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# Tissue vitamin C levels of guinea pig offspring are influenced by maternal vitamin C intake during pregnancy

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*The effect of a low to excessive maternal vitamin C intake during pregnancy on the vitamin C concentration of plasma and tissues of guinea pig dams and their pups was evaluated on the day of birth of the litter. Dams were provided low (0.15 g/kg diet) (n = 7 litters), normal (0.50 g/kg) (n = 2 litters), or excess (10 g/kg) (n = 8 litters) dietary vitamin C levels. Maternal weight gain during pregnancy, gestational length, and weight and crown-rump length of pups were similar among vitamin C groups. For plasma, vitamin C values were 5 fold higher for pups and dams of the excess vitamin C group, compared with pups and dams of the low vitamin C group (P < 0.005, P < 0.001, respectively). For liver, vitamin C values were 4 to 5 fold higher for pups and dams of the excess maternal group, compared with pups and dams of the low vitamin C group (P < 0.005, P < 0.005, respectively). For adrenals, values were 2 to 3.5 fold higher for pups and dams of the excess vitamin C group, compared with the low ascorbic acid group (P < 0.01, P < 0.005, respectively). For cerebellum, values were 150% higher for pups of the excess vitamin C group compared with pups of the low vitamin C group (P < 0.01). Vitamin C levels of plasma and tissues, for pups and dams, were similar between the low and normal vitamin C groups and between the normal and excess vitamin C groups. Thus, plasma and tissue vitamin C concentration of pups, but not litter outcomes, are influenced by maternal vitamin C intake.* (J. Nutr. Biochem. 7:524–528, 1996.)

**Keywords:** vitamin C; guinea pigs; pregnancy; fetal development

## Introduction

Vitamin C is widely distributed in the plasma and tissues of human fetuses,[1,2] and those of experimental animals.[3–5] The concentration of vitamin C of human umbilical cord blood has been reported to be higher than that of the maternal venous blood at delivery and the magnitude of difference for vitamin C concentration between fetal and maternal blood may be influenced by the maternal vitamin C intake.[6–11] However, there is considerable variability reported in the relationship of vitamin C concentration for the offspring and mother at delivery.

A study using experimental animals indicates that the fetal plasma and tissue vitamin C levels was less than normal when the maternal vitamin C intake was low during pregnancy. Wegger and Palludan,[5] using a strain of swine that cannot synthesize vitamin C (od/od), compared with those that synthesized vitamin C (OD/OD), evaluated maternal and fetal tissue and plasma vitamin C concentrations during pregnancy. After 3 to 4 weeks of vitamin C depletion, plasma and tissue vitamin C concentrations of the od/od dams and their offspring were generally 3 to 18 fold lower than of OD/OD dams and their offspring. Also, the vitamin C concentration of fetal plasma and tissues were similar to those of the dams. Consequently, vitamin C depletion occurred in both maternal and fetal plasma and tissues when the maternal vitamin C intake was severely restricted, indicating that the vitamin C status of the developing fetus may become very low with a low maternal vitamin C intake.

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The vitamin C status of the fetus may also be influenced by an excessive maternal vitamin C intake during pregnancy. The extent that excessive maternal vitamin C intake during pregnancy influences the vitamin C concentration of plasma and tissues of the offspring is not known. Metabolism of vitamin C by guinea pig offspring after birth was accelerated after a high maternal vitamin C intake during pregnancy,[12] although this issue is equivocal[13] suggesting that there may be negative effects of an excess maternal vitamin C intake on the developing fetus in utero.

The focus of this study, therefore, was to evaluate the impact of maternal vitamin C intake during pregnancy on litter outcome and plasma and tissue vitamin C levels of guinea pig dams and their pups on the day of birth of the litter.

## Methods and materials

### *Animals and diet*

Virgin female guinea pigs, 5 weeks of age and weighing 296 ± 3 g (Harlan Sprague Dawley, Haslett, MI USA) were provided free access to the powdered vitamin C-deficient guinea pig diet (*Table 1*), to which vitamin C was added. Vitamin C was added to the diet at a level that was low (0.15 g/kg diet) ($n$ = 7 litters), normal (0.50 g/kg) ($n$ = 2 litters), or excessive (10 g/kg) ($n$ = 8 litters). The experimental diet with the assigned vitamin C concentration was fed for at least 2 months before mating and throughout pregnancy. The low vitamin C intake has been used in previous studies to achieve tissue vitamin C concentrations that are below saturation, but high enough to prevent severe vitamin C deficiency in non-pregnant[14] and pregnant[a] guinea pigs. The low vitamin C intake was also adequate to achieve normal weight gain during pregnancy.[a] This low vitamin C intake was within the 2 to 5 mg/day recommended vitamin C range for reproduction in the guinea pig[15] because guinea pigs consume 20 to 25 g diet/day during early pregnancy and up to 40 g diet/day during later pregnancy.[a] The excessive vitamin C level was selected to exceed by at least 4 fold the vitamin C intake reported to saturate tissues in non-pregnant guinea pigs (0.8 to 1.5 g/kg diet).[14] Thus, the excess maternal vitamin C intake was well beyond the quantity needed to achieve tissue saturation. This excess vitamin C intake was used because in humans, self-supplementation with very high intakes of vitamin C has been reported.[16]

Animals were provided free access to food and fresh water daily. Animals were housed individually in plastic tubs with cedar chip bedding. The animal room was provided with a 12-hr light/dark cycle (lights on at 0600 hr) and was temperature controlled (22 to 24°C).

### *Breeding*

Animals were bred using established procedures,[17,18] with one female being placed with one male during the receptive time. For breeding, females were checked daily for dissolution of the vaginal membrane, which is an epithelial membrane covering the vagina. Opening of the membrane proceeds estrus and closure does not occur until after ovulation. The estrus cycle of the guinea pig ranges from 15 to 17 days. If pregnancy did not occur with the first mating, the female was placed with a male the next time she was receptive.

### *Sample collection and analysis*

Tissues from the dams and from all pups from each litter were collected within 24 hr after delivery. Dams and pups were weighed and then killed using an overdose of $CO_2$. Crown-rump length of pups was then measured.[19]

Blood was removed from the left ventricle of the heart using a heparinized syringe. Plasma was obtained and mixed with an equal volume of 1 M metaphosphoric acid (MPA) containing 0.54 mmol/L disodium ethylenediaminetetraacetate ($Na_2EDTA$) and frozen at −70°C for later analysis of vitamin C. A portion of the liver and both adrenals of the dams and pups and the cerebellum of the pups were removed, cleaned of adhering tissue, weighed, and placed in separate 10 mL aliquots of 0.5 M MPA containing 0.54 mmol/L $Na_2EDTA$. The tissues were homogenized using a Polytron homogenizer (setting 7, Brinkmann Instruments, Westbury, NY USA), centrifuged, and the protein-free supernatant fraction was stored at −70°C for later analysis of vitamin C using the dinitrophenylhydrazine method.[20]

### *Statistical analysis*

The data are expressed as mean ± SEM, with the litter mean as the unit of measure for the pups. Because all pups from the same litter were exposed to the same environment in utero and the plasma and tissue samples were evaluated within 24 hr of birth, evaluation of results using the litter means rather than the individual pup values was necessary.

The significance of differences among vitamin C groups for maternal weight gain, gestational length, and litter outcomes was evaluated using the Kruskal-Wallis test.[21] The significance of the trend of increasing tissue vitamin C concentration with increasing maternal vitamin C intake was separately evaluated for dams and for pups using The Jonckheere Test for Ordered Alternatives.[22] For both tests, Dunn's post-hoc analysis[23] was conducted to determine intergroup differences. Spearman rank correlation coefficients[21] were determined to evaluate the relationship among maternal weight gain, gestational length, and pup variables at birth.

Non-parametric analyses were used for all analyses because of the small group size to minimize the chance of type I error. Statistical analyses were conducted using BMDP Statistical Software

**Table 1** Diet composition

| Ingredient | Amount (g/kg) |
|---|---|
| Ground rolled oats | 400 |
| Wheat bran | 150 |
| Alfalfa meal | 80 |
| Whole milk powder | 200 |
| High protein casein | 100 |
| Cottonseed oil | 50 |
| Sodium chloride, iodized | 5 |
| Calcium chloride | 10 |
| Magnesium sulfite | 5 |

Vitamin C deficient diet for guinea pigs (Krehl formulation) (#TD69149, Teklad, Madison, WI USA).

[a] Kipp, D.E., McElvain, M.E., Kimmel, D.B., Doyle, K., Stewart, P.E., Pate, S.K., and Lukert, B.P. Low and excessive maternal ascorbic acid intakes during pregnancy result in altered bone mass and tissue collagen synthesis of 6 to 7-week-old guinea pig offspring. (Submitted for publication.)

**Table 2** Maternal weight gain, gestational length, and litter outcomes for guinea pig dams fed low, normal, or excess vitamin C diets throughout pregnancy

| | Low Vit C* ($n$ = 7 litters) | Normal Vit C ($n$ = 2 litters) | Excess Vit C ($n$ = 8 litters) |
|---|---|---|---|
| Maternal wt. gain during pregnancy (g) | 385 ± 123 | 445 ± 82 | 416 ± 81 |
| Gestational length (da) | 70 ± 1 | 66 ± 1 | 70 ± 1 |
| No. of pups/litter | 2.4 ± 0.2 | 3.5 ± 0.5 | 2.1 ± 0.4 |
| Average pup body wt (g) | 94 ± 6 | 89 ± 1 | 110 ± 9 |
| Crown-rump length (cm) | 11.7 ± 0.3 | 11.5 ± 0.3 | 12.2 ± 0.4 |

Data expressed as mean ± SEM. Means for group were averages of litter means. $P > 0.05$ for all parameters.
*Vit C = vitamin C.

(version PC90, Los Angeles, CA, USA). A $P < 0.05$ was considered significant.

## Results

### *Pregnancy outcomes*

Gestational length ranged from 65 to 73 days, with no difference among vitamin C groups (*Table 2*). Maternal weight gain during pregnancy, litter size, pup body weight, and crown-rump length of pups were also similar among vitamin C groups (*Table 2*). Gestational length, pup body weight, and crown-rump length were inversely related to litter size (*Table 3*).

### *Plasma and tissue vitamin C concentrations of dams and pups*

For dams and for pups, plasma and all tissue vitamin C concentrations significantly increased with increasing maternal vitamin C intake. For dams, plasma vitamin C values were 5 fold higher for the excess vitamin C group, compared with dams of the low vitamin C group ($P < 0.001$) (*Table 4*). For liver, vitamin C values for dams were 4.8 fold higher for the excess vitamin C group, compared with dams of the low vitamin C group ($P < 0.005$). For adrenals, values for dams were 3.5 fold higher for the excess vitamin C group, compared with the low vitamin C group ($P < 0.005$). For plasma, liver, and adrenals of dams, differences between the low and normal vitamin C groups and between the normal and excess vitamin C groups were not significant ($P > 0.05$) (*Table 4*).

For pups, plasma vitamin C values were 5 fold higher for the excess vitamin C group, compared with pups of the low vitamin C group ($P < 0.005$) (*Table 4*). For liver, vitamin C values for pups were 3.6 fold higher for the excess vitamin C group, compared with pups of the low vitamin C group ($P < 0.005$). For adrenals, values for pups were 2 fold higher for the excess vitamin C group, compared to the low vitamin C group ($P < 0.01$). For cerebellum, pup values were 150% higher for the excess vitamin C group compared with the low vitamin C group ($P < 0.01$). For pup plasma and tissues, differences between the low and normal vitamin C groups and between the normal and excess vitamin C groups were not significant ($P > 0.05$) (*Table 4*).

## Discussion

Results of this study indicate that maternal vitamin C intake influences vitamin C concentrations of the plasma and tissues of the dams and their pups on the day of birth, particularly at the low and excess maternal vitamin C intakes. Tissue vitamin C levels for dams and pups for all three vitamin C groups are consistent with prior reports in nonpregnant guinea pigs[14,24,25] and in young guinea pig offspring.[26] The large variability in results and small sample size for the normal vitamin C group preclude significant differences in results between the low and normal vitamin C groups or between the normal and excess vitamin C groups. However, major differences in plasma and tissue vitamin C levels, for dams and for pups between the low and excess vitamin C groups, were evident.

With a low maternal vitamin C intake, the pups did not concentrate vitamin C in plasma or tissues, because their values were similar to or lower than for dams. Consequently, the pup does not sequester vitamin C at the expense of the mother when the maternal vitamin C intake is low,

**Table 3** Correlation coefficients among litter outcome parameters for all animals ($n$ = 17 dams and their litters)

| | Maternal wt. gain | Gestational length | No. pups per litter | Av. pup body wt. | Crown-rump length |
|---|---|---|---|---|---|
| | | | $r$ | | |
| Maternal wt. gain | 1.00 | | | | |
| Gestational length | 0.16 | 1.00 | | | |
| No. pups per litter | 0.11 | −0.58[a] | 1.00 | | |
| Av. pup body wt. | 0.46 | 0.66[b] | −0.63[b] | 1.00 | |
| Crown-rump length | 0.41 | 0.74[b] | −0.57[a] | 0.89[b] | 1.00 |

[a]$P < 0.05$.
[b]$P < 0.01$.

**Table 4** Vitamin C concentration of plasma and tissues of guinea pig dams and pups on the day of birth of the litter

| | Low Vit C* (*n* = 7) | Normal Vit C (*n* = 2) | Excess Vit C (*n* = 8) |
|---|---|---|---|
| Dams | | | |
| Plasma (μmol/L) | 22.1 ± 3.8[a] | 59.1 ± 1.8[a,d] | 134 ± 2[d] |
| Liver (μmol/g wet wt) | 0.331 ± 0.075[a] | 0.910 ± 0.122[a,e] | 1.92 ± 0.37[e] |
| Adrenals (μmol/g wet wt) | 1.75 ± 0.39[a] | 5.16 ± 0.39[a,e] | 8.03 ± 0.76[e] |
| Pups | | | |
| Plasma (μmol/L) | 21.2 ± 4.0[a] | 97.7 ± 23.3[a,d] | 127 ± 15[d] |
| Liver (μmol/g wet wt) | 0.242 ± 0.089[a] | 0.716 ± 0.262[a,d] | 1.11 ± 0.19[d] |
| Adrenals (μmol/g wet wt) | 3.42 ± 1.01[a] | 8.67 ± 0.30[a,c] | 9.84 ± 1.12[c] |
| Cerebellum (μmol/g wet wt) | 1.68 ± 0.29[a] | 4.34 ± 0.04[b] | 3.98 ± 0.33[b,c] |

Data represent means ± SEM. Sample size is the number of dams or number of litters evaluated. Means in a row with different superscripts are significantly different (*a* vs. *b*, $0.05 < P < 0.10$; *a* vs. *c*, $P < 0.01$; *a* vs. *d*, $P < 0.005$; *a* vs. *e*, $P < 0.001$).
*Vit C = vitamin C.

which is consistent with results for vitamin C-requiring (od/od) swine.[5] Due to the integral role of vitamin C in collagen synthesis[27] and in bone remodeling,[28,29] the low maternal vitamin C intake during pregnancy may compromise in utero fetal development. Lower than normal total body bone density and collagen synthesis of tibial epiphysis and gastrocnemius and soleus tendons were observed in 6 to 7-week-old male guinea pig offspring after the dam was provided the low vitamin C intake through pregnancy and this same low vitamin C intake was continued by the offspring after weaning.[a]

With an excess maternal vitamin C intake, plasma and tissue vitamin C levels of pups and dams were saturated. Although plasma and tissue vitamin C concentrations of the pups were similar to those of the mother, there may still be a potential risk to the developing fetus due to exposure to a high vitamin C concentration in utero. Results of our recent study[a] suggest that collagen synthesis of the tibial epiphysis of guinea pig pups at 6 to 7 weeks of age was lower than normal when the pups were exposed in utero and after birth to the excess vitamin C intake.

In humans, the vitamin C concentration of fetal umbilical cord blood have been reported to be 100 to 300% higher than the maternal plasma vitamin C levels[6–10] although an even higher difference has been reported when the maternal vitamin C intake was low.[8,10,11] Braestrup[30] reported that the vitamin C concentration of umbilical cord blood at delivery was 4 fold higher than of the mothers. The vitamin C concentration of blood taken from the heel of the infant within 30 min of the umbilical cord blood collection, however, was 36% lower than the cord blood. The plasma vitamin C concentration of the infant was nearly 50% lower at 24 hr after delivery. Because the blood sample collected in the present study was taken within 1 day of delivery, plasma vitamin C levels of the pups may have been lower than if the vitamin C concentration was determined at birth.

The ability of tissues to concentrate vitamin C may be different during in utero development and immediately after birth than in the older guinea pig. The kinetic characteristics of vitamin C in different guinea pig tissues varies,[31] as does the total quantity of vitamin C that is taken up by individual tissues at saturation levels.[14] In the present study, vitamin C concentrations of the plasma and tissues (liver and adrenals) of the dams were 3.5 to 5 fold higher with the excessive maternal vitamin C intake compared to the low maternal vitamin C intake. For pups, the vitamin C concentration of the plasma and liver was 3.6 to 5 fold higher with the excessive maternal vitamin C intake compared with those of the low maternal vitamin C group. In contrast, the vitamin C concentration of adrenals and cerebellum was only 1.5 to 2 fold higher for pups for the excessive vitamin C intake than for pups from the low maternal vitamin C group. Consequently, the pup cerebellum, in particular, and the adrenals to a lesser extent, appear to retain their vitamin C concentration at the low maternal vitamin C intakes. This greater retention of vitamin C in the adrenals and cerebellum, compared to other tissues when vitamin C intake was low, was also reported by Wegger and Palludan[5] in fetal swine. Adlard and coworkers reported an at least 2 fold higher vitamin C level in fetal human forebrain than in adults[1] and 100% higher in rat pups within 1 week of delivery compared with their mothers.[32] Although the role of vitamin C in brain development is not known, the more avid retention of vitamin C by the brain than by other tissues in periods of vitamin C deprivation suggests an important role.

Litter size and fetal development, measured by pup body weight and crown-rump length at birth, were similar among vitamin C groups, which is consistent with prior reports,[a,4,26,33] Crown-rump length is a measure of pup size that has been used as an indicator of fetal development in guinea pigs.[20] The significant inverse relationship between litter size and gestational length, pup body weight, and crown-rump length for guinea pigs extends prior reports[18–20] to include our observation that the maternal vitamin C intake does not alter this relationship.

In summary, a low to excessive maternal vitamin C intake did not influence maternal weight gain during pregnancy, gestational length, or litter outcomes. The excessive maternal vitamin C intake during pregnancy resulted in vitamin C concentrations of plasma and tissues of the pups and dams that were significantly (1.5 to 5 fold) higher than of pups and dams from the low maternal vitamin C groups. Thus, the vitamin C levels of plasma and tissues of the guinea pig pup on the day of birth are influenced by the maternal vitamin C intake during pregnancy.

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