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## Hypocholesterolemic Effect of Ascorbic Acid in Maturity-Onset Diabetes Mellitus

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*Summary: A significantly lower vitamin C concentration has been found in the blood and particularly in the leukocytes of hypercholesterolemic diabetic patients than of healthy blood donors. Ascorbic acid administered in a dose of 500 mg per day for 12 months to metabolically stabilized hypercholesterolemic subjects with maturity-onset diabetes mellitus (diabetic diet without insulin or diabetic drugs) brought about a striking decline of cholesterolemia and a moderate decline of triglyceridemia. The serum lipid level in the control group given placebo remained unaltered. A daily administration of 500 mg of ascorbic acid for six months failed to affect the fasting level of serum immunoreactive insulin. It is assumed that the long-term administration of ascorbic acid to maturity-onset diabetics removed the tissue ascorbate deficiency and improved the liver ability to compensate the increased endogenous synthesis of cholesterol by its enhanced transformation to bile acids.*

BENNION and GRUNDY (1977) found an enhanced rate of endogenous cholesterol formation in subjects with uncontrolled maturity-onset diabetes mellitus. A state of chronic vitamin C deficiency is often reported in diabetic patients (OWENS *et al.* 1941, BESEDIN 1976). Experimentally induced diabetes provokes an ascorbate deficiency (ZEBROWSKI *et al.* 1976), a slowed down cholesterol transformation into bile acids (SADAHIRO *et al.* 1970), a depressed value of the

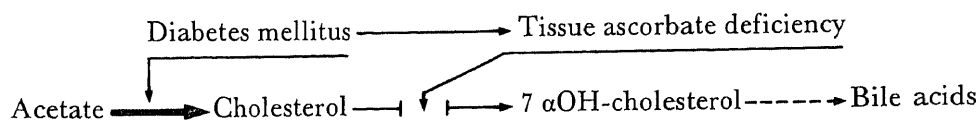
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rate constant for irreversible elimination of cholesterol from the organism (LEHNER *et al.* 1972), hypercholesterolemia (RUDAS *et al.* 1973, TUMAN and DOISY 1977, and numerous other authors), and an augmentation of the size of a rapidly exchanging cholesterol pool (LEHNER *et al.* 1972). A latent chronic vitamin C deficiency creates similar disorders in experimental animals, as it slows down the rate of cholesterol transformation into bile acids (GINTER 1973, HORNIG and WEISER 1976). Ascorbate is required for a normal course of the rate-limiting reaction of this process, 7  $\alpha$ -hydroxylation of cholesterol (GINTER 1975, BJÖRKHEM and KALLNER 1976). Hence, the metabolic situation in many diabetics probably corresponds to the scheme:



from which it ensues that the cause of the frequent occurrence of hypercholesterolemia in diabetes mellitus resides in the disbalance between the rate of synthesis and catabolism of cholesterol. As high doses of vitamin C tend to enhance cholesterol catabolism to bile acids in experimental animals (GINTER *et al.* 1978), the present study has been designed to ascertain whether ascorbate could be utilized to depress hypercholesterolemia in diabetes mellitus.

### *Materials and Methods*

From a group of 658 diabetic patients on the records of the Diabetological Outpatient Department at Počátky, 127 stabilized diabetics of both sexes (60% males) were selected, aged predominantly between 50 and 60 years with repeatedly determined hypercholesterolemia (above 7 mM/l). Towards the end of the winter period (MARCH 1977), fasting concentration of vitamin C in blood was determined in all the patients (ROE and KUETHER 1943) and in part of them (55 subjects – 31 males and 24 females) also in leukocytes (BESSEY *et al.* 1947). During the same period, the same parameters were determined in a matched group of 85 healthy blood donors.

From the diabetic group, 48 permanently hypercholesterolemic outpatients (29 males, 19 females), most of them obese (height:  $166 \pm 1$  cm, body weight:  $82 \pm 2$  kg), with stabilized maturity-onset diabetes mellitus were selected. The outpatients were kept on a diabetic diet, without insulin, oral diabetic drugs or any other drugs affecting lipid metabolism. Fasting values of serum total cholesterol (DREZGA and MIKAC-DEVIĆ 1969) and triglycerides (Boehringer Mannheim GmbH) and blood levels of vitamin C (ROE and KUETHER 1943) were determined in all the subjects. In part of them (20 persons), serum levels of immunoreactive insulin, too, were determined (IRI, INS-set, Institute of Nuclear Research, Poland). In a test designed as a double-blind experiment, 35 subjects received 500 mg of ascorbic acid per day (Celaskon effervesces Spofa) and 13 had placebo, produced for this purpose by Slovafarma Hlohovec. The same parameters as at the start were determined after 6 and 12 months of the experiment (IRI only after 6 months). The results were processed statistically by means of standard programmes (Student's t-test, paired t-test, linear correlation) on a computer.

## Results and Discussion

### 1. Vitamin C status in hypercholesterolemic diabetics

Vitamin C concentration in the blood of diabetic patients proved to be significantly lower than in healthy subjects (diabetics:  $0.39 \pm 0.03$ ; healthy controls:  $0.58 \pm 0.04$  mg %;  $P < 0.001$ ). As these data were obtained from subjects matched for age, and at the same season of the year (end of winter), the low ascorbemia in the diabetics is due not solely to a seasonal vitamin C deficit in the diet, but also to their specific health status. The vitamin C level in blood was not significantly affected by the mode of treatment (diabetic diet alone: 0.37; oral diabetic drugs: 0.38; insulin: 0.42 mg %). Ascorbemia was found to be lower in the male than the female patients (men:  $0.32 \pm 0.03$ ; women:  $0.46 \pm 0.04$  mg %;  $P < 0.01$ ).

Vitamin C concentration in the leukocytes was substantially lower in the diabetic patients than in healthy subjects (diabetics:  $9.8 \pm 0.7$ ; healthy controls:  $22.7 \pm 0.8$  mg %;  $P < 0.001$ ). Similarly close linear correlation was found between ascorbate concentration in blood and in leukocytes in the controls and the diabetic patients (the correlation coefficient in the two groups was 0.64,  $P < 0.001$ ). On the other hand, a comparison of the distribution of ascorbate levels in the blood and leukocytes shows that the ascorbate values in leukocytes of the diabetics were more depressed than those in blood (Fig. 1). This points to a lowered availability of ascorbate for storage in the tissues (LOH and WILSON 1971).

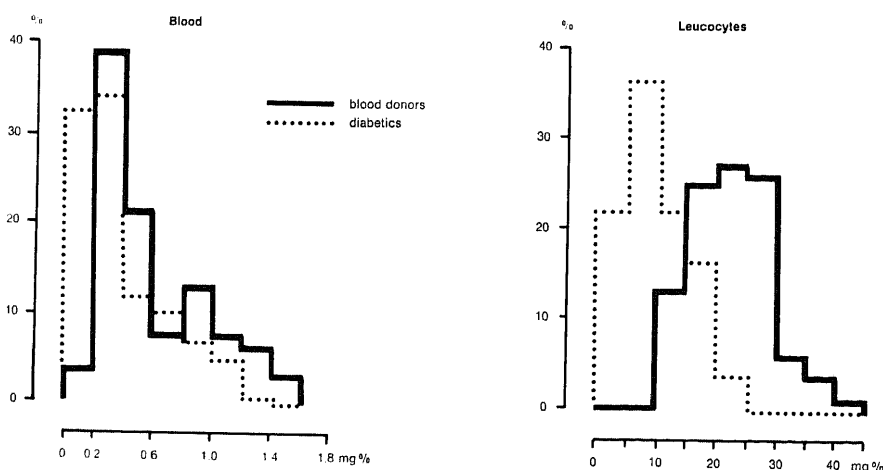


Fig. 1: Distribution pattern of ascorbic acid levels in blood and leukocytes of healthy blood donors ( $n = 85$ ) and hypercholesterolemic diabetic patients ( $n = 127$ ) at the end of the winter season.

Insulin and hyperglycaemia affect the membrane transport not only of glucose, but also of vitamin C (Cox *et al.* 1974, MANN and NEWTON 1975). On the basis of a structural similarity between glucose and vitamin C, MANN (1974) assumes that an impairment of insulin function, whether by its absence (juvenile diabetes), or by its inhibition (maturity-onset diabetes), will lead to impaired transport of vitamin C into the cells of certain tissues. Experiments on streptozotocin-diabetic and insulin-treated rats brought support to this assumption (ZEBROWSKI *et al.* 1976). It is therefore probable that our group of diabetic patients, too, suffered from a marked tissue deficiency of ascorbate brought about not only by a seasonal vitamin C deficit in the diet, but also by an impairment of insulin function.

### 2. Hypolipemic effect of ascorbic acid in maturity-onset diabetes

A daily administration of 500 mg of ascorbic acid to hypercholesterolemic diabetics with a latent vitamin C deficiency led to a substantial increase of ascorbemia, a striking decline of cholesterolemia and a moderate, though statistically significant decline of triglyceridemia after 6 and 12 months (Fig. 2). Student's t-test and the paired t-test proved the cholesterolemia decline to be of

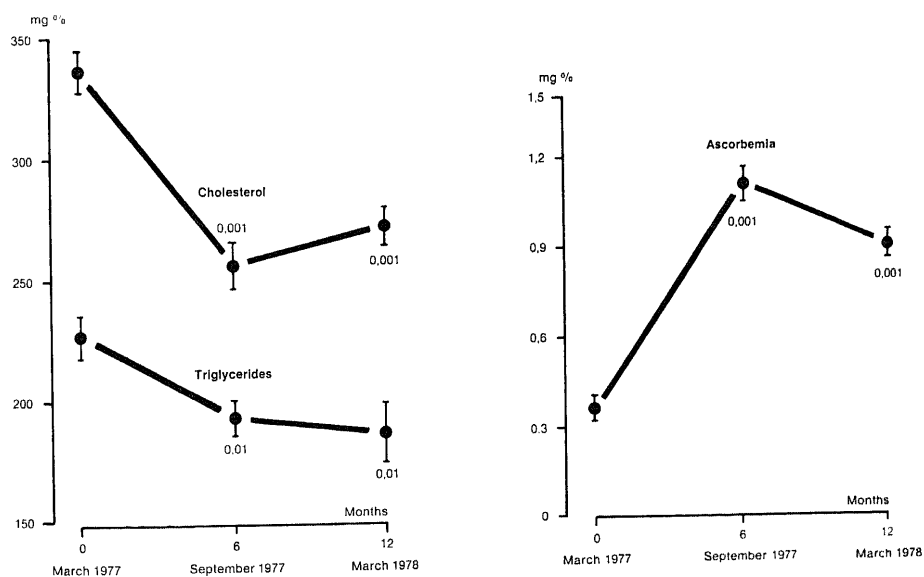


Fig. 2: Effect of a long-term administration of ascorbic acid (500 mg per day) on cholesterol and triglyceride levels in blood serum and on vitamin C in blood of hypercholesterolemic maturity-onset diabetic patients (means from 35 persons). Vertical bars for each value indicate  $\pm$  S. E. M. Statistical significance (t-test) in comparison with the initial state is given at each value.

high statistical significance ( $P < 0.001$  at both the time intervals). The decline of cholesterolemia was of clinical interest in the majority of the patients. After one year of vitamin C administration, cholesterolemia in 60% of the patients dropped minimally by 40 mg%; in some cases the decline exceeded 100 mg%. In about one-third of the patients, vitamin C proved without effect.

In the group receiving placebo, the serum lipid levels remained unchanged (Tab. I). A moderate increase of ascorbemia noted in this group in September

Tab. I: Cholesterolemia, triglyceridemia and ascorbemia in the control group of maturity-onset diabetics receiving placebo

| Parameter                                   | Startig values<br>(March 1977) | After 6 months<br>(September 1977) | After 12 months<br>(March 1978) |
|---|--------------------------------|------------------------------------|---------------------------------|
| Serum cholesterol (mg/100 ml) . . . . .     | 308 ± 11                       | 304 ± 14                           | 292 ± 24                        |
| Serum triglycerides (mg/100 ml) . . . . .   | 187 ± 4                        | 189 ± 3                            | 184 ± 27                        |
| Whole blood vitamin C (mg/100 ml) . . . . . | 0.38 ± 0.09                    | 0.61 ± 0.11                        | 0.45 ± 0.08                     |

Means from 13 patients ± S. E. M.

No change of statistical significance took place in either time interval in comparison with the starting values.

1977 was evidently due to a higher consumption of fresh vegetables and fruit during this period. Body weight of patients receiving daily 500 mg ascorbic acid underwent no significant change during the course of the experiment (start: 79.4 ± 2.4, end of experiment: 80.5 ± 2.4 kg). The fasting serum IRI level was not affected by the six-month administration of ascorbic acid (starting value: 54 ± 7 μU/ml; after 6 months: 58 ± 6 μU/ml;  $t = 0.465$ ). It thus seems plausible to assume that the mechanism of the hypocholesterolemic action of ascorbate was not conditioned by its effect on the secretion or turnover of insulin.

In our view, the mechanism of the hypocholesterolemic action of ascorbic acid is as follows: the extremely low ascorbate levels in leukocytes permit to presume very low ascorbate levels also in the liver of our patients (BEATTIE and SHERLOCK 1976). A long-term administration of ascorbic acid led to an enhanced ascorbate concentration in the liver, resulting in an enhanced rate of cholesterol transformation to bile acids. A similar phenomenon was repeatedly demonstrated in experimental animals (GINTER 1975, HORNIG and WEISER 1976, IWAMOTO *et al.* 1976, GINTER *et al.* 1978). An improvement of the disbalance between the synthesis of endogenous cholesterol and its transformation to bile acids led in a long-term experiment to a decreased quantity of cholesterol circulating in the blood.

The mechanism of a moderate hypotriglyceridemic action of vitamin C may perhaps be related to the lipolytic systems whose activity is depressed in both experimental and human diabetes (BAGDADE *et al.* 1968, BRUNZELL *et al.* 1975, REDGRAVE and SNIBSON 1977, NIKKILÄ *et al.* 1977, ELKELES and HAMBLEY 1977, VAN TOL 1977). High doses of ascorbic acid were found to stimulate postheparin lipolytic activity of plasma in monkeys and guinea pigs (KOTZÉ and SPIES 1976, BOBEK and GINTER 1978).

These data underline the necessity of monitoring vitamin C status in diabetic patients and in case the values are low, to increase the intake of vitamin C. The dose of ascorbic acid used in the present experiment (500 mg per day) cannot be considered as extremely high in hypercholesterolemic diabetics, for both hypercholesterolemia and diabetes mellitus lay enhanced claims of the organism on vitamin C (GINTER and ZLOCH 1972, MANN 1974, NAMBISAN and KURUP 1976, ZEBROWSKI *et al.* 1976).

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