CHEMISTRY & BIODIVERSITY

Accepted Article

Title: Investigation of the effects of squalene and squalene epoxides on the homeostasis of coenzyme Q10 in rats by UPLC-Orbitrap MS

Authors: Hongmei Yang, Wenjing Yu, Kaiju Sun, Liying Zhang, Changbao Chen, Rui Su, Shuying Liu, Hongfeng Wang, and Yifei Liu

To be cited as: *Chem. Biodiversity* 10.1002/cbdv.202000243

Link to VoR: https://doi.org/10.1002/cbdv.202000243

Chem. Biodiversity

Investigation of the effects of squalene and squalene epoxides on the homeostasis of coenzyme Q10 in rats by UPLC-Orbitrap MS

Wenjing Yu,[a,c] Kaiju Sun,[a,c] Liying Zhang,[a] Xilin Wan,[a] Changbao Chen,[a] Rui Su,[a] Yifei Liu,[b] and Corresponding Hongfeng Wang*[,a], Hongmei Yang*[,a]

[a] Changchun University of Chinese Medicine, 1035 Boshuo Road, Changchun, 130117, China, yanghm0327@sina.cn and ccwhf@126.com

[b] Anhui Pujia Medical Technology Co., Ltd, 2800 Chuangxin Road, Hefei, 230000, China

[c] These authors contributed equally

Squalene has been used as a dietary supplement for a long history due to its potential cancer-preventive function. However, the mechanism has not yet been investigated in detail. Therefore, the aim of this study is to see if the plasma coenzyme Q10 (CoQ10) level will be altered by gavage of squalene and oxidosqualenes to rats. In the present work, a sensitive and simple high-performance analytical method based on ultra-high-performance liquid chromatography coupled with an Orbitrap mass spectrometry (UPLC-Orbitrap-MS) was developed for the quantification of CoQ10 in rat plasma. Coenzyme Q9 (CoQ9) was employed as the internal standard. CoQ10 was determined after acetonitrile-mediated plasma protein precipitation using UPLC-Orbitrap-MS in negative ion mode. Intragastric administration of squalene and the two squalene epoxides into rats once daily for several days elevated the level of CoQ10 in their plasma, but there was no significant difference between high-dose (286 mg/kg) and low-dose (143 mg/kg) groups. Intragastric administration of squalene once a day for 5 consecutive days and oxidosqualens once a day for 3 consecutive days is necessary for reaching the steady-state level of CoQ10. Our present findings indicate that squalene and oxidosqualenes may be useful for stimulating the synthesis of CoQ10 in rats.

Keywords: UPLC-Orbitrap MS • Squalene • Squalene epoxides • CoQ10 • Protein precipitation

Introduction

Squalene is a polyunsaturated branched hydrocarbon with a formula of $C_{30}H_{50}$.[1] Squalene is found in the livers of all kinds of sharks, with higher levels in the livers of deep-sea sharks and lower levels in other animal fats such as tallow and lard.[2] Squalene is widely distributed in human skin, subcutaneous fat, liver, nail, brain and other organs,[3] and has a high concentration in human fat cells.[4] It is also widely distributed in plants, but its content is not high.[5] A small amount of squalene is found in oils and fats, especially in olive oil, palm oil and its deodorization distillate.[6-8] Squalene plays important roles in the following aspects:[9-11] (1) Promotion of blood circulation, (2) activation of the body functional cells, (3) improvement of immune capacity, and (4) anti-inflammatory and antimicrobial activities. Squalene is widely used in cosmetics, medicine, food, machinery, and many other industries. Up to now, there are no effective anti-cancer drugs with no side effects. Many research results have showed that squalene has certain biological activity for the treatment of tumors.[12-14]

CoQ10 is an important lipophilic antioxidant. The body can synthesize CoQ10 by itself,[15] so it is widely used in dermatology and cosmetics industry.[16] The amount of CoQ10 in the skin will decrease with the growth of age. The topical application of CoQ10 to human skin can effectively reduce wrinkles.[17] CoQ10 is also a popular antioxidant in skin care products, protecting the skin from free radical damage.[18]

Squalene has ever been analyzed mainly by gas chromatography and liquid chromatography.[19-21] In recent years, gas chromatography-mass spectrometry (GC-MS) has been widely used to analyze squalene.[22-25] So far, there are few reports on detection of CoQ10 in biological samples by MS. For example, Avery et al.[26] used LC-MS^2 to determine CoQ10 in rat urine, and their method was not very sensitive, with a lower limit of detection measured at 50 ng/mL. In addition, CoQ10 in mitochondria has been quantitatively determined in selective reaction monitoring mode by LC-MS with isotopic labeled CoQ10 as the internal standard, with the lower limit of quantification (LLOQ) at 0.5 nmol/L.[27] Much effort has been made on determination of CoQ10 by scientists. Recently, LLOQs of CoQ10 in

the biological matrices were reported to be 2 and 4 ng/mL by supercritical fluid chromatography-electrospray ionization tandem mass spectrometry and liquid chromatography coupled with tandem mass spectrometry, respectively.[28,29]

Squalene is biosynthesized via farnesyl-PP (pyrophosphate, a 15-carbon compound) with the assistance of squalene synthetase. Thus, the externally added squalene will alter the product/precursor balance to inhibit the biosynthetic process from farnesyl-PP to squalene. In addition, squalene is a precursor for cholesterol biosynsynthesis. We hope that the excess farnesyl-PP after administration of squalene will be preferentially used in the synthesis of non-steroidal isoprenoid compounds with potential antioxidant activity (e.g., coenzyme Q, vitamin E, heme ferrous).[30] But little research has been done on this effect. In this study, we want to check whether administration of squalene and oxidosqualenes will affect the steroidal homeostasis in rats by UPLC-Orbitrap-MS. Different doses of squalene and oxidosqualenes were also investigated. We found that squalene and the two epoxides induced CoQ10 synthesis in vivo, which provides deep insight into the action mechanism of squalene and its oxidants. To the best of our knowledge, this is the first study to report the effects of squalene and squalene epoxides on the homeostasis of coenzyme Q10 in rats by UPLC-Orbitrap MS.

Results and Discussion

Sample Preparation

In order to efficiently extract CoQ10 and CoQ9 from rat plasma, two extraction methods were compared: ethanol-hexane extraction and acetonitrile-mediated protein precipitation. LLOQ at 1 ng/mL and similar recovery were obtained by both methods. In contrast, the experiment operation for the latter is simpler. Therefore, acetonitrile-mediated protein precipitation method was employed in our study. The chosen composition of mobile phase gave adequate separation between the IS (t_R=4.15min) and analyte (t_R=4.70 min) (Fig. 1A). The peak at t_R of 4.15 min corresponded to the ion at *m/z* 794.62, as indicated in Fig. 1B. The mass spectrum of the fraction eluted at t_R of 4.70 min showed a strong signal at *m/z* 862.68 (Fig. 1C) in negative ion mode. The standard curve in Fig. 1D was obtained using blank rat plasma as matrix, followed by adding standard curve working solution, internal standard and being treated with protein precipitation method by UPLC-Orbitrap MS.

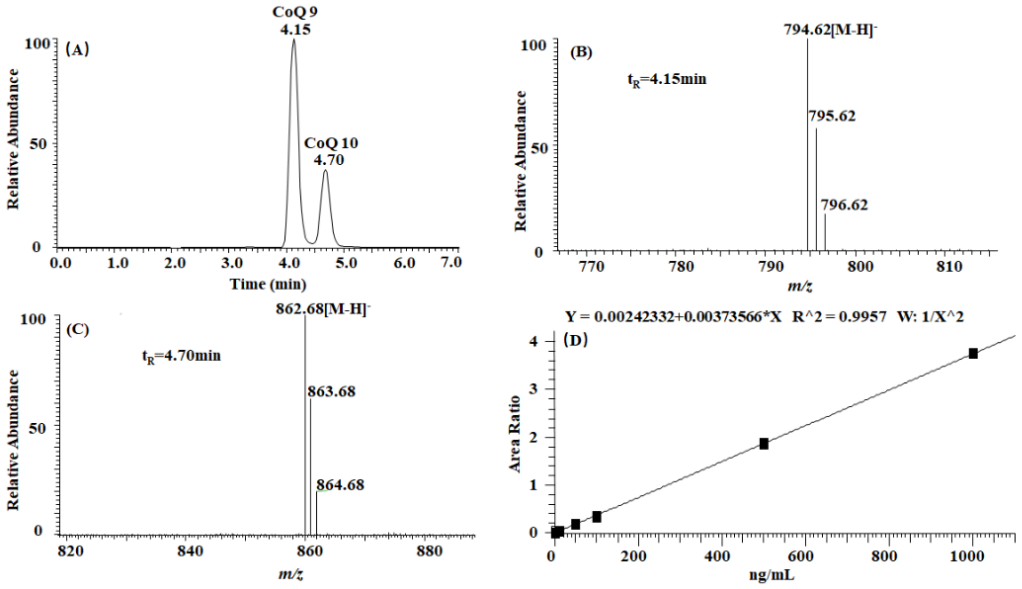


Figure 1 (A) The extracted ion chromatograms of IS CoQ9 and CoQ10 in negative ion mode. (B) and (C) The full-scan mass spectra of the eluted peaks in the negative ion mode. (D) The representative standard curve from the peak area ratio versus nominal CoQ10 concentration, using linear regression analysis with $1/X^2$ weighting.

Linearity, Assay Specificity and LLOQ

The CoQ10 responses were linear over the range 1~1000 ng/mL, with correlation coefficients greater than 0.995, as shown in Fig. 1D. The selectivity of the method was demonstrated by comparing extracted ion chromatograms (EICs) of blank samples with those of the corresponding samples spiked with CoQ10 and IS. Fig. 2 shows typical chromatograms for a blank rat plasma sample, a spiked plasma sample, and a rat plasma sample at 2.5 h after an intravenous administration of 143 mg/kg squalene. There were no significant endogenous peaks that directly interfered with the detection of analytes. Typical retention times for CoQ10 and the internal standard

were 4.7 and 4.2 min, respectively. No matrix effect was observed for the six different plasma pools. The peak areas of the six reconstituted samples had an RSD of 6.3%, indicating that the extracts were "clean" with no co-eluting compounds which could influence the ionization of CoQ10.Therefore, matrix effects can be ignored. In addition, the matrix effect recovery was 105.0 ± 2.7%.

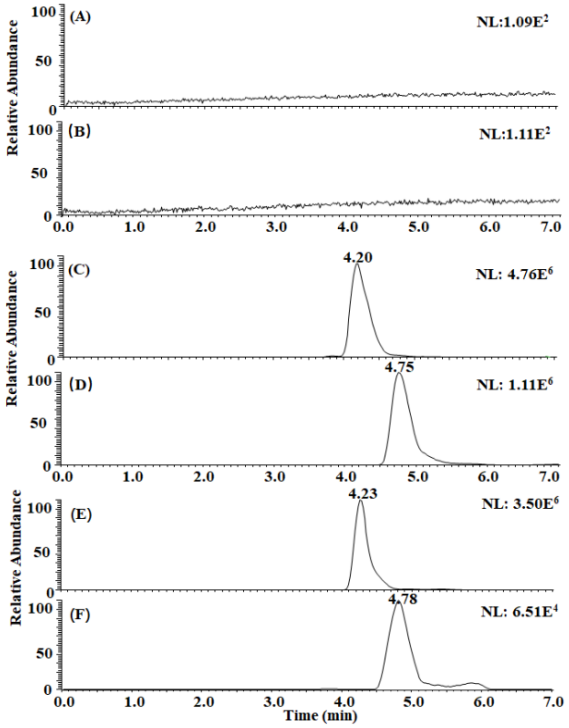


Figure 2 Representative EICs of plasma samples determined by the present UPLC-Orbitrap MS method: (A) CoQ9 in blank plasma; (B) CoQ10 in blank plasma; plasma spiked with 100 ng/mL (C) CoQ9 and (D) CoQ10; (E) spiked CoQ9 (100 ng/mL) and (F) CoQ10 in a plasma sample withdrawn at 7.5 h after intragastrical administration of 50 μL of squalene to a rat on the second day.

The present UPLC-MS method provided an LLOQ of 1 ng/mL with an accuracy of 6.2% in terms of relative error (RE) and a precision of 7.0% in terms of RSD (n=6). In comparison with the literature methods,[26-29] lower LLOQ was acquired in our study, which is indispensable for the following investigation of the effects of squalene and squalene epoxides on the homeostasis of coenzyme Q10 in rats.

Precision, Accuracy, and Recovery ratio

In order to evaluate of the validity of our method, we tested its precision, accuracy, and recovery ratio. The method showed very good precision and accuracy. Table 1 summarizes the intra-and inter-day precisions and accuracies for CoQ10 from QC samples. In this assay, the intra- and inter-run precisions were less than 8.9%, and accuracies were in the range of 99.4~100.6%. The results indicated that the values were within the acceptable range and the method was accurate and precise.

Table 1. The precision and accuracy of determination of CoQ10 in plasma by UPLC-MS (n = 3 days, six replicates per day).

	Coq 10 concentration in plasma (ng/mL)		
	1	50	500
Determined value (ng/mL)	1.0 ± 0.02	50.0 ± 0.6	499.0 ± 8
Accuracy (%)	100.2	100.6	99.4
Intraday precision (%)	8.9	6.8	7.0
Daytime precision (%)	2.0	2.0	2.5

The recoveries observed (value ± S.D., n = 6) were 72.4 ± 3.3, 72.4 ± 3.5 and 77.8 ± 2.3 % (1, 50, and 500 ng/ml, respectively) for CoQ10 and 75.5 ± 1.9% for CoQ9, as listed in Table 2. The relative standard deviation of different concentrations were all below 3.5%, which was considered acceptable, especially taking into account of the adequate LLOQ.

Table 2. Extraction recovery of CoQ10 plasma sample treatment

Recovery (%)	concentration of CoQ10 (ng/mL)			internal standard concentration (ng/mL)
	1	50	500	100
	73.5	73.8	79.5	74.1
	72.0	68.9	76.2	74.8
	75.7	74.1	75.5	77.0
	71.6	69.9	76.6	76.1
	69.1	75.9	78.9	73.6
	72.5	71.8	80.1	77.4
Determined value (%)	72.4 ± 3.3	72.4± 3.5	77.8 ± 2.3	75.5 ± 1.9

Characterization of Squalene Epoxides

Squalene was oxidized and eluted, and the oxidized compounds, A1 and A2, were obtained. The ESI mass spectra of A1 and A2 (Fig. 3A and 3C) showed the base peaks at *m/z* 425.38 which corresponded to the $[M-H]^-$ ions. With the goal of identification of the components, the experiment of MS/MS was performed. All masses observed differed by no more than ±2 ppm from the theoretical masses (Table 3). The peaks at *m/z* 424.48 in Fig. 3B and 3D arised from loss of one hydrogen atom from the corresponding quasimolecular ions. Based on the fragmentation ions at *m/z* 368.49 and 339.45 in Fig. 3B, this squalene epoxide is assigned as 2,3-oxidosqualene, as displayed in Fig. 3E. The ion at *m/z* 368.49 was generated by loss of C_3H_5O from the intact molecular ion of 2,3-oxidosqualene. This fragment ion further dissociated to produce a minor signal at *m/z* 339.45 which came from the loss of C_2H_5. The ions at *m/z* 275.15 and 231.20 are observed in Fig. 3D, belonging to the losses of $C_{13}H_{22}O$ and $C_{11}H_{16}$ from 10,11-oxidosqualene, respectively, as exhibited in Fig. 3F. Therefore, the squalene epoxides eluted by benzene and hexane were identified as 2,3-oxidosqualene and 10,11-oxidosqualene, respectively.

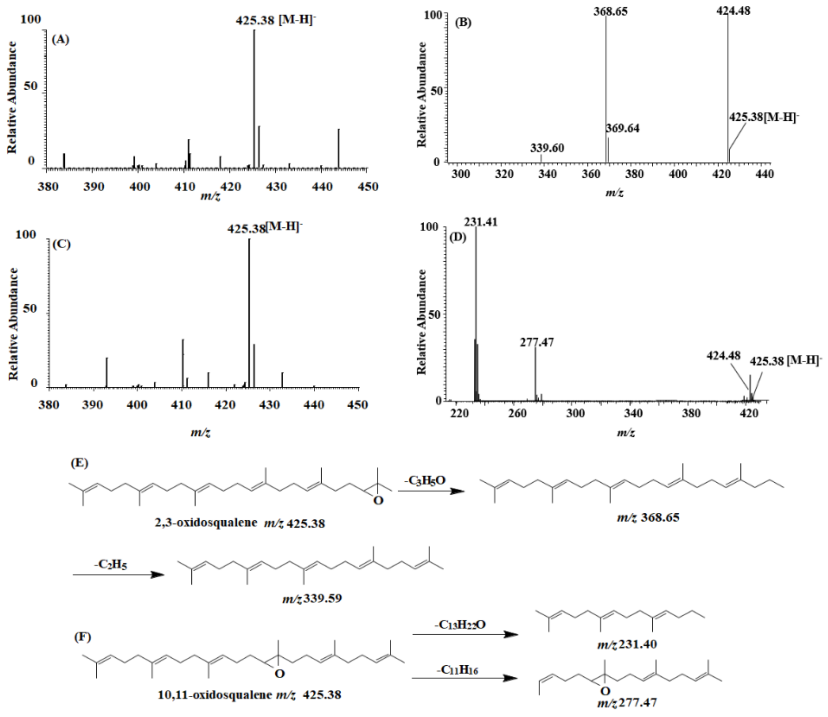


Figure 3 (A) The full-scan mass spectrum of the squalene epoxide eluted by benzene in negative ion mode. (B) The MS/MS spectrum of the squalene epoxide eluted by benzene. (C) The full-scan mass spectrum of the squalene epoxide eluted by hexane in negative ion mode. (D) The MS/MS spectrum of the squalene epoxide eluted by hexane. (E) The fragmentation pathway of 2,3-oxidosqualene. (F) The fragmentation pathway of 10,11-oxidosqualene.

Table 3. Mass spectrometric analysis of the squalene epoxides synthesized as described in experimental. The *m/z* values of monoisotopic quasimolecular ions $[M-H]^-$ were determined by Orbitrap MS.

Sample name	Chemical formula of molecular ion[M-H]$^-$	[M-H]$^-$ *m/z*	
		Calculated	Experimental ± error(ppm)
A1			
Epoxy squalene	$[C_{30}H_{50}O\text{-}H]^-$	425.3759	425.3763 ± 0.9
Product 1	$[C_{27}H_{45}\text{-}H]^-$	368.6489	368.6494 ± 1.0
Product 2	$[C_{25}H_{40}\text{-}H]^-$	339.5870	339.5990 ± 0.7
A2			
Epoxy squalene	$[C_{30}H_{50}O\text{-}H]^-$	425.3759	425.3766 ± 1.3
Product 3	$[C_{17}H_{28}\text{-}H]^-$	231.4030	231.4093 ± 1.7
Product 4	$[C_{19}H_{34}O\text{-}H]^-$	277.4722	277.4753 ± 0.8

Effects of Squalene and Squalene Epoxides on Levels of CoQ10

Rats were administrated intragastrically with different doses of squalene, 2, 3-oxidosqualene and 10,11-oxidosqualene once daily for several days, and plasma samples as described above were analyzed by UPLC-Orbitrap MS after such treatment. In order to ensure the accuracy of the method, in each analytical batch, the real samples, the standard curve samples, and the quality control samples were tested and analyzed in the same days. From Fig. 4A, it is clearly seen that, in the first 4 days, the concentration of CoQ10 in plasma increased after gavage administration of squalene, and then there was a decreasing trend with time. After gavage for 5 days, the concentration of CoQ10 reached steady state, becoming 50 times higher than that of the control value. This is consistent with the conclusion reported in the literature that squalene 2, 3-oxidosqualene can promote the increase of CoQ10 in the blood.[31] After repeated administration of 2,3-oxidosqualene and 10,11-oxidosqualene for 5 days, the levels of CoQ10 could be in a steady state, being 80 times higher than that of the control value (Fig. 4B and 4C). By t-test comparison between high- and low-dose groups, the fold change (FC) and p values are 1.05, 0.82 for squalene, 1.14, 0.15 for 2, 3-oxidosqualene, and 1.29, 0.28 for 10,11-oxidosqualene at the final steady state. There is no significant difference between high-dose group and low-dose group for the 3 analytes. Low doses of squalene and oxidosqualenes still increased the homeostasis of CoQ10 in rats.

The initial part of the mevalonate pathway is a sequence of reactions that forms farnesyl PP (FPP) from acetyl-CoA. FPP is the last common substrate for the biosynthesis of several end products including CoQ.[32] As we hypothesized in "Introduction' section that the accumulated farnesyl-PP after administration of squalene was preferentially used for CoQ10 biosynthesis. Furthermore, from Fig. 4, we found that the CoQ10 level was high after administration of squalene for 0.5 h, indicating that the synthetic speed is fast. In comparison with the reports in literatures,[33,34] the pharmacokinetic properties of CoQ10 after administration of squalene were different from dosing CoQ10 directly.

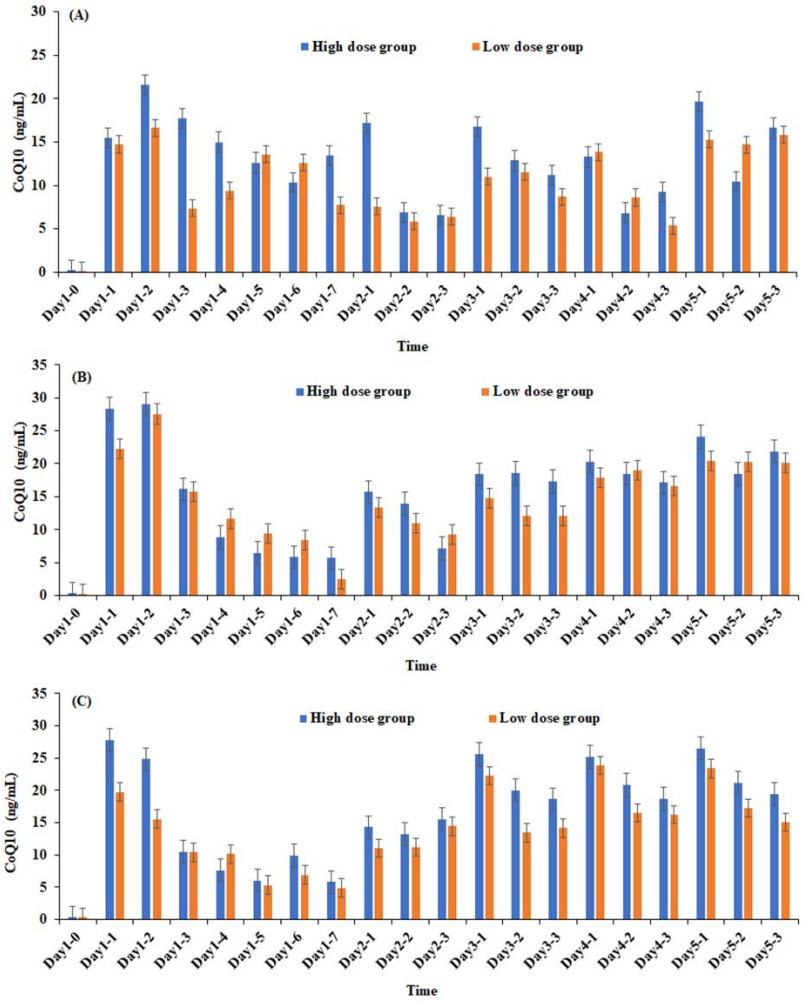


Figure 4. The influence of treatment with (A) squalene, (B) 2,3-oxidosqualene, and (C) 10,11-oxidosqualene on plasma levels of CoQ10 in rats. The values presented are the means ± S.E. (vertical bars) of three independent experiments. "Day1-2" indicates the second time point for plasma collection on the first day. Other annotations are performed similar to "Day1-2".

Therefore, we can conclude that both squalene and its epoxides can promote the synthesis of CoQ10 in rats, although there is no significant difference between squalene and its epoxide effects. For example, as for low-dose group at the final steady state, FC and p values between the two epoxides and squalene are 1.27, 0.06, and 0.96, 0.75, respectively.

Conclusions

In this study, a highly sensitive and rapid method based on UPLC-Orbitrap-MS combined with the precipitation protein method was established for the quantification of CoQ10 in rat plasma. Two squalene epoxides were successfully separated by column chromatography. The effects of different doses of squalene and oxidosqualenes on levels of CoQ10 in rats were investigated, and it was concluded that both squalene and oxidosqualenes could influence for the balance of CoQ10 in rats. No significant difference was found between high-dose group and low-dose group for each tested substance, indicating that low doses of squalene and oxidosqualenes were enough to synthesize CoQ10. If squalene or its oxidosqualenes were developed into health products, it will result in a very important economic and social significance. Our work potentially provides novel information on understanding the interaction between squalene/oxidosqualenes and CoQ10 in vivo, which may be of future value for treatment of CoQ10 deficiency in human.

Experimental Section

Chemicals and Reagents

Squalene (batch number: 137-10279) and CoQ10 (batch number: 211-01431) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); 3-Chloroperoxybenzoic acid and CoQ9 (batch number: C135068) were bought from Aladdin Chemistry Co., Ltd. (Shanghai, China); Heparin sodium was provided by Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China); 230-400 column chromatography silica gel was purchased from Qingdao Ocean Chemical Co., Ltd. (Qingdao, China); Dichloromethane (AR grade), benzene (AR grade), n-hexane (AR grade) were obtained from Tianjin Damao Chemical Reagent Factory (Tianjin, China); Ether (AR grade) was obtained from Tianjin Tiantai Fine Chemicals Co., Ltd. (Tianjin, China); Nitrogen was obtained from Changchun Juyang Gas Co., Ltd. (Changchun, China); Methanol, isopropanol and acetonitrile (HPLC grade) were all imported from Fisher Chemical Company (Shanghai, China). Ultrapure water (specific conductivity, 18.2 MΩ/cm) was produced by a Milli-Q device (Millipore, Milford, MA).

Preparation of Standard and Quality Control Solutions

Stock solutions and working solutions were stored at -20 °C. Standard CoQ10 was dissolved in ether solution at a final concentration of 5000 ng/mL. Calibration standards were prepared at concentrations of 1000, 500, 100, 50, 10, and 1 ng/mL by diluting stock solution of CoQ10 in 50% methanol-ether. The quality control (QC) samples were prepared at concentrations of 1, 50, and 500 ng/mL in 50% methanol-ether,[35] by a separate weighing of the pure standard. Internal standard CoQ9 was dissolved in methanol at a final concentration of 100 ng/mL. Stock solutions and working solutions were stored at 4 °C.

Oxidation of Squalene

To achieve squalene epoxides, squalene was first dissolved in dichloromethane and then mixed with 3-chloroperoxybenzoic acid (77%) dissolved in dichloromethane to obtain a 1:2 molar ratio of squalene:3-chloroperoxybenzoic acid.[36] Following incubation at room temperature for 30 min, the solvent was removed by evaporation under nitrogen, and the residue was redissolved in hexane. Subsequently, the individual epoxides were separated by column chromatography on silica gel 60 (230–400 mesh) utilizing a gradient from hexane to a mixture of hexane and diethyl ether (96:4) for elution. Further separation of the individual compounds was obtained by elution from a second silica column with benzene, followed by elution of hexane. The two fractions eluted successively contained monoepoxides with identical molecular weights determined by MS. Squalene epoxides eluted by benzene and hexane were referred as A1 and A2, respectively.

Animal Studies

13 adult male and 13 adult female Wistar rats (200~259g, SPF grade) were purchased from Liaoning Changsheng Biotechnology Co., Ltd. (License No: SCXK (Liao) 2015-0001). The animals were housed in normal cages under controlled environmental conditions (21-25 °C, humidity of 40%-70%, a 12-hour light and dark cycle, and noise less than 60 decibels). All animal treatments were strictly in accordance with the National Institutes of Health Guide of the Care and Use of Laboratory Animals. The experiments were carried out under the approval of the Committee of Experimental Animal Administration of the University (No: 20190107). Rats were fasted for 12 h before dosing but with free access of water. 26 experimental animals were randomly divided into six groups. 100 μL (286 mg/kg) of squalene was administrated intragastrically to seven rats (four females and three males) as a high-dose group; seven randomly selected rats (three females and four males) were intragastricly administrated with 50 μL (143 mg/kg) of squalene as a low-dose group. 100 μL and 50 μL of A1 (the oxidosqualene eluted by benzene) were intragastricly administrated to three rats as the high-dose and low-dose groups, respectively. As for A2 (the oxidosqualene eluted by hexane), It has the same dosing operation as A1.
Blood samples were collected into heparinized tubes from the retro-orbital sinus of rats at 0 (before dosage), 0.5, 2.5, 5, 8, 10, 12, 24 h after intragastric administration of analytes on the first day. In the case of the other days, blood was taken at 0, 3.5, 7.5, and 10 hours after gavage administration.

Sample Preparation

Plasma was obtained from each sample by centrifugation at 4000 rpm for 10 min, and kept frozen at -80 °C until analysis. Matrix-matched calibration standards and QC samples of squalene and squalene epoxides were prepared by spiking 400 μL acetonitrile into 100 μL of the working solutions, 100 μL of CoQ9, and 100 μL of blank rat plasma, and the mixture was then vortex-mixed for 60 s followed by centrifugation at 4000 rpm for 10 min. An aliquot of 20 μL of the supernatant was taken and then injected into UPLC-Orbitrap MS. The plasma samples were treated as described above. As for the ethanol-hexane extraction method, in the EP tube, 100 μL of CoQ9 internal standard working solution, 100 μL of CoQ10 standard curve working solution and 100 μL of blank plasma were added in turn. Then the mixture was mixed well, followed by adding 3 mL of ethanol-hexane (1:5), vortexing for 1 min, shaking for 10 min,

centrifuging at 6000 rpm at 4°C for 10 min, and taking the supernatants. 3 mL of ethanol-hexane (1:5) was added again, followed by repeating the step mentioned above. The supernatants twice were combined, blowed dry with nitrogen at 40°C, redissolved in 200 µL of isopropanol-methanol (45:55). The sample was injected into MS after passing the membrane.

Method Validation

Plasma samples were quantified using the peak area ratios of CoQ10 to that of the I.S. Peak area ratios were plotted against concentrations of CoQ10. The sample concentrations were calculated using weighted ($1/x^2$) least squares linear regression.
To evaluate linearity, plasma calibration curves were prepared and were assayed in duplicate on three separate days. Accuracy and precision were also assessed by determining QC samples at three concentration levels on three different validation days. The accuracy was expressed by (mean observed concentration)/(spiked concentration) × 100%, and the precision was evaluated by relative standard deviation (R.S.D.).
The extraction recoveries of CoQ10 at three QC levels were evaluated by comparing peak areas of CoQ10 obtained from plasma samples with CoQ10 spiked before extraction to those spiked after the extraction. The matrix effect experiments were carried out by extracting blank plasma from six different sources, reconstituting the final extract in mobile phase containing a known amount of CoQ10, analyzing the reconstituted extracts and then comparing the peak area of CoQ10 with those of standard solutions in the mobile phase.

Liquid Chromatography and Mass Spectrometry

The ultra-performance liquid chromatography was performed on an UltiMate 3000 system (Dionex, Sunnyvale, CA, USA). The analytes were separated by a Thermo C18 column (100 mm ×2.1 mm i.d., 1.79 µm, Thermo, USA). The isocratic elution system consisted of solvent A (isopropanol) and solvent B (methanol), delivered at a flow rate of 0.15 mL/min, and the injected sample volume was 20 µL. For the analysis of samples, the isocratic elution was used as follow: 0-7 min 45% A.
Mass spectrometric detection was carried out on a Q-Exactive Orbitrap mass spectrometer (Thermo, San Jose, CA) equipped with an electrospray ionization (ESI) source in negative ion mode. Nitrogen was supplied as the sheath gas and auxiliary gas at flow rates of 0.53 and 4 L/min, respectively. Capillary temperature was set at 300 °C. The optimized spray voltage and S-lens voltage were set at 4000 and 55 V, respectively.
Analytical data were acquired using Xcalibur 3.0 software, and quantification processing was performed using Xcalibur Quan Browser software package (Thermo).

Direct Infusion ESI-MS

The eluent of squalene oxide were dissolved in ether/methanol (20:80, v/v) and then infused into the Q-Exactive mass spectrometer by a syringe pump at a flow rate of 20 µL/min. All the data were acquired in the negative ion mode. The spray voltage and the tube lens offset voltage were set to 3800 V and 55 V, respectively. The capillary temperature was set at 300 °C, and the flow rates of the sheath gas (nitrogen) and auxiliary gas (nitrogen) were set to 0.4 and 3.2 L/min, respectively.

Statistical Analyses

The results were expressed as means ± SD. Differences between two groups were analyzed by a pairwise t-test. P values of less than 0.05 and fold change (FC) more than 1.5 were considered statistically significant.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 81874502), the Project from Beijing Institute of Technology "Determination of a Series of Iodine and Nitrites by MS", Jilin Provincial Key Laboratory of Ginseng Chemistry and Pharmacology, and the Health Technology Innovation Project of Jilin Province (No. 2016J098).

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.
Compliance with Ethics Requirements This article does not contain any studies with human or animal subjects.
Informed Consent Informed consent is not applicable.

Author Contribution Statement

Wen-jing Yu, Kai-ju Sun and Li-ying Zhang performed the experiments; Wen-jing Yu, Chang-bao Chen, Rui Su, Hong-feng Wang, Xi-lin Wan, Hong-mei Yang analyzed the data and wrote the manuscript; Hong-mei Yang and Yi-fei Liu designed the study and revised the manuscript.

References

[1] S. K. Kim, F. Karadeniz, 'Biological Importance and Applications of Squalene and Squalane', *Adv. Food Nutr. Res.* **2012**, *65*, 223-233.

[2] P. P. Deprez, J. K. Volkman, S. R. Davenport, 'Squalene content and neutral lipis composition of Livers from Deep-sea sharks caught in Tasmanian waters', *Mar. Freshwater Res.* **1990**, *41*, 375-387.

[3] Y. Wu, G. Chen, C. Ji, M. Hoptroff, A. Jones, L. Z. Collins, H. G. Janssen, 'Gas chromatography-mass spectrometry and Raman imaging measurement of squalene content and distribution in human hair', *Anal. Bioanal. Chem.* **2016**, *408*, 2357-2362.

[4] R. Tilvis, P. T. Kovanen, T. A. Miettinen, 'Metabolism of squalene in human fat cells. Demonstration of a two-pool system', *J. Biol. Chem.* **1982**, *257*, 10300-10305.

[5] B. I. Wu, Z. D. Zhao, L. L. Han, D. W. Li, 'Review on Some Potential Plant Squalene Resources', *Chem. Ind. For. Prod.* **2011**, *31*, 102-108.

[6] H. L. Newmark, 'Squalene, Olive Oil, and Cancer Risk: Review and Hypothesis ', *Ann. N. Y. Acad. Sci.* **2006**, *889*, 193-203.

[7] M. A. C. Yunus, Z. B. Idham, N. A. Morad, in *2015 10th Asian Control Conference (ASCC)*, **2015**.

[8] A. A. Pokrovski, N. A. Pisareva, T. M. Ushakova, K. I. Eller, F. A. Medvedev, 'Method of determining and the makeup of the aliphatic hydrocarbon fractions of sunflower, cottonseed and soya oils and of oil cakes', *Vopr. Pitan.* **1979**, *56*, 60.

[9] K. H. S. Farvin, A. Surendraraj, R. Anandan, 'Protective Effect of Squalene on Endogenous Antioxidant Vitamins in Experimentally Induced Myocardial Infarction in Rats', *Asian J. Biochem.* **2009**, *4*, 133-139.

[10] Z. D. Zhao, Z. Sun, 'Research progress on natural resources and application of the bioactive substance-squalene', *Chem. Ind. For. Prod.* **2004**, *3*, 109-114.

[11] B. Boughton, R. M. Mackenna, V. R. Wheatley, A. Wormall, 'Studies of sebum. VIII. Observations on the squalene and cholesterol content and the possible functions of squalene in human sebum', *Biochem. J.* **1957**, *66*, 32.

[12] H. Y. Zhang, H. M. Li, Z. Yu, X. Y. Yu, K. Guo, 'Expression and significance of squalene epoxidase in squamous lung cancerous tissues and pericarcinoma tissues', *Thorac. Cancer.* **2014**, *5*, 275-280.

[13] H. Narayan Bhilwade, N. Tatewaki, H. Nishida, T. Konishi, 'Squalene as Novel Food Factor', *Curr. Pharm. Biotechnol.* **2010**, *11*, 875-880.

[14] D. T. Bui, J. Nicolas, A. Maksimenko, D. Desmaële, P. Couvreur, 'Multifunctional squalene-based prodrug nanoparticles for targeted cancer therapy', *Chem. Commun.* **2014**, *50*, 5336-5338.

[15] F. Mcardle, L. E. Rhodes, R. A. Parslew, G. L. Close, C. I. A. Jack, P. S. Friedmann, M. J. Jackson, 'Effects of oral vitamin E and beta-carotene supplementation on ultraviolet radiation-induced oxidative stress in human skin', *Am. J. Clin. Nutr.* **2004**, *80*, 1270-1275.

[16] Y. Ashida, H. Yamanishi, T. Terada, N. Oota, K. Sekine, K. Watabe, 'CoQ10 supplementation elevates the epidermal CoQ10 level in adult hairless mice.', *BioFactors* **2005**, *25*, 175-178.

[17] K. Muta-Takada, T. Terada, H. Yamanishi, Y. Ashida, S. Inomata, T. Nishiyama, S. Amano, 'Coenzyme Q10 protects against oxidative stress - induced cell death and enhances the synthesis of basement membrane components in dermal and epidermal cells', *Biofactors* **2009**, *35*, 435-441.

[18] Y. Yue, H. Zhou, G. Liu, Y. Li, Z. Yan, M. Duan, 'The advantages of a novel CoQ10 delivery system in skin photo-protection', *Int. J. Pharm.* **2010**, *392*, 57-63.

[19] Y. L. Wei, M. F. Yang, A. Y. Liu, X. Zou, G. University, 'Determination of squalene in Cordyceps cicadae by capillary gas chromatography', *Chin. J. Pharm. Anal.* **2014**, *11*, 71-74.

[20] C. Yuan, Y. Xie, R. Jin, L. Ren, L. Zhou, M. Zhu, Y. Ju, 'Simultaneous Analysis of Tocopherols, Phytosterols, and Squalene in Vegetable Oils by High-Performance Liquid Chromatography', *Food Anal. Method.* **2017**, *10*, 3716-3722.

[21] A. Salvo, G. L. La Torre, A. Rotondo, V. Mangano, K. E. Casale, V. Pellizzeri, M. L. Clodoveo, F. Corbo, N. Cicero, G. Dugo, 'Determination of Squalene in Organic Extra Virgin Olive Oils (EVOOs) by UPLC/PDA Using a Single-Step SPE Sample Preparation', *Food Anal. Method.* **2017**, *10*, 1377-1385.

[22] C. X. Jia, D. B. Mao, J. Yang, X. L. Sun, 'Analysis of Squalene and Vitamin E in Supercritical CO2 Extracts from Staphylea bumalda DC.Seed by GC-MS', *Nat. Prod. Res. Dev.* **2007**, *9*, 9-11.

[23] B. P. Ezhilan, R. Neelamegam, 'GC-MS analysis of phytocomponents in the ethanol extract of Polygonum chinense L', *Pharmacogn. Res.* **2012**, *4*, 11-14.

[24] J. V. Goodpaster, B. C. Drumheller, B. A. Benner, 'Evaluation of extraction techniques for the forensic analysis of human scalp hair using gas chromatography/mass spectrometry (GC/MS)', *Expert Opin. Ther. Pat* **2003**, *48*, 299.

[25] J. V. Goodpaster, J. J. Bishop, B. A. Benner, 'Forensic analysis of hair surface components using off-line supercritical fluid extraction and large volume injection', *J. Sep. Sci.* **2003**, *26*, 137-141.

[26] L. Li, D. Pabbisetty, S. Zhu, M. A. Avery, B. A. Avery, 'Urinary Excretion Study of Coenzyme Q10 in Rats by Ultra-Performance Liquid Chromatography-Mass Spectrometry', *J. Chromatogr. Sci.* **2008**, *46*, 215-219.

[27] O. Itkonen, A. Suomalainen, U. Turpeinen, 'Mitochondrial Coenzyme Q10 Determination by Isotope-Dilution Liquid Chromatography-Tandem Mass Spectrometry', *Clin. Chem.* **2013**, *59*, 1260-1267.

[28] R. Yang, Y. Li, C. Liu, Y. Xu, L. Zhao, T. Zhang, 'An improvement of separation and response applying post-column compensation and one-step acetone protein precipitation for the determination of coenzyme Q10 in rat plasma by SFC-MS/MS', *Journal of Chromatography B.* **2016**, *1031*, 221-226.

[29] S. Yang, 'Preparation, in vitro characterization and pharmacokinetic study of coenzyme Q10 long-circulating liposomes', *Drug research.* **2018**, *68 (05)*, 270-279.

[30] L. H. Reddy, P. Couvreur, 'Squalene: A natural triterpene for use in disease management and therapy', *Adv. Drug Delivery Rev.* **2009**, *61*, 1412-1426.

[31] M. Bentinger, M. Kania, W. Danikiewicz, E. Kaczorowska, J. Wojcik, K. Brismar, G. Dallner, T. Chojnacki, E. Swiezewska, M. Tekle, 'Effects of various squalene epoxides on coenzyme Q and cholesterol synthesis', *Biochim. Biophys. Acta.* **2014**, *1841*, 977-986.

[32] S. Shukla, K. K. Dubey, 'CoQ10 a super-vitamin: review on application and biosynthesis', *3 Biotech.* **2018**, *8*, 249.

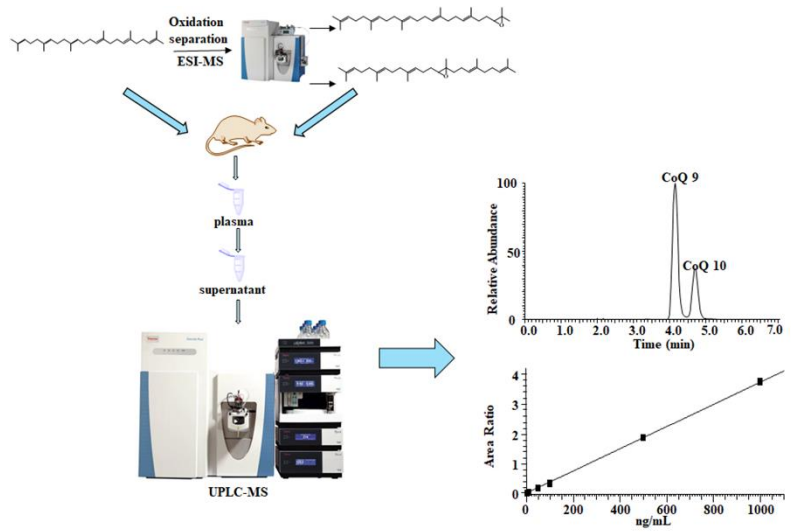
[33] N. E. Masotta, C. Höcht, M. Contin, S. Lucangioli, A. M. Rojas, V. P. Tripodi, 'Bioavailability of coenzyme Q10 loaded in an oleogel formulation for oral therapy: comparison with a commercial-grade solid formulation', *Int J Pharm.* **2020**, *582*, 119315.

[34] A. Martucci, D. Reureanpintilei, A. Manole, 'Bioavailability and Sustained Plasma Concentrations of CoQ10 in Healthy Volunteers by a Novel Oral Timed-Release Preparation', *Nutrients* **2019**, *11 (3)*, 527.

[35] Y. Y. Li, Y. Y. Song, C. H. Liu, X. T. Huang, X. Zheng, N. Li, M. L. Xu, S. Q. Mi, N. S. Wang, 'Simultaneous determination of esculin and its metabolite esculetin in rat plasma by LC–ESI-MS/MS and its application in pharmacokinetic study', *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2012**, *907*, 27-33.

[36] P. K. Duitsman, A. B. Barua, B. Becker, J. A. Olson, Effects of epoxycarotenoids, beta-carotene, and retinoic acid on the differentiation and viability of the leukemia cell line NB4 in vitro, *Int. J. Vitam. Nutr. Res.* **1999**, *69*, 303.

Entry for the Graphical Illustration



Twitter Text

A highly sensitive and rapid method based on UPLC-Orbitrap-MS combined with the precipitation protein method was established for the quantification of CoQ10 in rat plasma. Two squalene epoxides were successfully separated by column chromatography. The effects of different doses of squalene and oxidosqualenes on levels of CoQ10 in rats were investigated, and it was concluded that both squalene and oxidosqualenes could compensate for the lack of CoQ10 in rats. The effect of oxidosqualenes was more distinct than that of squalene. No significant difference was found for each analyte between high-dose group and low-dose group, indicating that low doses of squalene and oxidosqualenes were enough to synthesize CoQ10. If the oxidosqualenes were developed into health products, it will result in a very important economic and social significance. Our study on the interaction between squalene/oxidosqualenes and CoQ10 in vivo provides novel insights on the treatment of CoQ10 deficiencies in human.