10} has the potent antioxidant action in the body (*8, 9*). This highly lipid-soluble substance is not easily absorbed by body. Even when taken with food, the absorption rate is about 3% (*10*). Furthermore, the high lipid solubility means that use in food products is limited. PureSorb-Q^{TM}40 (water-soluble type powder. CoQ_{10} content is 40 w/w%) is a product that was developed to improve processability and absorption in the fasting state while focusing on safety.

To ascertain the effects of food on the absorption of PureSorb-Q^{TM}40, the present study compared serum CoQ_{10} levels following oral intake of CoQ_{10} between PureSorb-Q^{TM}40 tablets and commercially available lipid-soluble CoQ_{10} soft-gel capsules.

Materials and Methods

Materials. Male Sprague-Dawley rats (6- to 7-wk-old; body weight: 190–230 g) were purchased from Japan SLC Inc. (Shizuoka, Japan). The PureSorb-Q^{TM}40 (Water-soluble type CoQ_{10} powder. CoQ_{10} content is 40 w/w%, hereafter P40) developed by Nisshin Pharma Inc. (Tokyo, Japan) was used. In a human oral intake study, P40 tablets, made by Nisshin Pharma Inc., and commercially available lipid-soluble CoQ_{10} (Q10 oil) soft-gel capsules (30 mg of CoQ_{10} per capsule) were used. Each P40 tablet weighed 300 mg and contained 30 mg of CoQ_{10}, and other ingredients included starch, reduced maltose, agar, dextrin, cellulose, sucrose ester, glycerin and fine silicon dioxide.

Subjects. Subjects in the human study comprised 20 healthy male volunteers at Miyawaki Orthopedics Hospital (Eniwa, Hokkaido). Mean age was 23.3 y (range, 20–40 y) and mean BMI was 21.2 kg/m^2 (range, 18–26 kg/m^2).

Rat single-dose study. Rats were randomly divided

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into the following 4 groups based on body weight: control group (n=4, food+water); prandial P40 group (n=12, ad libitum access to food and P40 administration); fasting P40 group (overnight fasting (12 h from 20:00 to 08:00 the next morning) and P40 administration); prandial Q10 oil group (ad libitum access to food and Q10 oil administration); and fasting Q10 oil group (overnight fasting (12 h from 20:00 to 08:00 the next morning) and Q10 oil administration). P40 was directly administered to the stomach using an oral tube by dissolving CoQ_{10} at 3.33 mg/kg body weight (for humans, 200 mg/60 kg body weight). Q10 oil was administered as follows: A commercially available soft-gel capsule was cut using scissors to remove the contents. Water was added, and the resulting solution was warmed for 10 min in a 50˚C water bath, then subjected to ultrasonication to prepare a homogenized solution. Intake volume and methods for Q10 oil were the same as those for P40. After CoQ_{10} administration, no food was given to any group, but the rats had ad libitum access to water. At 6, 10 and 24 h after administration, a blood sample was collected for measuring serum CoQ_{10} by killing the rats under anesthesia. Rats were fed using Lab MR Stock (Japan SLC).

Human single-dose study. The study was conducted on consenting volunteers under the supervision of the chief investigator and collaborators in accordance with the Declaration of Helsinki, with approval from the Investigational Review Board (Miyawaki Orthopedic Clinic IRB). Screened subjects were randomly divided into 2 groups: fasting group (n=10); and postprandial group (n=10). In each group, P40 and Q10 oil were administered in a crossover fashion. In other words, a single oral dose 2-group 2-period crossover study was conducted. In the single-dose study, either 2 P40 tablets or 2 Q10 oil capsules (60 mg CoQ_{10}) were administered with food or in the fasting state. Fasting-state subjects were instructed to fast for 12 h, and were then orally administered CoQ_{10} with 200 mL of water. Conversely, postprandial subjects were orally administered CoQ_{10} immediately after breakfast with 200 mL of water. In both groups, subjects ate the same lunch 4.5 h after CoQ_{10} intake. When taking CoQ_{10} with food, a typical Japanese breakfast was served (600–700 kcal, protein : fat : carbohydrate (PFC) ratio, 15 : 25 : 60) to minimize the effects of dietary differences on CoQ_{10} absorption. Physical tests, clinical tests (hematological and blood biochemical tests) and doctor interviews were performed before taking CoQ_{10} and after collecting the final blood sample. Blood samples were collected before CoQ_{10} intake and 2, 4, 6, 8, 12 and 24 h after intake to measure serum CoQ_{10} levels.

Measurement of serum CoQ_{10} levels. Rat serum CoQ levels were measured by HPLC using an electrochemical detector according to the methods of Okamoto et al. (*11*). With each human blood sample, 2 mL of blood was centrifuged at 800×g for 10 min, and the resulting serum was subjected to the above-mentioned methods to quantify reduced and total CoQ levels.

Data analysis. In the rat single-dose study, basic statistics (mean and SD) were calculated for each group, and numerical data were expressed as mean±SE. Uptake rate was calculated based on the slope of serum CoQ_{10} level at 6 h after intake. Unpaired t-tests were used to compare serum CoQ_{10} levels at 6 h after intake and uptake rate among different groups. In the human single-dose study, basic statistics (mean±SD) were calculated for fasting and postprandial groups, and numerical data were expressed as mean±SE. An unpaired t-test was used to compare uptake rate at 6 h after intake and area under the blood drug concentration-time curve for up to 8 h after intake (ΔAUC) between the fasting and postprandial groups, and paired t-tests were used to compare P40 and Q10 oil intake within each group. Values of $p<0.05$ were considered statistically significant.

Results

Rat serum CoQ_{10} levels following CoQ_{10} intake

Figure 1 shows changes in serum CoQ_{10} levels up to 24 h after oral administration of P40 or Q10 oil.

Following fasting administration, serum CoQ_{10} levels peaked 6 h after intake for the P40 group at 0.208±0.014 μg/mL, but, for the Q10 oil group, no serum CoQ_{10} was observed at 6, 10 or 24 h after intake. Following prandial administration, serum CoQ_{10} levels peaked 6 h after intake for the P40 group at 0.270±0.044 μg/mL, but for the Q10 oil group serum CoQ_{10} levels peaked at 10 h after intake, at 0.183±0.017 μg/mL, which is comparable to serum CoQ_{10} levels at 10 h after intake for the P40 group (0.175±0.010 μg/mL). As shown in Table 1, on the other hand, the endogenous CoQ_9 levels were not changed after oral administration of either P40 or Q10 oil.

CoQ_{10} uptake rates at 6 h after intake were compared among the groups. CoQ_{10} uptake rate for the prandial P40 group (0.031±0.008 μg/mL/h) was 4.4-times greater than that for the prandial Q10 oil group (0.007±0.002 μg/mL/h) ($p<0.05$). In addition, CoQ_{10} uptake rate for the fasting Q10 oil group was zero, com-

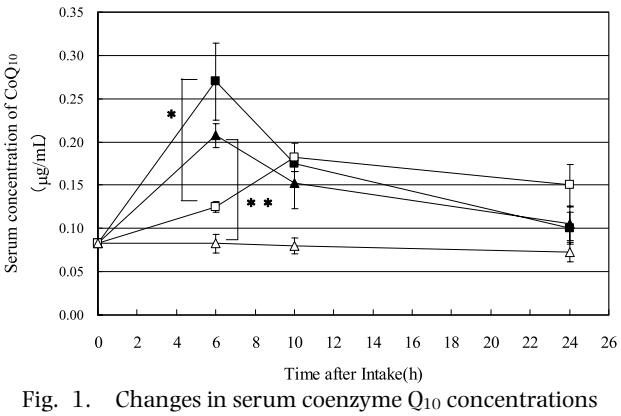

Fig. 1. Changes in serum coenzyme Q_{10} concentrations in rats after single oral intake of 3.33 mg/kg. Values represent mean ±SE (n=4). *$p<0.05$ versus Q10 oil intake (Feed). **$p<0.01$ versus Q10 oil intake (Fasting). ■, Feed+P40; ▲, Fasting+P40; □, Feed+Q10 oil; △, Fasting+Q10 oil.

Table 1. Changes in serum coenzyme Q_9 concentration in rats after single oral intake of 3.33mg/kg.

	6 h after the start of intake	10 h after the start of intake	24 h after the start of intake
Fasting+P40	0.353±0.030	0.368±0.025	0.390±0.025
Feed+P40	0.370±0.015	0.380±0.032	0.368±0.011
Fasting+Q10 oil	0.360±0.032	0.370±0.018	0.390±0.026
Feed+Q10 oil	0.353±0.011	0.370±0.021	0.378±0.040

Serum coenzyme Q_9 concentration of control group is 0.373±0.011 μg/mL (n=4).
Values represent mean±SE (n=4).
Unit is μg/mL.

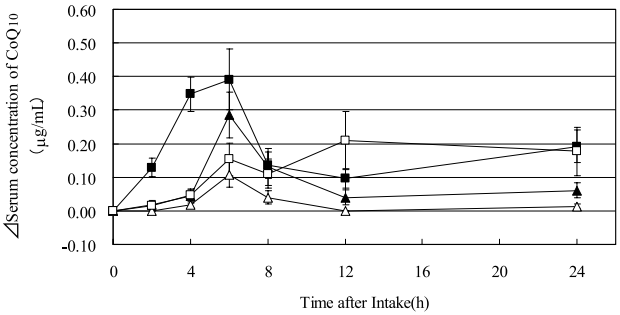

Fig. 2. Changes in serum coenzyme Q_{10} concentrations in humans after single oral intake of 60 mg. Values represent mean ±SE (n=10). ■, P40 (After meals); ▲, P40 (Fasting); □, Q10 oil (After meals); △, Q10 oil (Fasting).

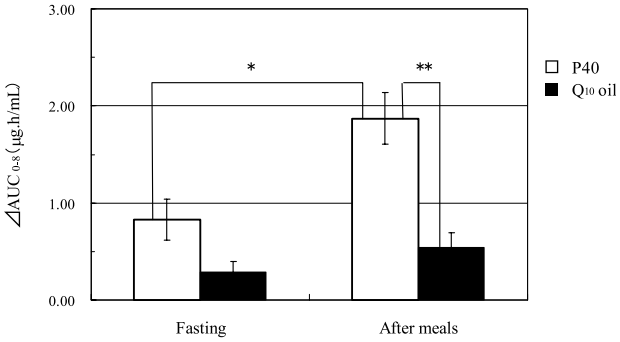

Fig. 3. Comparison of ΔAUC_{0-8} between fasting and after-meals groups in humans. Values represent mean ±SE (n=10). *p<0.01 versus Fasting (P40). **p<0.01 versus Q10 oil (After meals)

pared to 0.021±0.003 μg/mL/h for the fasting P40 group.

Human serum CoQ_{10} levels following CoQ_{10} intake (Figs. 2, 3)

The difference in uptake rate between P40 and Q10 oil in rats was compared in the human study. Figure 2 shows changes in serum CoQ_{10} levels for 24 h after P40 and Q10 oil intake in humans (increase level: Δ).

Unlike in rats, serum CoQ_{10} levels peaked at 6 h after intake for fasting P40, postprandial P40 and fasting Q10 oil groups, and as with rats, serum CoQ_{10} levels quickly increased for the postprandial P40 group. Uptake rate up to 6 h after intake for the fasting P40 group was 0.048±0.011 μg/mL/h, and tended to be faster than that for the fasting Q10 oil group (0.018± 0.006 μg/mL/h) or postprandial Q10 oil group (0.026±0.008 μg/mL/h), but no significant differences were identified. In addition, uptake rate up to 6 h after intake for the postprandial P40 group (0.065± 0.015 μg/mL/h) was 2.6-fold faster than that for the postprandial Q10 oil group (p<0.05).

To confirm the uptake volume of orally ingested exogenous CoQ_{10}, ΔAUC for 8 h after intake was calculated (Fig. 3). The ΔAUC was 0.828±0.211 μg·h/mL for the fasting P40 group, 2.9-fold higher than for the fasting Q10 oil group (0.288±0.109 μg·h/mL), but no significant differences were identified.

The ΔAUC for the postprandial P40 group was 1.869±0.267 μg·h/mL, 2.3-fold greater than that for the fasting P40 group (p<0.01) and 3.4-fold greater than that for the postprandial Q10 oil group (p<0.01).

In the present study, subjective symptoms, objective symptoms, physiological test findings and clinical laboratory test findings did not indicate any undesirable symptoms resulting from consumption of the investigational products.

Discussion

Exogenous CoQ_{10}, such as dietary CoQ_{10}, is absorbed by epithelial cells in the small intestine and transported via the lymphatic system to the liver, where most CoQ_{10} stays. CoQ_{10} absorption and its metabolic pathway are believed to be broadly similar in rats and humans (*12*). Exogenous and endogenous CoQ_{10} in the liver is bound to very low density lipoprotein (VLDL) and then secreted into the blood (*13*). The serum of healthy adults contains about 1 μM of CoQ_{10}, and most CoQ_{10} exists as reduced CoQ_{10} in the lipoprotein fraction (*14*). Serum samples are often used to measure the absorption of exogenous CoQ_{10} or CoQ sufficiency in the body. However, marked differences have been seen in increased serum CoQ_{10} levels due to intake of exogenous CoQ_{10}. This may be attributable to individual differences and dietary oil contents, and these factors have been shown to markedly affect serum CoQ_{10} levels (*15*). To alleviate the effects of diet on CoQ_{10} absorption, PureSorb-Q™40 was developed, and the present study investigated CoQ_{10} uptake in rats and humans. The present results clarified that, for the administration of P40, the effects of food intake were small and CoQ_{10} was absorbed promptly even when administered in a fasting state. CoQ_{10} absorption is improved by postprandial P40 intake compared to postprandial lipid-soluble CoQ_{10} intake. The results of each study are described in

the following sections.

In rats, CoQ_9 is the predominant homologue, and CoQ_{10} accounts for its several to a dozens of percent (*11*). CoQ_9 and CoQ_{10} are widely distributed in the spleen, kidney, heart, liver and plasma. In the liver and plasma, the ratio of reduced CoQ to total CoQ (reduced and oxidized CoQ) is 70–80%, compared to 30% in other tissues (*16*). The present results did not show any marked differences in serum CoQ_9 levels following oral intake of P40 or Q10 oil, and the ratio of reduced CoQ for CoQ_9 and CoQ_{10} was 70–80%. This indicates that exogenous CoQ_{10} intake does not affect the ratio of reduced CoQ in the body. Furthermore, for the fasting Q10 oil group, little chronological change in serum CoQ_{10} levels was observed, suggesting that in rats, lipid-soluble CoQ_{10} is not absorbed if not consumed with food. Conversely, under the same conditions, the P40 group displayed a favorable uptake rate of CoQ_{10}, and P40 improved absorption of CoQ_{10} in the fasting state, while CoQ_{10} uptake was higher for postprandial P40 than for postprandial lipid-soluble CoQ_{10}.

In the human study, uptake rate for the fasting P40 group was almost the same as for the postprandial Q10 oil group. This means that P40 improves the uptake rate for the fasting state. Hence, although most currently available CoQ_{10} supplements need to be taken with food, P40 does not, and P40 sufficiently increases serum CoQ_{10} levels whether taken in the fasting state or with food. Serum CoQ_{10} levels for the postprandial P40 group were higher than those for the postprandial Q10 oil group, and as with rats, this was attributed to the fact that the uptake rate for the postprandial P40 group 6 h after intake was 2.6-fold faster compared to the postprandial Q10 oil group.

In the present study, the second peak in serum CoQ_{10} levels was seen at 12 h after intake for the Q10 oil group (Fig. 2). This was attributed to enterohepatic circulation of exogenous CoQ_{10} uptake by the small intestine, followed by secretion into plasma together with endogenous CoQ_{10} synthesized in the liver. In a study where deuterium-labeled CoQ_{10} was administered to humans, serum CoQ_{10} levels peaked at 6 h after intake, but a second peak was seen 24 h after intake (*12*), suggesting a similar mechanism was at work for the second peak observed in the present study. For the prandial Q10 oil group in rats, however, the second peak in serum CoQ_{10} levels was not observed. The difference of the peak between humans and rats might be affected by the collecting interval of blood samples and/or the lunch intake in humans.

When improving the absorption of minimally soluble drugs, reducing particle size is a common technique. P40 is a water-soluble powder with a mean particle size of about 0.19 μm during water dispersion, and was designed so that easy emulsification could occur with bile acid in the intestinal tract, followed by rapid absorption by the intestinal mucosal layer. The tablets used in the present human single-dose study disintegrate within 20 min of oral administration, and changes in serum CoQ_{10} levels were comparable to the results of a rat study where powdered P40 was orally administered, suggesting that regardless of dosage form, the particle design of P40 is maintained in the small intestine following oral consumption. These findings clarify that compared to lipid-soluble CoQ_{10}, P40 is quickly and reliably absorbed by the body when consumed in the fasting state or with food. P40 may thus represent a CoQ_{10} product that is quickly and reliably absorbed by the body regardless of dosage form or intake time.

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