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Serum Bile Acids in Man during Vitamin C Supplementation and Restriction

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ABSTRACT. A daily dosage of 5 g ascorbate was given to 14 persons during one month. Various routine biochemical parameters were studied and the concentrations of individual bile acids in serum were determined. A significant increase in chenodeoxycholic acid concentration was found on interruption of vitamin C supplementation, whereas no other changes in bile acid concentrations were significant. One person, used to a large ascorbate intake (1 g/day), was deprived of ascorbate. On resuming the high ascorbate intake, serum bile acid concentrations showed an increasing trend. Urinary oxalate excretion and concentrations were impressively increased during vitamin C supplementation but no effects on kidney function were observed.

Bile acids are produced in the liver by oxidation of cholesterol. The bile acids are distributed between two main pools, the liver and the intestine including the gallbladder. In a recent review, Krumdieck and Butterworth (14) summarized several investigations dealing with the effect of ascorbate on cholesterol metabolism. It was shown in a recent study (2) that when guinea pigs were given a vitamin C free diet, the activity of the 7α-hydroxylating system of liver microsomes was decreased. A similar approach to investigate the effect of vitamin C on cholesterol metabolism in man is virtually impossible.

In the present report, two experimental models were used. In one, the daily dosage of ascorbate was increased far above the normal and changes in the bile acid pattern in serum were followed during one month. In the other, one subject was on a large daily dosage for three months and the daily intake was then drastically reduced.

SUBJECTS

Vitamin C, 5 g (1.25 g×4 C-vimin®, Astra, Södertälje), was given to 14 male volunteers, below 25 years of age, for 4 weeks. To the best of our knowledge they were healthy and all laboratory test values were within normal limits. No restrictions in diet were applied. One subject was given 1 g/day of vitamin C for 3 months and the supplementation was then discontinued. During a period of one week vitamin C rich food was avoided and the supplementation was then resumed. Samples were taken from all subjects after a night's fasting. Samples from vitamin C supplemented subjects were investigated before supplementation, and at two and four weeks. After interruption of supplementation, samples were taken on days 1, 2, 5 and 9. The samples are numbered I, II, III, IV, V and VI, respectively. Samples were taken daily from the other test persons under identical premises.

METHODS

Chemical methods

Triglycerides, cholesterol, calcium, creatinine and aminotransferases were determined by routine methods in the Department of Clinical Chemistry. Phosphate was determined by a malachite-green method (12). Ascorbate in serum and urine was determined as described by György (8). This method determines the serum concentration of ascorbate and dehydroascorbate. Oxalate in urine was determined by a commercial laboratory (17) by a titrimetric method after precipitation.

Bile acids were determined by gas–liquid chromatography after isolation and concentration on XAD-2 (Serva) according to the method of Sjövall and Masuri (19). Recovery of bile acids was calculated by addition of a tracer amount of 24-^{14}C-tauro-cholate (Radiochemical Center, Amersham) to the serum sample before dilution and chromatography. Recoveries were between 92 and 97%. Lithocholic acid was used as internal standard in gas-chromatographic analysis, added before derivation of bile acids. The areas under the peaks were measured by planimetry and the calculated concentration was corrected with regard to recovery data in each case.

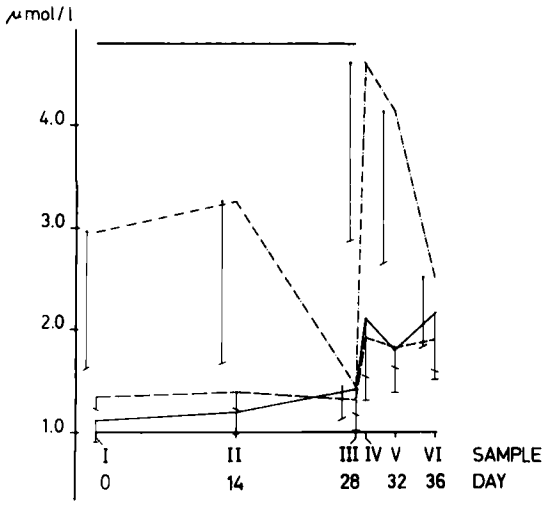

Fig. 1. Concentrations of serum bile acids during vitamin C supplementation. —=cholic acid, ---=deoxycholic acid, -·-=chenodeoxycholic acid. Vertical bars to the left of the value for chenodeoxycholic acid=S.E.M. Horizontal bar=period of vitamin C supplementation.

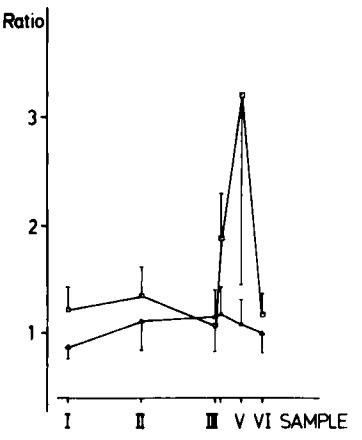

Fig. 2. Ratios C/D (△—△) and CD/D (□—□) during vitamin C supplementation. Abbreviations as in Table II.

Statistical methods

t-Values were calculated using Student's *t*-test for correlated data.

RESULTS

Routinely determined serum and urine parameters before and during administration of vitamin C are shown in Table I. After two weeks of supplementation no values showed increases except those directly dependent on the compound administered (ascorbate and oxalate). Decreases were found in creatinine and phosphate. Phosphate rose again during the second half of the test period to the same level as before the supplementation. Creatinine increased to a level significantly above the initial level but still within normal limits.

The bile acid concentrations in serum in the group supplemented with ascorbate are given in Table II and Figs. 1 and 2. A significant increase, judged by the sign test (20), was found for chenodeoxycholate between the last day of vitamin C supplementation and the first day without (samples III and IV). A return to previous values was obtained during the following week. The ratios of cholic acid and chenodeoxycholic acid to deoxycholic acid were calculated but no significant changes were recorded during the period of observation.

Serum bile acid concentrations in the deprivation test with only one subject are shown in Fig. 3. All

Table I. *Serum and urine concentrations before (I), after 2 (II) and 4 (III) weeks of vitamin C supplementation (mean±S.E.M.)*

Differences are significant at the 95% level for $t \geq 2.16$

	Reference value	I	II	III	*t* I/II	*t* II/III	*t* I/III
S-cholesterol (mmol/l)	<7.8	4.83±0.23	4.78±0.23	4.99±0.23	0.64	−1.51	−1.29
S-triglycerides (mmol/l)	<1.7	0.9±0.08	0.9±0.07	0.9±0.08	0.63	−0.15	0.35
S-creatinine (μmol/l)	<120	88±2	79±2	97±3	3.68	−4.25	−2.74
S-calcium (mmol/l)	2.20–2.70	2.53±0.02	2.51±0.0	2.54±0.0	0.20	−0.22	−0.08
S-phosphate (mmol/l)	0.7–1.6	1.3±0.04	1.2±0.03	1.3±0.02	2.97	−3.12	0.70
S-ASAT (μkat/l)	<0.70	0.27±0.01	0.29±0.02	0.26±0.02	−1.26	2.07	0.54
S-ALAT (μkat/l)	<0.70	0.27±0.03	0.33±0.05	0.26±0.03	−1.56	2.16	0.27
S-ascorbate (μmol/l)	30–85	79±3	130±6	102±6	−8.92	3.27	−3.54
U-ascorbate (μmol/24 h)	–	942	11 695	13 115			
U-creatinine (mmol/24 h)	7–19	19±4	19±4	20±4	0.43	−1.76	−1.04
U-oxalate (μmol/24 h)	–	49±16	1 295±744	1 540±601	−6.21	−1.12	−9.22

Table II. *Serum bile acid concentrations (μmol/l) at different times during vitamin C supplementation (mean±S.E.M.)*

Sample no.	Cholic acid (C)	Deoxycholic acid (D)	Chenodeoxycholic acid (CD)	C/D	CD/D
I	1.11±0.208	1.34±0.123	2.96±1.350	0.87±0.11	1.22±0.20
II	1.19±0.220	1.38±0.166	3.25±1.592	1.11±0.26	1.35±0.26
III	1.40±0.392	1.31±0.153	1.43±0.318	1.15±0.25	1.07±0.22
IV	2.10±0.808	1.91±0.382	4.59±1.732	1.17±0.26	1.88±0.42
V	1.79±0.416	1.81±0.204	4.13±1.503	1.08±0.23	3.20±1.75
VI	2.15±0.649	1.89±0.306	2.50±0.688	1.00±0.19	1.17±0.18

Table III. *Serum bile acid concentrations (μmol/l) during vitamin C restriction in one subject*

Sample no.	Cholic acid (C)	Deoxycholic acid (D)	Chenodeoxycholic acid (CD)	C/D	CD/D
1	0.60	3.57	5.09	0.18	1.43
2	0.76	1.35	1.27	0.58	0.94
3	1.32	1.86	2.22	0.74	1.19
4	1.37	1.90	1.45	1.10	1.12
5	0.81	1.30	1.94	0.65	0.88
6	0.93	1.35	1.32	0.72	0.98
7	0.73	1.40	0.97	0.55	0.69
8	1.08	1.22	0.97	0.92	0.79
9	1.38	1.81	2.32	0.80	1.28
10	1.91	1.30	1.50	1.53	1.16

bile acids decreased to new levels, which were not essentially changed until supplementation was resumed. Concentrations and ratios are shown in Table III.

DISCUSSION

Spittle (21) observed that vitamin C supplementation lowered serum cholesterol to various degrees in different age groups and proposed that this could be explained by an increased metabolism of cholesterol and a mobilization of cholesterol deposits. Ginter et al. (6) have shown that the higher the initial cholesterolemia, the greater the hypocholesterolemic effect after a prolonged administration of ascorbic acid. Also, Davies and Newson (4) have shown a positive correlation between serum cholesterol and both plasma and leucocyte ascorbate. In a short-term experiment (6 weeks), Fix et al. (5) found no significant change in the cholesterol levels.

The rate-limiting step in the conversion of cholesterol to bile acids, the 7α-hydroxylation, is specifically reduced in ascorbate-deficient guinea pigs (2). On the other hand, the activity of HMG

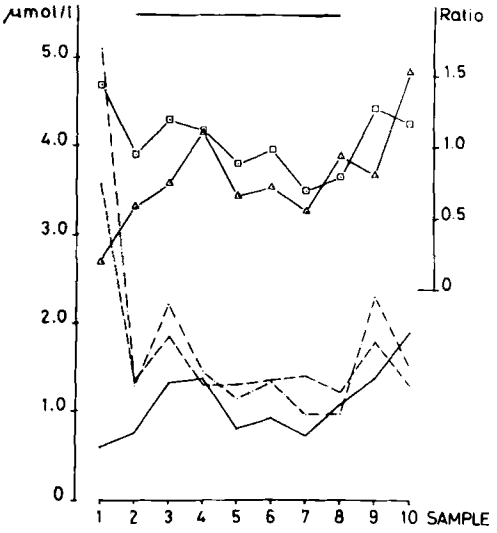

Fig. 3. Concentrations of serum bile acids and ascorbate in one subject during vitamin C restriction. Horizontal bar=period of vitamin C restriction. Symbols as in Figs. 1 and 2.

coenzyme A reductase, which catalyzes the rate-limiting step in the cholesterol biosynthesis, was also decreased in these animals (3). The content of cholesterol in the liver of these animals was increased, which could be interpreted as illustrating the total effect of both rate-limiting steps. There was no change in the serum levels of cholesterol.

The level of serum bile acids might be a sensitive indication of liver function (13) and it has been suggested that the liver is capable of removing a constant fraction of bile acids in the enterohepatic circulation (16).

Quantitative or qualitative changes in the bile acid synthesis might be reflected in the bile acid pattern of serum. A significant increase of short duration in chenodeoxycholate concentration was found in the present study after discontinuation of vitamin C supplementation. The individual changes are great and minor trends may be obscured by the biological variations.

The formation of gallstones in hamsters can be decreased by high doses of ascorbate (7). This could rather be a general effect of the cholesterol bile acid ratio than of an endogenous increase in chenodeoxycholic acid synthesis. The effect of ascorbic acid on biliary lipid composition in man is not significant but Pedersen (18) only determined the total bile acid content in bile, not the individual bile acids.

There is evidence that dihydroxy bile acids and in particular chenodeoxycholic acid are absorbed from the intestine at a higher rate than cholic acid (6). It has been reported recently (11) that ascorbate lowers the absorption of bile acids from the intestine in guinea pigs, in particular the absorption of chenodeoxycholic acid. This is in agreement with the findings in the present study that a decrease in the ascorbate intake causes an increase in the serum chenodeoxycholate level. On the other hand, the above authors obtained a large increase in the biliary output of chenodeoxycholic acid on intraperitoneal administration of ascorbate.

Only one of the present subjects was studied after a sudden reduction of the ascorbate intake, from a comparatively high daily dosage to almost nought. Such a rapid, large change in dosage might possibly enhance the effects of a lowered dosage and simulate a stage of relative deficiency. A decrease in the dihydroxy bile acid concentration in serum was indicated when the high ascorbate intake was discontinued.

One of the metabolites of ascorbic acid in man is oxalate and with the dose given in this study, relatively large amounts would be expected in the urine (15, 22). On a molar basis, about 5% of the ascorbate given was recovered in the urine. After administration of 1-^{14}C-ascorbate, about 2% of the isotope has been recovered in the oxalate fraction of urine (9). It is unlikely that a high oxalate concentration per se would form precipitates (1).

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