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# Dietary pyrroloquinoline quinone (PQQ) alters indicators of inflammation and mitochondrial-related metabolism in human subjects☆☆

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**Abstract**

Pyrroloquinoline quinone (PQQ) influences energy-related metabolism and neurologic functions in animals. The mechanism of action involves interactions with cell signaling pathways and mitochondrial function. However, little is known about the response to PQQ in humans. Using a crossover study design, 10 subjects (5 females, 5 males) ingested PQQ added to a fruit-flavored drink in two separate studies. In study 1, PQQ was given in a single dose (0.2 mg PQQ/kg). Multiple measurements of plasma and urine PQQ levels and changes in antioxidant potential [based on total peroxyl radical-trapping potential and thiobarbituric acid reactive product (TBAR) assays] were made throughout the period of 48 h. In study 2, PQQ was administered as a daily dose (0.3 mg PQQ/kg). After 76 h, measurements included indices of inflammation [plasma C-reactive protein, interleukin (IL)-6 levels], standard clinical indices (e.g., cholesterol, glucose, high-density lipoprotein, low-density lipoprotein, triglycerides, etc.) and $^{1}$H-nuclear magnetic resonance estimates of urinary metabolites related in part to oxidative metabolism. The standard clinical indices were normal and not altered by PQQ supplementation. However, dietary PQQ exposure (Study 1) resulted in apparent changes in antioxidant potential based on malonaldehyde-related TBAR assessments. In Study 2, PQQ supplementation resulted in significant decreases in the levels of plasma C-reactive protein, IL-6 and urinary methylated amines such as trimethylamine *N*-oxide, and changes in urinary metabolites consistent with enhanced mitochondria-related functions. The data are among the first to link systemic effects of PQQ in animals to corresponding effects in humans.



*Keywords:* PQQ; Mitochondria; Inflammation; Biofactor; nutrient; Human metabolism

## 1. Introduction

Pyrroloquinoline quinone (PQQ) acts as a novel growth factor in both plants [1] and animals [2,3]. The mechanism of action in animal models and cultured cells involves the activation or expression of factors, such as peroxisome proliferator-activated receptor alpha; cAMP response element-binding; nuclear respiratory factors 1 and 2; transcription factor A, mitochondrial; and peroxisome proliferator-activated receptor-γ coactivator 1-alpha [4,5]. Each of these transcription factors and coactivators plays a central role in the regulation of cellular energy metabolism (e.g., promotion of β-oxidation) and mitochondrial biogenesis [4–10] and are related to the remodeling of muscle tissue to a fiber-type composition that is metabolically more oxidative and less glycolytic [11,12]. In addition, genes important to cellular stress (e.g., thioredoxin), cell signaling (Janus kinase-, MAPK- and STAT-related pathways) and nutrient transport are affected [5], which suggests that PQQ may act initially through cell surface cytokine and/or growth factor receptors [11,12] or by influencing the phosphorylation of key components [13,14].

When added to nutritionally complete, semipurified diets devoid of PQQ, as little as 300 μg PQQ per kg diet improves reproduction and enhances neonatal rates of growth in rats and mice compared to the response from diets devoid of PQQ [5–10]. PQQ exposure (3 mg PQQ/kg body weight) also results in the protection of cardiac and neural tissue following experimentally induced ischemia in rodent models [15–17].

However, no reports are available regarding whether PQQ evokes similar physiological responses in humans. Humans consume from 0.1 to 1.0 mg PQQ and its derivatives per day based on available food compositional data [18–20]. Animal and human tissues contain from 1 to 3 ng of nonderivatized PQQ per gram tissue or milliliter of fluid, i.e.,

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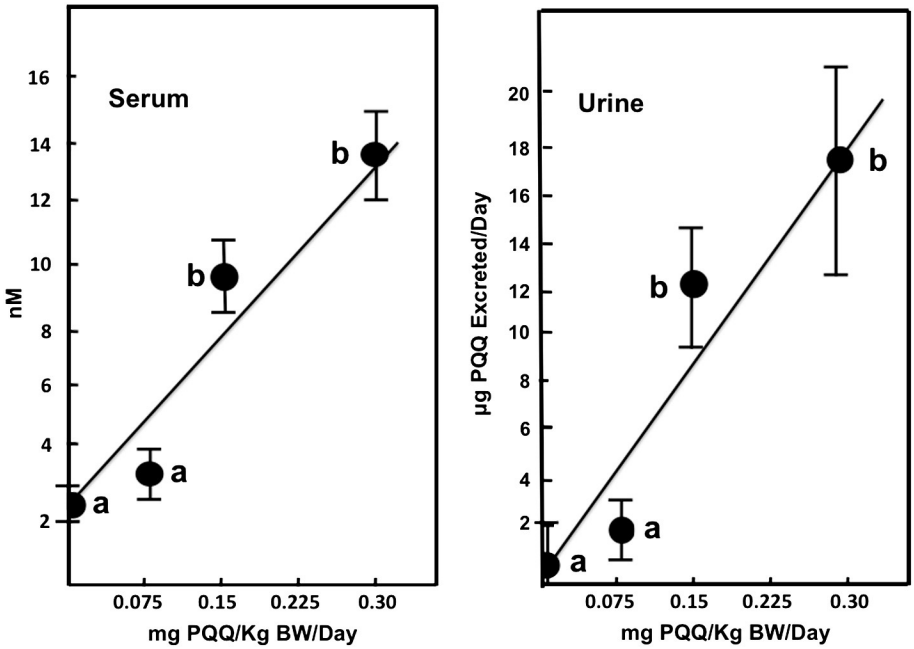


Fig. 1. Dietary PQQ, serum, and urine PQQ levels. PQQ serum concentrations increased directly in response to dietary intake, and the daily excretion of PQQ in urine was related to both the serum levels of PQQ and the daily PQQ intake. At each dose of PQQ, approximately 0.1% of the parent compound (nonderivatized PQQ) was recovered in urine based on a glucose dehydrogenase assay specific for PQQ (see text). Values with differing superscripts are significant at $P<.01$ (ANOVA using a Tukey correction).

about 10% the levels typically found in plants. Accordingly, dietary PQQ appears sufficient to maintain nanomolar concentrations in animal tissues, measured as the parent compound [18]. Organisms common to the gut do not produce PQQ in amounts that are easily determined [21]. Moreover, the levels of PQQ in animal tissues are responsive to changes in diet [5–10].

The reduced form of PQQ is a potent antioxidant and capable of catalyzing continuous and repeated oxidation and reduction reactions in chemical assays [13,22–27]. For example, PQQ is 100–1000 times more efficient than other quinone biofactors in assays designed to assess redox cycling [22]. Other quinone biofactors also tend to either self-oxidize or condense into inactive forms. In contrast, PQQ is relatively stable and does not easily polymerize. It is also noteworthy that there may have been a constant exposure to PQQ-like compounds throughout biological evolution based on the observations that stellar dust contains PQQ-like compounds [28].

Given such findings, the studies reported herein were designed to assess whether PQQ promotes biological responses in humans consistent to those reported in experimental animals. Data are presented that PQQ does improve antioxidant potential and alters markers of inflammation, such as C-reactive protein (CRP). In addition, indirect estimates of oxidative metabolism, based on changes in urinary metabolites, indicate that PQQ ingestion influences mitochondrial function.

## 2. Materials and methods

### 2.1. Human studies

Subjects (5 female and 5 male) were recruited (mean age: 28.1 years; range: 23–33 years, female; 21–34 years, male). All participants were employed or students at the University of California, Davis. Subjects were asked to abstain from alcohol, analgesics and nutritional supplements, but otherwise consume their normal diets.

To assess potential dosages for subsequent clinical studies, the relationship between the dietary intake of PQQ and changes in PQQ plasma and urinary concentrations was first examined in a preliminary study. Following basal estimates, PQQ was administered at 0.075 mg PQQ/kg body weight per day for 7 days, followed by 0.15 mg PQQ/kg body weight per day for 7 days and 0.3 mg PQQ/kg body weight per day for 7 days. At the end of each of these periods, plasma PQQ and urinary PQQ were measured.

Next, two separate studies were conducted using a crossover design. For each study, baseline plasma and urine samples were obtained, i.e., prior to PQQ supplementation. PQQ was administered in ~6–8 fluid ounces (~180–240 ml) of an orange drink. For assays using plasma, 18 ml of venous blood was drawn into Vacutainer tubes containing EDTA or sodium heparin (Becton Dickinson, Franklin Lakes, NJ, USA). Plasma was separated by low-speed centrifugation (1500×g at 4°C for 10 min) and stored at −80°C until analysis. For assays using urine, 100-ml samples were obtained in acid-washed containers at each time point and frozen immediately.

In Study 1, PQQ was administered in a single dose at 0.2 mg PQQ/kg body weight. Plasma and urine samples were then obtained at 0 (baseline), 2, 4, 8, 24 or 48 h following PQQ supplementation. Measurements included assessments of PQQ in plasma and urine and plasma antioxidant potential plus standard clinical indices [e.g., aspartate aminotransferase (AAT), cholesterol, glucose, high- (HDL) and low-density lipoprotein (LDL) levels, uric acid, triglycerides (TG), uric acid and protein; see descriptions that follow].

In study 2, PQQ was administered daily at 0.3 mg PQQ/kg body weight for 3 days. Blood samples were obtained by venipuncture at baseline (0 h) and the morning of day 4, i.e., following 72 h of daily PQQ supplementation. Indices related to PQQ status, inflammation [CRP, interleukin (IL)-6 and matrix metalloproteinase (MMP)-9] and urinary metabolites reflecting oxidative metabolism (e.g., various amino acid-, carbohydrate-, or fatty acid-derived products) were measured.

All studies were reviewed and approved by the Institutional Review Board (Federal-wide assurance 00004557) under the auspices of IRB 200816307, UC Davis.

### 2.2. PQQ

PQQ was measured in serum and urine using a glucose-dehydrogenase-based assay system [29–31]. PQQ was extracted from tissue and plasma as described by Suzuki et al. [31]. Recoveries based on samples (serum samples spiked with PQQ) were routinely 85% or greater.

### 2.3. Plasma antioxidant activity

Antioxidant activity was based on measurements of plasma thiobarbituric acid reactive products (TBARS) and the intensity of luminol-induced chemiluminescence by radicals derived from the thermolysis of 2,2'-azo-bis[2-amidinopropane], as a measure of total peroxyl radical-trapping potential (TRAP). For the TBARS assay, 100 µl of plasma was mixed with 200 µl of 3% sodium dodecyl sulfate, 800 µl of 0.1 mol/L HCl, 100 µl 10% (wt/v) phosphotungstic acid and 400 µl of 0.7% (wt/vol) 2-thiobarbituric acid and incubated (95°C for 30 min). Samples were cooled on ice and mixed with 1 ml of *n*-butanol. After centrifugation (1800×g, 10 min, 4°C), a 200-µl aliquot of the *n*-butanol phase was separated and analyzed spectrofluorometrically (excitation=515 nm and emission=555 nm) using a plate reader attachment (Perkin-Elmer Cetus, Norwalk, CT, USA). TBARS are expressed as malondialdehyde equivalents [32,33].

For TRAP, plasma samples (5–10 μl) were assayed for their ability to inhibit the chemiluminescence produced by a mixture of 3 ml of 5.4 mg/ml 2,2'-azo-bis [amidinopropane] in 0.1 mmol/L phosphate-buffered saline (PBS), pH 7.4 (GIBCO BRL, Life Technologies, Grand Island, NY, USA), and 10 μl of 1 mg/ml luminol [34]. The lag time was measured that proceeds the increase in luminol-induced chemiluminescence derived from the thermolysis of 2,2'-azo-bis[2-amidinopropane]. The lag time is proportional to antioxidant capacity. A reference lag time was obtained by using (10 μM Trolox in 1× PBS) of 6-hydroxy-2,5,7,8-tetramethoxychroman-2-carboxylic acid (Trolox; Aldrich Chemical Co., Milwaukee, WI, USA).

### 2.4. Inflammation-related assays

CRP was measured using a commercial assay kit (ALPCO, Keewaydin Drive, Salem, NH, USA; 03079 Kit 30-9710S) following the protocol provided with the kit (http://www.alpco.com/pdfs/30/30-9710s.pdf). For IL-6, an optimized enzyme-linked immunosorbent assay (ELISA) kit specific for human IL-6 was used (Thermo Fisher Scientific, Rockford, IL, USA; 61105 Kit EH2IL6) based on the colorimetric enzyme-linked quantification of human IL-6 in serum. The assay standard was calibrated to an international reference standard. The sensitivity was less than 2 pg IL-6/ml. MMP-9 (associated with tissue remodeling at sites of inflammation) was also estimated using an ELISA assay (Invitrogen MMP-9 Immunoassay Kit, Catalog #KHC3062/KHC3061; Invitrogen, Camarillo, CA, USA) as an additional estimate of inflammatory response [35,36].

### 2.5. Other clinical parameters

Aspartate amino acid transferase, cholesterol, creatinine, glucose, HDL, LDL, TG, uric acid and total protein were measured in plasma using a COBAS c111 analyzer (Roche Diagnostics, Indianapolis, IN, USA) using methods and reagents supplied by the manufacturer.

### 2.6. Urinary metabolites and nuclear magnetic resonance (NMR)

NMR spectra were acquired as previously described [37,38] using a Bruker Avance 600-mHz NMR spectrometer equipped with a sample jet autosampler using the NOESY-presaturation pulse sequence at 25°C. Samples were prepared for NMR spectroscopy by centrifuging to remove particulate matter followed by the addition of 65 μl of internal standard containing 5 mM 3-(trimethylsilyl)-1-propanesulfonic acid-d6 and 0.2% $NaN_3$ in 99.8% $D_2O$ to 0.585 ml of urine supernatant. The pH of each sample was adjusted to 6.8 ±0.1. Spectra were acquired with a spectral width of 12 ppm, a mixing time of 100 ms, 32 transients, 8 dummy scans and an acquisition time of 2.5 s. Water suppression was accomplished through application of a low-power pulse during the prescan delay (2.5 s) and mixing time (100 ms). Once acquired, all spectra were zero-filled to 128k data points, Fourier transformed with a 0.5-Hz line broadening, phased and baseline corrected using NMR Suite 6.1 Processor (Chenomx Inc., Edmonton, Canada). Identification and quantification of metabolites were accomplished using the 600-MHz library from the Chenomx NMR Suite 6.1 Profiler, which uses the concentration of a known reference signal (DSS) to determine the concentration of compounds [37,38].

### 2.7. Statistical analysis

A Student's *t* test (paired) was used for direct comparisons. For multiple comparisons, an analysis of variance (ANOVA) employing a Tukey or Dunnett *post hoc* correction was performed.

## 3. Results

### 3.1. Dietary PQQ, serum PQQ levels, urinary excretion of PQQ and changes in antioxidant potential

To establish an oral dose of PQQ that would result in a 5–10-fold increase in the plasma levels of PQQ, the relationship between the PQQ intake, corresponding plasma levels and urinary excretion was assessed. A close relationship was observed between intake, the concentration of PQQ in serum and the amounts of PQQ excreted in urine (Fig. 1). At each dose (0 to 0.3 mg/kg/day), ~0.1% of the PQQ

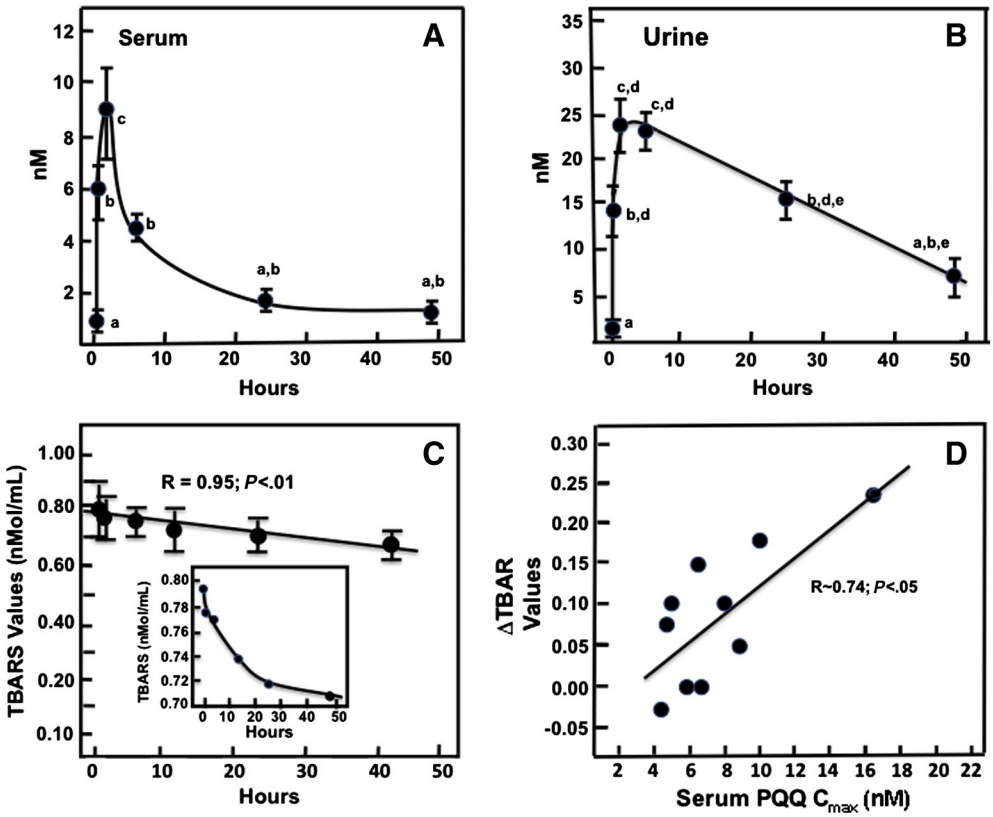


Fig. 2. Levels of PQQ in serum and urine and serum TBAR in response to a single oral supplement of PQQ. A single dose of PQQ [0.2 mg PQQ/kg body weight (BW)] was administered, and the levels of PQQ were measured in serum and urine. PQQ peaked in serum at ~2–3 h. The rise and clearance of PQQ paralleled the changes in urine. Values are means (n=10)±1 S.E.M. (B). For panels (A) and (B), values with differing superscripts are significantly different from each other at $P<.01$ (ANOVA using a Tukey *post hoc* correction). Changes in plasma TBARS were also observed in response to the PQQ supplementation (C). The initial value (equivalent to 0.79 nmol/ml as malondialdehyde) differs from the final value (0.7 nmol/ml malondialdehyde) at $P\sim.06$ based on a paired *t* test. The gradual decrease in malondialdehyde over time appeared linear ($R=0.95$) and significant ($P<.01$). The correlation coefficient for change in TBAR values and peak serum PQQ values (D) was $R=0.74$ ($P<.5$).

ingested was recovered in urine as nonderivatized PQQ. Serum concentrations of nonderivatized PQQ increased in response to dietary intake ($R^2$>0.9, $P$<.05), and the daily excretion of PQQ in urine was directly related to serum levels ($R^2$>0.9, $P$<.05).

Following a single dose of PQQ (0.2 mg PQQ/kg body weight; Study 1), levels of PQQ peaked in serum at ~2 h (Fig. 2A). The rise and clearance of PQQ in serum paralleled the changes in urine (Fig. 2B). We also assessed if the changes in plasma PQQ concentrations following oral administration would result in measurable and corresponding changes in reactive oxidant products. Changes in TBARS, which measure malondialdehyde generated from lipid hydroperoxides, and TRAP values were chosen as indices. Although no significant changes were observed in the TRAP values, there was a slight but significant decrease in TBAR values over the time course of Study 1 (Fig. 2C). Further, individual values expressed as ΔTBAR values (0 $h_{TBARvalue}$ minus 48 $h_{TBARvalue}$) were correlated with plasma $C_{max}$ values for PQQ (Fig. 2D, $P$<.05).

### 3.2. CRP, IL-6 and MMP-9

The levels of CRP, MMP-9 and the cytokine IL-6 are given in Fig. 3A–C. The serum CRP level was reduced for each of the 10 subjects in response to oral administration of PQQ ($P$<.05). The serum CRP concentrations were also positively associated with IL-6 levels (Fig. 3D). The positive relationship between IL-6 and CRP was taken in part as validation that PQQ was capable of attenuating an inflammatory response [39–42]. There was no change in MMP-9 levels.

### 3.3. Clinical indices

To determine whether a brief exposure to PQQ influences standard clinical indices (Studies 1 and 2), estimates of total cholesterol, creatine, glucose, LDL, HDL, TG, uric acid, total protein, and AAT isozyme activity [35,36] were measured at 48 h after a single dose of PQQ (Study 1) or 72 h after a continuous daily dose of PQQ. As indicated in Table 1, the various values were all within normal ranges [43].

### 3.4. Urinary metabolites related to mitochondrial function

In Figs. 4–6 and Table 2, the relative changes in concentrations of urinary metabolites are presented (Study 2; response to 0.3 mg PQQ/day × 3 days). Significant differences were observed for urinary lactate ($P$~.03) following PQQ supplementation (Fig. 4). Pyruvic acid was also reduced ($P$~.07), and the initial ratio of lactate to pyruvate was reduced from 4.5±0.5 to 3.6±0.35. Although no significant changes were observed for other citric acid cycle intermediates, when their respective urinary concentrations were summed (citrate +isoaconitate+2-ketoglutarate+succinate), the values following PQQ supplementation were reduced from baseline by ~15%, with a reduction in the urinary fumarate level of 18% ($P$<.07). These data were taken to suggest that PQQ might influence mitochondrial efficiency, assuming the relative reduction in urinary concentrations reflects increased oxidation and flux of citric acid cycle intermediates via mitochondria [44–46].

Fig. 5 shows data for methylated amine products, which are derived largely from gut microbial metabolism [47–51]. Methylated amine compounds can be eventually metabolized to trimethylamine *N*-oxide (TMAO) primarily in the liver by flavin-containing monooxygenases (derived from the FMO3 gene) [50]. Changes in TMAO are of particular interest because of their use as urinary markers in atherosclerosis [47–50], diabetes [51] and perturbations in energy metabolism in animal models [52]. We previously observed that PQQ-deprived rats have lower levels of expression of FMO3 based on gene

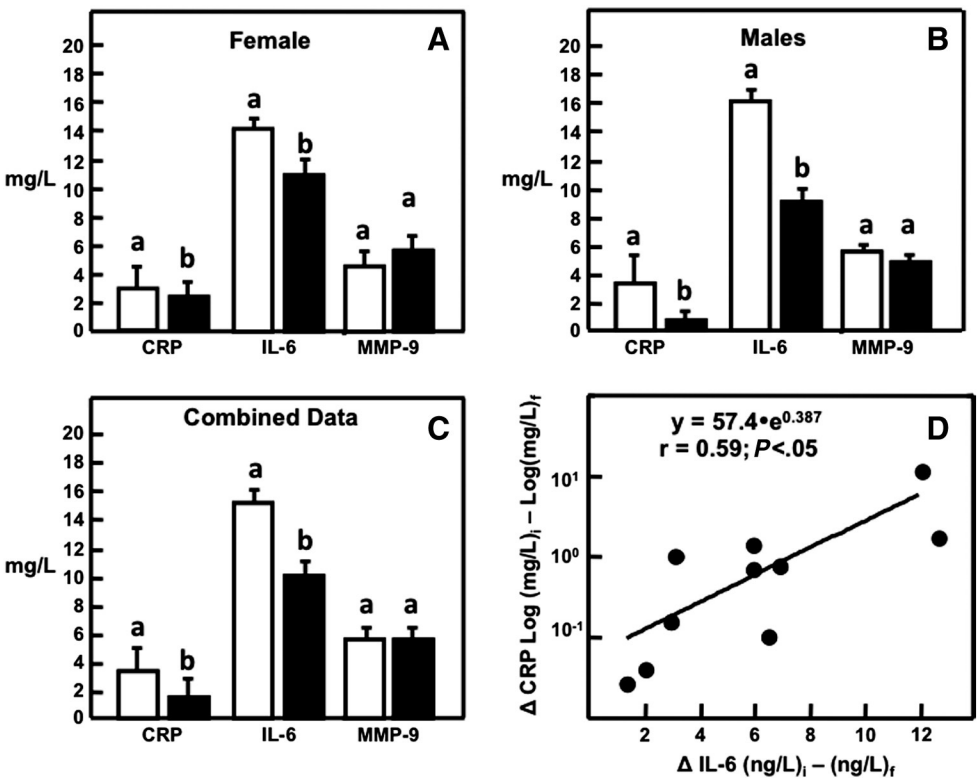


Fig. 3. PQQ, plasma CRP, IL-6, and MMP-9 levels. Plasma CRP and IL-6 levels were decreased in each of the 10 subjects in response to oral administration of PQQ. Open bars represent the baseline values. The solid bars represent values following PQQ supplementation at 0.3 mg/kg BW for each of 3 days. Corresponding bars with differing letter designation are significant at $P$<.05. The changes in levels for male and female subjects and the total group of subjects are shown in panels (A) to (C). Both IL-6 and CRP levels were measured because they have related roles in the inflammatory response. The positive relationship between IL-6 and Δ log CRP values (D) was taken as an internal validation that PQQ was capable of attenuating an inflammatory response involving CRP and IL-6 (see Discussion). No changes were observed in MMP-9.

Table 1
Effect of PQQ supplementation on serum clinical indices

| Study 1[a] | | |
|---|---|---|
| Indices | Day 0 | Post 48 h |
| Aspartate transaminase (U/L) | 29±1.1 | 24±1.1 |
| Cholesterol (mg/L) | 175±6 | 177±6 |
| Creatine (mg/dl) | 0.8±0.06 | 0.8±0.05 |
| Glucose (mg/L) | 89±6 | 94±6 |
| HDL (mg/L) | 52±2 | 53±3 |
| LDL (mg/L) | 110±9 | 109±9 |
| TG (mg/L) | 89±11 | 91±12 |
| Uric acid (mg/dl) | 4.7±0.5 | 4.5±0.6 |
| Total protein (g/L) | 7.2±0.2 | 7.3±0.3 |
| Study 2[a] | | |
| Indices | Day 0 | Post 72 h |
| Aspartate transaminase (U/L) | 29±1.1 | 24±1.1 |
| Cholesterol (mg/L) | 169±7 | 183±9 |
| Creatine (mg/dl) | 0.8±0.07 | 0.8±0.08 |
| Glucose (mg/L) | 90±3 | 85±4 |
| HDL (mg/L) | 51±3 | 54±2 |
| LDL (mg/L) | 102±11 | 112±13 |
| TG (mg/L) | 81±14 | 94±12 |
| Uric acid (mg/L) | 4.9±0.4 | 4.5±0.3 |
| Total protein (g/L) | 7.1±0.2 | 7.2±0.2 |

[a] PQQ was supplemented at 0.2 mg/kg/day (Study 1) and at 0.3 mg/kg/day (Study 2). Values are means±1 S.E.M.

array data derived from hepatic tissue [9]. In this regard, the data in Fig. 5 suggest that short-term PQQ exposure in humans may also result in reduced TMAO production.

In Table 2, values for urinary glucose, selected amino acid, and ketone-related metabolites are summarized. Attention was paid to amino acids that are metabolized predominantly in resting muscle; for example, leucine, isoleucine, asparagine, aspartate, valine and glutamate. These amino acids provide the amino groups and ammonia required for synthesis of glutamine and alanine [53]. In addition, serine was examined because of its important role in pyruvate metabolism, and 4-hydroxyphenylacetate and 4-hydroxyphenyllactate were included because their respective urinary levels decrease when β-oxidation is enhanced [46].

Regarding specific amino acids, although changes in the amounts of most of the amino acid-related metabolites that were detected by $^1$H-NMR (approximately 30 total) were not influenced following PQQ supplementation, collectively, there was a significant reduction of ~15% in the total amount of amino acids excreted (baseline vs. PQQ supplementation, $P<.01$). Further, significant differences or strong trends were observed for serine, isoleucine, asparagine and aspartic acid, four of the six amino acids that are metabolized predominantly in resting muscle (Table 2). Significant differences were also observed in 4-hydroxyphenylacetate plus 4-hydroxyphenyllactate urinary levels (baseline vs. PQQ supplementation, $P<.05$).

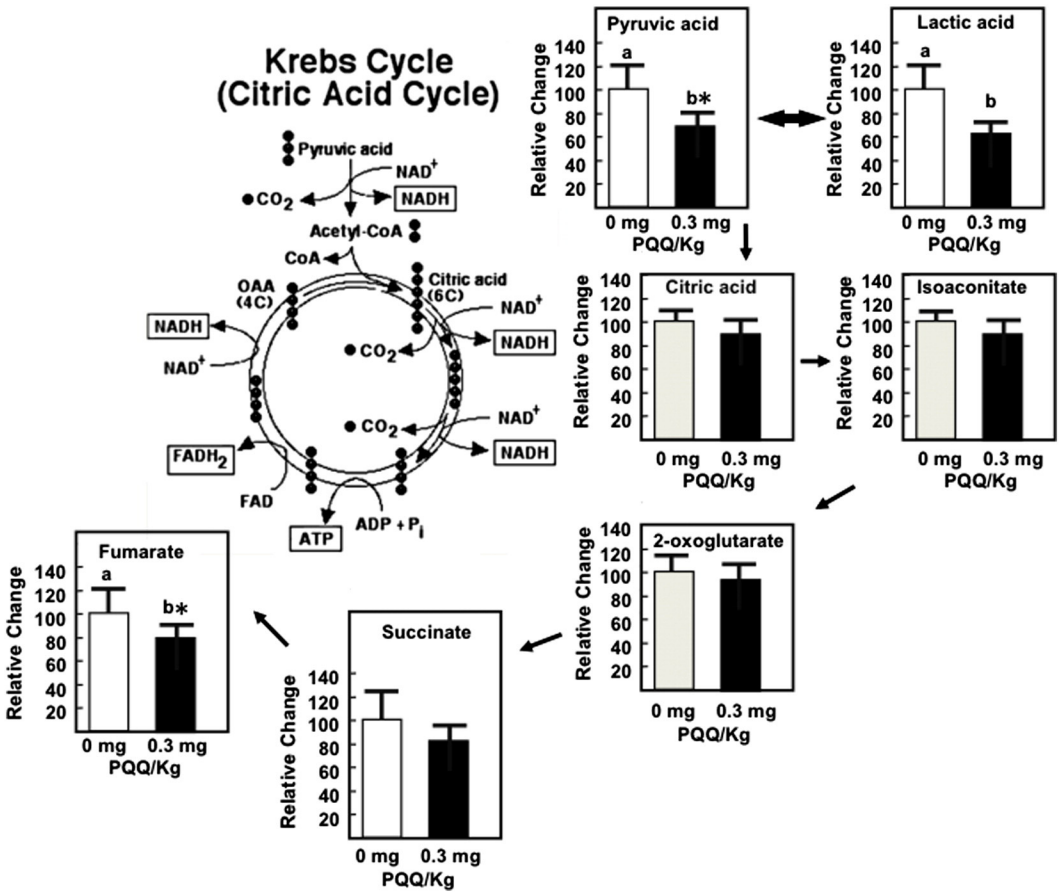


Fig. 4. PQQ and relative changes in urinary TCA cycle metabolites. In Study 2, differences were observed for lactate ($P<.03$) and pyruvate ($P\sim.07$) after 3 days of PQQ exposure. The mean values (μmol/L) were 45±6 (PQQ+) vs. 35±4 (PQQ−) for pyruvate and 195±20 (PQQ+) vs. 121±15 (PQQ−) for lactate. The initial ratio of lactate to pyruvate was 4.5±0.5 to 3.6±0.35 and was reduced to 3.6±0.35 following PQQ supplementation ($P\sim.1$). Values are means±1 standard mean error. Corresponding values with differing superscripts are significant at $P<.05$. The inclusion of an asterisk (*) indicates a $P$ value of>.05 but <0.1. Regarding the other TCA cycle intermediates that are shown, the mean values (μmol/L) were as follows: citrate, 3570±666 [baseline (BL)] vs. 3278±512 [PQQ supplemented (PS)]; *cis*- plus *trans*-aconitate, 506±112 (BL) vs. 385±53 (PS); 2-ketoglutarate, 202±28 (BL) vs. 192±53 (PS); succinate, 93±18 (BL) vs. 78±18 PS; and fumarate, 5.1±0.5 (BL) vs. 4.3±0.4 (PS).

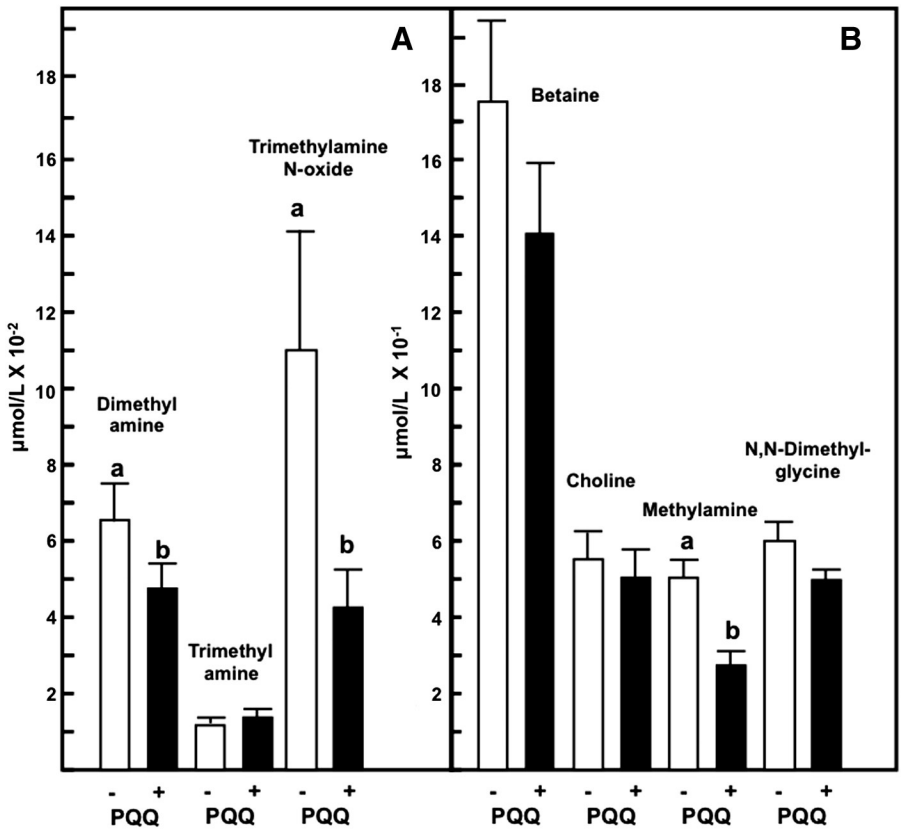


Fig. 5. PQQ and urinary methylated amines. Important methylated amines are shown. A major end product of methylated amine metabolism is TMAO, which is produced in the liver by the action of monooxygenase (isozyme 3). Values are means±1 standard mean error. Corresponding values with differing superscripts are significant at $P<.05$.

Lastly, as measures of the internal precision and validation, known relationships, such as the relationship between glutamine and glutamate and creatinine and the methylhistidines, were examined. Significant correlation coefficients were observed when the amounts of glutamate excreted were plotted vs. glutamine excreted ($R=0.94$, $P<.001$, Fig. 6A) and the amounts of creatinine vs. methylhistidines were plotted, particularly 3-*N*-methylhistidine ($R=0.95$, $P<.001$, Fig. 6B and C). Other changes that suggested perturbations in nitrogen metabolism were decreases in urea excretion (Fig. 6 D, $P<.01$).

In contrast, as a measure of purine-related metabolites, no differences were observed for hypoxanthine (baseline vs. following PQQ supplementation, 115±17 [SEM] vs. 123±35 μmol/L, respectively) or uracil (baseline vs. following PQQ supplementation, 102±9 vs. 102±13 μmol/L, respectively). The amounts of urinary *myo*-inositol were also not changed (baseline vs. following PQQ supplementation, 325±96 vs. 263±73 μmol/L, respectively), nor were the levels of hippuric acid (baseline vs. following PQQ supplementation, 2097±1195 vs. 2037±2020 μmol/L, respectively), which is often used as a marker for changes in the microbiome [55].

## 4. Discussion

The data reported are among the first to link systemic effects of PQQ in animals to related or corresponding effects in humans. For example, PQQ influences the immune response in mice through mechanisms that involve changes in interleukin levels [9]. In humans, PQQ appears to promote anti-inflammatory potential, which could be related to changes in plasma CRP and IL-6 levels [39–42]. Reductions of both CRP and IL-6 following PQQ ingestion were observed, important because CRP expression is dependent on IL-6. IL-6 helps to coregulate B cell differentiation, plasmacytogenesis and the acute phase reactions [55,56], leading to the production and release of CRP into circulation. Normal levels of CRP in healthy human serum are usually substantially lower than 10 mg/L [40]; accordingly, it is noteworthy that PQQ supplementation reduced further CRP levels by ~45%. Moreover, although subtle, the changes in plasma TBARS values (~ 0.2% decrease in relative TBAR values/h following supplementation, $P<.05$) and its association with changes in plasma PQQ levels (in the 6–12-nM range) suggest that PQQ has potential as an antioxidant. For comparison, Rein et al. [57] have reported that the consumption ~560 mg of procyanidins in the form of 80 g of dark chocolate results in the appearance of 200–300 nM of epicatechin in plasma with an accompanying decrease (25%–35%) in TBAR levels in as few as 2 h.

The data were also consistent with features associated with mitochondrial-related oxidative metabolism [2–8,10–14]. A potentially important finding was the relative decreases in mitochondrial-related intermediates and metabolites in urine following PQQ exposure. These changes are consistent with increased mitochondrial efficiency and in keeping with other observations wherein $^{1}$H-NMR data of urinary metabolite profiles have been used to assess differences in metabolic efficiency or disease relationships when mitochondrial function is important [45–52].

We previously have shown that, in animal models, dietary PQQ exposure improves various indices related to oxidative metabolism, for example, parameters that reflect changes in the rates of β-oxidation [6,7], mitochondrial-related amino acid metabolism [10] and the amounts of mtDNA [4–10]. Similar changes have been reported when streptozotocin-treated diabetic rats are compared to rats with normal glycemic control [45] or rats fed diets containing 0.2% or more resveratrol vs. a standard control diet [58,59]. For example, the relative levels of urinary and serum pyruvate, various

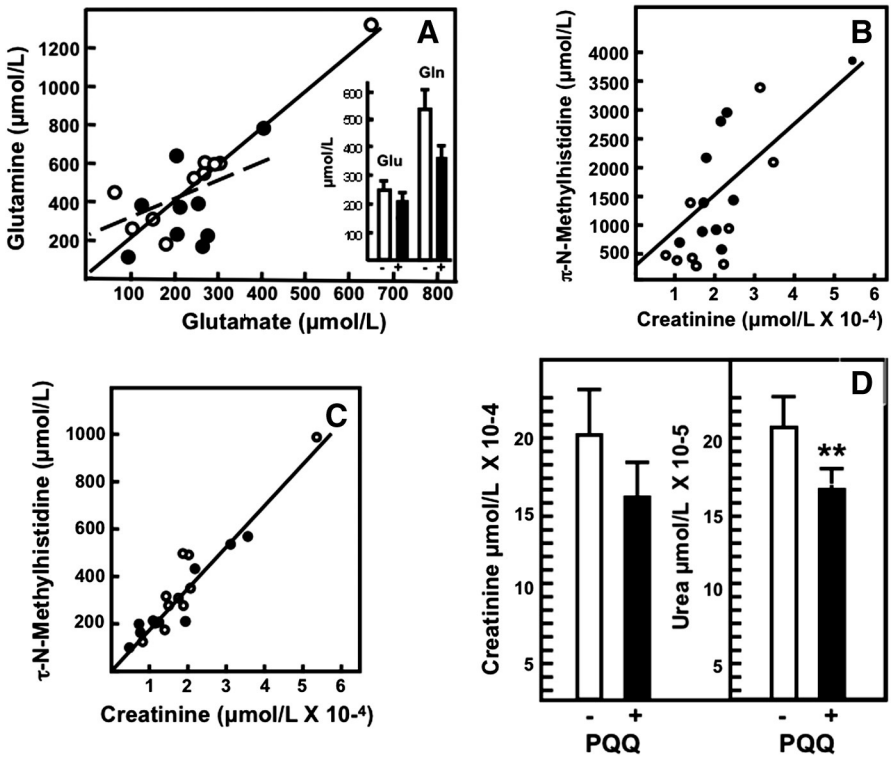


Fig. 6. PQQ and indices of muscle protein retention. The relationships for glutamate vs. glutamine (A), creatinine vs. 1-*N*-methylhistidine (B), creatinine vs. 3-*N*-methylhistidine (C), and urinary creatinine and urea levels (D) are shown. Baseline values are indicated by open circles, and values following PQQ supplementation are indicated by closed circles. It was expected that glutamine and glutamate would be related. The correlation coefficient between glutamate and glutamine prior to PQQ supplementation was $R=0.94$ ($P<.001$). The value for $R$ for the combined data was 0.77 ($P<.05$) (A). As shown in panels (B) and (C), the relative concentrations of creatinine, 1-*N*-methylhistidine and 3-*N*-methylhistidine were also correlated. The combined correlation coefficients between creatinine and 1-*N*-methylhistidine (B) and between creatinine and 3-*N*-methylhistidine (**C**) were $R=0.55$ ($P<.05$) and $R=0.95$ ($P<.001$), respectively. The levels of urinary creatinine and urea are given in panel (D). There was a significant reduction in the amounts of urea excreted ($P<.05$).

citric acid cycle intermediates, and TMAO are elevated in streptozotocin-diabetic rats compared to rats in which oxidative metabolism is not compromised [45]. With regard to humans, subjects with compromised β-oxidation or type II diabetics also have elevated values for plasma and/or urinary pyruvate and various citric acid cycle intermediates and, particularly in diabetes, elevated TMAO urinary levels [51], metabolites that are influenced by PQQ supplementation. Bennett et al. [60] have recently reported that TMAO levels are strongly associated with atherosclerosis. Increased TMAO levels are associated with inhibition of the reverse cholesterol transport pathway [60]. Consequently, it may be of clinical significance and potential importance that PQQ supplementation reduces the levels of urinary TMAO.

Of the six amino acids that are metabolized predominately by mitochondria in resting muscle (leucine, isoleucine, valine, asparagine, aspartate and glutamate) [61], the levels of urinary isoleucine and asparagine/aspartate were significantly reduced following PQQ supplementation. Further, given the overall decrease in amino acid excretion, it would be expected that such phenomena may result in some degree of nitrogen retention. In this regard, Walsh et al. [62] has examined phytochemical intake and its influence on urinary metabolic profiles. A low-phytochemical diet (no fruits or vegetables) was compared to a phytochemical-enriched diet using the subject's conventional diet as a control. The consumption of a high level of polyphenolic compounds compared to lower levels of exposure for 2 days was characterized by decreased excretion of creatinine, creatine,

Table 2
PQQ and mitochondrial-related metabolites, urinary glucose and ketone-related products[a]

| Metabolite | Initial values (µM/L) | Values following PQQ supplementation (µM/L) | *P* value |
|---|---|---|---|
| *Urinary amino acids* | | | |
| Serine | 551±98 | 356±63 | .08 |
| Asparagine | 146±18 | 99±10 | .03 |
| Aspartic acid | 143±75 | 95±33 | .08 |
| Total | 289±40 | 194±16 | .03 |
| Isoleucine | 27±4.0 | 12±2.1 | .045 |
| 4-Hydroxyphenylacetate | 192±51.6 | 137±49.3 | .03 |
| 4-Hydroxyphenyllactate | 37±10.7 | 20±4.3 | .12 |
| Total | 229±56 | 157±51 | .04 |
| *Urinary glucose and ketone-related products* | | | |
| Glucose | 340±37 | 328±46 | .87 |
| 3-Hydroxybutyrate | 121±38 | 83±14 | .32 |
| Acetate | 94±16 | 75±10 | .24 |

[a] PQQ was supplemented at 0.3 mg/kg/day (Study 2). Values are means±1 S.E.M.

TMAO, and 1- and 3-methylhistidine, which is consistent with our observations following supplementation with PQQ. Clearly, more data are needed to identify other components responsible for N retention in addition to skeletal muscle. The microbiome is one possibility, although a direct effect on microbial populations was not clear. For example, the levels of methylamines were variable, and hippuric acid, which is sometimes used as a marker for changes in the microbiome [54], did not appear to be influenced by PQQ supplementation. Moreover, urinary choline concentrations, an important source of microbially derived methylamines, were not significantly different after supplementation with PQQ. This is an area to be addressed in future studies.

In summary, PQQ influences a wide range of systemic responses ranging from the stimulation of reproductive performance and neonatal growth in animal models fed highly refined diets to modulation in mitochondrial content and β-oxidation potential at dietary levels of exposure that are one to two orders of magnitudes below those required for biofactors, such as resveratrol (cf. [59] and references cited). Further, when the responses to PQQ are compared to other vitamins with redox cycling activity (e.g., ascorbic acid), the actions are often the opposite of those observed for PQQ, i.e., a reduction or no change in mitochondrial biogenesis or function [63,64]. The data herein support the hypothesis that human subjects respond rapidly to PQQ supplementation with changes in urinary metabolites consistent with enhanced mitochondria-related functions.

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