The Role of Ascorbic Acid in the Mosaic of Coronary Heart Disease: Lipid Metabolism and Antioxidant Functions

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Summary Ascorbic acid (AA) is purported to have a preventative role in coronary heart disease (CHD) incidence. Studies in animal models support an interaction of AA in lipid metabolism, while results from epidemiological and supplementation trials conducted in humans are inconsistent. However, AA may mediate its protective action against CHD via its function as an antioxidant, inhibiting lipid peroxidation and regenerating α -tocopherol. Although AA can exert potentially beneficial effects, excessive AA administration can lead to damaging effects via interactions with transition metals, such as iron and copper.

Key Words: ascorbic acid, coronary heart disease, lipid metabolism, antioxidants, copper, iron

Epidemiological evidence is suggestive of a protective role for ascorbic acid (AA) against coronary heart disease (CHD). While a high dietary AA intake has been linked with decreased incidence of coronary mortality [1, 2], epidemiological evidence has associated low plasma AA levels with increased risk of CHD [3–5]. AA has a demonstrated protective effect *in vitro* against oxidative modification of lipoproteins [6], which are implicated in atherogenesis [7]. AA may also enhance the antioxidant protection provided by α -tocopherol, the major lipid-soluble chain-breaking antioxidant present in low density lipoprotein (LDL). This is mediated via AA's regeneration of α -tocopherol from its oxidation product, the tocopheroxyl radical [8]. The purpose of this review is to examine the suggested protective effects of AA against CHD. It will primarily focus on AA's function in lipid metabolism and its antioxidant properties. The potential adverse effects of excessive AA consumption will also be discussed briefly.

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EFFECT OF AA ON LIPID METABOLISM

Studies in animals. The role of AA in lipid metabolism has been studied extensively in guinea pigs as, like humans, they are incapable of synthesizing AA. Long term hypovitaminosis C resulted in the accumulation of cholesterol in blood serum and/or a number of tissues, including the liver and the thoracic aorta [9], possibly due to a decreased rate of cholesterol catabolism [10, 11]. In genetically modified rats unable to synthesize AA, AA deficiency resulted in elevated serum and liver concentrations of cholesterol regardless of whether rats were fed a normal or cholesterol-containing diet [12]. In other studies involving transgenic rats, AA deficiency caused serum total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-C) levels to be elevated and high density lipoprotein-cholesterol (HDL-C) levels to be lowered [13, 14]. In contrast, a decrease in the cholesterol level of the serum, liver and aorta was observed in weanling rats fed a large dose of AA for one month [15]. Similarly, in guinea pigs receiving an atherogenic diet, high doses of AA resulted in reductions in liver and aorta cholesterol levels and serum triglyceride (TG) levels [16].

Epidemiological studies. The relationship between AA and HDL-C is of considerable interest as HDL-C is inversely associated with CHD [17]. Several [18–26] but not all [27] studies have reported a positive correlation between plasma AA and HDL-C concentrations (Table 1). This has been demonstrated in groups of varying ages, genders and cultural backgrounds. Similarly, while most studies have reported a positive correlation between dietary AA intake and plasma HDL-C levels [28, 29], another found no association between these two parameters [30]. The balance of the epidemiological studies suggests an inverse association between AA and HDL-C (Table 1).

Inverse associations have been reported between plasma AA and plasma TC [19, 22, 25, 31, 32], LDL-C [25] and TG [22, 25, 26, 31] levels (Table 1). In contrast, a positive correlation was shown to exist between serum TC and both plasma and leucocyte AA levels in Kenyan tribespeople [33]. Interestingly, Hallfrisch and colleagues [23] found a negative association between plasma AA and plasma TC, LDL-C and TGs in men, but the opposite association in women. However, as for the relationship with AA and HDL-C, a number of investigators have not demonstrated a significant association between plasma or leucocyte AA and TC, LDL-C, and TG concentrations (Table 1). Consequently, the epidemiological studies demonstrate that the relationships between AA and TC, LDL-C and TGs are inconsistent.

Supplementation studies. Supplementation trials have been conducted to test the epidemiological association between plasma AA and plasma lipids (Table 2). The results of some trials demonstrated a beneficial influence [35–38], while in some cases, AA administration has been shown to have no effect on different plasma lipids [39–52], despite the wide range of doses (0.15–6 g/day) administered

Table 1. Epidemiological studies investigating the relationship between AA and plasma lipids.

Subjects	Results	Comments [reference]
254 F (Wales)	No effect.	— [34]
23 M, 11 F (Kenya)	Positive correlation between serum cholesterol and both PAA and LAA.	AA levels low [33].
12 M, 11 F; healthy elderly, 72–86 y	Positive correlation between PAA and WBC-AA and plasma HDL-C in M.	Correlation based on average of three-monthly values taken over 18 months [18].
300 M, 300 F; blood-donors, 25–55 y	Negative linear correlation between LAA levels and levels in M and F for TC and TG.	— [31]
97 M; 30–69 y	PAA negatively associated with TC and weakly but positively associated with HDL-C. No association between PAA and TG.	Random sample; controlled for age; known alteration of AA metabolism in the elderly [19].
100 M+F; 70–84 y	Significant negative correlation between PAA and TC in M. No correlation between PAA and TG.	Seasonal variation in PAA evident; altered AA metabolism in the elderly [32].
13 M, 16F; 20–53 y	Positive correlation between HDL-C and AA intake in F.	Serum AA levels not measured [28].
1,166 M (Eastern Finnish); 54 y	PAA not associated with HDL-C or LDL-C.	— [27]
146 F, 92 M; Chinese-Americans, ≥ 60 y	Partial correlation between PAA and plasma HDL-C in predominantly non-smoking F.	Altered AA metabolism in the elderly [20].
148 M, 93 F; elderly Chinese-Americans	Positive correlation between PAA and HDL-C.	Abstract only [21].
79 M, 96 F (Japan); ≥ 60 y	Positive correlation between HDL-C and AA found in M and in F. PAA is negatively correlated with TG. PAA is negatively correlated with plasma TC in F.	Adjusted for several confounding variables; altered AA metabolism in the elderly [22].
17 M, 17 F; body-builders	AA intake was positively associated with HDL-C for M.	PAA not measured [29].
2,379 girls; 9–10 y	Negative association between dietary AA intake and serum TC in a subgroup with TC levels ≥ 200 mg/dl. Dietary AA intake associated inversely with LDL-C.	Diet controlled; PAA not measured [30].
316 F, 511 M; 19–95 y	Positive correlation between PAA with: HDL-C in M; TC, LDL-C, and TG in F. A negative association between PAA and plasma TC, LDL-C and TG in M.	Adjusted for several confounding factors; correlations given for a subsample of the population [23].
256 M, 221 F; 20–65 y	Positive association between AA and HDL-C, and inverse association between AA and TC, LDL-C and TG in M. AA positively associated with HDL-C in F.	Adjusted for several confounding factors [25].

Table 1 (Continued)

Subjects	Results	Comments [reference]
835 M, 1,025 F; 45-75 y	Serum AA not correlated with serum TC or LDL-C. Serum AA positively correlated with HDL-C and negatively correlated with TGs in F.	Non-fasting samples; diet not included as a variable [26].

AA=ascorbic acid; F=female; HDL-C=high density lipoprotein-cholesterol; LAA=leucocyte ascorbic acid; LDL-C=low density lipoprotein-cholesterol; M=male; PAA=plasma ascorbic acid; TC=total cholesterol; TG=triglycerides; WBC-AA=white blood cell ascorbic acid.

(Table 2). Interestingly, an exception to the above observations was a study conducted by Spittle [53], who showed that while AA supplementation of 1 g/day over 6 weeks resulted in a significant increase in serum TC in atherosclerotic patients, no significant effect was observed in patients without atherosclerosis.

While there is a plethora of evidence from studies in animals to substantiate the postulated influence of AA on lipid metabolism, the evidence derived from studies in humans has been inconsistent. Consequently, studies in humans have failed to demonstrate a link of AA with CHD. Overall, the quality of both epidemiological and supplementation studies has been variable. The majority of trials reviewed have suffered from limitations. Some of these include the lack of a control group or randomization, low statistical power, poor dietary control, failure to blind, no evidence of compliance, a heterogeneous population with respect to pathological conditions and AA status, and the use of elderly subjects who have a recognized age-related alteration in the metabolism of AA. Further controlled human studies of appropriate design are warranted to elucidate the relationship between AA and CHD.

Postulated mechanisms for the effect of AA on lipid metabolism. AA may influence lipid metabolism in different ways. In guinea pigs, the catabolism of cholesterol to bile acids is hindered by low AA levels [11, 54-57]. A significant linear correlation has been observed between the rate of cholesterol conversion to bile acids and the AA concentration in the liver of guinea pigs [11, 55]. AA-deficient guinea pigs have reduced bile acid synthesis due to a reduction in the activity of cholesterol 7 α -hydroxylase, the rate-limiting enzyme in bile acid synthesis [9, 57-60]. Bjorkhem and Kallner [58] found that AA affected cytochrome P-450 whose main function is to activate the oxygen used by 7 α -hydroxylase during the hydroxylation of cholesterol [36]. AA may mediate a reduction in plasma LDL-C concentrations, as AA supplementation has been shown to increase the number of LDL receptors in cultured smooth muscle cells [61]. The manner in which AA influences HDL-C metabolism has not been ascertained. It appears that the lipoprotein lipase (LPL) activity is elevated by AA, and that this enzyme is subsequently involved in the regulation of HDL metabolism [62]. It has been

Table 2. Supplementation trials investigating the relationship between AA and plasma lipids.

Subjects	Intervention period (AA dose)	Results	Comments [reference]
8 M, 6 F; hypercholesterolemia with defined arteriosclerosis, 9–56 y	5–16 weeks, 1–6 g/d	No effect.	Serum AA not measured; not diet-controlled; no control group or randomization [39].
58 volunteers; 33 with history of atherosclerosis	6 weeks, 1 g/d	Significant increase in serum TC in patients with atherosclerosis; no effect in other groups.	No placebo control; serum AA not measured; not diet-controlled [53].
18 M, 16 F; healthy subjects, 19–40 y	3 months, 1 g/d	No effect.	Non-fasting blood samples; serum AA not measured; randomized, not blinded and no placebo control [40].
14 F, 108 M; 18–25 y	4 months, 1 g/d	No effect.	Randomized, placebo-controlled, single-blinded study; not diet-controlled [41].
6 M, 3 F; with hyperlipidemia, 26–57 y	8 weeks, 4 g/d	No effect of AA supplementation on plasma TC and TG levels or their distribution in lipoproteins.	Diet-controlled; no control group or randomization [42].
Hyperlipidemic subjects with low PAA levels at baseline	12 months, 1 g/d	Decrease in plasma TC and TG levels.	No control group; AA-deficient patients [35].
40 M; with CAD	6 months, 0–2 g/d	Supplementation with 2 g AA/d caused the serum cholesterol levels to decrease by 12%, but did not change TG levels.	Randomized, double-blinded, placebo-controlled trial with control group; all subjects had low to normal AA levels at the initiation of the study [43].
9 M; 23–36 y	6 weeks, 1 g/d	No effect.	— [44]
13 F; 21–28 y	4 weeks, 1 g/d	No effect.	— [45]
40 M; healthy, free-living, 21–35 y	6 weeks, 2 g/d	The subject with the lowest baseline PAA and WBC-AA levels had the sharpest decline in TC and LDL-C coupled with an elevation in HDL-C	Diet-controlled; other interventions apart from AA supplementation also examined; blinded, placebo-controlled trial [46].
20 M; normocholesterolemic	3 weeks, 3 g/d	Decreased plasma TC and TG levels in all subjects.	PAA not measured [36].
20 M; hypercholesterolemic, 44–79 y			
8 M, 1 F; with hypertriglyceridemia, 41–65 y	1 month, 2 g/d	No effect.	AA levels and AA status not determined [47].

Table 2 (Continued)

Subjects	Intervention period (AA dose)	Results	Comments [reference]
3 groups: 14 M and 15 F; 21–61 y	0–12 months, 0–2 g/d	Fall in serum TC following supplementation with 1 and 2 g AA/d.	Age-related delay in cholesterol-lowering by AA [37].
130 M; 65–74 y	6 weeks, 150 mg/d	No effect.	Initial AA levels low (≤ 0.23 mg/dl) [48].
27 F; hospital patients with low PAA levels, 68–93 y	6 weeks for each dose, 0.2 g/d; 2 g/d	No effect.	Randomized, placebo-controlled trial; known alteration of AA metabolism in the elderly and patients where AA-deficient (< 6.0 mg/liter) [49].
138 M,F: healthy adults, 20–65 y	8 months, 1 g/d	AA supplementation significantly increased HDL-C among subjects with lower baseline PAA levels.	— [50].
10 F; nonsmokers	4 weeks, 1 g/d	AA supplementation resulted in a significant decline in plasma LDL-C levels.	Carry-over effect of AA [38].
59 M; smokers, 36–65 y	2 months, 0.5 g/d	LDL-C decreased significantly in slow release AA group.	Randomized, single-blinded, placebo-controlled trial; not diet-controlled [51].
8 M; smokers	2 weeks, 1 g/d	AA supplementation produced no changes in plasma TC, TGs, or the distribution of cholesterol among the lipoprotein fractions.	Randomized, single-blinded, placebo-controlled, crossover trial; subjects served as their own control; diet-controlled; small number of subjects [52].

AA=ascorbic acid; F=female; HDL-C=high density lipoprotein-cholesterol; LAA=leucocyte ascorbic acid; LDL-C=low density lipoprotein-cholesterol; M=male; PAA=plasma ascorbic acid; TC=total cholesterol; TG=triglycerides; WBC-AA=white blood cell ascorbic acid.

reported that LPL deficiencies are related to low levels of HDL-C [63]. Alternatively, it has been suggested that HDL production may be elevated by the cytochrome P-450 system, whose activity may be inducible by AA [64].

Oxidation of HDL has been shown to modify the structure of its major protein component, apolipoprotein A-I (apoA-I) [65]. In doing so, HDL has a depleted capacity to stimulate efflux of total cholesterol and cholesterol esters from foam cells [66, 67]. Supplementation with antioxidant vitamins such as AA has been shown to be protective against HDL oxidation [68], although further studies are warranted. HDL-C may also be indirectly affected by AA, as a reduction in the expression and subsequent plasma concentration of apoA-I has been found in transgenic rats unable to synthesize AA [69].

It has been proposed that the reduction of TG levels by AA may also be attributable to an effect on LPL [70]. In rats and rabbits fed cholesterol, Sokoloff

et al. [70] found a gradual decline in LPL activity, accompanied by an increase in TG levels. However, additional AA inhibited the reduction in LPL activity and the associated elevation of TG levels. In contrast, Nambisan and Kurup [16] found the LPL activity in the aorta to be independent of the AA concentration.

EFFECT OF AA ON LIPID PEROXIDATION

Lipid peroxidation and oxidative modification of LDL have been proposed as important steps in the atherosclerotic process. Consequently, the antioxidant action of AA means that it may facilitate inhibition of atherosclerotic development. Although the methodology used to assess this process requires further research, *in vitro* studies support the idea that AA is effective in protecting lipids from peroxidative damage induced by a variety of sources [71-76].

Studies in animals have shown that high levels of AA added to the diet have reduced lipid peroxidation in iron-overloaded rats [77] and guinea pigs [78], and in rabbits when combined with vitamin E supplementation [67]. Similarly, AA therapy in AA-deficient guinea pigs [79] has resulted in decreased lipid peroxidation.

Supplementation with AA in smokers has led to inconsistent results. Some studies have shown a reduction in lipoprotein oxidation [80, 81], but others have not [51, 52, 82-84]. In human studies where AA has been administered with other antioxidants, decreased lipid peroxidation resulted [68, 85-88]. Supplementation of smokers with dietary sources of AA and β -carotene supported these findings [89]. Consequently, although *in vitro* and animal studies support the action of AA as an antioxidant, human studies which have examined the effect of dietary/supplemental AA on lipid peroxidation are inconclusive.

AA may also demonstrate its antioxidant functions via its putative role in the regeneration of vitamin E (α -tocopherol) from the α -tocopheryl radical [8]. In studies where high doses of AA have been administered to animals, some investigators have shown elevated vitamin E concentrations in plasma [90] and in tissues [91], but others have not [92, 93]. There is also a lack of controlled human studies to support the *in vitro* observations. Previous studies in smokers and healthy women have not demonstrated an effect of AA supplementation on plasma tocopherol levels [51, 80, 81, 83, 94, 95]. This is despite variations in AA doses (300 to 1,500 mg/day) and duration of supplementation (14 days to 2 months). In contrast, Benzie and Strain [96] found that supplementation in 10 apparently healthy men and women with 1,000 mg AA/day for 4 weeks resulted in significant increases in total vitamin E concentrations and a highly significant increase in median lipid standardized vitamin E concentration. Similarly, a study in a healthy elderly population showed that AA supplement use was significantly correlated with plasma α -tocopherol levels in females [97]. While to date, AA has not been consistently shown to enhance α -tocopherol levels *in vivo*, other mechanisms by which it can exert its antioxidant functions have been reported [98].

Table 3. Summary of literature on AA and copper indices in humans.

Subjects	Study design	AA dose	Results
11 M	3 weeks (metabolic unit)	0.07–9 mg/kg BW/d. 605 mg/d	Cu absorption, excretion and serum levels were unaffected by AA dose. Cp oxidase activity reduced by 21% with the highest AA dose [99].
10 F	4 weeks (crossover)	17 mg/kg BW/d. 1,000 mg/d	Reduction in E-Cu by 23% and E-SOD by 14.7% during AA supplementation [100].
13 M	64 days (parallel)	21 mg/kg BW/d. 1,500 mg/d	Fall in serum Cu and Cp activity after 28 days of AA dose. This effect was reversed 20 days after AA was withdrawn [101].
8 F	42 days (metabolic ward)	1.5–25 mg/kg BW/d. 1,500 mg/d	Cp activity was reduced due to a slight increase in Cp protein [102].
32 M, 21 F	8 weeks (parallel)	29–33 mg/kg BW/ d. 2,000 mg/d	Transient rise in Cp [103].

AA=ascorbic acid; BW=body weight; Cp=ceruloplasmin; Cu=copper; E-Cu=erythrocyte copper; E-SOD=erythrocyte superoxide dismutase; F=female; M=male.

POTENTIAL ADVERSE EFFECTS OF AA

AA administration should be viewed with caution, as AA can exert potentially adverse effects via its interaction with transition metals. Animal and human studies [99–103] suggest that AA reduces copper status (Table 3). A reduction in copper status may include a reduction in the activity of copper dependent enzymes such as superoxide dismutase, a free radical quenching enzyme [100, 104].

AA is also a powerful reductant and is known to catalyze the reduction of non-heme ferric iron to ferrous iron which in turn aids its absorption [105, 106]. Whilst this is beneficial to those who are at risk of iron deficiency, enhancing iron absorption is not a favorable event for those with or at risk of hemochromatosis. Approximately 10% of Caucasians are born as carriers of hemochromatosis while 0.3% are born with the disease which is the most common autosomal recessive disorder amongst Caucasians [107]. AA consumption in the presence of iron can cause mobilization of iron from the spleen which is then deposited into the liver. Excess iron can damage tissue by peroxidation of membrane lipids [108]. AA supplementation is also likely to precipitate earlier phenotypic expression of hemochromatosis [109].

CONCLUSION

Clinical trials conducted to examine the epidemiological relationship between AA and plasma lipids have produced equivocal results which suggests that AA may be a surrogate marker [110] for other dietary constituents such as flavonoids.

The latter are being studied increasingly because of their potential roles in the prevention of the major chronic diseases [111-113].

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