Plasma Ascorbate in a Population of Children: Influence of Age, Gender, Vitamin C intake, BMI and Smoke Exposure

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The objective of this study is to determine the influence of several personal and lifestyle factors on the levels of circulating vitamin C in a population of children. To accomplish this objective, blood samples were collected from 511 healthy children residing in the Greater San Juan area. The population was stratified into 4 percentile groups (approaching quartiles) according to plasma ascorbate levels from lowest to highest concentrations. Comparisons were made between percentile groups on the basis of age, gender, body mass index (BMI), dietary intake of vitamin C (corrected and uncorrected for energy intake) and exposure to environmental tobacco smoke (ETS). Smoke exposure was determined using urinary cotinine, which is a highly sensitive bioindicator for ETS. Dietary vitamin C was determined via one 24hr recall questionnaire. When all 4 percentile groups were used as a basis of comparison, no differences were noted for any of the factors between groups, however when comparing percentile group 1 (lowest blood ascorbate) to an aggregate value of percentile, groups 2-4, it was found that vitamin C intake (corrected for energy intake) paralleled blood values with a statistically significant association. Among personal and environmental factors only exposure to ETS showed a significant difference in blood levels between groups 2-4 and group 1. No differences between percentile groups were noted for age gender or BMI. These results emphasize that ETS is strongly associated with lowered blood ascorbate levels with the obvious implication of reduced antioxidant protection and increased risk of adverse health consequences.

Key words: Vitamin C, Ascorbate, Environmental tobacco exposure, ETS, Cigarette smoking, Children, 24 hr dietary questionnaires

Current research shows vitamin C to be the most effective aqueous-phase antioxidant in human plasma and a physiological antioxidant of major importance for protection against diseases and degeneration processes caused by oxidative stress (1). Vitamin C in blood increases linearly with increasing ascorbate intake but plateaus at levels between 1.2 and 1.8 mg/dl (2). Blood levels can be further influenced by several personal and lifestyle factors. It is the objective of this study to determine the contribution of age, gender, body mass index (BMI) and environmental smoke exposure (ETS) on the level of circulating vitamin C in a population of children. Each of the factors has previously been shown to modify blood vitamin C but documentation has been primarily restricted to adult (ages 15-65) and elderly (ages 61-90) populations (3-7).

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The project was supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service; grant no 2003-35200-13590, the RCMI Clinical Research.
Infrastructure Initiated Award RCR11, award no. 1P20RR11126 and the RCMI Program 612RR03051

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Methods

Subjects. Our study group included 511 healthy children aged 2-13 y that routinely visited the Pediatric Care Clinic of the Cataño Health Center, a satellite program of the University of Puerto Rico Pediatrics Department. Cataño is an industrial city of >42 000 inhabitants that is located across the harbor from San Juan. Blue-collar workers make up the bulk of the population. The city is highly homogeneous in terms of socioeconomic factors and children visiting the clinic are representative of children residing in the community as a whole. However, the method of subject selection was basically by convenience, constituting a no probability sample, and therefore no

inferences should be drawn regarding the generalizability of the findings to a broader population. The study protocol was approved by the Institutional Review Board of the University of Puerto Rico, Medical Sciences Campus.

Data Collection. Dietary data were obtained by using a 24-h-recall method (8). Mothers supplied this information for the youngest children, and children aged ≥ 8 y also contributed some information. Detailed descriptions of all foods and beverages consumed, including cooking methods and brand names, were recorded by the interviewers. Use of vitamin and mineral supplements was also noted. Because the study focused on food sources of vitamin C, those persons taking vitamin C supplements were excluded from the analysis.

Clinical and laboratory procedures: blood and urine samples. Eligible children provided fasting blood and urine samples, which were collected from 08 to 10am at the clinic immediately before administration of the 24-h dietary recall. Urine samples were collected to measure cotinine, which is a sensitive biomarker of smoke exposure (9). Samples were refrigerated and cotinine concentrations were determined within 48 h by using an enzyme-linked immunosorbent assay (Solar Care Technologies, Bethlehem, PA). In a previous study, this assay was verified against gas-liquid chromatography and was found accurate for measuring cotinine concentrations ≥ 3 ng/mL (10). To adjust for urine dilution, urinary cotinine concentrations were standardized to creatinine concentrations and were expressed as ratios of cotinine to creatinine. Creatinine was measured colorimetrically by using picric acid in an alkaline environment (11).

Blood samples were obtained by venipuncture and were drawn into EDTA-coated tubes. Plasma was separated by centrifugation at 2000 x g for 20 min at 4°C. Supernatant fluids were analyzed for ascorbic acid content by derivatization with 2,4-dinitrophenyl hydrazine (12) within 6 h of collection. Values obtained with this method correlate well with values obtained by high-pressure liquid chromatography (13). The results are expressed as mg/dl (to convert to µM/L multiple mg/dL by 57).

Determination of body mass index. The heights and weights of the children were obtained according to published assessment methods (8). The BMI, also called Quetelet's index, was calculated as weight in kg divided by the square of the height in m (kg/m^2); BMI was used as a measure of obesity. Weight is a determining factor in plasma ascorbate concentrations because weight is inversely related to vitamin C concentration independent of the amount of smoke exposure (14).

Determinations of dietary ascorbate. Vitamin C intakes were estimated from the 24-h dietary recall interviews. To determine the vitamin C contents of the foods consumed, we used the Minnesota Nutrition Data System 32, which contains > 6000 brand-name foods, fast foods, and > 16000 other foods. In addition, it is a comprehensive nutrient database including data derived from the US Department of Agriculture tables, food manufacturers, the scientific literature, and foreign food consumption tables; hence, it contains many ethnic foods that are commonly eaten in Puerto Rico.

Statistical analysis. Subjects were divided into percentile groups based on blood ascorbate levels. Each percentile group approached 1/4 of the total population however, overlap of blood values made it impossible to include equal numbers in each group. Percentile group, cut off values and percent of population in each group were set at: Percentile group 1: 0.32-0.74 mg/dl ascorbate (23.3%); Percentile group 2: 0.75 – 0.89 mg/dl ascorbate (26.2%; Percentile group 3: 0.90 – 1.02 mg/dl ascorbate (25.6%) and Percentile group 4: 1.03 – 1.46 mg/dl ascorbate (24.9%).

Frequency distributions were obtained for categorical variables (age, gender and BMI). The Pearson's Chi-square test was used to describe the statistical association between categorical variables. Descriptive statistics including mean, standard deviation, median and range (min-max) were computed for continuous variables (blood ascorbate, total vitamin C intake, total caloric intake, vitamin C intake/Kcal ratio and cotinine/creatinine ratio). The Shapiro Wilk test was used to verify the normal assumption of these variables (15). Because these continuous variables were non-normal, the Kruskal Wallis test was used to compare the medians between vitamin C percentile groups (15).

The level of significance was $p < 0.05$. All statistical tests were one-sided. Data entry was performed using MS Access. The Sigma Plot software was used to prepare the graphs (16). The STATA package was used to perform the statistical analysis (17).

Results

Blood values for ascorbic acid were normally distributed (Figure 1). Concentrations were classified into clinically based categories (18). The number and percent of total subjects in each category were as follows: < 0.2 mg/dl (scorbutic range) = zero subjects (0%), 0.2-0.39 mg/dl (marginal range)) = 5 subjects (1%), 0.4-0.99 mg/dl (normal range) = 348 subjects (68%) and 1.0-3.0 mg/dl (saturated range) = 158 subjects (31%).

Frequency distributions and percent of total subjects for categorical variables are shown in Table 1. There were no differences for age, gender or BMI between percentile groups (p values are 0.148, 0.096 and 0.090 respectively).

It should be noted BMI has been treated as both a categorical variable and a continuous variable. In Table 1 the values of 16.7 is the midpoint for the BMI of our population. This value would not necessarily be the same for other populations because age, gender, ethnicity and maturation stage all of which can independently contribute to percent body fat (19).

Table 1. Percent of Subjects in each Percentile Group according to categorical variables (total n=511)

	Percentile Group				
Variable	1	2	3	4	p value
Age (yrs)					
2-4	26.9	25.4	25.2	24.4	0.148
5-8	29.4	32.8	43.5	44.1	
9-13	43.7	41.8	31.3	31.5	
Gender					
Boys	38.7	52.2	56.5	42.7	0.0958
Girls	61.3	47.8	43.5	57.3	
BMI					
≤ 16.7	51.3	41.0	50.4	56.7	0.0910
> 16.7	48.7	59.0	49.6	43.3	

Values for continuous variables are shown in Table 2. Comparison between the 4 percentile groups showed no statistically significant differences for any of the variables. A priori, one would expect a significantly positive relationship between vitamin C intake and ascorbate blood

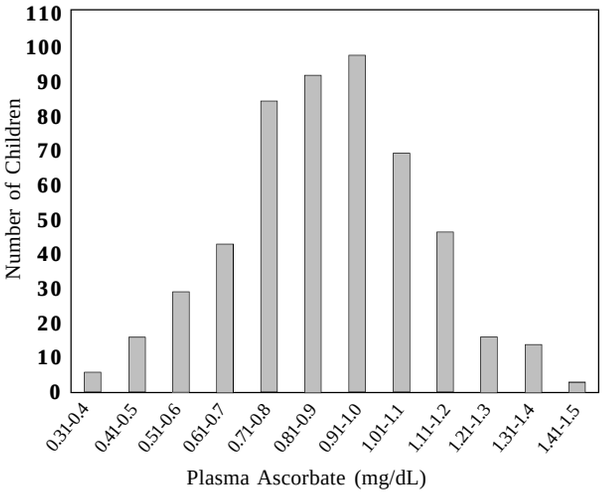

Figure 1. Plasma ascorbate concentrations in a population of children ages 2 – 13 years

levels. Indeed, a relationship has been well documented in many diverse populations (4,20,21). These graphs however were based on studies (almost always in adults) in which populations ingest from almost no vitamin C to megadose quantities. It is known that plasma levels increase linearly up to an intake in excess of 100 mg/day. Because of the non-linearity of intestinal absorption and renal excretion of the vitamin, plasma concentrations (and total body pool size) tend to plateau (18).

Based on these studies as well as others (22,23), current National Research Council (NRC) dietary reference intakes for adults have been set at: Estimated Average Requirement (EAR) of 75 mg/day for men and 60 mg/day for women

Table 2. Values for Continous Variables in Each Percentile Group

	Percentile Group				
Variable	**1**	**2**	**3**	**4**	**p value**
Vitamin Intake (mg/day)					
Mean ± SO	135.1 + 8.60	150.9 ± 100.0	159.8 ± 113.3	150.9 ± 88.2	0.3927
Median	122.1	135.9	133.3	127.4	
Range	6.2 – 440.9	9.2 - 573.2	4.4 - 573.3	4.3 - 488.2	
Energy Intake (Kcal/day)					
Mean ± SD	1888.4 ± 469.6	1853.0 ± 494.0	1841.9 ± 500.5	1815.3 ± 479.4	
Median	1919.0	1789.6	1848.7	1750.1	0.5284
Range	540.0 - 2924.1	804.7 – 3214.9	804.7 – 3013.3	772.8 - 2905.9	
Vitamin Intake/Energy Intake (mg/Kcal/day)					
Mean ± SD	69.9 ± 37.7	81.3 ± 46.3	86.0 ± 56.6	83.6 ± 42.8	
Median	68.1	76.1	76.7	77.7	0.0570
Range	6.2 – 198.2	7.9 – 237.4	2.7 – 363.9	4.4-219.3	
Cotinine/Creatinine (ng/mg)					
Mean ± SD	21.1 ± 36.0	14.4 ± 24.0	14.9 ± 26.2	11.3 ± 13.3	
Median	9.5	7.1	7.4	7.8	0.0904
Range	0 – 266.1	0 – 202.0	0 – 231.3	0 – 73.4	

(20). Likewise Recommended Dietary Allowances (RDA's) have been set at 90 mg/day for men and 75 mg/day for women (20). Our population, on the other hand, is composed exclusively of children ages 2-13. Consequently, EAR and RDA values are proportionally lower with EAR's set at 13 mg/day for children (1-3 y), 22 mg/day for children (4-8 y) and 39m/day for boys and girls (9-13 y) (20). Similarly RDA's are set at 15mg/day for children (1-3 y), 25mg/day for children (4-8 y) and 45 mg/day for boys and girls (9-13 y) (20). Our dietary questionnaire data (not shown) indicated only 1.3% of subjects consumed less than 13 mg/day, 3.1% of subjects consumed between 13 and 25 mg/day and 3.9% of subjects consumed between 25 and 45 mg/day. Therefore more than 91% of our population met NRC dietary requirements. It is therefore not surprising that a graph of vitamin C intake and plasma ascorbate levels does not resemble a sigmoidal curve but is a straight line with slightly positive slope (Figure 2). This data

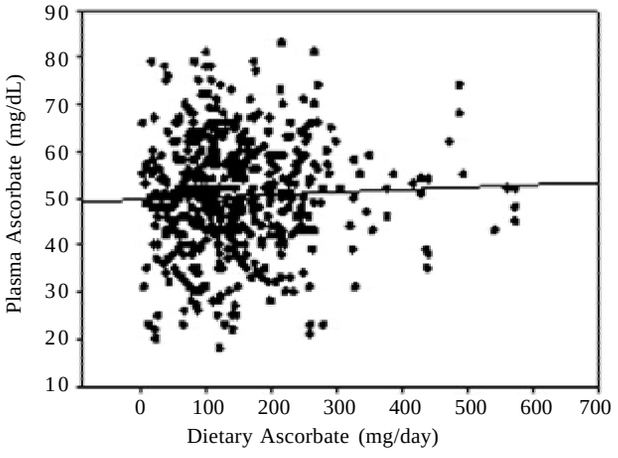

Figure 2. Relation between dietary ascorbate (vitamin C) intake and plasma ascorbate in children ages 2 – 13 years

suggests that ingestion levels have essentially reached the plateau region thereby masking the expected positive relationship between dietary intake and corresponding plasma levels. This observation has been noted by us in a previous study (24) as well as by others with vitamin C supplemented men (25) and in a vitamin C adequately fed adult population (26).

Upon closer inspection of data in Table 2, it is noted that while mean and median values of variables in percentile groups 2-4 seem similar, there appears to be distinctly different values when compared to values in percentile group 1. It is difficult to distinguish between individuals who have a moderately high intake as compared to those who have a still higher intake (percentiles 2-4). Consequently, we feel justified in using aggregate data for comparative purposes. Therefore Table 3 was prepared which compares variables in percentile group 1 to an aggregate from percentile groups 2-4. Results from Table 3 show that both vitamin C intakes corrected for energy intake and cotinine/creatinine ratio to be significantly different between the first percentile group and the aggregate group with p values of 0.0082 and 0.0143 respectively.

Table 3. Comparison of Values between First Percentile Group and Aggregate of Percentile Groups 2 – 4

	Percentile Group		
Variable	**1**	**2 - 4**	**p value**
Age (yrs)			
Mean ± SD	7.3 ± 3.4	6.9 ± 2.9	
Median	7	7	0.1775
Range	2 – 13	2 – 13	
BMI			
Mean ± SD	18.1 ± 5.2	17.9 ± 4.2	
Median	16. 7	16.8	0.8399
Range	11.1 – 57.0	10.9 – 38. 2	
Vitamin C Intake (mg/day)			
Mean ± SD	135.1 ± 86.0	153.9 ± 101.2	
Median	122.1	131.8	0.0897
Range	6.2 – 440.0	4.3 – 573.3	
Energy Intake (Kcal/day)			
Mean ± SO	1888.4 ± 469.6	1837.1 ± 490.5	
Median	1919.0	1803.1	0.1835
Range	540.0 – 2924.1	722.8 – 3214.9	
Vitamin Intake/Energy Intake (mg/Kcal/day)			
Mean ± SD	69.9 ± 37.7	83.6 ± 48.9	
Median	68.1	76.7	0.0082
Range	6.2 – 198.2	2.7 – 363.9	
Cotinine/Creatinine(ng/mg)			
Meam ± SD	21.1 ± 36.0	13.5 ± 22.0	
Median	9.5	7.3	0.0143
Range	0 - 266.1	0 – 231.3	

Discussion

Any discussion of diet and blood values needs to address the experimental design by which the data was collected. In this case, results were obtained from one blood sample and one 24 hr dietary recall questionnaire per subject. Both methods present limitations as to the generalization of the information collected, however, this being said, most large-scale nutritional surveys are conducted in this exact or similar manner (27,28) so data can be representative of populations as a whole.

Our population of children ages 2-13 y consumed well in excess of NRC required amounts of vitamin C. Blood

levels were overwhelming in the normal and saturated categories. For comparative purposes, it is unfortunate that broad scale surveys with children in this age bracket are limited. Data is more plentiful in the newborn range (20) and in adult populations (7,20) which suggest that suboptimal vitamin C consumption may be a larger problem than is generally acknowledged.

The principal findings of this study are that when blood levels of ascorbate are examined from lowest to highest percentile that higher levels are associated with increased vitamin C intake corrected for energy intake and are reduced when the urinary cotinine/creatinine ratio is high. Age, gender, BMI and total energy intake are not important contributors and vitamin C intake unadjusted for calorie intake as only of borderline importance. We do not rule out other factors that may influence blood levels such as genetics, physical activity, stress, existing vitamin stores, etc. but consideration of all of these possible modulators of blood ascorbate are well beyond the scope of this investigation.

Results reported here are not novel nor unexpected. The positive association between dietary vitamin C and blood ascorbate is fundamental knowledge and will not be further mentioned. Likewise, the effect of (ETS) on lowering blood ascorbate has also been documented in both children (24,29) and in adults (30). The unique aspect of this study is in its design. In all previous investigations (mentioned above), subjects were classified as either exposed or not exposed to ETS via a questionnaire. They were then characterized as to the level of smoke exposure, dietary, intake, blood ascorbate, etc. In the study reported here, subjects are classified solely by percentile of blood ascorbate, which is independent of any questionnaire information. Furthermore, smoke exposure is determined by urinary cotinine, a bio-indicator uninfluenced by information provided by the subjects. Finding statistical agreement between chemically based categories of blood ascorbate and ingested vitamin C across a full range of intakes can be problematic due to saturation effects. (2) Nevertheless among the continuous and categorical variables examined, exposure to ETS is salient in relation to a lower level of circulating ascorbate.

These findings add weight to the argument that ETS is a major factor in determining the probability of finding a low ascorbate blood concentration in children. Health consequences of such phenomena have been amply documented (31,32,33,34) and are of continued concern.

Acknowledgements

We acknowledge the support to the following individuals and institutions: Cynthia Pérez, Tomas Matos and Orville Disdier for statistical consultation, Luis Ramos and Cindy Calderon for concepts and ideas, the Department of Pediatrics, University of Puerto Rico for providing access to patients at their satellite facility, the Cataño Health Center personnel for their help in sampling, and Magaly Ramos for manuscript preparation.

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