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Vitamin C and Plasma Cholesterol

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I. INTRODUCTION

There has been a long-lasting controversy about whether vitamin C has any significant effect on plasma cholesterol levels in human beings. Some early Russian studies suggested that vitamin C may decrease elevated cholesterol levels, and so the vitamin was used to some extent in the treatment of hypercholesterolemia (for review see Reference 1). However, the studies were not well controlled, and duplications have yielded conflicting results. Nevertheless, animal studies have consistently found that vitamin C has substantial effects on cholesterol metabolism.

The purpose of this review is to analyze the published intervention studies in order to identify the factors that may have resulted in the discordance in the results. Several of the studies have used subjects with initially low cholesterol levels. Such studies do not test the hypothesis that a low level of the vitamin may decrease the rate of cholesterol catabolism, and thereby enhance hypercholesterolemia in some people. Accordingly, studies with hypercholesterolemic subjects are more relevant for testing this hypothesis. Most of the studies in the latter group have reported a significant decrease in the cholesterol level with vitamin C supplementation. Furthermore, such results indicate that in certain people low vitamin C status may be one of the factors that lead to the elevation of cholesterol levels.

II. CHOLESTEROL METABOLISM

A. Guinea Pigs

The role of vitamin C in cholesterol metabolism has been studied extensively in guinea pigs (for review see References 2 through 4). Guinea pigs are the experimental animals of choice for physiological studies dealing with vitamin C because they do not synthesize the vitamin themselves, unlike most other mammals. Complete lack of vitamin C causes scurvy, a complicated pathological state characterized by anorexia, weight loss, hemorrhages, and finally death. In guinea pigs, a long-term marginal vitamin C intake also results in low levels of the vitamin in the body.[3,5] Marginal vitamin C deficiency is a relevant model when studying the effects of low vitamin levels, since it is a stable physiological state and may allow more reasonable extrapolation in regard to humans with low levels of vitamin intake.

In guinea pigs, a marginal vitamin C deficiency increases cholesterol levels in plasma and various tissues.[6–31] Synthesis of bile acids is decreased and the composition of bile is changed in marginal[24–33] and in acute[34–36] deficiency. Cholesterol-containing gallstones may also be formed in vitamin-deficient guinea pigs.[3,32,33] Even though the cholesterol levels are elevated when the feed is normal except for the low level of the vitamin, the increase is more pronounced when vitamin-

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deficient guinea pigs are fed an atherogenic diet (cholesterol, coconut oil).[17,18,20,21,37–39]

The increase in cholesterol levels by vitamin C deficiency is not caused by a significant increase in the rate of cholesterol synthesis. Cholesterol synthesis is not affected or may be decreased by vitamin deficiency[6,40–42] (Figure 1). 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is the rate-limiting enzyme in cholesterol synthesis. The activity of HMG-CoA reductase, *in vivo*, is decreased or unaffected in guinea pigs that have been fed low amounts of the vitamin.[11,14,43] High concentrations of vitamin C inhibit the activity of HMG-CoA reductase *in vitro*,[44] but it is not clear whether this effect is physiologically significant. Finally, vitamin C deficiency does not increase the absorption of cholesterol from the intestines, but rather the absorption appears to decrease.[3,45]

Cholesterol 7α-hydroxylase is the rate-limiting enzyme[46] in bile acid synthesis (i.e., cholesterol catabolism). *In vivo*, the activity of 7α-hydroxylase is considerably decreased (−30 to −80%) in vitamin C deficiency.[11,15,30,35,47,48] Consistently, vitamin C deficiency does not affect the catabolism of 7α-hydroxycholesterol, which is the end product of cholesterol 7α-hydroxylase.[9,49] The effect on 7α-hydroxylase appears to be the major mechanism whereby vitamin C affects cholesterol levels and bile acid synthesis. The major effect of the vitamin on cholesterol 7α-hydroxylase is indirect: vitamin C is not a cofactor for the enzyme and the vitamin does not markedly affect 7α-hydroxylase activity *in vitro*.[47,50] However, the precise mechanism of the effect has not yet been defined.[4]

When guinea pigs are fed very large quantities of vitamin C, no reduction is observed in the cholesterol levels when compared with animals fed a normal diet;[13,18,22,51] instead, cholesterol levels may actually increase.[11,19,23,27,30,31] In fact, the activity of cholesterol 7α-hydroxylase is also decreased by very high amounts of the vitamin.[11,15,30,48] However, when guinea pigs are fed an atherogenic diet, high doses of the vitamin may lead to a decrease in the cholesterol levels.[7,15,16,18,51,52]

The studies with marginally deficient guinea pigs have very consistently found an increase in

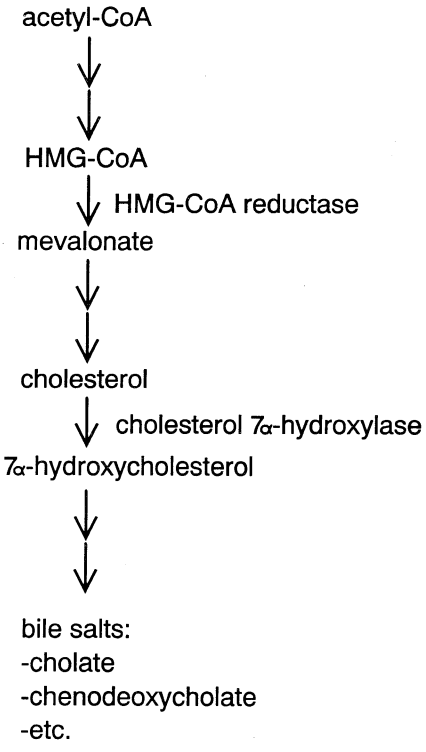

FIGURE 1. Schematic presentation of cholesterol metabolism. HMG-CoA reductase is the rate-limiting enzyme in cholesterol synthesis, and cholesterol 7α-hydroxylase is the rate-limiting enzyme in cholesterol catabolism. Two arrows are used to indicate several consecutive enzymatic steps. HMG-CoA: 3-hydroxy-3-methylglutaryl CoA.

cholesterol levels in various tissues, but the studies with acute deficiency, scurvy, have yielded ambiguous results.[2,3] In acute deficiency, with no dietary vitamin C, cholesterol levels have been reported to decrease[6,16,35,53] or increase[37–39,54–58] in plasma, and decrease[6,35,37] or increase[16,53,57,59] in the liver. Apparently, the variable effects of acute deficiency on cholesterol levels may depend on coincident effects of the vitamin on the synthesis and catabolism of cholesterol. Interestingly, Willis[60] observed in an early report that acute vitamin C deficiency caused the formation of lipid deposits in the aorta of scorbutic guinea pigs, but the deposits disappeared within a few days of the animals being fed the vitamin. However, advanced atherosclerotic type lesions were considerably more resistant to resorption.

B. Rabbits, Rats, and Pigs

The effect of vitamin C on cholesterol metabolism has been studied also in rabbits, rats, and pigs that synthesize the vitamin themselves. Administration of cholesterol to these species increases cholesterol levels in plasma and tissues, but the elevation is significantly smaller when vitamin C is supplemented.[61–69] One study with rabbits found only an insignificant decrease in the cholesterol levels when the vitamin was supplemented.[70] However, much larger quantities of cholesterol were fed compared with another, more thorough study,[64] and thus any modest effects produced by the vitamin could be overshadowed. It has also been reported that extra vitamin C, in the absence of dietary cholesterol, decreases the cholesterol level in the serum, liver, and aorta of rats,[71,72] although one study found no effect.[73] Since rats synthesize vitamin C by themselves, any effect of extra vitamin C may depend on several experimental variables, and a discrepancy in the results is not surprising.

Furthermore, the role of vitamin C has been studied in mutant rats that were unable to synthesize this vitamin. Vitamin deficiency had only minor effects on cholesterol metabolism in these rats when a normal diet is administered. However, when they were fed cholesterol, vitamin deficiency resulted in the accumulation of cholesterol in the plasma and liver, a lower activity of cholesterol 7α-hydroxylase, and lower excretion of fecal bile acids, when compared with mutant rats fed normal amounts of the vitamin,[74–76] or to normal rats.[77]

C. Effect of Cholesterol on Vitamin C Metabolism

Administration of cholesterol to guinea pigs decreases the tissue-levels of vitamin C,[7,18,20,21,37,78] which indicates increased consumption of the vitamin. Increased catabolism of vitamin C was directly observed in one study,[79] and it has also been reported that the feeding of cholesterol increases the amount of vitamin C required for normal growth.[7,79]

It seems reasonable to conclude that under normal conditions rats and rabbits synthesize vitamin C in amounts close to the optimal levels.[80,81] However, the feeding of cholesterol could increase the optimal amount of the vitamin, and thus affect the rate of its synthesis. For example, several drugs cause a dramatic increase in the synthesis of vitamin C in rats.[82,83] In one study, the administration of cholesterol to rats and rabbits increased the level of vitamin C in several tissues,[78] and this was presumed to be due to an increase in vitamin synthesis. In another experiment, cholesterol-feeding led to a decrease in vitamin levels in the tissues of rats,[84] with no effect being found on the rate of vitamin synthesis, although the rate of vitamin C catabolism was increased. However, among other experimental differences, an amount of cholesterol 2.5 times as high was used in the latter experiment, and such differences could possibly explain the discrepancy in the vitamin levels. Apparently, the rate of vitamin C synthesis is not increased enough in the animals that synthesize the vitamin when they are fed an atherogenic diet because supplemental vitamin causes a decrease in the cholesterol levels.[61–69] Novitskii[85] observed that in two groups of rabbits that responded to dietary cholesterol by either increasing or maintaining plasma cholesterol levels there were respective differences in vitamin C metabolism, and he speculated that the differences in the effects on dietary cholesterol could be due to the differences in vitamin C metabolism.

D. Lipoproteins and Lipid Oxidation

Vitamin C deficiency decreases HDL-cholesterol levels in guinea pigs,[17,20,21,38,39,56] and vitamin supplementation increases the HDL-cholesterol levels in guinea pigs fed an atherogenic diet.[51] When vitamin-dependent rats were put on a vitamin C-deficient diet, their plasma HDL-cholesterol level became lower than in mutant rats that were given the vitamin,[74,76] or in normal rats.[77] The mechanism whereby vitamin C could affect the level of HDL-cholesterol is not clear, but it is noteworthy that vitamin C seems to increase the activity of lipoprotein lipase (see Sections III and V.C), which in turn appears to participate in the regulation of HDL metabolism.[86,87]

In guinea pigs, vitamin C deficiency decreases the rate of catabolism of LDL-cholesterol,[88] and increases the level of LDL-cholesterol in plasma.[20,37,38,56] High levels of the vitamin significantly decrease LDL cholesterol levels in guinea pigs that are fed an atherogenic diet.[51] In one study using vitamin-dependent rats, LDL-cholesterol levels were about three times higher in the group receiving a vitamin-deficient diet than in the control group.[89] Furthermore, vitamin C increases the number of LDL receptors in cultured arterial smooth muscle cells.[90] It has also been suggested that the vitamin plays an important role in the metabolism of lipoprotein(a), which has been linked with atherosclerosis.[91–93]

Lipid oxidation has emerged as a potential factor in the etiology of atherosclerosis.[94,95] Vitamin C is the major water-soluble antioxidant,[96–99] and it may decrease lipid oxidation, either directly or indirectly, by regenerating vitamin E, the major lipid soluble antioxidant.[99–102] It has been reported recently that vitamin C may protect low-density lipoproteins[103–105] and plasma lipids[96,97] against free radical-mediated oxidation. Previous data have indicated that vitamin C deficiency increases CCl_4-induced lipid oxidation *in vivo* in guinea pigs,[106] and that vitamin C supplementation decreases lipid peroxidation *in vivo* in rats that have an iron overload.[107] Furthermore, it has been reported that people with low vitamin C levels have higher amounts of lipid peroxides in plasma than do people with high vitamin levels.[108] Accordingly, vitamin C could also affect cholesterol metabolism through the antioxidant effect.

Because of the similarity among mammals at the biochemical level, vitamin C may play a role in the cholesterol metabolism of human beings also. However, there may be large quantitative differences between the metabolism of different species, and, therefore, studies in man are required in order to estimate whether vitamin C affects cholesterol metabolism in man to a degree that is quantitatively significant.

III. TRIGLYCERIDE METABOLISM

Vitamin C appears to affect triglyceride metabolism. Several studies have reported an increase in plasma triglyceride levels in guinea pigs that have been administered a diet containing a minimal amount of vitamin C.[16–23,28,35,37–39,52,109–112] When guinea pigs,[16,18,51] hamsters,[3] rats, or rabbits[64,69] are administered extra vitamin C, the increase in triglyceride levels caused by an atherogenic diet is partially prevented. Also, a statistically significant decrease in plasma triglyceride level was found when vitamin C was given to rats that were fed on an otherwise normal diet.[71] Finally, low vitamin C levels have been associated with increased triglyceride levels in baboons[113] and rhesus monkeys.[114,115]

Sokoloff et al.[64] suggested that the lowering of triglyceride levels by vitamin C could be due to an effect on lipoprotein lipase, which is the major enzyme that degrades plasma triglycerides.[116] They found that concomitantly with the elevation of triglyceride levels, lipoprotein lipase activity steadily decreased in rats and rabbits that were fed cholesterol. Extra vitamin C completely prevented the decrease in lipoprotein lipase activity and partially prevented the increase in triglyceride levels.[64] Furthermore, in guinea pigs vitamin C deficiency decreased,[37,110] and in baboons vitamin C supplementation increased[117] lipoprotein lipase activity. So far, the mechanism whereby vitamin C could affect the activity of lipoprotein lipase has not been determined.

Vitamin C also affects fatty acid metabolism by participating in the synthesis of carnitine.[118,119] Carnitine plays a crucial role in the transport of long-chain fatty acids into mitochondria where β-oxidation takes place.[119,120] Carnitine deficiency has been suspected to play a role in some diseases including hyperlipidemia.[121] In some studies with human subjects, administration of carnitine has led to decreased triglyceride levels.[121] In scorbutic guinea pigs, the carnitine levels of various tissues decrease[122–126] and, moreover, administration of carnitine to scorbutic guinea pigs slowed down weight loss and increased the survival time by 10%.[127] Marginal vitamin C deficiency also decreases carnitine levels.[22,112]

IV. PHYSIOLOGICAL STUDIES IN MAN AND PRIMATES

A. Acute Scurvy

In scorbutic human beings plasma cholesterol levels are reduced, apparently due to a decrease in synthesis and intestinal absorption.[128–130] Administration of vitamin C to scorbutic people caused an increase in cholesterol levels.[128,129] Still, acute scurvy may affect cardiac functions: some scorbutic patients have ECG-abnormalities[131–133] and cardiomegaly,[134] which disappear after vitamin treatment. It has also been reported that atherosclerotic changes are reversed in certain subjects by vitamin C supplementation, but the initial vitamin status of the subjects was not determined.[135]

The effects of a vitamin C-deficient diet on cholesterol metabolism has been studied in monkeys. In baboons[113] and rhesus monkeys[114,136] plasma cholesterol level decreased, but in marmoset monkeys it increased.[137] Liver homogenates from scorbutic baboons incorporated [^{14}C]-acetate into cholesterol at a reduced rate.[138,139] It should be noted that these studies do not test the effect of long-term marginal vitamin C deficiency. In guinea pigs, acute vitamin C deficiency has caused variable effects, but a long-term marginal deficiency has consistently increased cholesterol levels in plasma and tissues (see Section II.A).

B. Supplementation and Depletion in Man

Two studies have been conducted to examine the effects of vitamin C supplementation (1 to 5 g/d) on bile acid metabolism in healthy, well-nourished humans.[140,141] Vitamin supplementation had no clear effects on bile acid levels. On the other hand, results from animal studies suggest that the most significant effects should be expected when a long-term marginal deficiency of vitamin C is compared with good vitamin status, and not by supplementation of subjects that have initially good vitamin status (see Section II.A).

The effect of dietary restriction of vitamin C was examined in one study, with no consistent effects being observed in plasma cholesterol, biliary lipid composition, or bile acid synthesis rate.[142] However, the study lasted for quite a short period: the vitamin C level in plasma barely reached low levels (10 μM) during the test period of 2 months. In contrast, the studies with guinea pigs that had reached low vitamin C levels before the start of the trial usually lasted for 2 to 5 months.[6–31] Also, four out of five subjects in the study[142] had a high initial vitamin C level (over 45 μM). Quite a long depletion period may be required to observe whether low vitamin levels have noticeable effects in subjects with such high initial vitamin levels. Interestingly, vitamin depletion did cause a reduction in bile acid synthesis in the subject who had the lowest initial vitamin level (27 μM), and the synthesis returned to base line levels when the vitamin was readministered to him.[142]

Even if vitamin C affects cholesterol metabolism in humans, it seems obvious that not all people respond to low vitamin levels by developing an elevated cholesterol level. In general, the correlations between vitamin C and cholesterol levels are quite low, even though the negative correlation has reached statistical significance in several studies (see Section VI.A). Still, it is possible that in certain subgroups low vitamin C level tends to elevate cholesterol level, which could, in turn, be lowered through vitamin C supplementation.

V. INTERVENTION STUDIES

The effect of supplemental vitamin C on plasma cholesterol has been analyzed in a number of intervention studies (Table 1). The results are quite conflicting, yet a moderately consistent picture may be obtained when taking into account some of the apparent differences in the studies.

TABLE 1
Vitamin C and Plasma Cholesterol: Intervention Studies

Study	No. subjects; age, mean/ range (y)[a]	Vitamin dose (g/d)	Duration of study (weeks)	Control group	Pretreatment		Change in cholesterol[b] (%)	Ref.
					Vitamin C (μ*M*)	Cholesterol (m*M*)		
Low initial plasma cholesterol (average below 5 m*M*)								
Anderson et al. 1972	41, 21	1	14	P	—	4.8	+5[c]	143
Crawford et al. 1975	18, 25	1	12	C	—	4.9	+7 (+4)	144
Menne et al. 1975	122, 18–25	1	16	P	74	4.5	+9 (+5)	145
Horsey et al. 1981	14, 82	1	6	—	—	4.6	+6	146
Johnson 1981	9, 20–40	1	6	P	74	4.3	+5 (+6)	147
Joshi et al. 1981	27, 17–20	2	3	—	—	4.2	+14	148
Khan 1981	13, 21–28	1	4	—	60	4.6	0	149
Buzzard et al. 1982	20, 21–35	2	6	P	78	4.1	0 (+4)	150
	20, 21–35	2[d]			84	4.1	+8 (+7)	
Average, group 1:						4.4	+6.1	
Medium initial plasma cholesterol (average 5–6.5 m*M*):								
Sokoloff et al. 1967	40, 17–36	2	22–26	P	—	5.3	−2 (+2)	64, 151
	28, 44–70	2	34			6.4	−3 (+3)	
Spittle 1971	58, —	1	6	—	—	5.5	−1	152
	25, 54[e]					6.3	+8 ***	
Hanck 1973	10, 25–45	4	3	—	58	5.5	−8 **	17, 153
Crawford et al. 1975	16, 25	1	12	C	—	5.1	+2 (+6)	144
Ginter et al. 1977	82, 64	1	12	—	23	5.4	−5	154
Kothari and Jain 1977	20, 20–30	1	4	C	43	5.3	−13 (+7) **	155
Vijayakumar 1980	30, 30–45	3	1	—	—	5.0	−6 ***	156
Horsey et al. 1981	11, 82[e]	1	6	—	—	5.2	0	146
Fidanza et al. 1982	20, 44–79	3	3	—	—	5.8	−14 **	157
Dobson et al. 1984	19, 29	1	26	C	48	5.9	−17 (+5) **	158
Koh 1984	23, >35	1	12	—	90	6.2	−10	159
Burr et al. 1985	130, 65–74	0.15	6	C	9	5.3	+10 (+10)	160
Erden et al. 1985	25, 21	2	8	—	—	5.2	−8 **	161
Aro et al. 1988	27, 81	2	6	DB	24	5.4	0 (+5)	162
		0.2					+4 (+5)	
O'Brien 1988	20, 45	1.5	12	C	56	5.1	−1[c]	163
		1.5[d]				5.4	0[c]	
Salonen et al. 1991	39, 54	0.6[f]	20	DB	28	5.6	−2[c]	164
Average, group 2:						5.6	−5.2	
High initial plasma cholesterol (average over 6.5 m*M*):								
Vitamin C level was determined								
Ginter et al. 1970	42, >40	0.3	7	C	35	6.6	−7 (+5) **	165
	13,[g]					7.4	−12 **	
Peterson et al. 1975	9, 46	4	8	—	85	7.9	0	166
Ginter et al. 1977	35, 62	1	52	C	23	6.8	−13 (−4) ***	154
Kothari and Jain 1977	20, 31–50	1	4	C	42	6.6	−12 (−2) **	155
Ginter et al. 1978	48, 50–60[h]	0.5	52	DB	22	8.6	−19 (−5) ***	167
Ginter et al. 1979	21, 44	0.45[i]	6	—	48	7.2	−9 ***	168
	11, 44[j]					9.4	−19 ***	

TABLE 1 (continued)
Vitamin C and Plasma Cholesterol: Intervention Studies

Study	No. subjects; age, mean/ range (y)[a]	Vitamin dose (g/d)	Duration of study (weeks)	Control group	Pretreatment Vitamin C (μ*M*)	Pretreatment Cholesterol (m*M*)	Change in cholesterol[b] (%)	Ref.
High initial plasma cholesterol (average over 6.5 m*M*):								
Vitamin C level was determined								
Bordia 1980	40, 40–60[e]	2	26	DB	30	7.4	−11(0)	169
		1					NS[k]	
Dobson et al. 1984	10, 58	1–2	52	—	59	7.0	−14 *	158
Bishop et al. 1985	50, 56[h]	0.5	8	DB	41	7.0	−5 (−5)	170
Gulenc 1988	38, —	2	8	—	43	6.9	−16 ***	171
Ginter 1989	14, —[l]	0.8[i]	18	—	44	11.4	−7 **	172
	13, —[m]				28	9.3	−9 ***	
	7, —[h]				43	9.6	−11 *	
Vitamin C level not reported								
Samuel 1964	14, 42	1–6	5–16	—	—	9.7	−2	173
Sokoloff et al. 1967	109, 47–78[n]	2–3	44–104	P	—	8.0	−21 (−3) °	64, 151
Ginter 1975	24, 62	1	26	—	—	6.6	−17 ***	10
Filho et al. 1978	19, 75	2	2	—	—	6.7	−43 °	174
Heine and Norden 1979	63, 57[e]	1	68	—	—	8.5	−11 °	175
Fidanza et al. 1982	20, 63	3	3	—	—	8.1	−18 **	157
Wahlberg 1982	9, 55	2	4	P	—	7.4	0 (−2)	176
Mamianetti et al. 1988	14, 48	1.5	3	—	—	6.5	−9	177
Average, group 3:						7.8	−12.4	

Note: control groups: DB, double-blind placebo control; P, placebo control; C, control group without placebo; —, no control group. Averages for the three groups: three studies[143,163,164] are not included because they did not report the change from the pretreatment level. Only the higher dose group of two studies[162,169] is included in the average, and one study[160] was not included because the vitamin dose was only 0.15 g/d.

[a] The number of subjects includes the control group.
[b] In parenthesis: the change in the control group. The level of significance for the vitamin group as reported in the publications: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.
[c] Comparison to control group.
[d] Subjects received three eggs per day.
[e] Patients with atherosclerosis.
[f] 300 mg vitamin E, 27 mg β-carotene, and 75 μg selenium were also given each day.
[g] Subgroup with serum cholesterol values over 6.2 m*M*.
[h] Patients with diabetes.
[i] Pectin (15 g/d) was administered along with the vitamin C.
[j] Hyperlipemic outpatients, six of them with diabetes.
[k] No significant effect; data not provided.
[l] Familial hypercholesterolemia.
[m] Polygenic hypercholesterolemia.
[n] Groups 3 to 6 pooled from Reference 151.
[o] No statistical analysis was made by the authors.

A. Pretreatment Cholesterol Level

Several studies in this area have used subjects with initially low levels of plasma cholesterol (group 1 in Table 1). The animal experiments and the biochemical data of the mechanism do not suggest that supplemental vitamin C would considerably decrease an initially low cholesterol

level. Furthermore, while elevated cholesterol levels (above 5 m*M*) are associated with a progressive increase in mortality, especially due to coronary heart disease (CHD), an increase in mortality is also noted with low cholesterol levels (below 4.5 m*M*).[178] Low levels of cholesterol have been associated with increased risk of cancer[179] and hemorrhagic stroke,[180] and such effects appear to overshadow the benefits of a minor decrease in CHD that may occur by lowering cholesterol levels below 5 m*M*.[178]

Accordingly, there is no clear biochemical basis for the expectation, and no apparent medical reason for the hope, that rather low cholesterol levels would be further decreased by vitamin C supplementation. Therefore, it seems quite surprising that several of the intervention studies have used subjects with initially low cholesterol levels (group 1). None of these studies found an actual decrease in the cholesterol level by the vitamin.

A number of studies have used subjects with initially medium levels of plasma cholesterol (group 2). These studies have mostly found minor or moderate decreases in cholesterol levels, which have been statistically significant in a few cases (Table 1). However, one of these studies reported a statistically significant increase in cholesterol level in one of the study groups.[152]

If low vitamin C status decreases cholesterol catabolism, and increases cholesterol levels even in a subgroup of people, then one may expect the greatest effects of supplemental vitamin on a group with high cholesterol levels. Such a group could contain subjects in whom the cholesterol catabolism is limited by low levels of vitamin C. Several trials have used hypercholesterolemic subjects, and these studies have quite consistently reported a substantial decrease in the cholesterol level by vitamin C supplementation (group 3 in Table 1). Only two studies[166,176] in this group did not report any absolute decrease caused by the vitamin, and one study found no difference between the effects of vitamin and placebo.[170]

There has been a large variation in the technical quality of the intervention studies. Several of the studies did not use any kind of control group, and in many cases the control group did not receive a placebo (Table 1). In two studies the control group was not comparable to the vitamin group.[154,165] Furthermore, the experimental design was poorly described in one of the placebo controlled studies that has shown a notable decrease in cholesterol level.[64,151] Obviously, the calculation of statistical significance is rather doubtful in the absence of a proper control group that would give a satisfactory estimate of the placebo effect. However, for the technical details of the three dozen studies, the reader is referred to the original publications.

In a further analysis of the intervention studies, the placebo-controlled trials were considered separately (Figure 2). There is a significant correlation between the initial cholesterol level and the change in cholesterol level in the placebo groups ($r = -0.90$; $p < 0.001$) and in the vitamin C groups ($r = -0.86$; $p < 0.001$). According to these studies, vitamin C does not differ from placebo if the initial cholesterol level is about 4.5 m*M*; at this level the placebo increases plasma cholesterol by some 5%. The difference between vitamin C and placebo progressively increases with the increase in the pretreatment cholesterol level (Figure 2). In subjects with plasma cholesterol of 9 m*M*, the linear regression lines for the placebo and vitamin C group predict decreases of 6 and 18%, respectively, in the cholesterol level.

Only three of the study groups match poorly with the regression lines (Figure 2). First, the cholesterol level (4.1 m*M*) did not increase in another of the vitamin groups of Buzzard et al.[150] Second, the decrease in cholesterol level (from 7.0 m*M*) by placebo in the study of Bishop et al.[170] was significantly larger than expected by the regression line: −5 vs. 0%, respectively. Third, Wahlberg and Walldius[176] observed absolutely no effect by vitamin C on the cholesterol level (7.4 m*M*), whereas the regression line predicts a decrease of 10%. Still, the agreement of the results with the two regression lines is striking, when taking into consideration the large variations in the studies with respect to the selection of the subjects, the design of the study, etc.

It is noteworthy that placebo causes an increase in low cholesterol levels, but a decrease in high levels (Figure 2). The increase in low cholesterol levels by placebo has in general not

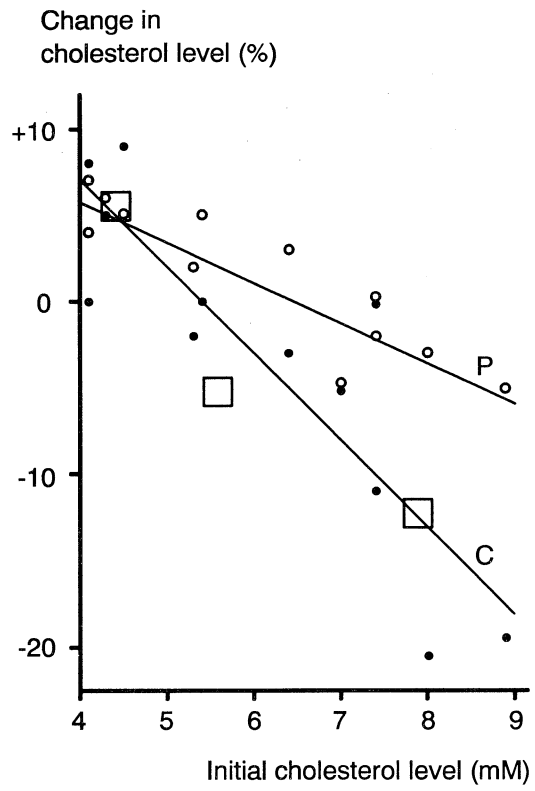

FIGURE 2. Change in cholesterol levels in placebo groups (o) and vitamin C groups (•). The studies are taken from Table 1 (control group: P or DB). Two studies[143,164] are not included because only the difference between the vitamin and placebo groups was reported, and thus the changes from the pretreatment cholesterol level are not known. Only the higher vitamin C dose groups of two other studies[162,169] were included. Linear regression lines for the placebo (P) and vitamin C (C) groups are shown.

been considered appropriately. For example, Aro et al.[162] found no effect at all on cholesterol by vitamin C, whereas the placebo caused an increase by 5%. This was considered to be an indication that vitamin C does not affect cholesterol metabolism. However, the pretreatment cholesterol level was 5.4 m*M*, and at this level the regression lines suggest no effect of vitamin C, but a small increase by the placebo (Figure 2). Thus, paradoxically, in this case a zero effect by vitamin C may indicate a meaningful difference from placebo. However, the observation was not statistically significant because such a small number of subjects were used.[162] The behaviour of the placebo groups (Figure 2) may be due to regression toward mean.

The regression lines are not markedly affected if those studies that used control groups that were not administered a placebo are also included. Furthermore, the averages for the three groups of Table 1 fall quite close to the vitamin C-regression line (Figure 2). An inspection of the placebo groups (Table 1 and Figure 2) indicates that the decreases in cholesterol that were reported in the uncontrolled studies of group 3 are not explainable by just a placebo effect. So far, the largest decreases in cholesterol level by a placebo have been only −5%, whereas 13 studies in group 3 have reported larger decreases in plasma cholesterol than that (Table 1).

Accordingly, the pretreatment level of cholesterol seems to be an important factor in determining whether supplemental vitamin C causes a decrease in the plasma cholesterol levels of the subjects. The importance of pretreatment cholesterol level was previously suggested by the Russian authors,[1] and by Ginter, who observed the role of initial cholesterol level in two studies[154,165] and in an analysis of several of his own trials.[181] Still, there are also other factors that differ between and may affect the discordance of the results.

B. Other Factors Possibly Affecting the Results

On biochemical grounds one may expect that the effect of vitamin C is saturable. Thus, if the initial vitamin C level is very high one should not expect any noticeable effect of additional vitamin, since in such a case hypercholesterolemia may not be caused, even partially, by a marginal vitamin deficiency. It is noteworthy that the study by Peterson et al.,[166] which found no effect of the vitamin, used subjects that had the highest pretreatment vitamin C level of group 3a. Apparently, with such high vitamin C levels (85 μ*M*), the vitamin may not be rate limiting in cholesterol catabolism. In fact, such a high base line vitamin level suggests that the diet of the subjects contained over 300 mg/d of vitamin C,[147,155,162–164,167–170,182] which is much higher than the RDA recommendation (60 mg/d; Reference 183).

The vitamin dose may also affect the results. Bordia[169] observed a notable decrease in plasma cholesterol level with 2 g/d of vitamin C, but not

with the smaller amount of 1 g/d. Aro et al.[162] found that when compared with the placebo, 2 g/d of vitamin C decreased plasma cholesterol level by −5%, whereas 0.2 g/d decreased it by only −1%. Thus, the effect of the vitamin seems to be dose dependent. The amount of vitamin used by Burr et al.,[160] Salonen et al.,[164] and Bishop et al.,[170] which did not show any benefit compared with the placebo, were among the smallest of all studies (0.15 to 0.6 g/d). However, decrease in the plasma cholesterol level by a small dose of the vitamin (0.3 to 0.5 g/d) has been reported in some trials,[165,167,168] and there is no obvious correlation between the dose and the decrease in the cholesterol level in studies of group 3a. Thus, no clear dose-effect relationships may be inferred from the results available, aside from the two studies referred to above.[162,169]

The duration of the study may also affect the results. Dobson et al.[158] observed that in older subjects (average 58 years), vitamin C slowly decreased the cholesterol levels over a period of 1 year, while the decrease was much faster in younger subjects. Similarly Sokoloff et al.[64,151] observed a slow decrease in plasma cholesterol levels in elderly people (average 60 years) over a 1 year time period. This suggests that the length of the trial may be an important factor in the case of elderly subjects. A few trials with elderly people have lasted for a relatively short period,[152,170,176] which could affect the results.

Several studies have indicated that there are large individual variations in the response to vitamin C supplementation. Ginter et al.[154,167,168] noted that approximately one third of their subjects did not show any benefit from the vitamin. Sokoloff et al.[64,151] found no effect in 20% of the hypercholesterolemic subjects used, but found varying benefits for others. Samuel and Shalchi[173] found a statistically significant decrease in plasma cholesterol levels in 3 persons out of 14, whereas none of the subjects experienced a significant increase. Such large differences in individual responses may depend on several factors. The groups are heterogeneous with respect to pretreatment levels of cholesterol and vitamin C. Furthermore, there may be large differences in individual responses to low vitamin C levels in human beings, in the same manner as has been observed in guinea pigs.[184,185] Accordingly, certain individuals could be sensitive to low levels of vitamin C regarding the catabolism of cholesterol, even if the majority were not.

Moreover, there are also other factors affecting plasma cholesterol levels. For example, nutritional factors such as dietary palmitate, cholesterol and fiber,[186–189] and genetic factors[190,191] are important in determining plasma cholesterol levels. Holloway et al.[31] found that in guinea pigs the effect of marginal vitamin C deficiency on cholesterol levels and bile acid metabolism depended on the type of feed given, thus underscoring the role of basic diet. Unfortunately, the diets used in the intervention studies have not been described in such detail that the role of, for example, dietary fats, cholesterol, or fiber could be evaluated as a possible explanation for some of the variation in the results. A combination of vitamin C and pectin was used in two groups,[168,172] but there were no control groups to determine whether the observed effect differs from that of vitamin C alone (or pectin alone). Also, there is a limited amount of information describing the subjects in the studies. Thus, one cannot conclude whether the effect of vitamin C depends on the type of hyperlipidemia that the patients suffer; one would expect only a small effect in patients with familial hypercholesterolemia.

The inaccuracy in the measurement of vitamin C and cholesterol levels, as well as within-person variation in the level from day to day may also partially account the differences among studies. These factors are most important in studies using a small number of subjects. On the other hand, the role of these and other random variables should be markedly decreased when averaging the results of several studies; for that purpose the averages for the three groups was calculated (Table 1, Figure 2).

Of the factors discussed, the pretreatment level of cholesterol seems to be by far the most important in predicting the effect of vitamin C. High pretreatment vitamin C level and low vitamin dose indicate that only a small effect may be expected, but the data are too limited for any quantitative conclusions with respect to these factors. Furthermore, there may be large individual variations in the effects of vitamin C on choles-

terol metabolism, and the duration of the study may be important, especially in the case of elderly subjects.

C. HDL-Cholesterol and Triglycerides

HDL-cholesterol levels are inversely related to the risk of CHD, and HDL-cholesterol appears to be a stronger indicator for that risk than total plasma cholesterol.[86,193,194] The level of HDL-cholesterol has been measured in several intervention studies. In some studies the level of HDL-cholesterol was increased by vitamin C supplementation and this reached statistical significance in six study groups (Table 2). Furthermore, Buzzard et al.[150] found a considerable increase in the HDL-cholesterol levels in three individuals that had the lowest base line vitamin C levels. So far, no obvious biochemical mechanism is known whereby one should expect vitamin C to increase the HDL-cholesterol level specifically, but the apparent effect of the vitamin on lipoprotein lipase could be such a link (see Sections II.D and III).

An elevated triglyceride level appears to be a risk factor for CHD, but it is usually associated with other risk factors, such as low HDL-cholesterol, and thus it has been difficult to evaluate its precise significance.[195] The effect of vitamin C on triglyceride levels has been studied in 27 groups of subjects (Table 2). It is notable that a decrease in triglyceride levels has been reported in 20 study groups. The triglyceride level was increased only in three study groups: in one study the change in triglyceride levels was −8% after 3 weeks, but +20% after 6 weeks,[168] and in another study the increase in the placebo group was even higher.[170] Bishop et al.[170] found a notable, but not statistically significant, decrease in triglyceride levels in subjects with initially very low vitamin C levels. The results of Sokoloff et al.[151] suggest that the effect of vitamin C could be more pronounced in subjects with elevated triglyceride levels (Table 2). Interestingly, Geoly and Diamond[196] observed in a brief report that the elevated triglyceride levels (9 and 12 m*M*) of two patients were decreased by 60% with the administration of vitamin C (3 g/d).

Vitamin C could decrease triglyceride levels by affecting lipoprotein lipase activity or carnitine synthesis (see Section III). Sokoloff et al.[64,151] found that concomitantly with the decrease in triglyceride levels by the vitamin, the activity of lipoprotein lipase steadily increased in the patients. Davies et al.[197] found that supplementation of elderly men with vitamin C (0.2 g/d) resulted in an increase of urinary excretion of carnitine, and ending of supplementation reduced the excreted carnitine level. They found also a positive correlation between levels of leukocyte vitamin C and urinary carnitine ($r = 0.5$, $p = <0.01$). However, in another study no effect of vitamin C on urinary carnitine was found.[198]

Thus, the intervention studies suggest that in some cases vitamin C may increase HDL-cholesterol levels and decrease triglyceride levels. However, because the results are not quite consistent, and most of the studies have lacked a proper control group, the conclusions may be only preliminary. Possibly more pronounced and less-disputable effects could be observed in properly controlled studies with subgroups that have, for example, low HDL-cholesterol levels or elevated triglyceride levels.

VI. EPIDEMIOLOGICAL STUDIES

A. Cholesterol, HDL-Cholesterol, and Triglycerides

Numerous cross-sectional studies have been carried out to determine whether there is a correlation between plasma vitamin C level and the levels of total cholesterol or HDL-cholesterol (Table 3). If a low vitamin level tends to increase plasma cholesterol levels, then a negative correlation between vitamin C and cholesterol should be observed, which has been the case in a majority of the studies. In several cases, the negative correlation has been significant statistically (Table 3). A single notable exception to this general pattern is the study by Saha and Tan,[215] which found a statistically significant positive correlation between the levels of vitamin C and cholesterol.

TABLE 2
The Effect of Vitamin C on HDL Cholesterol and Triglyceride Levels

Study	No. subjects	HDL-cholesterol (m*M*)	Change (%)	Triglycerides (m*M*)	Change (%)	Ref.
Sokoloff et al. 1967	40	—		0.76	−3 (−1)	151
	28			1.1	−5 (+2)	
	35			1.4	−37 (+1)	
	12			1.8	−6 (−3)	
	36			2.0	−41 (−8)	
	26			2.4	−51 (−5)[a]	
Menne et al. 1975	122	—		1.3	−25 (−22)	145
Peterson et al. 1975	9	0.67	−34	1.7	−13	166
Ginter et al. 1978	48	—		2.6	−18 (−2)*	167
Ginter et al. 1979	21	1.5	−5	2.2	+20	168
Heine and Norden 1979	63	—		3.8	0	175
Bordia 1980 (2 g/d)	30	—		1.8	−5 (0)	169
Horsey et al. 1981	7	0.80	+19 *	1.1	+7	146
	7	1.5	−5	1.5	0	
	6[b]	0.67	+31 *	1.8	−14	
	5[b]	1.2	+21 *	2.2	−27 *	
Johnson and Obenshain 1981	9	1.1	+5 (+4)	1.1	0 (+8)	147
Joshi et al. 1981	27	1.8	−3	—		148
Khan and Seedarnee 1981	13	1.5	0	0.67	−5	149
Buzzard et al. 1982	40	1.2	+2 (−2)	—		150
Fidanza et al. 1982	20	—		2.0	−12 *	157
Wahlberg and Walldius 1982	9	1.1	0 (+4)	4.8	0 (−14)	176
Koh 1984	23	1.1	0	2.5	−35 *	159
Bishop et al. 1985	50	—		2.8	+11 (+19)	170
Erden et al. 1985	15	1.5	+27 *	1.7	−21 *	161
Aro et al. 1988 (2 g/d)	27	1.2	−2 (0)	1.3	−8 (+6)	162
Gulenc and Nebioglu 1988	31	1.4	+13 *	1.6	−14 *	171
O'Brien and McMurray 1988	20	1.1	−9[c]	1.4	−10[c]	163
		1.0	+7[c]	1.5	−15[c]	
Ginter 1989	14	1.4	+7 *	—		172
	13	1.4	+2			
	7	1.1	+9			
Salonen et al. 1991	39	1.3	−5[c]	—		164

Note: For the age of subjects, vitamin dose, and the duration of the study see Table 1. Number in parenthesis shows the change in the control group. Statistical significance: *, $p < 0.05$. Triglyceride levels in (mg/dl)-units were converted to (m*M*)-units, assuming that 1 m*M* corresponds to 88 mg/dl.[192]

[a] Control group was poorly matching: average triglyceride level was 1.9 m*M*.
[b] Patients with CAD.
[c] Comparison to control group.

TABLE 3
Vitamin C, Plasma Cholesterol and HDL-Cholesterol: Cross-Sectional Studies

Study	No. of subjects; age, mean/range (y)	Plasma vitamin C, average (μM)	Cholesterol, correlation coefficient[a]	HDL-cholesterol, correlation coefficient[a]	Ref.
Masek 1960	35, —	40	−0.42 *	—	199
	36, —	50	−0.26	—	
Cheraskin and Ringsdorf 1968	127, 22	25	−0.08 +0.27[b] **	—	200
Kajaba and Bucko 1968	>450, 7–11	60	−neg ***	—	201
Elwood et al. 1970	154, adults	35	+0.02	—	202
Jain et al. 1976	150, 18–50	40	−0.31 **	—	203
Bates et al. 1977	11, 72–86, female	30	0.0	−0.28	204
	12, 72–86, male	15	+0.30	+0.71 **	
Cerna and Ginter 1978	600, 25–55	—	−0.18[c] ***	—	205
Koh and Stewart 1978	304, 5–80	—	−0.05	—	206
Bates et al. 1979	228, 77, female	20	NS	+0.13 *	207
	109, 76, male	15	NS	+0.12	
Olusi et al. 1979	57, 32	90	−0.34	—	208
	30, 35[d]	50	−0.61 *	—	
	32, 33[d]	50	−0.49 *	—	
Koh and Chi 1980	245, >34, female	—	+pos		209
	184, >34, male		−neg		
Dwyer et al. 1981	530, 21	—	—	NS[e]	210
Horsey et al. 1981	12, 87, female	—	—	NS[c]	146
	13, 77, male	—	—	+0.60[c] *	
Burr et al. 1982	97, 51, male	45	−0.23 *	+0.21 *	211
Burr et al. 1982	97, 46, female	50	+0.12	+0.21 *	212
Greco and Rocca 1982	70, 70–84	30	−0.27 *	—	213
Hooper et al. 1983	270, 72	70	NS	NS	214
Saha and Tan 1983	198, 24	65	+pos *	—	215
Church et al. 1984	16, 34, female	100 mg/d[e]	—	+0.54[e] *	216
	13, 35, male	140 mg/d[e]		+0.53[e]	
Yoshioka et al. 1984	194, 30–39	40	−0.07	−0.02	217
Mayet et al. 1986	382, 44	—	−0.02[c]		218
Jacques et al. 1987	680, 73	>55[f]	+0.03[g]	+0.09[g] *	219
Kothari et al. 1988	104, 0–85	50	−0.66 **	—	220
Aro et al. 1988	27, 81	—	−0.05	—	162
O'Brien and McMurray 1988	20, 45	—	NS	+pos *	163
			−neg[c] *	NS[c]	
Salonen et al. 1988	1132, 54	40	—	NS	221
Dallal et al. 1989	146, 60–96, female	60	—	+0.21[g] * +0.22[g,h]	222
	92, 60–96, male	45	—	+0.10[g] +0.43[g,h] **	
Itoh et al. 1990	96, 71, female	60	−0.31 **	+0.37 **	223
	79, 71, male	50	−0.03	+0.24 *	

TABLE 3 (continued)
Vitamin C, Plasma Cholesterol and HDL-Cholesterol: Cross-Sectional Studies

Study	No. of subjects; age, mean/range (y)	Plasma vitamin C, average (μM)	Cholesterol, correlation coefficient[a]	HDL-cholesterol, correlation coefficient[a]	Ref.
Riemersma et al. 1990	131, 46[i]	20	+0.06	+0.12	224
	99, 44[j]	30	−0.07	−0.04	
	85, 44[k]	35	−0.19	+0.07	
	80, 46[l]	40	+0.01	+0.05	

Statistical significance according to the original articles except Reference 199, which was calculated by this author: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Value of correlation coefficient not reported: −neg, negative; +pos, positive; NS, not indicated.
Lingual vitamin C test; the value is inversely related to plasma vitamin C level.
White blood cell vitamin C level.
Asthmatic patients.
Daily vitamin C intake, correlation to vitamin C intake.
Serum vitamin C level in 62% of males and in 82% of females over 55 μM.
Partial correlation coefficient.
Apoprotein A-I.
Scotland.
North Karelia, Finland.
Southwest Finland.
Italy.

Vitamin C levels decrease and cholesterol levels increase with age,[203,220,225] which could be due to the former process effecting the latter. However, there are other factors that may also increase cholesterol levels with age, and therefore the significance of the decrease in vitamin C level is not clear. Although several of the epidemiological studies have used both young and old subjects, the role of age has not been considered. However, some studies have used subjects of a small age range,[200,201,213,223] and the results of these studies indicate that the correlation between vitamin C and cholesterol is not just secondary to the effect of age on both factors.

Various studies have also examined the relationship between vitamin C and HDL-cholesterol (Table 3). Except for three study groups,[204,217] a positive correlation has been found consistently, and in several cases the correlation has reached a level of statistical significance (Table 3). It is notable that the positive association between vitamin C and HDL-cholesterol is more consistent in the cross-sectional studies than in the intervention studies (Table 2).

Five studies have reported a negative correlation between the levels of vitamin C and triglycerides,[205,209,213,217,223] and two of these correlations were statistically significant.[205,223] As far as this author is aware, no study has reported a positive correlation between vitamin C and triglyceride levels.

The cross-sectional studies probably underestimate the true magnitude of the role of vitamin C, since the relationship may be attenuated by several factors such as the inaccuracy of measurements, within-person fluctuations in the levels of cholesterol[226,227] and vitamin C,[147,228] and variations in other factors affecting cholesterol levels (diet, genetics, etc.). In addition, the relationship between vitamin C level and cholesterol metabolism may differ among individuals. The large variations caused by such factors could result in observed correlational coefficients that underestimate the true relationship in the case of sensitive individuals (for an analogous case see Reference 229). Accordingly, vitamin C could be a moderately important determinant for the plasma level of cholesterol and HDL-cholesterol in some subgroups of normal populations. It is interesting that Olusi et al.[208] found a low negative correlation between the vitamin C and cholesterol levels in a control group, whereas in two

groups of asthmatic patients the negative correlation was significant.

B. Mortality and Morbidity

Some epidemiological studies have analyzed the connection of vitamin C levels to mortality and morbidity from atherosclerosis. In different regions of Great Britain, the mortality from CHD and cerebrovascular disease was found to be negatively correlated with the average vitamin C intake that varied in the range of 48 to 57 mg/d.[3,230] A similar inverse correlation[231] was also found with CHD and the amount of fruits and green vegetables eaten, which are good sources of vitamin C. A comparison of 16 populations of European countries found that low vitamin C levels were more common in regions with high mortality rate from CHD.[232] However, the correlation with vitamin E level was stronger. A comparison of 22 districts of Scotland found low vitamin C levels as the most notable dietary factor associated with mortality from CHD.[233]

In one case-control study,[234] it was found that subjects with abnormal coronary arteries had significantly lower leukocyte vitamin C levels than subjects of the control group. In another case control study, patients with angina pectoris were found to have significantly lower vitamin C concentrations than controls.[235]

A steady fall in mortality rate from CHD has been observed in the U.S. It has been pointed out that concomitantly the gross production of vitamin C,[236] and the consumption of fruits[237] have increased. Whether this is due to a causal relationship or to chance is an open question. Lastly, a negative correlation between vitamin C level and blood pressure has been reported in some studies.[159,206,209,217,230,238–240]

VII. CONCLUSIONS

Animal studies have shown that vitamin C participates in cholesterol metabolism, and the most significant effect of the vitamin appears to be on the catabolism of cholesterol. Several intervention studies suggest that vitamin C may also have a substantial role in human cholesterol metabolism. However, epidemiological studies have usually found a low correlation between vitamin C status and plasma cholesterol, and, thus, it is possible that only a subgroup of people respond to low vitamin C levels with a notable elevation in plasma cholesterol levels. In animal studies, the effects of low vitamin levels are more pronounced when the animals are fed an atherogenic diet. This invites speculation that low vitamin C levels could be more critical for people that, for example, eat foods that are rich in saturated fat and cholesterol.

A. Nutritional Recommendations

Although no final conclusions about the medical significance of vitamin C in cholesterol metabolism may be made from the intervention studies carried out so far, the results evoke thoughts about the basis of nutritional recommendations. With respect to vitamin C, the nutritional recommendations are concerned only with the prevention of frank scurvy.[183,241–247]

The RDA recommendations[183] conclude that vitamin C does not affect plasma cholesterol level. This is based on the study of Peterson et al.,[166] which did not use any kind of control group, but used subjects that had a very high initial vitamin C level (85 μM). Still, this study is provided as a disproof of one study by Ginter et al.,[154] which is the only reference that is mentioned to suggest that vitamin C might decrease elevated plasma cholesterol levels. This latter study is not placebo controlled either. However, ten placebo-controlled studies were published before the recommendations (Table 1), and five of these used subjects with elevated cholesterol levels. Nevertheless, none of these studies is referred to in the RDA recommendations.[183] Accordingly, the selection of references may give a misleading impression of the number, quality, and results obtained in the intervention studies carried out prior to the publication of the RDA recommendations.

The recommendations consider that amounts greater than 1 g of vitamin C falls into the category of pharmacological use.[183] However, the

animals that synthesize vitamin C by themselves make amounts of 1 to 10 g/d when extrapolated to human size.[243] Furthermore, an evolutionarily close relative of ours, the gorilla, eats approximately 4 g/d of vitamin C,[248] and the diet of our remote ancestors probably contained 0.4 to 2 g/d of the vitamin.[81,249] Therefore, such levels do not appear to be unfamiliar to human physiology. Consequently, the levels that have been used in most of the intervention studies (0.3 to 2 g/d) should not be considered, *a priori*, as strictly pharmacological, even if they are considerably larger than the RDA recommendation of 60 mg/d.

The recommendations are based on a poorly defined concept of "nutrient need", which appears to lack a valid biochemical basis.[241,242,247] Recommendations are only intended to provide reasonable reserves to protect against frank scurvy, and they are not based on any studies suggesting that such a level would be best for long-term health.[183,241–246] In fact, several studies have suggested that low levels of vitamin C may be associated with increased morbidity and mortality due to causes that are unrelated to scurvy.[230–235,245,250–270] Even though the significance of these observations is not quite clear, a complete neglect of them seems unwise.

Scurvy is mostly attributed to a decrease in collagen synthesis.[119,243] However, vitamin C also participates in several other enzymatic[119,243,246,271–273] and nonenzymatic[96–101,270,274,275] functions, in addition to collagen metabolism. These other reactions may not necessarily proceed with optimal rates, even if the obvious signs of scurvy are absent. Amounts of vitamin C surpassing the recommendation have been suggested to be beneficial for the protection against nitrite[275] and oxygen radicals,[97] and for the degradation of cholesterol and xenobiotic substances.[246] Consequently, the lack of frank scurvy should not be used as the principal criterium upon which to base a decision as to whether vitamin C levels are adequate for long-term health.

B. Prevalence of Low Vitamin C Levels

When the signs of scurvy appear, the plasma concentration of vitamin C has fallen to approximately 5 μM, but there are marked individual variations in the emergence of the symptoms.[129,131,263] With a daily intake corresponding to the recommendation of 60 mg/d, the median vitamin C level in plasma is 25 μM in elderly men.[182] On the other hand, the intake of gram amounts of the vitamin may increase the plasma concentration to 100 to 200 μM.[145,158,159,162,166,276–278] Thus, even in the absence of frank deficiency the variation in the vitamin C intake may cause over twentyfold variation in the plasma vitamin level, which may be reflected on the rates of several vitamin C-dependent reactions.

Low vitamin C levels are not infrequent. In one study,[224] the median concentration of plasma vitamin C in randomly chosen adult men from Italy, Finland, and Scotland was 38, 33, and 18 μM, respectively. Five percent of the subjects studied had a vitamin level below 10, 6, and 6 μM, respectively, in the three countries. Furthermore, in some groups of elderly people[160,263] the mean vitamin C level may be as low as 10 μM. Accordingly, considerable segments of industrialized societies appear to have low vitamin C status, and therefore it is not only an academic question whether long-term marginal vitamin C deficiency increases cholesterol levels in certain susceptible individuals. It is possible that intakes larger than the recommended 60 mg/d would be beneficial for health, even though no definite conclusions may be made from the data available. Vitamin C is a cheap and safe nutrient, and several of the suspected side-effects of gram amounts have appeared to be unfounded;[279] yet, diarrhea[266] seems to be a common consequence in healthy people if they ingest 4 to 10 g/d.

Simonson and Keys[1] concluded in their review of the early Russian studies that, "the reports reviewed here require a careful consideration. Repetition of some of the studies, with more attention to controls and statistical analysis, would be essential for critical evaluation". Much work has been done since their review. Vitamin C has been shown to affect cholesterol catabolism in several animal species. However, the significance of vitamin C in cholesterol metabolism in humans is not settled, and the suggestions of the above authors are still valid. Obviously, further studies are required that are better planned with

respect to the selection of experimental subjects, and the use of proper control groups, in order to provide more reliable data about the role of vitamin C in the cholesterol metabolism of humans.

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