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Status of lipids, lipid peroxidation, and antioxidant systems with Vitamin C supplementation during aging in rats

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Molecular mechanisms responsible for age-dependent deterioration of biochemical functions remain unclear. We determined the effect of ascorbic acid supplementation (20 mg/100 g body weight/day) in young and aged rats for 15, 30, and 60 days. In the aged animals cholesterol, triglycerides, phospholipids, and lipid peroxidation were considerably high, whereas, antioxidants superoxide dismutase, catalase, glutathione peroxidase, glutathione, ascorbic acid, and α-tocopherol were low. Administration of ascorbic acid reverted these age-associated differences to the status comparable to young rats. (J. Nutr. Biochem. 7:270–275, 1996.)

Keywords: Ascorbic acid; lipid peroxidation; antioxidants; aging

Introduction

Aging is a universal biological phenomenon associated with histological, biochemical, and functional alterations. The free radical theory of aging proposes that aging occurs as a consequence of the deleterious effects of free radicals produced during cellular metabolism.[1]

Aging is attributable to several alterations in lipid metabolism. The cholesterol, triglyceride, and phospholipid levels increase with aging.[2] Lipid peroxidation is characteristically a free radical chain reaction initiated by the abstraction of a hydrogen atom from polyunsaturated fatty acid side chain. The free radical-mediated lipid peroxidation has been proposed to be critically involved in several disease states including cancer, cardiovascular diseases, immune function decline, brain dysfunction, and cataract as well as in the degenerative processes associated with aging.[3] A number of enzymatic and non-enzymatic antioxidants have, however, been evolved to protect living organisms against free radical-mediated cellular damage.[4]

Ascorbic acid is the most widely cited form of water-soluble antioxidant and prevents oxidative damage to cell membranes induced by aqueous radicals.[5] Hydrophilic ascorbic acid however, is not able to scavenge lipophilic radicals within the interior of the membrane. Tocopherol and ubiquinol are the primary scavengers of radicals within the membrane. Ascorbic acid causes the regeneration of tocopherol from its oxidized form, as a result of which, tocopherol can continue to scavenge free radicals within the membrane.[5] The blood and leucocyte ascorbic acid levels decrease with aging in rats;[6] also the content of ascorbic acid in rat liver and muscle declines with aging.[7] As a result, the requirement of ascorbic acid increases with aging.

The major aim of this study has been to observe and compile the biochemical changes that occur during the process of aging. Measuring changes in the specific enzyme activities in various tissues with increasing age has been an important approach in gerontological research. In this respect, the present study was undertaken to determine the effect of ascorbic acid supplementation on the status of lipids, lipid peroxidation, and enzymatic and non-enzymatic antioxidants in the plasma, liver, and kidney of rats with aging.

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Methods and materials

Animals and diet

Male albino rats of Wistar strain were divided into two major groups: Group I: normal young rats (3 to 4 months old) and Group II: normal aged rats (about 24 months old). These groups of animals were fed a normal diet. Groups I and II were subdivided into four groups: one control group (Group I, II) and three experimental groups based on the contemplated duration of ascorbic acid supplementation for 15 days (Groups Ia, IIa), 30 days (Groups Ib, IIb), and 60 days (Groups Ic, IIc). Each group consisting of six animals were housed under standard laboratory conditions and allowed for feed and water *ad libitum*. Experimental animals received a daily oral dose of ascorbic acid (20 mg/100 g body weight) by gastric intubation. For this purpose, ascorbic acid solution was prepared in water just before administration. Control groups (Groups I, II) received the same amount of water as that of experimental animals by the same route.

Monitoring biochemical function

On completion of 15, 30, and 60 days of ascorbic acid supplementation, blood was collected from the jugular vein into heparinised tubes. After sufficient amount of blood had been collected, the animals were killed. Liver and kidney were excised, immersed in physiological saline, blotted with filter paper, and weighed accurately for the preparation of 10% homogenate in 0.01 M Tris-HCl buffer (pH 7.4). Blood, plasma, and liver and kidney homogenates were used for the estimation of protein,[8] lipid peroxidation,[9] total lipids,[10] cholesterol,[11] triglycerides,[12] phospholipids,[13] superoxide dismutase,[14] catalase,[15] glutathione peroxidase,[16] glutathione,[17] ascorbic acid,[18] and α-tocopherol.[19]

Statistical analysis

Data are expressed as mean ± SD and were analyzed by one-way analysis of variance (ANOVA).[20] Each experimental group was compared with respective control groups. When ANOVA indicated significant differences, data were further analyzed by Student Newman Keul's (SNK) Test for multiple comparison.[21] Differences were considered significant at $P < 0.05$.

Statistical significance of differences between the young control (Group I) and aged control (Group II) animals was determined by Student's t test. The levels of significance were evaluated with P values.

Results

Table 1 gives the body weight, the status of lipids, and lipid peroxidation in plasma and of blood ascorbic acid in young and aged rats fed with normal diet and ascorbic acid supplementation. Lipids and lipid peroxides in plasma were considerably high, whereas blood ascorbic acid was markedly low in aged rats. Ascorbic acid administration brought down the levels of cholesterol, triglycerides, phospholipids, and lipid peroxides considerably and elevated blood ascorbic acid markedly in aged rats (*Table 1*). In young rats ascorbic acid supplementation exhibited a lowering of plasma cholesterol and lipid peroxides.

The status of lipids, lipid peroxidation, enzymatic and

Table 1 Body weight, plasma lipids, lipid peroxidation, and blood ascorbic acid in young and aged rats fed normal diet and ascorbic acid supplementation

	Young rats				Aged rats			
	Group I	Group Ia	Group Ib	Group Ic	Group II	Group IIa	Group IIb	Group IIc
Mean body weight								
Initial	158.0	158.0	158.1	158.0	260.6	260.8	260.6	260.8
Final	158.1	158.3	158.8	159.1	260.0	261.3	261.5	262.0
Cholesterol (mg/dl)	73 ± 7.26	68.6 ± 5.84	63.5 ± 5.50	59.6[a] ± 6.53	99.2 ± 8.80###	89.3 ± 7.20	83.5[a] ± 7.90	75.8[a] ± 7.10
Triglyceride (mg/dl)	81 ± 8.20	77.5 ± 9.20	72.3 ± 6.40	70 ± 7.70	104 ± 8.70##	99 ± 8.50	90.8 ± 7.50	83[ab] ± 8.80
Phospholipids (mg/dl)	89 ± 9.80	84 ± 9.30	78 ± 8.20	73 ± 8.70	115 ± 10.70##	108 ± 9.80	101 ± 9.40	93[a] ± 8.70
Lipid peroxide (nmoles of MDA released/ mg protein)	2.87 ± 0.31	2.64 ± 0.24	2.54[a] ± 0.22	2.44[a] ± 0.19	4.22 ± 0.39###	3.92[a] ± 0.32	3.42[ab] ± 0.29	2.93[abc] ± 0.22
Blood ascorbic acid (mg/dl)	1.13 ± 0.11	1.14 ± 0.09	1.17 ± 0.13	1.22 ± 0.12	0.77[b] ± 0.13###	0.85[ab] ± 0.12	1.2 ± 0.11	1.25[a] ± 0.14

Each value is expressed as mean ± SD of six animals.
Each experimental group was compared with respective control group.
[a]Group I/II versus respective other groups.
[b]Group Ia/IIa versus respective other groups.
[c]Group Ib/IIb versus Group I/II.
Young (Group I) versus aged (Group II)
##$P < 0.01$, ###$P < 0.001$

non-enzymatic antioxidants in rat liver is presented in *Table 2*. Total lipids, cholesterol, triglycerides, phospholipids, and lipid peroxidation were quite high in aged rats; the activities of superoxide dismutase, catalase and glutathione peroxidase and non-enzymatic antioxidants, glutathione, ascorbic acid, and α-tocopherol were found remarkably low (*Table 2*). Administration of ascorbic acid for aged animals increased the liver content of antioxidants markedly; in consonance the lipids and lipid peroxidation were lowered considerably (*Table 2*). In young rats the level of cholesterol and lipid peroxidation decreased markedly and of antioxidants increased slightly after ascorbic acid supplementation (*Table 2*).

Table 3 depicts the profile of lipids, lipid peroxidation, and enzymatic and non-enzymatic antioxidants in the kidney of young and aged rats. Lipids and lipid peroxidation were significantly high, whereas the antioxidants were remarkably low in aged rats. The total lipids, cholesterol,

Table 2 Liver lipids, lipid peroxidation, and enzymatic and non-enzymatic antioxidants in young and aged rats fed normal diet and ascorbic acid supplementation

	Young rats				Aged rats			
	Group I	Group Ia	Group Ib	Group Ic	Group II	Group IIa	Group IIb	Group IIc
Total lipids (mg/g wet tissue)	58.2 ± 5.80	56.2 ± 4.90	53.3 ± 4.40	49.9 ± 3.90	85.5 ± 7.30$^{\#\#\#}$	79.7 ± 6.80	74.6 ± 6.20	68.3^{a} ± 5.80
Cholesterol (mg/g wet tissue)	4.8 ± 0.52	4.4 ± 0.59	3.9 ± 0.51	3.6^{a} ± 0.55	9.2 ± 0.62$^{\#\#\#}$	8.5 ± 0.67	7.8^{a} ± 0.79	6.7^{ab} ± 0.68
Triglyceride (mg/g wet tissue)	15.3 ± 1.80	14.2 ± 1.40	13.6 ± 1.60	12.8 ± 0.99	20.8 ± 2.40$^{\#\#}$	18.7 ± 1.90	17.6 ± 1.60	15.9^{ab} ± 1.50
Phospholipids (mg/g wet tissue)	33.5 ± 3.20	32.6 ± 3.50	30.5 ± 2.70	28.6 ± 2.40	42.5 ± 4.60$^{\#\#}$	38.2 ± 4.20	36.9 ± 3.90	34.6^{a} ± 3.20
Lipid peroxidase (nmoles of MDA released/ mg protein)	2.6 ± 0.38	2.4 ± 0.27	2.2 ± 0.24	1.9^{ab} ± 0.18	4.1 ± 0.48$^{\#\#\#}$	3.9 ± 0.43	3.3^{ab} ± 0.36	2.8^{ab} ± 0.32
Superoxide dismutase (units/min/ mg protein)	8.3 ± 0.78	9.3 ± 0.94	9.8 ± 1.30	10.2 ± 1.20	6.2^{b} ± 0.49$^{\#\#\#}$	6.9^{ab} ± 0.59	7.8 ± 0.68	8.6^{a} ± 0.67
Catalase (μ moles of H_2O_2 consumed/min/mg protein)	52.7 ± 4.50	54.8 ± 4.40	55.8 ± 5.10	58.8 ± 4.80	44.1 ± 3.90$^{\#\#}$	46.8 ± 4.30	49.4 ± 4.20	53.4^{a} ± 4.60
Glutathione peroxidase (μ moles of GSH oxidised/ min/mg protein)	7.7 ± 0.72	8.2 ± 0.67	8.5 ± 0.86	9.1 ± 0.96	5.3^{bc} ± 0.56$^{\#\#\#}$	6.2^{a} ± 0.64	6.8^{a} ± 0.63	7.8^{a} ± 0.67
Glutathione (μg/mg protein)	12.1 ± 1.30	12.6 ± 1.20	13.2 ± 1.60	14.1 ± 1.40	8.8 ± 0.76$^{\#\#\#}$	9.6^{a} ± 0.82	10.2^{a} ± 0.98	11.7^{a} ± 1.30
Ascorbic acid (μg/mg protein)	2.7 ± 0.34	2.8 ± 0.38	3.2 ± 0.59	3.4 ± 0.49	1.4^{b} ± 0.32$^{\#\#\#}$	1.8^{a} ± 0.34	2.3 ± 0.39	2.6^{a} ± 0.37
α-tocopherol (μg/mg protein)	1.7 ± 0.32	1.9 ± 0.31	2.1 ± 0.42	2.3 ± 0.39	0.98^{b} ± 0.18$^{\#\#\#}$	1.2^{ab} ± 0.26	1.6 ± 0.28	1.9^{a} ± 0.26

Each value is expressed as mean ± SD of six animals.
Each experimental group was compared with respective control group.
[a]Group I/II versus respective other groups.
[b]Group Ia/IIa versus respective other groups.
[c]Group Ib/IIb versus Group I/II.
Young (Group I) versus aged (Group II).
$^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$.

Table 3 Kidney lipids, lipid peroxidation, and enzymatic and non-enzymatic antioxidants in young and aged rats fed normal diet and ascorbic acid supplementation

	Young rats				Aged rats			
	Group I	Group Ia	Group Ib	Group Ic	Group II	Group IIa	Group IIb	Group IIc
Total lipids (mg/g wet tissue)	46 ± 6.80	44 ± 5.10	41 ± 4.60	38 ± 5.20	62 ± 6.30$^{\#\#}$	57 ± 5.60	54 ± 5.80	48^{a} ± 5.20
Cholesterol (mg/g wet tissue)	4.4 ± 0.48	4.2 ± 0.42	3.9 ± 0.39	3.6 ± 0.41	8.6 ± 0.81$^{\#\#\#}$	7.8 ± 0.67	6.8^{ab} ± 0.63	5.3^{abc} ± 0.64
Triglyceride (mg/g wet tissue)	6.2 ± 0.82	5.8 ± 0.66	5.3 ± 0.76	4.9 ± 0.52	10.3 ± 1.20$^{\#\#}$	9.7 ± 1.30	8.2^{a} ± 0.98	6.9^{ab} ± 0.82
Phospholipids (mg/g wet tissue)	29.3 ± 3.10	28.2 ± 3.40	27.1 ± 2.80	26.3 ± 2.30	38.5 ± 3.50$^{\#\#}$	36.8 ± 3.20	34.7 ± 3.60	32.4 ± 3.30
Lipid peroxide (nmoles of MDA released/ mg protein)	2.1 ± 0.24	1.9 ± 0.21	1.7 ± 0.28	1.5^{a} ± 0.23	3.8 ± 0.36$^{\#\#\#}$	3.4 ± 0.38	2.9^{a} ± 0.36	2.4^{ab} ± 0.31
Superoxide dismutase (units/min/ mg protein)	5.4 ± 0.45	5.7 ± 0.43	6.0 ± 0.58	6.3 ± 0.62	4.4 ± 0.42$^{\#\#}$	4.8 ± 0.51	5.1 ± 0.49	5.5^{a} ± 0.53
Catalase (μ moles of H_2O_2 consumed/ min/mg protein)	44.3 ± 5.10	44.8 ± 4.90	45.3 ± 4.60	46.8 ± 5.30	33.8 ± 3.80$^{\#\#}$	37.8 ± 3.50	39.2 ± 3.90	42.6^{a} ± 4.30
Glutathione peroxidase (μ moles of GSH oxidised/ min/mg protein)	9.8 ± 0.92	10.2 ± 0.96	11.1 ± 1.80	12.0 ± 1.70	5.2^{b} ± 0.760$^{\#\#\#}$	6.3^{a} ± 0.93	7.5^{a} ± 0.89	8.8^{a} ± 0.85
Glutathione (μg/mg protein)	8.7 ± 0.93	9.6 ± 0.88	10.1 ± 1.30	10.6 ± 1.20	3.8^{bc} ± 0.33$^{\#\#\#}$	4.5^{ab} ± 0.41	5.6^{a} ± 0.48	6.8^{a} ± 0.62
Ascorbic acid (μg/mg protein)	1.3 ± 0.14	1.4^{a} ± 0.16	1.6 ± 0.19	1.8^{a} ± 0.22	0.61 ± 0.09$^{\#\#\#}$	0.73^{a} ± 0.08	0.81^{a} ± 0.11	1.1^{a} ± 0.13
α-tocopherol (μg/mg protein)	0.95 ± 0.15	1.0 ± 0.14	1.1 ± 0.18	1.3^{a} ± 0.21	0.71 ± 0.08$^{\#\#}$	0.78 ± 0.09	0.84 ± 0.11	0.93^{a} ± 0.13

Each value is expressed as mean ± SD of six animals.
Each experimental group was compared with respective control group.
[a]Group I/II versus respective other groups.
[b]Group Ia/IIa versus respective other groups.
[c]Group Ib/IIb versus Group I/II.
Young (Group I) versus aged (Group II).
$^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$.

triglyceride, and lipid peroxidation were decreased markedly after ascorbic acid supplementation in aged rats; there was a considerable elevation in the status of superoxide dismutase, catalase, glutathione peroxidase, glutathione, ascorbic acid, and α-tocopherol after ascorbic acid supplementation (*Table 3*). The kidney content of lipid peroxides decreased markedly, whereas the levels of ascorbic acid and α-tocopherol increased remarkably after ascorbic acid supplementation in young rats (*Table 3*).

Discussion

The present study focuses on the status of lipids, lipid peroxidation, and antioxidant systems in the plasma, liver, and

kidney of rats with ascorbic acid supplementation in the modulation of the aging process. The concept that Vitamin C might affect serum lipid levels was first presented on the basis of experimental studies in rabbits and man.[22] The concentration of cholesterol was considerably high in aged rats in this study, which may be due to decreased concentration of ascorbic acid. It has been pointed out that ascorbic acid is necessary for the activation of 7α-hydroxylase and that ascorbic acid deficiency causes the depression of hepatic cholesterol-7α-hydroxylase, which is a rate-limiting step in cholesterol transformation to bile acids.[23] In this light, the decrease in plasma and tissue cholesterol due to ascorbic acid supplementation in the present study may be attributed to the activation of 7α-hydroxylase by ascorbic acid.

Aged rats in the present study showed high content of triglycerides. Lipoprotein lipase is the rate-limiting enzyme in triglyceride metabolism. It has been reported that low ascorbic acid concentration reduces the activity of lipoprotein lipase[24] though the mechanism by which ascorbic acid deprivation suppresses the activity of lipoprotein lipase is not known. The elevated triglyceride concentration in plasma and tissues of aged rats may therefore be due to the low level of ascorbic acid in the aged rats. Ascorbic acid is also required as a cofactor in two hydroxylation reactions in the pathway of carnitine biosynthesis.[25] Carnitine stimulates β-oxidation of fatty acids without affecting the overall rate of fatty acid uptake, resulting in lower cellular triglycerides.[26] Ascorbic acid deficiency can lead to a decrease of tissue carnitine and an increase of triglycerides in guinea pigs.[26] The progressive decrease of triglycerides after supplementation with ascorbic acid in our study may be due to increased activity of lipoprotein lipase and also increased β oxidation of fatty acids.

Age-associated increase in phospholipids has been reported in humans[2] and the present observations in aged rats corroborate this. Phospholipids, however, decreased significantly in the present study after supplementation with ascorbic acid. Phospholipases hydrolyse the phospholipid structure and phospholipase A_2 is one such phospholipase. It cleaves the second position of fatty acid side chain in the phospholipid structure. It has been observed that ascorbic acid enhances the activity of phospholipase A_2.[27] The reduction of phospholipids observed immediately after ascorbic acid supplementation may be a reflection of the enhanced activity of phospholipase.

Lipid peroxidation increased considerably in aged rats in the present study. Age-related lipid damage has long been of interest to investigators seeking clues to the progressive cellular injury that purportedly occurs in aging organisms.[28] Indeed, age-related lipid modifications have been confirmed by many investigators. Grinna for instance, supplied strong evidence that lipids undergo substantial changes as a function of age in experimental animals.[29] Cellular damage caused by free radicals have been associated with the aging process.[30] Antioxidants are essential in preventing the cellular damage caused by free radicals and free radical-mediated lipid peroxidation.

The antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase and non-enzymatic antioxidants, glutathione, ascorbic acid, and α-tocopherol were significantly low in aged rats. Superoxide dismutase catalytically scavenges the superoxide radicals and this renders cytoprotection against free radical damage. Catalase is an antioxidant defense enzyme that primarily works to catalyze the decomposition of H_2O_2 to H_2O, sharing this function with glutathione peroxidase. The non-enzymatic antioxidant, glutathione plays an important role in a variety of detoxification processes, including nullification of peroxide damage.[28] The antioxidant action of α-tocopherol is highly effective in protecting against membrane lipid peroxidation by reacting with peroxy and alkoxy radicals. Low ascorbic acid intake reduces plasma glutathione concentration in humans.[31] The decreased activity of superoxide dismutase, catalase, glutathione peroxidase, and the lowered levels of glutathione and Vitamin E in aged rats in our study are, therefore, obviously due to increased lipid peroxidation and decreased ascorbic acid concentration with aging.

Vitamin C is a hydrophilic antioxidant and functions better in an aqueous environment than antioxidants. Ascorbic acid directly reacts with $O_2^{\cdot-}$, $OH^{\cdot}$ and various lipid hydroperoxides. In addition, it can restore the antioxidant properties of Vitamin E.[28] Ascorbic acid is a more effective antioxidant than protein thiols, bilirubin, urate, and tocopherol in plasma in in vitro systems.[4] The blood and leucocyte ascorbic acid concentrations have been observed to increase after supplementation with ascorbic acid.[6] In the present investigation, the enzymatic and non-enzymatic antioxidants increased remarkably in aging rats after supplementation with ascorbic acid. Ascorbic acid deficiency has also been shown to increase lipid peroxidation in the plasma and liver of rats.[32] The increase of antioxidants observed presently after supplementation with ascorbic acid may be due to effective radical scavenging action of ascorbic acid in the aqueous phase.

In conclusion, Vitamin C supplementation reduces age-associated lipid alteration and also suppresses the free radical mediated damage, thereby retarding aging and age-associated degenerative diseases.

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