Pyrroloquinoline Quinone Improves Growth and Reproductive Performance in Mice Fed Chemically Defined Diets

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Growth, reproductive performance, and indices of collagen maturation and expression were investigated in Balb/c mice fed chemically defined, amino acid-based diets with or without the addition 6 μM pyrroloquinoline quinone (PQQ)/kg diet. The diets were fed to virgin mice for 8 weeks before breeding. At weaning, the pups from successful pregnancies were fed the same diet as their respective dams. Reproductive performance was compromised in mice fed diets devoid of PQQ, and their offspring grew at slower rates than offspring from mice fed diets supplemented with PQQ. Successful mating (confirmed vaginal plugs) was not affected by the presence or absence of PQQ; however, pup viability (number of pups at parturition/number of pups at Day 4 of lactation) was decreased in PQQ-deprived mice. Conception (percentage of females giving live births) and fertility (percentage of births) were also decreased in PQQ-deprived mice. The slower rates of growth in offspring from PQQ-deprived mice were associated with decreased steady-state mRNA levels for Type I procollagen α_1-chains in skin and lungs from neonatal mice. Values for lysyl oxidase accumulation as protein in PQQ-deficient mice also tended to be lower than corresponding values from PQQ-supplemented or -replete mice. Skin collagen solubility was increased in PQQ-deprived mice. These results indicate that PQQ supplementation can improve reproductive performance, growth, and may modulate indices of neonatal extracellular matrix production and maturation in mice fed chemically defined, but otherwise nutritionally complete diets. Exp Biol Med 228:160–166, 2003

Key words: pyrroloquinoline quinone; lysyl oxidase; collagen; murine reproduction

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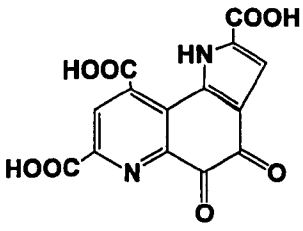
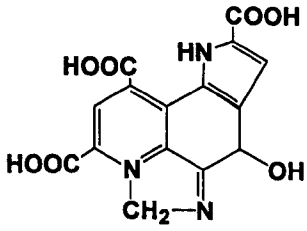
Pyrroloquinoline quinone (PQQ) is often used in bacteria as a dehydrogenase co-factor (1–3). In animals, PQQ is present in mammalian tissues and in milk (4–7). Although there is no known enzymatic function that directly involves PQQ in eukaryotic cells, PQQ can replace FAD in heme reductase and β-hydroxy, β-methylglutaryl coenzyme A reductase (8, 9). PQQ is also beneficial to animal growth and development (10). Mice fed nutritionally adequate diets, but devoid of PQQ, have decreased fertility and slower rates of neonatal growth in first- and second-generation mice compared with corresponding mice supplemented with PQQ (10). Signs of gestational and neonatal PQQ deprivation often include friable skin, general unfitness, and a hunched posture in severely affected mice. However, the role that PQQ may play in the etiology of these characteristics has not been investigated. Defects in immune function also occur, specifically a reduction in interleukin-2 (IL-2) levels and decreased B- and T-cell sensitivity to mitogens (10).

In biological fluids and amino acid-enriched solutions, PQQ rapidly forms adducts such as imidazolopyrroloquinoline quinone (IPQ, see Refs. 6 and 11). Attributes that result from exposure of selected cells to PQQ or IPQ include increased production of nerve growth factor (NGF) and protection of N-methyl-D-aspartate (NMDA) receptors (13–20). PQQ protects neuronal cells from NMDA toxicity (13–20) and stimulates the production of NGF. Moreover, Jensen *et al.* (15) have extended these observations *in vivo* by showing that PQQ protects against the likelihood of severe stroke in an experimental animal model for stroke and brain hypoxia. PQQ has also been shown to have radical scavenging ability and protective capacity following *in vivo* and *in vitro* oxidative insults (21–25). The incorporation of [^{3}H]thymidine into human fibroblasts is increased *in vitro* when PQQ or IPQ is provided in media at concentrations as low as 3 or 15 n*M*/l, respectively (6, 11, 12).

The purpose of this report is to expand on some of our previous findings regarding PQQ, reproductive outcome, and neonatal growth. The extent to which dietary PQQ addition modulates changes in extracellular matrix (ECM) ex-



PQQ_{Ox}



IPQ

pression was examined because parameters important to ECM production and maturation are closely linked to growth.

Materials and Methods

Chemicals. Chemicals and reagents were obtained from Fisher Scientific (Pittsburgh, PA) or from Sigma Chemical Corporation (St. Louis, MO). PQQ was a gift from Dr. James Mah (University of South California School of Dentistry, Los Angeles, CA). The PQQ, used as standards and in diets, was chemically characterized by mass spectrometry as described previously (6). Amino acids used in preparing mouse diets were obtained from Ajinomoto Co. (Tokyo, Japan).

Animal Care and Diets. Male and female Balb/c mice (Charles River Laboratories, Wilmington, MA) were housed in plastic cages with recycled fiber bedding devoid of inks or dyes (Carefresh Total Clean Bedding; Absorption Corp., Bellingham, WA). To decrease bacterial and chemical contamination, mice were housed in a Bioclean incubator (Duo-Flo; Bioclean Lab Products, Maywood, NJ). Food cups and cages were changed twice weekly and mice were provided food and water *ad libitum*. The water supply was filtered through an activated carbon cartridge (Carbon Capsule; Gellman Sciences, Ann Arbor, MI) and a 0.2-μm bacterial filter (Mini Capsule; Gellman Sciences). The care and housing of mice met current U.S. Department of Agriculture and National Institutes of Health guidelines. The mice were housed in an AALAC approved facility.

Mice were fed an amino acid-based diet (Table I) that provided all known essential nutrients in amounts sufficient to provide maximal growth, reproduction, and lactation (10, 26). The PQQ concentration in the basal amino acid diet was determined to be below 5 f*M*/g diet (Ref. 27, c.f. PQQ determination section).

Reproduction and Growth. Four-week-old virgin female Balb/c mice were adapted (for 8 weeks) to the basal diet devoid of PQQ or to the basal diet supplemented with PQQ at 6 μ*M*/g of PQQ/kg of diet. The dams were then mated (no more than three times) with different males until successful births were achieved. The experimental protocol was repeated four times over the course of 1 year. Measurements and definitions used in the assessment of mating, fertility, conception, and pup viability are as follows:

1. Mating: the number of plugs divided by the number of females exposed to proven fertile males;
2. Fertility: the number of births divided by the number of females exposed to proven fertile males;
3. Conception: the number of births divided by the number of plugs; and
4. Pup viability: number of viable pups at parturition divided by the number of pups viable pups on lactation Day 4.

Data are expressed as percentages.

Additional experiments were performed in which tissue weight and ECM protein expression were measured For these experiments, one-half of the pups from PQQ-deprived dams were switched from the PQQ-deficient diet to the PQQ-supplemental diet after birth (designated Day 1). This resulted in three groups: pups from PQQ-deficient dams (–PQQ), pups from PQQ-deficient dams switched to the PQQ-supplemented diet at Day 1 (±PQQ), and pups from PQQ-supplemented dams (+PQQ). Pups from PQQ-deficient dams were also injected i.p. with PQQ at 1, 2, 4, or 16 mg/Kg body weight. Each dose was administered sequentially (every 4 days) to the same mouse (total of eight mice were used).

PQQ Determination. The relative concentrations of PQQ and related redox cycling substances were estimated in mouse serum, milk, and experimental diets by the redox cycling assay as described by Gallop and colleagues (27–30). Although shortcomings of this assay are that PQQ-derived products (e.g., IPQ) are not detected, and other quinones and enediol-containing compounds may interfere, the assay can be used to provide a relative measure of the PQQ concentration in purified samples or isolates. The assay is

Table I. Diet Composition

Diet ingredient	Percent of diet
Amino acid mix[a]	18%
Sucrose	31.5%
Corn starch	27.5%
Corn oil	10%
Mineral mix[b]	10%
Cellulose	2.5%
Vitamin mix[c]	0.5%

[a] Amino acids (g/Kg diet): L-arginine, 12; L-proline. 10; L-serine. 12; L-histidine. 4; L-isoleucine, 8; L-leucine, 12; L-lysine, 14; L-methionine, 6; L-phenylalanine, 5.0; L-tyrosine, 5; L-threonine, 8; L-tryptophan, 2; L-valine, 8; L-alanine, 12; L-asparagine, 11.5; L-cystine, 4; L-glutamate, 12.5; glycine, 12.

[b] Minerals (g/Kg diet): sodium carbonate, 3.0; calcium phosphate, 28.0; potassium phosphate dibasic, 9.0; sodium chloride, 8.8; sodium bicarbonate, 10.0; magnesium sulfate·H_2O, 1.85; manganese sulfate·H_2O, 0.650; ferric citrate, 0.5; zinc carbonate, 0.1; copper sulfate·$5H_2O$, 0.05; boric acid, 0.005; sodium molybdate·$2H_2O$, 0.005; sodium fluoride, 0.005; nickel(II) chloride·$6H_2O$, 0.005; potassium iodide, 0.001; cobalt (III) sulfate·$7H_2O$, 0.001; chromium (III) chloride·$6H_2O$, 0.001; sodium selenite (as mg/Kg), 0.250; and corn starch to 100.0 g.

[c] Vitamins (mg/Kg diet): choline·HCl, 2000; thiamine·HCl, 100; niacin, 50; riboflavin, 15; calcium pantothenate, 30; vitamin B-12, 0.2; pyridoxine·HCl, 30; biotin, 3; folic acid, 20; myoinositol. 100; ascorbic acid, 250; retinyl acetate (I.U.), 5200; cholecalciferol (I.U.), 90; L-α-tocopherol acetate, 40; phylloquinone, 10; and glucose to a total of 5.0 g.

based on the observation that quinones and related quinoid substances catalyze redox cycling at pH 10 in the presence of excess glycine as a reductant, and nitroblue tetrazolium (NBT) as a hydrogen ion acceptor. Product formation (formazan from NBT reduction) is monitored at 550 nm with a sensitivity of 5 p*M* for PQQ (28–30).

To improve selectivity and specificity, the following procedure was used. PQQ and IPQ are nonspecifically bound to protein and peptides. Accordingly, samples (250 μl of milk or serum) were first adjusted to pH 1.0 with 1 *M*/l HCl and acetonitrile (1:1, v/v) to reduce PQQ/protein/peptide interactions. Precipitated material was sedimented by centrifugation (10,000*g* for 20 min). Next, the supernatant fraction was adjusted to pH 7.0 and was passed through a trimethylaminopropyl anion exchange cartridge (Bond-Elut SAX; Analytichem International, Harbor City, CA). The SAX cartridge was washed with 0.01 *M*/l potassium phosphate buffer, pH 7.0 (5–6 column volumes), and PQQ was eluted with 1 *M* potassium phosphate buffer, pH 2.0. This eluate was then passed through an octadecyl reverse-phase cartridge (Bond-Elut C-18; Analytichem International). Bound PQQ was eluted with HCl at 0.01 *M*/l in methanol (1:1, v/v). The recovery for this procedures was >95% for 0.1 μg or less of authentic PQQ. The eluate was then dried under vacuum and an aliquot was assayed. PQQ as assessed by this protocol is operationally defined as a substance that absorbs to both C-18 and SAX columns and catalyzes redox cycling in the presence of appropriate electron acceptors and donors at pH 10. Most polyphenolic derivatives (e.g., dopamine) easily polymerize at pH 10 and are not effective as catalysts in the assay. Enediols, such as ascorbic acid, do not effectively carry out redox cycling in the presence of sodium borate, which was used as buffer (3, 6).

To determine recovery and the reactivity of PQQ when added to the basal diet, 100-mg aliquots of diet containing 0 or 30 n*M* PQQ g^{-1} were suspended in 5 ml of sodium phosphate buffer (0.05 *M*/l), agitated, and assayed at 2.5-, 17-, 43-, 77-, 108-, 170-, 241-, and 308-min intervals. Two pH conditions were examined, pH 2.5 and 7.0.

ECM Protein Expression and Maturation. For lysyl oxidase and type I-procollagen-α_1-chain (I-procollagen-α_1) mRNA levels, RNA was extracted from 1-week-old mouse skin and lung (0.25- to 0.5-g samples) using a modification of the guanidine thiocyanate-phenol-chloroform procedure as described by Chomcynski and Sacchi (32). RNA was separated on 1% agarose, 1 *M*/l formaldehyde gels and was transferred to Zeta-Probe GT membranes (Bio-Rad, Richmond, CA) for use in initial Northern assays to assess cDNA probe specificity. Next, the relative levels of lysyl oxidase, I procollagen-α_1, and β-actin mRNA were estimated in dot blot assays. The dot blot assays used total RNA blotted onto Zeta-Probe GT membranes at concentrations of 1.0, 0.5, 0.1, and 0.05 μg of RNA per sample. The RNA was fixed by transillumination with UV light for 5 min and was then dried at 80°C (for 1–2 hr).

The cDNA probe for lysyl oxidase in RNA was identical to that described and used in Gacheru *et al.* (33) and Tchaparian *et al.* (34). For I-procollagen-α_1 mRNA, the cDNA probe was identical to that described by Genovese *et al.* (35). Values were normalized to β-actin. Probes were randomly primed using the multiprime DNA system (Amersham, San Francisco, CA).

Membranes containing RNA samples were prehybridized at 65°C for 10 min. For hybridization, the concentration of each denatured probe (labeled with ^{32}P-dCTP) was 10^6 dpm/ng per milliliter of 0.5 *M*/l sodium phosphate buffer, pH 7.6, containing 1 m*M*/l EDTA and 7% SDS (w/v). Hybridization was carried out overnight at 65°C. Membranes were next washed twice at 65°C for 30 min each, with a solution of 40 m*M*/l sodium phosphate buffer containing 1 m*M*/l EDTA and 5% SDS, followed by two additional washes with the same solution, but with the SDS concentration adjusted to 1%. X-OMAT AR film (Eastman-Kodak, Rochester, NY) with an intensifying screen (Fisher Scientific, Santa Clara, CA) was used for autoradiographic analysis. Films were exposed to the labeled membranes for periods ranging from 18 hr to 1 week, depending on the intensity of the signal. Autoradiography films were analyzed using an Ultroscan XL enhanced laser densitometer (LKB, Bromma, Sweden). Data from the dot blots was expressed as the ratio of the slope of the regression line for concentration of lysyl oxidase or I-procollagen-α_1 mRNA hybrids relative to the slope for the corresponding signals for β-actin mRNA.

The concentration of lysyl oxidase protein in skin samples was also measured by a direct enzyme-linked immunoabsorbant assay (ELISA) using mice (PQQ^+ and PQQ^- groups) at 8 weeks of age. A partially purified sample of lysyl oxidase, isolated as described by Romero-Chapman *et al.* (36), was used to calibrate the assays; values are expressed as arbitrary units. Samples were prepared by extracting pulverized skin samples twice into 0.1 *M*/l sodium borate buffer containing 0.15 m*M*/l sodium chloride (pH 8.0 at 4°C) followed by centrifugation (25,000*g* for 20 min). The resulting pellet was then reextracted twice with 4 *M*/l urea at 4°C (4–8 hr each). The supernatant fractions were pooled and the urea concentration was adjusted with sodium borate buffer to less than 0.5 *M*/l. The extracted samples were analyzed by ELISA. Each assay was performed in triplicate.

Skin collagen solubility was measured according to the method of Monetta and Martinol (31). Samples of skin were first extracted into a solution of NaCl containing 0.05 *M*/l sodium phosphate adjusted to pH 7.0. Samples were extracted for 24 hr at 4°C with continuous agitation followed by centrifugation (10,000*g* for 30 min). The amount of collagen in the supernatant fractions was then determined spectrophotometrically.

Statistical Analysis. The Statview 5.0 (SAS Insti-

tute, Cary, NC) statistical analysis program was used to analyze the data. All parametric data were analyzed using one-way analysis of variance (ANOVA) and the Tukey-Kramer test at $P < 0.05$. Chi-square testing was used to test nonparametric data. Data were considered significantly different at $P < 0.05$.

Results

PQQ and Reproduction. Reproductive indices representing measures of mating, fertility, conception, and pup viability were used to assess the effects of PQQ on reproductive outcome (Table II). There were no differences in the percentage of confirmed vaginal plugs observed among groups. However, conception was decreased ($P < 0.001$) and fertility tended to decrease in response to PQQ deprivation. An important consideration in reproduction studies is the number of offspring born and surviving to weaning. An indicator of pup survival, viability (number of pups at parturition/number of pups on lactation Day 4), was significantly ($P < 0.001$) increased when PQQ was added to diets.

PQQ, Growth, and Body Composition. Mice fed amino acid-based diets devoid of PQQ grew poorly compared with mice born from dams fed diets supplemented with PQQ (Table III). The improved growth was observed when mice were fed the supplemented diet continuously from birth or were switched to the PQQ-containing diet at weaning. Regarding the latter, two experiments were performed in which the body weights for mice fed continuously from dams fed either a PQQ-deficient, PQQ-supplemented, or PQQ-deficient group that was switched at birth to the PQQ-supplemented diet were compared. Determining whether changes occurred in gross body composition (e.g., relative organ weight changes) was the objective of the initial experiments that compared three groups. The body weights were 8.7 ± 0.4, 9.1 ± 0.8, or 10.4 ±1.2 g, respectively at Day 35 postparturition. The weights for liver, heart, kidney, and spleen (expressed as a percentage of the body weight) were not affected by the dietary treatments (data not shown). In the accompanying experiment, changes in ECM expression were assessed (see the next section). The body weights are given in Figures 1 and 2. In both experiments, a period of *in utero* PQQ deprivation followed by supplementation caused a greater relative body weight change than continuous supplementation. Administration of PQQ by injection i.p. resulted in growth comparable to mice fed 6 nmol/g diet (Table III). No signs of toxicity were observed during routine autopsies.

Table II. PQQ and Reproductive Performance

Group	Reproductive indices[ab]			
	Mating	Fertility	Conception	Pup viability
PQQ (–)	70%	28.0%	40%	67.0%
PQQ (+)	56%	56.0%	100.0%	96.8%
	$P < 0.20$	$P < 0.08$	$P < 0.01$	$P < 0.001$

[a] See text for definitions.
[b] Data pooled from four separate experiments (see Table IV). Inter-experimental variance was <±20%. The level of significance was established using chi-square analysis. Data are expressed as a percentage (ratio × 100%). For each experiment, four to eight virgin females were assigned to the basal group, and four virgin females were assigned to the PQQ-supplemented group.

Table III. Growth of BALB/C Mice in Response to PQQ Supplementation

Experiment	PQQ (nmol/g diet)	Weight at 35 days g ± SEM (number of mice)
1	0	9.7 ± 0.3 (3)[a]
	6	12.7 ± 0.3 (8)
2	0	10.7 ± 0.4 (9)**
	6	13.5 ± 0.9 (9)
3	0	10.7 ± 0.7 (6)***
	6	12.2 ± 0.5 (6)
4	0	7.0 ± 0.2 (18)*
	6	9.2 ± 0.2 (4)

[a] Values with an asterisk are statistically different. *$P < 0.01$; **$P < 0.08$, and ***$P < 0.15$. (n). Some mice were randomly selected and used as a tissue source in other experiments to be reported elsewhere, which accounts for the variability in the numbers of mice remaining at 35 days.

ECM Expression. Lung lysyl oxidase and I-procollagen-α_1 mRNA levels were obtained from mice at 1 week of age, i.e., pups from the PQQ^-, PQQ^+, or $PQQ^{+/-}$ groups). The relative levels of lysyl oxidase and I-procollagen-α_1 mRNA were positively related to body weight (Fig. 1). In lung, lysyl oxidase and I-procollagen-α_1 mRNA levels were also correlated. In Figure 2, data for the expression of I-procollagen-α_1 and lysyl oxidase in mouse skin are given, respectively. In the most stunted mice, I-procollagen-α_1 mRNA levels were related to the rate of growth, although there was no apparent relationship between changes in weight and lysyl oxidase mRNA levels.

When estimated by ELISA, lysyl oxidase protein tended to be less in skin from mice deprived of PQQ. Skin collagen maturation (as measured by increased collagen solubility into neutral salt solutions), also tended to appear less mature in PQQ deprived mice compared with mice fed a PQQ-supplemented diet (Table IV).

PQQ in Diets, Serum, and Milk. Values for PQQ and PQQ-like substances in serum and milk are given in Table V. For these assays, the redox cycling assay was used after absorption and elution from SAX and C-18 preparatory columns. PQQ was reduced in milk (taken at Day 7 of lactation) and serum samples (taken at Week 5) in mice deprived of PQQ compared with PQQ-supplemented mice.

Data related to the fate of PQQ added to the basal diet are presented in Figure 3. PQQ disappeared in an apparent first-order fashion, so that by 60 min, less than one-half of added PQQ was detected when the samples were incubated at pH 2.5, and less than one-half of the sample was detected as PQQ after approximately 2 hr at pH 7.0 (see "Discussion").

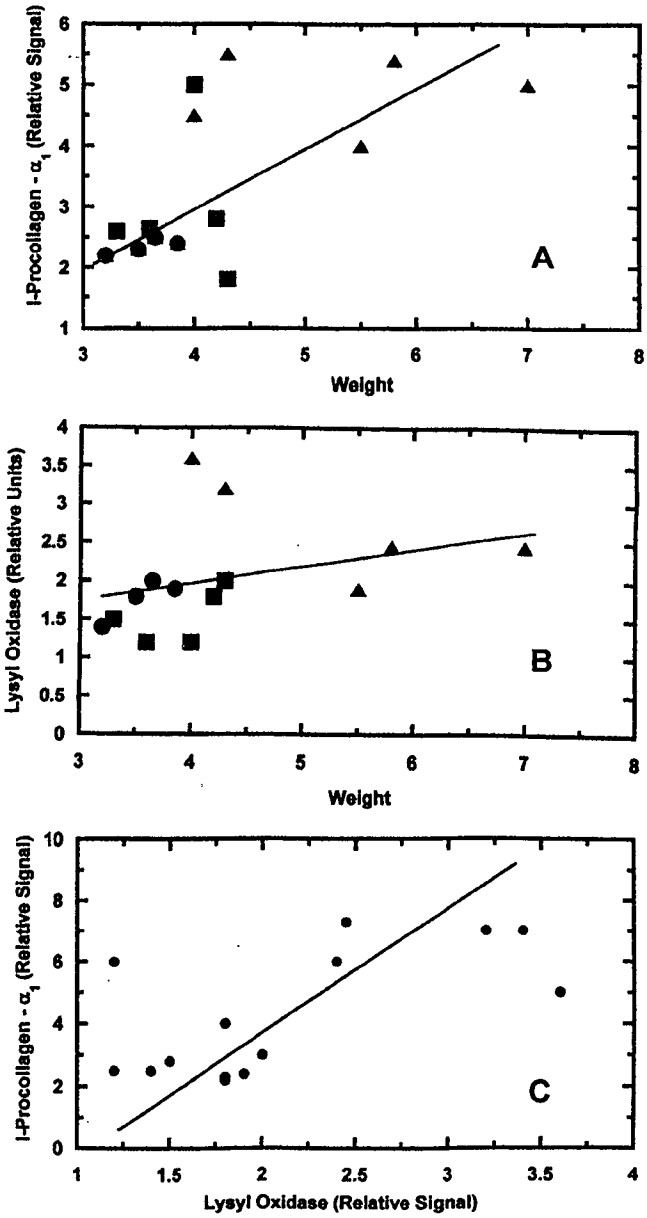


Figure 1. I-Procollagen-α_1 (A) and lysyl oxidase (B) mRNA levels in 1-week-old mouse lung. ●, PQQ mice; ■, PQQ^+ mice, and ▲, $PQQ^{-/+}$ mice (see text). Values were normalized relative to the level of β-actin in a given sample. Levels of mRNA for I-procollagen-α_1 in lung were positively correlated with body weights up to 6–7 g ($r^2 \approx 0.24$, $P < 0.15$). There was significant and positive correlation between lysyl oxidase and I-procollagen-α_1 (C; $r^2 \approx 0.66$, $P < 0.05$).

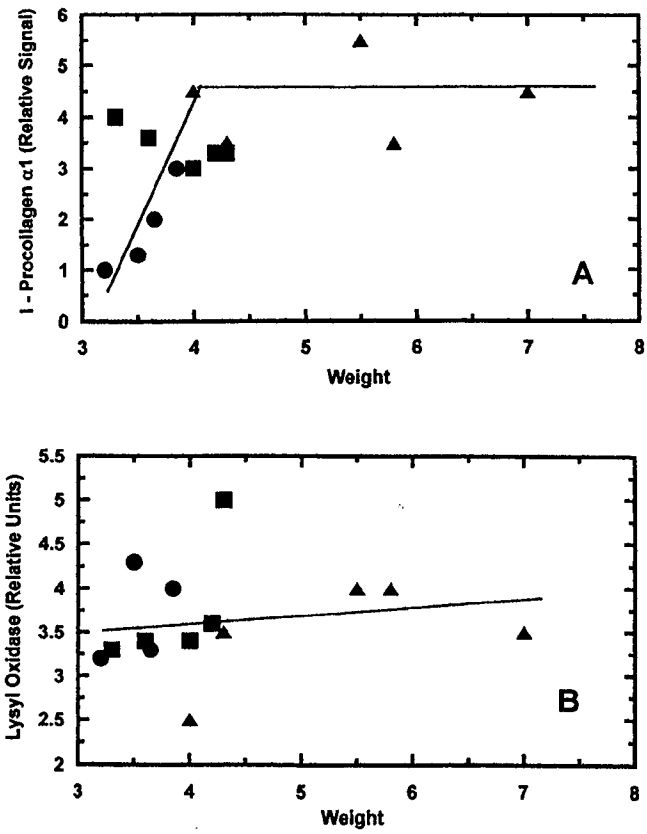


Figure 2. I-Procollagen-α_1 mRNA levels in skin in 1-week-old mice (A). ●, PQQ^- mice, ■, PQQ^+ mice, and ▲, $PQQ^{-/+}$L mice. The mRNA levels for I-procollagen-α_1 were positively correlated with body weight up to 6–7 g. In contrast to lung, no relationship was observed between lysyl oxidase mRNA levels and neonatal growth in mouse skin (B).

Table IV. Lysyl Oxidase[a] and Skin Collagen Solubility[b]

Diet	Lysyl oxidase (relative units/g skin)	Collagen soluble in 1 mol/l NaCl (mg/g skin[b])
PQQ –	30 ± 1*	74 ± 8**
PQQ –/+	41 ± 16	N.D.
PQQ +	58 ± 22	63 ± 5

Note. N.D., not detected.
[a] Lysyl oxidase is expressed as arbitrary units because a partially purified fraction of lysyl oxidase from rat skin was used to calibrate the ELISA assay. Rat skin normally contains 50–150 μg of lysyl oxidase per gram of fresh skin (36). *$P < 0.08$ or **$P < 0.2$.
[b] Milligrams of fibrillar collagen extracted from pulverized skin samples.

Discussion

Steinberg *et al.* (10) reported that mice fed amino acid-based diets containing PQQ in amounts <0.3 n*M* PQQ/g diet have impaired reproduction performance. Herein, PQQ deficiency is associated with specific indices of impaired reproductive performance, e.g., reduced fertility, viability, and conception, compared with PQQ-supplemented mice. Moreover, growth was improved when PQQ was added to the basal diet or by injection i.p. In this regard, the basal diet in the current studies was modified from the diet that was used previously (10) to comply with the most recent National Research Council recommendations (26). In addition, the concentrations of α-tocopherol and ascorbic acid were reduced and antibiotics were not added (see Ref. 10).

We examined features of ECM expression and maturation because of their importance to growth and neonatal development (37, 38). In neonatal mouse lung and skin, I-procollagen-α_1 mRNA levels were positively correlated with growth as influenced by dietary PQQ intake. Such measures of collagen expression are consistent with an ear-

Table V. PQQ Equivalents in Murine Serum and Milk

Diet	Serum[ab] (pmol/ml)	Milk[b] (pmol/ml)
PQQ supplemented	46 ± 6 (n = 4)	54, 67
PQQ deficient	28 ± 12 (n = 3)*	N.D.

[a] n, Number of determinations.
[b] Average ± 1 SEM, $P < 0.1$ for serum. PQQ was measured in two milk samples from PQQ-supplemented mice. PQQ was not detected (N.D.) in milk from PQQ-deprived mice.

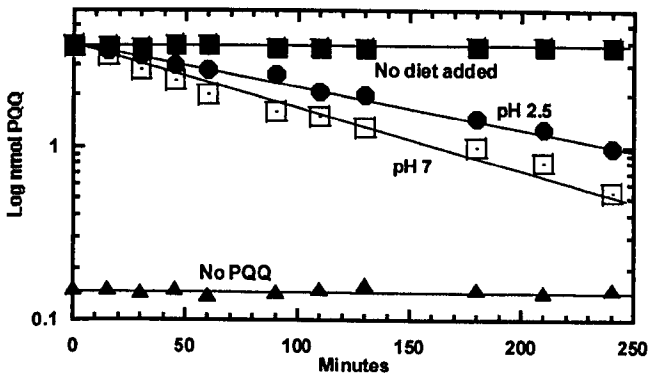


Figure 3. PQQ in diets. Diet samples were suspended in sodium phosphate buffer (0.005 *M*/l), and samples were assayed at the times indicated using the redox cycling assay described by Flückinger *et al.* (28). The rate of PQQ disappearance appeared first-order with a $t_{1/2}$ of 45–60 min. See text for additional details. No diet added (■-■); pH 7.0 (▣ - ▣), pH 2.5 (●-●); no PQQ added (▲-▲ n).

lier report by Spanheimer *et al.* (37), who demonstrated that collagen synthesis could be modulated at the mRNA level in response to under nutrition and changes in growth in rats. We have also examined collagen biosynthesis in mice homozygous for the high-growth locus (38). The collagen concentration, expressed per weight of tissue, was significantly increased in all tissues examined, as was collagen cross-linking, expressed as moles of cross-link per mole of collagen. Types I and III collagen, lysyl oxidase, and lysyl hydroxylase were increased in all tissues analyzed in the high-growth mice compared with wild-type controls. Consequently, we interpret the findings reported herein, i.e., the indices of matrix protein expression and maturation, to be largely growth related and not specific to PQQ.

With regard to tissue and dietary levels of PQQ, a reduction in the levels of PQQ (or PQQ-like substances) was observed in mouse milk and plasma samples from PQQ-deprived mice. As a caveat, we have previously shown that IPQ is the major product when PQQ is incubated with amino acids at neutral pH (e.g., a molar ratio of glycine/PQQ of >5 can result in >98% conversion of PQQ to IPQ, see Ref. 6). Moreover, IPQ is the major form of PQQ in human milk (7–9 times the PQQ concentration, see Ref. 6). Regrettably, straightforward and precise assays for IPQ are not available because unequivocal detection and quantification of IPQ isomers require more rigorous separation and analysis, e.g., mass spectrometry. Accordingly, the values for PQQ given in Table IV should be viewed as relative and do not reflect the contribution of IPQ or related isomers. As may be inferred from the data presented in Figure 3, PQQ in an amino acid based-diet rapidly forms adducts when made liquid. Therefore, we assume that PQQ, as IPQ or other PQQ adducts, are the most likely absorbed products.

How important is PQQ and IPQ to neonatal growth and development and what is its function? The effects of PQQ on reproductive performance are impressive and occur at nanomolar concentrations. Although the effects of PQQ have been ascribed to its potential as an antioxidant, near micromolar amounts of PQQ are required per gram of diet for effective antioxidant functions (21–25), in contrast to the nanomole per gram of diet concentrations used here. Other suggestions include its potential function as a redox catalyst (or co-factor) or, as IPQ, interaction with melatonin- or imidazoline-related receptors (39). PQQ may also be acting as a pre- or probiotic, given its known role as a bacterial chemotactic agent and co-factor for prokaryotic organisms (40). Although the exact mechanism remains to be clarified, PQQ or a derivative can potentially play an important role in reproductive performance and may improve indices of neonatal growth.

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