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The Effect of Ascorbic Acid on Cholesterolemia in Healthy Subjects with Seasonal Deficit of Vitamin C

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Abstract. The effect of vitamin C on cholesterolemia in a selected group of persons above 40 years of age with a seasonal deficit of ascorbic acid and with an initial level of serum cholesterol in the upper permitted limit, or hypercholesterolemia, was studied. A nutritional situation in the examined area can be characterized by a high consumption of animal fats and sucrose, all-the-year-round deficit of vitamin A, B_2 and iron and an evident seasonal deficit of vitamin C. Administration of 300 mg of ascorbic acid daily for 47 days significantly decreased the cholesterolemia. The effect of ascorbic acid was most pronounced in persons with hypercholesterolemia. In the control group not receiving ascorbic acid, the cholesterol levels showed no significant changes throughout the examined period. In the discussion it is assumed that mechanism of hypocholesterolemic effect of ascorbic acid is based on increasing catabolism of cholesterol by vitamin C.

Among the relatively numerous references found about the effect of ascorbic acid on cholesterolemia in men there is no agreement concerning the influence of vitamin C on the level of cholesterol in blood. In normocholesterolemic persons vitamin C does not affect serum cholesterol level [TJAPINA, 1952; BUKOVSKAJA, 1957; ANDERSON *et al.*, 1958; HRUBÁ and MAŠEK, 1962; BRONTE-STEWARD *et al.*, 1963]. In persons with increased levels of serum cholesterol, some papers indicate in a part of the examined subjects a hypocholesterolemic effect of the ascorbic acid (TJAPINA, 1952; SEDOV, 1956; BUKOVSKAJA, 1957; GRABENKO, 1958; FEDOROVA, 1960; SOKOLOFF *et al.*, 1966], however, some authors maintain that the vitamin C is without any effect [KRIVORUCHENKO, 1963, 1965; SAMUEL and SALCHI, 1964]. The most of the papers, trying to prove the hypocholesterolemic effect of ascorbic acid, are criticised on the ground that this effect has been attained under simultaneous effect of further factors with hypo-

cholesterolemic influence, as e.g. protective diet, curative regime, etc. Another shortcoming of the all previously cited papers is the fact, that the state of vitamin C saturation has not been studied in the patients subjected to investigations.

Data obtained by epidemiological study of two population sets in Eastern Slovakia showed a statistically highly significant negative correlation between some lipid metabolism parameters, especially the level of cholesterolemia and that of vitamin C in blood [KAJABA, 1964; KAJABA and BUČKO, 1968]. These data indicated that an increase of vitamin C in blood is accompanied with a decrease of average cholesterolemia in population groups. Similarly, experimental studies performed on guinea-pigs revealed the close relationship between vitamin C and cholesterol metabolism (GINTER *et al.*, 1965, 1967].

The presented study is based on the assumption [GINTER, 1968], that eventual hypocholesterolemic effect of the ascorbic acid could be most clearly evident at an intentional selection of a group of persons with higher levels of serum cholesterol and at the same time with biochemically proved low vitamin C saturation. The objective of the study is to test this assumption by a model epidemiological experiment on selected individuals.

Material and Methods

In the preceeding years we have performed a year-round study of food-stuffs consumption by 18 selected typically agricultural families in the study area Voderady in the Southwest part of Slovakia with an objective of obtaining a picture about the method and seasonal variability of nutrition within the locality under study. Since this is an area, where a further broadly based observation of nutritional state of inhabitants did not reveal any important changes of the style of living and nutritional habits, the obtained data on caloric and biological value of consumption can be accepted as material reliably illustrating the situation in nutrition of the given region also in the period of our experiment.

For dietetic research, the inventory method [BUDLOVSKY, 1960] and data obtained about food-stuffs were calculated from commonly used biological indexes using Czechoslovak Tables of Nutritional Value of Food-stuffs [KRONDLOVÁ-ŠKOPKOVÁ and ŠMRHA, 1965]. The food-stuffs consumption is presented both in absolute values of calculations in relation to nutritional factors, both in comparison with the found consumption of the currently accepted Czechoslovak recommended doses [MAŠEK *et al.*, 1962], in the form of percentage maintenance of a dose, as well as the structure of consumption of basic nutrients and their triple relationship.

In the clinical and biochemical research of the so-called healthy population in the Voderady area (the village of Voderady, Cífer, Slovenská Nová Ves and Pác), age

group above 40 years, one of the authors was examining by the end of 1967 apart from other indexes the level of cholesterol and vitamin C in blood by a relatively large group of persons. From the whole group of 256 persons under investigation (181 women, 175 men) 50 persons were selected with low levels of vitamin C in blood (about 0.6 mg%) and cholesterolemia at the upper limit of the standard or beyond it (232–312 mg%).

The selected subjects were divided into two groups, experimental and a control one. Persons in the control group (villages Cífer and Pác) were unaware of taking part in an experiment; only at the end of the experiment, were blood samples taken for biochemical examination. The control group persons (villages Voderady, Slovenská Nová Ves, Pác) had blood samples for determinations of cholesterol and vitamin C content in blood taken on January 23, 1968. Each person received a container with 141 tablets (100 mg of vitamin C in each tablet) of Celaskon Spofa, and were instructed to take, 3 times daily, 1 tablet of the 'vitamin preparation'. The daily dose of vitamin C was calculated for exactly 47 days, so that a real intake of tablets could be checked at the end of the experiment. They all received instructions about not changing their nutritional habits.

After 7 weeks of vitamin C administration (March 12, 1968), blood samples were taken from each member of the experimental group and the number of tablets, not taken, were checked. Persons, having at that time more than 30 tablets (about 20% of the total dose of vitamin C) were excluded from further experiments. Following the blood sampling, a saturation test was performed: after intravenous administration of 100 mg of ascorbic acid, the elimination of vitamin C in 3-h urine was examined. In the control group, a similar examination was carried out a day after the taking of blood samples. After excluding the individuals which either did not use the required number of tablets or developed a more serious disease during the experiment, the experimental group consisted of 24 persons (13 men and 11 women) and there were 18 persons in controls (10 men and 8 women), in an age group of 42 to 65 years.

The analytical methods employed: vitamin C in total blood [BESSEY, in GYÖRGYI, 1950], vitamin C in urine [ROE and KUETHER, 1943], cholesterol in blood serum [PEARSON *et al.*, 1953]. The variation coefficient obtained by analysis of 10 parallel samples was shown to be for the method of vitamin C in blood determination $\pm 2{,}6\%$, for the method of cholesterolemia determination $\pm 0.5\%$.

The results were statistically evaluated following the calculation SE by Student's t-test and the pair test, evaluating differences in the studied parameters by individual persons.

Results and Discussion

Tables I and II present the results of a year-round study of alimentation of selected typically agricultural families in the area under investigation, both of these are expressed in an average all-year-round measuring, both quarterly in an effort to record seasonal differences in the nutrition. Consumption of basic nutrients is relatively satisfactory, proteins reaching in total estimates about 10% less value than is the recommended rate, nevertheless, animal proteins are in-

Table I. Caloric and nutrient intake in the rural population (peasants) of the investigated area Voderady in the course of one year, as compared with recommended allowances

Seasons	Total calories		Total proteins		Animal proteins		Plant proteins		Fats		Carbohydrates total		Carbohydrates sugar		Ratio		
	abs.	%[1]	abs.	%[1]	abs.	%[1]	abs.	%[1]	abs.	%[1]	abs.	%[1]	abs.	%[2]	P:F:C	Pa:P pl	Fa:F pl
	g		g		g		g		g		g		g				
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
I. trimester	3,010	100	86	86	45	89	41	82	105	105	427	100	89	21	11.6:31.6:56.8	52.2:47.8	69.3:30.7
II. trimester	2,982	99	86	86	47	94	39	78	108	108	417	98	95	23	11.5:32.6:55.9	54.6:45.4	70.0:30.0
III. trimester	2,949	98	87	87	46	92	41	82	101	101	427	100	87	20	11.6:30.6:57.8	52.9:47.1	72.2:27.8
IV. trimester	3,241	108	94	94	51	102	43	86	124	124	439	103	92	21	11.6:34.2:54.2	54.1:45.9	62.7:37.3
Daily average per year	3,045	102	88	88	47	94	41	82	109	109	428	101	91	21	11.5:32.3:56.2	53.5:46.5	68.4:31.6

[1] Percent of recommended Czechoslowak allowances
[2] % of carbohydrate
P proteins, *P–a* proteins animal, *P–p* proteins plant, *F* fats, *C* carbohydrates

Table II. Vitamin and mineral intake in the rural population (peasants) of the investigated area Voderady in the course of one year, as compared with recommended allowances

Seasons	Ca		Fe		P	Vitamin A		Vitamin B_1		Vitamin B_2		Vitamin PP		Vitamin C	
	abs.	%[1]	abs.	%[1]	abs.[2]	abs.	%[1]	abs.	%[1]	abs.	%[1]	abs.	%[1]	abs.	%[1]
	mg		mg		mg	J.U.		mg		mg		mg		mg	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
I. trimester	819	102	11.75	84	1,456	2,222	44	1,624	108	1,112	56	16.62	111	40.2	54
II. trimester	876	110	11.25	80	1,493	2,326	46	1,488	99	1,062	53	15.31	102	37.1	49
III. trimester	859	107	11.61	83	1,488	3,859	77	1,487	99	1,134	57	16.30	109	86.4	115
IV. trimester	813	102	13.12	94	1,545	3,402	68	1,557	104	1,270	64	19.44	130	53.4	71
Daily average per year	842	105	11.93	85	1,496	2,952	59	1,539	103	1,114	57	16.92	113	54.3	72

[1] Per cent of recommended Czechoslowak allowances
[2] Per cent of recommended allowances according to SHERMAN

cluded in this to a smaller degree than proteins of plant origin. Carbohydrates according to their balanced value of consumption throughout the year kept at the level of the recommended supply. This does not apply to structure of their consumption, where the ratio of sugar participating in carbohydrate calories attains higher values similarly as was found during our previous findings in Eastern Slovakia [KAJABA and BUČKO, 1968a]. The nutrition of the inhabitants of the studied area is with an exception of summer months characterized moreover by an excessive consumption of animal fats.

Consumption of protective substances is characterized by a year-round deficiency of vitamin A, B_2 and iron. The intake of vitamin C shows typical seasonal variations, depending on access to these sources. This is seen by insufficient maintenance of the recommended supply during the critical nutrient period in the winter and spring months. From the point of view of the objective of our study it is important to state, that in the experimental period the consumption of vitamin C over the given area was attaining only about 50% of the recommended values.

Table III lists the cholesterolemia data in the experimental and control group prior to administration of vitamin C, recorded from November 28th to December 8th, 1967 and after the end of the experiment March 12th–13th, 1968. The initial cholesterol level in blood serum was in both groups practically the same. The administration of vitamin C resulted in the experimental group in a slight, but sta-

Table III. The influence of vitamin C on cholesterolemia (mg%). In the table means ± SE are given

Group	December 1967	March 1968	Statistical significance	
			Student's t-test	t-test of pair sample
Control group	251±3	263±6	P>0.1	P<0.1
Vitamin C treated group	255±2	238±8	P<0.05	P<0.02
Statistical significance				
Student's t-test (control × treated group)	P>0.5	P<0.05		
t-test of pair sample	–	P<0.01		

tistically significant decrease of cholesterolemia. This decrease was evident both by comparing the final and initial cholesterol levels by the experimental group and when comparing the level of cholesterolemia between the control and experimental group at the end of the experiment. When evaluating differences using the method of the pair t-test, even statistically more persuasive results were observed. On the contrary in the control group, when comparing the data from December and March a trend towards a modest increase of cholesterolemia was observed, however the increase was not statistically significant.

Determination of the level of cholesterolemia in the experimental group immediately prior to starting with vitamin C administration in January 1968, revealed that there was no difference in comparison with initial values determined in December 1967 (average values in December 255 ± 2, in January 258 ± 8 mg%). A decrease in the cholesterol level occurred under the effect of vitamin C only at a later period. The decrease of cholesterolemia caused by administration of ascorbic acid was also significant, when data from the end of the experiment were evaluated (March 1968) by the pair t-test ($P<0.02$) in comparison with data recorded in January 1968.

The hypocholesterolemic effect of vitamin C was most pronounced in persons with serum cholesterol values over 240 mg% (table IV). In this group the cholesterolemia level was decreased due to the effect of the vitamin C supply with a considerable significance, on the average by 34 mg%, the observed decrease being exceptionally great in some people.

Table V shows a concentration of vitamin C in total blood in control and experimental subjects at the beginning and at the end of the experiment. In December 1967, there were no differences in levels of vitamin C of control and experimental groups. At the end of the experiment in March 1968, this differences was statistically evident in favour of the experimental group receiving vitamin C. This difference is influenced by the fact, that in the course of winter months from December to March, the vitamin C level in the blood of the control group decreased significantly ($P<0.02$, evaluated by the pair t-test), while in the experimental group, the administration of vitamin C prevented this decrease.

A very surprising fact is, that daily intake of 300 mg of vitamin C for 47 days did not result in a more pronounced increase of ascorbin-

Table IV. The influence of vitamin C on serum cholesterol levels (mg%) in persons with hypercholesterolemia

Person examined	January 1968	March 1968	Difference
K.M. ♀	340	260	—80
K.M. ♀	320	260	—60
R.A. ♀	313	321	+ 8
Č.A. ♂	305	275	—30
M.J. ♂	305	248	—57
P.J. ♂	291	199	—92
K.A. ♂	291	260	—31
O.J. ♂	291	283	— 8
B.L. ♂	269	283	+14
M.H. ♀	255	217	—38
F.F. ♂	254	220	—34
Š.A. ♂	247	231	—16
P.J. ♂	243	239	— 4
Mean ± SE	286±9	253±9	—33±9
Statistical significance:			P<0.01

Table V. Blood vitamin C levels (mg%) in control and vitamin C treated group

Group	December 1967	March 1968
Control group	0.56±0.07	0.40±0.09
Vitamin C treated group	0.62±0.05	0.67±0.06
Statistical significance	P>0.5	P<0.02

emia in persons belonging to the experimental group. A saturation test after the intravenous administration of ascorbic acid however has shown, that saturation of tissues of persons included in the experimental group, is considerably higher (figure 1). The obtained data in spite of a considerable individual variability demonstrate, that elimination of vitamin C in urine was in the experimental group higher by nearly ten times. The results obtained agree well with the authors of the

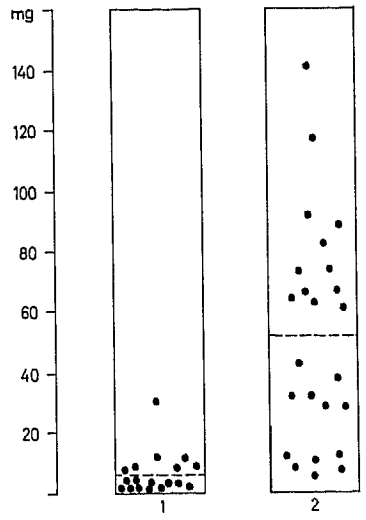

Fig. 1. Vitamin C excretion (mg) in 3 h urine after intravenous injection of 100 mg ascorbic acid. *1* Control group, *2* vitamin C treated group. The difference is statistically highly significant ($P<0.001$).

the saturation test [PLENERT and HEINE, 1967], who report that in saturated persons the elimination of vitamin C in 3-h urine should be about 50% of the administered dose. The values under 15% are considered as defective. Taken from this standpoint, the saturation in the control group persons was extremely low, since from 18 observed persons, only in one case a value higher than 15% was found; this is an indication of unsatisfactory saturation of the Czechoslovak population by vitamin C in winter [BUDLOVSKÝ, 1966; KAJABA, 1966; HEJDA *et al.*, 1965; HEJDA, 1967].

The data obtained demonstrate the causal relationship between saturation of an organism with vitamin C and cholesterolemia. In recent years various authors pointed out the seasonal fluctuation of cholesterolemia in men [THOMAS *et al.*, 1961; TOCHOWICZ *et al.*, 1962; THORP, 1963]: the highest concentrations of serum cholesterol could be observed in the period of minimal intake of vitamin C (winter, spring), lowest in summer and autumn. A similar fluctuation of cholesterolemia was observed in children, too [KAJABA and BUČKO, 1968], with an inverse trend of changes of vitamin C levels in blood (maximum in summer, minimum in winter months). Statistical anal-

ysis of this material has shown the existence of a significant negative correlation between ascorbinemia and cholesterolemia. Therefore it is probable, that a cause of seasonal fluctuations of cholesterolemia is – not excluding other factors – also the different intake of vitamin C in winter, or summer, respectively.

Further evidence on the participation of vitamin C in cholesterol metabolism was obtained by experimental animals. In guinea-pigs with chronic hypovitaminosis C a significant increase of cholesterol concentration in tissues occurs, especially in liver [GINTER *et al.*, 1967] and an increase of beta-lipoprotein level in blood serum [GINTER *et al.*, 1965]. In guinea-pigs fed on an atherogenic diet, the chronic deficit of ascorbic acid results in an increased accumulation of cholesterol in a whole range of tissues, including aorta walls [GINTER *et al.*, 1969]. Indirect evidence obtained by a study of cholesterol metabolism in hypovitaminic guinea pigs indicates that a shortage of vitamin C results in a decrease in cholesterol catabolism. Therefore it is likely that the hypocholesterolemic effect of the ascorbic acid is based on an increase of cholesterol catabolism, which is finally manifested by a decrease of cholesterolemia.

The fact, that various authors [KRIVORUCHENKO, 1963, 1965; SAMUEL and SALCHI, 1964] were not successful in proving the hypocholesterolemic effect of ascorbic acid, is most likely to be explained by a high saturation of the examined persons with vitamin C, due to which any further supply of ascorbic acid remained ineffective. The evident hypocholesterolemic effect of ascorbic acid, found in the presented study, is undoubtedly influenced by the fact that persons under investigation were showing a clear-cut seasonal vitamin C hyposaturation. We do not consider as impossible that a strictly controlled intake of higher doses of ascorbic acid will under similar conditions result in an even more extensive decrease of cholesterolemia, as that described in this study.

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