

A Co-Twin Control Study of the Effects of Vitamin C

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INTRODUCTION

In recent years, a number of benefits of prophylactic pharmacologic doses of vitamin C have been suggested. In addition to claims by Linus Pauling [1971] and others of a reduction in the morbidity due to the common cold and upper respiratory infections, other authors have subsequently suggested that serum cholesterol levels may be lowered [Spittle, 1971]. Some investigations have suggested that this effect may be observed only in hypercholesterolemic individuals and not those with normal cholesterol levels [Sokoloff et al, 1966; Ginter et al, 1970]. It has also been suggested that exogenous vitamin C might improve mental alertness as measured by IQ test performance [Kubala and Katz, 1960]. The present study was designed to test these hypotheses by means of a double-blind co-twin control study.

MATERIALS AND METHODS

Eighteen male and 26 female monozygotic twin pairs, aged 6 to 15 years, were enrolled in the five-month double-blind co-twin control study. Zygosity, determined by dermatoglyphic analysis and extensive genotyping, yielded a median probability of monozygosity of 0.9964 with a range of 0.9488 to 0.9999. The sample was divided into three dosage groups on the basis of body weight and the numbers in each group are shown in Table I. The result of this effort to equalize dosage on the basis of body weight was a division which was also approximately correlated with age. The dosage levels employed were 1,000, 750, and 500 mg/day administered twice daily in a divided dose. Assignment of twins to treated or control groups was done at random and the control twins received a capsule which was identical in appearance to that of the treated twin.

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TABLE I. Distribution of Twin Pairs by Sex and Dosage

Dose (mg/day)	Number of pairs		
	Male	Female	Total
1,000	5 ^a	7	12
750	6 ^a	6	12
500	7	13	20
Total	18	26	44

^aOne pair from each of these groups was omitted from the analysis of cold symptoms. Reprinted from Miller et al [1977]: Therapeutic effect of vitamin C: A co-twin study. *JAMA* 237:248–251, with permission.

Daily observations of the presence of 14 symptoms known to be associated with the common cold were recorded by the twins' mothers over the five-month period. Compliance was monitored by the monthly collection of morning urine samples for the measurement of vitamin C excretion. Overall, excretion of vitamin C by the control twin was invariably less than that of the treated twin.

Multiple blood chemistries, including SMA-12 (cholesterol, protein, albumin, uric acid, alkaline phosphatase, sodium, potassium, calcium, glucose, BUN, and SG01) and complete blood counts (CBC), and 24-hour urine samples were obtained prior to both the initiation and conclusion of the study to look for possible treatment effects. Anthropometric measurements were also made at this time, including height, weight, blood pressure, and clinical observations of tonsil and cervical node size. At the conclusion of the study several psychological tests were administered to measure accuracy and speed, as well as tests for motor control, reaction time and time sense.

Ascorbic acid and its major metabolites, dehydroascorbic acid and diketogulonic acid, were measured by the method of Aeschbacher and Brown [1972] adapted for a Technicon Autoanalyzer. Serum cholesterol was determined by the method of Abell et al [1952]. Free and esterified cholesterol were separated on Silica gel G thin layer chromatography plates before analysis by this method. Twenty-four-hour urine samples were prepared for ammo acid analysis following a modification of the procedure outlined by Benson and Patterson [1965]. Separation and elution of ammo acids were accomplished by a modification of the method of Perry et al [1969].

All 44 pairs completed the study, but two male pairs were omitted from the analysis of cold symptoms due to incomplete data.

RESULTS AND DISCUSSION

For the purposes of analysis of illness data, a cold was defined as an illness lasting at least two days with at least two different symptoms, and separated by at least two days from a previous incident. A single day was counted as an incident if there was more than one symptom of moderate or worse severity. The detailed results of this analysis have been published elsewhere [Miller et al, 1977]. Analysis by means of the paired comparison t test showed no significant overall treatment effects. However, there were significant dose \times sex interactions for average severity ($p < 0.01$) and total duration ($p < 0.05$). Consequently the data dosage groups of females showed significant treatment effects at the 5% level on five of the six variables of duration and severity, while the lowest dosage group, males and females together, had significant treatment effects on total severity. It is important to note that all investigators remained blinded as to which twin received the vitamin until after this part of the analysis was completed.

Similar analysis of anthropometric measurements showed no overall treatment effect on actual height or weight, nor on tonsil and cervical node size. However, analysis of the change in height, or growth, during the five-month period of the study showed an entirely unexpected dose \times sex Interaction ($p < 0.05$). Inspection of the subgroups showed a greater increase in growth during the study in males receiving 500 mg of vitamin C daily (1.61 ± 0.64 inches) compared to their co-twins (1.09 ± 0.65 inches). In six of the seven twin pairs in this group the treated twin grew more than his co-twin, and in the seventh case the growth was the same.

Overall analysis of the biochemical data available from CBC and SMA-12 likewise showed no consistently significant treatment effect on white blood cell count, red blood cell indices, uric acid, cholesterol, electrolytes, liver function tests, total protein, or albumin. It was interesting that several variables from the SMA-12 and blood count determinations, including cholesterol, calcium, uric acid, and hemoglobin, did show significant changes in comparison of mean values before and after the study in both treated and control twin groups. The data from the total cholesterol determinations by the method of Abell et al [1952] are presented in Table II. As can be seen, both control and treated twins within a group changed in the same direction on the average, showing a slight decrease in cholesterol values overall in both treated and control twins. Free and esterified cholesterol fractions showed an increase on the average for both treated and control groups. Whether the differences in these values are attributable to seasonal variation, growth, or systematic laboratory error is unknown. Had a "self control been used in the analysis of these variables, one would have erroneously attributed this effect to the treatment. However, analysis of the intrapair difference of the change showed no significant differences between mean changes for the treated and for the untreated twin.

TABLE II. Mean Total Serum Cholesterol Values*

Dose	Males			Females		
	Before	After	Difference \pm SE ^a	Before	After	Difference \pm SE
1,000 mg/day						
Vitamin C	138.35	134.90	3.45 \pm 13.55 (2) ^b	131.40	141.45	-10.05 \pm 5.82 (6)
Placebo	121.95	136.65	-14.70 \pm 9.74 (3)	133.68	144.50	-10.82 \pm 5.98 (6)
750 mg/day						
Vitamin C	163.42	152.07	11.35 \pm 9.28 (6)	150.35	168.22	-17.87 \pm 7.89 (4)
Placebo	159.17	153.62	5.55 \pm 10.04 (6)	160.08	164.75	- 4.67 \pm 4.39 (4)
500 mg/day						
Vitamin C	149.27	161.33	-12.06 \pm 8.12 (7)	156.08	149.16	6.92 \pm 7.28 (13)
Placebo	151.30	159.21	- 7.91 \pm 5.55 (7)	148.78	158.62	- 9.84 \pm 7.20 (13)

*mg/100 ml.

^aStandard error of difference.^bNumbers in parentheses are numbers in each group.

Analysis of variables designed to test mental alertness also showed no treatment effect on accuracy, speed, reaction time, time sense, or fine muscle control. A sex effect ($p < 0.01$) was detected in the most sensitive tremor measure, which showed that treated males had less tremor than their co-twin, while the opposite was generally true in females.

Overall analysis of the 24-hour urinary excretion of amino acids ($\mu\text{moles}/24$ hours) yielded only two amino acids, lysine and glutamine, which showed significantly decreased excretion in the treated twins compared to the controls ($p < 0.05$). However, in the subgroup receiving 1,000 mg/day treated twins showed decreased excretion in the 13 amino acids shown in Table III. The other 18 ninhydrin-positive compounds which we were able to measure consistently were not significantly different in this group, although in all but three the mean excretion of the treated twin was less than his control.

SUMMARY

The effects of pharmacologic doses of vitamin C appear to be sufficiently complex that very sensitive methods are required for their detection and analysis. In this study, the efficiency of the co-twin control method was 2 to 14 times greater than the random block design. Using this technique, we found evidence for significant dose \times sex interactions in several of the treatment effects that were examined. Treated young female twins appeared to have shorter and less

TABLE III. Amino Acids With Significant 24-Hour Urinary Excretion in Twins Receiving 1,000 mg/day^a

Amino acid	After treatment mean values		
	Vitamin C	Placebo	Difference \pm SE
Lysine	211.54	322.45	-110.91 \pm 25.04**
Taurine	628.10	1,009.40	-381.30 \pm 167.73*
Threonine	114.20	162.53	- 48.33 \pm 14.24**
Serine	309.48	449.65	-140.17 \pm 39.33**
Glycine	810.86	1,381.07	-570.21 \pm 134.16**
Alanine	322.11	492.55	-170.44 \pm 42.82*
Valine	26.42	44.69	- 18.27 \pm 7.42*
Cystine	85.00	120.58	- 35.58 \pm 7.37**
Leucine	31.70	46.77	- 15.07 \pm 3.85**
Tyrosine	141.62	194.46	- 52.84 \pm 13.82**
Phenylalanine	51.54	66.01	- 14.47 \pm 3.21***
Methylhistidine	233.50	301.06	- 67.56 \pm 24.91*
Cystathionine	7.73	16.80	- 9.07 \pm 3.50*

^a $\mu\text{moles}/24$ hours.

* $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

severe upper respiratory infections than their untreated co-twin, while in young male twins receiving 500 mg/day there appeared to be a consistent treatment effect on growth. Our results do not support the contention that pharmacologic doses of vitamin C lower serum cholesterol levels in normocholesterolemic individuals. Also, there appeared to be significantly decreased excretion of 13 amino acids in twins receiving 1,000 mg/day when compared with their controls. It is possible that the age and sex effects detected in this study may in part account for the inconclusive results of previous investigations.

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