INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND DEVELOPMENT (IJPRD)
Platform for Pharmaceutical Researches & Innovative Ideas
www.ijprd.com

THE BUCCAL ABSORPTION OF ASCORBIC ACID USING CENTRIFUGED & UNCENTRIFUGED SALIVA

Navneet Kumar Verma*[1],
Nitin Chitransi[1], P.K.Tripathi[1]

[1]Department of Pharmacy, Rameshwaram Institute of Technology and Management,Lucknow (U.P) India

ABSTRACT

Vitamin C, also referred to as ascorbic acid (AA) or ascorbate, belongs to the water-soluble class of vitamins. Humans are one of the few species who lack the enzyme to convert glucose to vitamin C. Transport of vitamin C is a saturable and dose dependent process that occurs by active transport. At the intestine and cells AA is oxidized to Dihydro Ascorbic Acid (DHAA), which is more quickly transported across the cell membrane. Here we have reported the absorption of AA in the centrifuged and uncentrifuged saliva from the normal young adult man. The standard curve was prepared with different concentration ranging from 2-10 mcg/ml. The percentage of AA absorption was to be 93.85% in the uncentrifuged saliva which was significantly higher than the centrifuged saliva. AA absorption into the buccal mucosa was held in the mouth for periods of 1-9 minutes. For a constant mouth contact time, increase in pH of the loading solutions resulted in reduction of buccal absorption, and diminishing transfer into the buccal epithelium. The degree of intestinal absorption decreases as intake of AA increases.

Correspondence to Author

Navneet Kumar Verma

Department of Pharmacy, Rameshwaram Institute of Technology and Management,Lucknow (U.P) India

Email
navneet_its04@rediffmail.com

Key Words
ascorbic acid Buccal absorption; Oral cavity; Buccal cavity; Perfusion cell; Absorption kinetics

INTRODUCTION

Vitamin C, also known as L-ascorbic acid, is a water-soluble vitamin that is naturally present in some foods, added to others, and available as a dietary supplement. Humans, unlike most animals, are unable to synthesize vitamin C endogenously, so it is an essential dietary component[1].

Vitamin C is required for the biosynthesis of collagen, L-carnitine, and certain neurotransmitters; vitamin C is also involved in protein metabolism [1,2]. Collagen is an essential component of connective tissue, which plays a vital role in wound healing. Vitamin C is also an important physiological antioxidant [3] and has been shown to regenerate other antioxidants within the body, including alpha-tocopherol (vitamin E) [4]. Ongoing research is examining whether vitamin C, by limiting the damaging effects of free radicals through its antioxidant activity, might help prevent or delay the development of certain cancers, cardiovascular disease, and other diseases in which oxidative stress plays a causal role. In addition to its biosynthetic and antioxidant functions, vitamin C plays an important role in immune function [4] and improves the absorption of nonheme iron [5], the form of iron present in plant-based foods. Insufficient vitamin C intake causes scurvy, which is characterized by fatigue or lassitude, widespread connective tissue weakness, and capillary fragility [1,2,4,6-9].

The intestinal absorption of vitamin C is regulated by at least one specific dose-dependent, active transporter [4]. Cells accumulate vitamin C via a second specific transport protein. In vitro studies have found that oxidized vitamin C, or dehydroascorbic acid, enters cells via some facilitated glucose transporters and is then reduced internally to ascorbic acid. The physiologic importance of dehydroascorbic acid uptake and its contribution to overall vitamin C economy is unknown. Oral vitamin C produces tissue and plasma concentrations that the body tightly controls. Approximately 70%–90% of vitamin C is absorbed at moderate intakes of 30–180 mg/day. However, at doses above 1 g/day, absorption falls to less than 50% and absorbed, unmetabolized ascorbic acid is excreted in the urine [4]. Results from pharmacokinetic studies indicate that oral doses of 1.25 g/day ascorbic acid produce mean peak plasma vitamin C concentrations of 135 micromol/L, which are about two times higher than those produced by consuming 200–300 mg/day ascorbic acid from vitamin C-rich foods [10]. Pharmacokinetic modeling predicts that even doses as high as 3 g ascorbic acid taken every 4 hours would produce peak plasma concentrations of only 220micromol/L [10]. The total body content of vitamin C ranges from 300 mg (at near scurvy) to about 2 g [4]. High levels of vitamin C (millimolar concentrations) are maintained in cells and tissues, and are highest in leukocytes (white blood cells), eyes, adrenal glands, pituitary gland, and brain. Relatively low levels of vitamin C (micromolar concentrations) are found in extracellular fluids, such as plasma, red blood cells, and saliva [4].

Recommended Intakes

- Recommended Dietary Allowance (RDA): average daily level of intake sufficient to meet the nutrient requirements of nearly all (97%–98%) healthy individuals.
- Adequate Intake (AI): established when evidence is insufficient to develop an RDA and is set at a level assumed to ensure nutritional adequacy.
- Tolerable Upper Intake Level (UL): maximum daily intake unlikely to cause adverse health effects [8].

Table 1 lists the current RDAs for vitamin C [8]. The RDAs for vitamin C are based on its known physiological and antioxidant functions in white blood cells and are much higher than the amount required for protection from deficiency [4,8,11]. For infants from birth to 12 months, the FNB established an AI for vitamin C that is equivalent to the mean intake of vitamin C in healthy, breastfed infants.

Table 1: Recommended Dietary Allowances (RDAs) for Vitamin C [8]

Age	Male	Female	Pregnancy	Lactation
0–6 months	40 mg*	40 mg*		
7–12 months	50 mg*	50 mg*		
1–3 years	15 mg	15 mg		
4–8 years	25 mg	25 mg		
9–13 years	45 mg	45 mg		
14–18 years	75 mg	65 mg	80 mg	115 mg
19+ years	90 mg	75 mg	85 mg	120 mg
Smokers	Individuals who smoke require 35 mg/day more vitamin C than nonsmokers.			

MATERIAL AND METHOD

MATERIAL;

Ascorbic acid, H2SO4, Ammonium Molybdate and Distilled Water was obtained from well reputed research laboratory and U.V.Specrophotometer.

METHOD-

100mg of Ascorbic acid was weighed and taken into 100ml of volumetric flask and diluted with distilled water till the mark.10 ml solution was taken from above solution and diluted to 100ml with distilled water(100mg/ml).Aliquot were prepared by using 0.5ml,1.0ml,2.0ml,3.0ml,4.0ml and 5.0ml of solution in 25.0ml volumetric flask.

2.0ml of sample+2.0ml H2SO4(10%w/v)+4ml of Ammonium Molybdate(10%w/v) solution kept at room tempreture for 60 minuts. Absorbance was measured at 650nm.

SAMPLE SOLUTION-

Ascorbic acid 25ml solution,concentration 100mcg/ml was taken in a beaker and kept it in mouth and collected 35 times for 5min.Expelled the solution and collected in beaker,again mouth was rinsed with 10ml distilled water and expelled in beaker(volume become 35ml).2.0ml of above solution was taken and added 0.8ml H2SO4(5%w/v) and 1.0ml Ammonium Molybdate in 10.0ml of volumetric flask and vlume was made up 10.0ml with distilled water.solution was kept in dark room.Absorbance was was measured at 650nm.

EVALUATION

Absorption and body stores

Intestinal absorption of vitamin C depends on the amount of dietary intake, decreasing with increasing intake levels. At an intake of 30 to 180 milligrams, about 70% to 90% is absorbed; about 50% of a single dose of 1 to 1.5 grams is absorbed; and only 16% of a single dose of 12 grams is absorbed. Up to about 500 milligrams are absorbed via a sodium-dependent active transport process, while at higher doses simple diffusion occurs.

The storage capacity of water-soluble vitamins is generally low compared to that of fat-soluble ones. Humans have an average tissue store of vitamin C of 20 mg/kg body weight. The highest concentration is found in the pituitary gland (400 mg/kg); other tissues of high concentration are the adrenal glands, liver, brain and white blood cells (leukocytes).

Measurement

Vitamin C can be measured in the blood plasma and other body tissues by various techniques. Also dipstick tests for estimation of vitamin C levels in the urine are available. Less satisfying, however, is the evaluation of the analytical data concerning the true reflection of the body status. Threshold values are difficult to define and the subject of controversial discussion. Typical blood plasma levels are in the range of 20 to 100 µmol/L

Stability

Vitamin C is sensitive to heat, light and oxygen. In food it can be partly or completely destroyed by long storage or overcooking. Refrigeration can substantially diminish vitamin C loss in food.

Positive interactions

The presence of other antioxidants, such as vitamin E and beta-carotene, supports the protective antioxidant action of vitamin C. Other vitamins, such as those of the B-complex (particularly B6, B12, folic acid and pantothenic acid) and some pharmacologically active substances, as well as the naturally occurring

compounds known as bioflavonoids, may have a sparing effect on vitamin C.

Negative interactions

Due to toxic compounds in smoke, the vitamin C requirement for smokers is about 35 mg/day higher than for non-smokers. Also several pharmacologically active compounds, among them some anti-depressants, diuretics, birth control pills and aspirin, deplete the tissues of vitamin C. This is also true of certain habits, for example alcohol consumption.

Disease prevention and therapeutic use

Dozens of prospective studies suggest that vitamin C plays a role in preventing a variety of diseases. It is also used to treat certain diseases in orthomolecular medicine. As this nutrient is important for a variety of diseases, only a selection of them are presented here in detail.

Cardiovascular diseases (CVD) (heart disease and stroke)

The data for the CVD protective benefits of vitamin C are inconsistent. While some studies have failed to find significant reductions in the risk of coronary heart disease (CHD), numerous prospective cohort studies have found inverse associations between dietary vitamin C intake or vitamin C plasma levels and CVD risk. Vitamin C may protect coronary arteries by reducing the build-up of plaque, as this helps to prevent the oxidation of LDL cholesterol (the “bad” cholesterol), especially in combination with vitamin E. Some data has shown that vitamin C may also boost blood levels of HDL cholesterol (the “good” cholesterol), which is also considered positive for the prevention of heart diseases. The risk of stroke may be reduced by an adequate intake of vitamin C through fruits, vegetables and supplements. However, due to the inconsistency of the data and its lack of specificity to vitamin C, the interpretation of these results is difficult.

Cancer

The role of vitamin C in cancer prevention has been studied extensively, and until now no beneficial effect has been shown for breast, prostate, or lung cancer. However, a number of studies have associated higher intakes of vitamin C with decreased incidence of cancers of the upper digestive tract, cervix, ovary, bladder, and colon. Studies finding significant cancer risk reduction by dietary intake recommended at least 5 servings of fruits and vegetables per day. Five servings of most fruits and vegetables provide more than 200 mg vitamin C per day. Just significant cancer risk reductions were found in people consuming at least 80 to 110 mg of vitamin C daily.

Common cold

Numerous studies have shown a general lack of effect of prophylactic vitamin C supplementation on the incidence of common cold, but they do show a moderate benefit in terms of the duration and severity of episodes in some groups, especially those who are exposed to substantial physical and/or cold stress. The improvement in severity of colds after vitamin C supplementation may be due to the antihistaminic action of mega doses of vitamin C.

Wound healing

During a postoperative period, or during healing of superficial wounds, supplemental vitamin C contributes to the prevention of infections and promotes skin repair.

Blood pressure

Several studies have shown a blood pressure lowering effect of vitamin C supplementation at about 500 mg per day due to improved dilation of blood vessels.

Recommended Dietary Allowance (RDA)

The recommended daily intake of vitamin C varies according to age, sex, risk group and criteria applied in individual countries. The RDAs in the USA for vitamin C were recently revised upwards to 90 mg/day for men and 75 mg/day for women, based on pharmacokinetic data. For smokers, these RDAs are increased by an additional 35 mg/day. Higher amounts of vitamin C are also recommended for pregnant (85 mg/day) and lactating women (120 mg/day). The RDAs are in a similar range in other countries. Recent evidence sets the estimate for the maintenance of optimal health in the region of 100 mg daily.

Safety

As much as 6-10 g vitamin C per day (more than 100 times the RDA) has been ingested regularly by many people with no evidence of side effects. Although a number of possible problems with very large doses of

vitamin C have been suggested, none of these adverse health effects have been confirmed, and there is no reliable scientific evidence that large amounts of vitamin C (up to 10 g/day in adults) are toxic. In the year 2000 the US Food and Nutrition Board recommended a tolerable upper intake level (UL) for vitamin C of 2 g (2,000 mg) daily in order to prevent most adults from experiencing osmotic diarrhoea and gastrointestinal disturbances.

Uses in food technology

The food industry uses ascorbic acid as a natural antioxidant. This means that ascorbic acid, added to foodstuffs during processing or prior to packing, preserves colour, aroma and nutrient content. This use of ascorbic acid has nothing to do with its vitamin action. In meat processing, ascorbic acid makes it possible to reduce both the amount of added nitrite and the residual nitrite content in the product. The addition of ascorbic acid to fresh flour improves its baking qualities, thus saving the 4-8 weeks of maturation flour would normally have to undergo after milling.

RESULT AND DISCUSSION

Vitamin c is an essential vitamin for human life and it is most beneficial for human health in the formation of immunity.It work against the various micro-organisms and treatment of disease like cancer,blood pressure,wound healing,cardiovascular disease,common cold and prevant various disease.It is also used as a nutrients.Itis also act as a natural antioxidant.Absorption of ascorbic acid was found to be 93.85% in the uncentrifuged saliva which was significantly higher than the centrifuged saliva.vitaminC absorption into the buccal mucosa was held in the mouth for periods of 1-9 minutes. For a constant mouth contact time, increase in pH of loading solutions resulted in reduction of buccal absorption, and diminishing transfer into the buccal epithelium. The degree of intestinal absorption decreases as intake of vitaminC increases.

REFERENCES

1. Li Y, Schellhorn HE. New developments and novel therapeutic perspectives for vitamin C. J Nutr 2007;137:2171-84. [PubMed abstract]
2. Carr AC, Frei B. Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. Am J Clin Nutr 1999;69:1086-107. [PubMed abstract]
3. Frei B, England L, Ames BN. Ascorbate is an outstanding antioxidant in human blood plasma. Proc Natl Acad Sci U S A 1989;86:6377-81. [PubMed abstract]
4. Jacob RA, Sotoudeh G. Vitamin C function and status in chronic disease. Nutr Clin Care 2002;5:66-74. [PubMed abstract]
5. Gershoff SN. Vitamin C (ascorbic acid): new roles, new requirements? Nutr Rev 1993;51:313-26. [PubMed abstract]
6. Weinstein M, Babyn P, Zlotkin S. An orange a day keeps the doctor away: scurvy in the year 2000. Pediatrics 2001;108:E55. [PubMed abstract]
7. Wang AH, Still C. Old world meets modern: a case report of scurvy. Nutr Clin Pract 2007;22:445-8. [PubMed abstract]
8. Institute of Medicine. Food and Nutrition Board. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington, DC: National Academy Press, 2000.
9. Stephen R, Utecht T. Scurvy identified in the emergency department: a case report. J Emerg Med 2001;21:235-7. [PubMed abstract]
10. Padayatty SJ, Sun H, Wang Y, Riordan HD, Hewitt SM, Katz A, Wesley RA, Levine M. Vitamin C pharmacokinetics: implications for oral and intravenous use. Ann Intern Med 2004;140:533-7. [PubMed abstract]
11. Francescone MA, Levitt J. Scurvy masquerading as leukocytoclastic vasculitis: a case report and review of the literature. Cutis 2005;76:261-6. [PubMed abstract]
12. U.S. Department of Agriculture. USDA National Nutrient Database for Standard Reference Release 23. 2010.

13. Bates CJ. Bioavailability of vitamin C. Eur J Clin Nutr 1997;51 (Suppl 1):S28-33. [PubMed abstract]
14. Mangels AR, Block G, Frey CM, Patterson BH, Taylor PR, Norkus EP, et al. The bioavailability to humans of ascorbic acid from oranges, orange juice and cooked broccoli is similar to that of synthetic ascorbic acid. J Nutr 1993;123:1054-61. [PubMed abstract]
15. Gregory JF 3rd. Ascorbic acid bioavailability in foods and supplements. Nutr Rev 1993;51:301-3. [PubMed abstract]
16. Johnston CS, Luo B. Comparison of the absorption and excretion of three commercially available sources of vitamin C. J Am Diet Assoc 1994;94:779-81. [PubMed abstract]
17. Moyad MA, Combs MA, Vrablic AS, Velasquez J, Turner B, Bernal S. Vitamin C metabolites, independent of smoking status, significantly enhance leukocyte, but not plasma ascorbate concentrations. Adv Ther 2008;25:995-1009. [PubMed abstract]
18. Moshfegh A, Goldman J, Cleveland L. What We Eat in America, NHANES 2001-2002: Usual Nutrient Intakes from Food Compared to Dietary Reference Intakes. Washington, DC: U.S. Department of Agriculture, Agricultural Research Service, 2005.
19. Radimer K, Bindewald B, Hughes J, Ervin B, Swanson C, Picciano MF. Dietary supplement use by US adults: data from the National Health and Nutrition Examination Survey, 1999-2000. Am J Epidemiol 2004;160:339-49. [PubMed abstract]
20. Picciano MF, Dwyer JT, Radimer KL, Wilson DH, Fisher KD, Thomas PR, et al. Dietary supplement use among infants, children, and adolescents in the United States, 1999-2002. Arch Pediatr Adolesc Med 2007;161:978-85. [PubMed abstract]
21. Levine M, Conry-Cantilena C, Wang Y, Welch RW, Washko PW, Dhariwal KR, et al. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. Proc Natl Acad Sci U S A 1996;93:3704-9. [PubMed abstract]
22. Levine M, Rumsey SC, Daruwala R, Park JB, Wang Y. Criteria and recommendations for vitamin C intake. JAMA 1999;281:1415-23. [PubMed abstract]
23. King, CG, Waugh, WA. The chemical nature of vitamin C. Science 1932;75:357-358.
24. Svirbely J, Szent-Györgyi A. Hexuronic acid as the antiscorbutic factor. Nature 1932;129: 576.
25. Svirbely J, Szent-Györgyi A. Hexuronic acid as the antiscorbutic factor. Nature 1932;129: 690.
26. Hoffman FA. Micronutrient requirements of cancer patients. Cancer. 1985;55 (1 Suppl):295-300. [PubMed abstract]
27. Deicher R, Hörl WH. Vitamin C in chronic kidney disease and hemodialysis patients. Kidney Blood Press Res 2003;26:100-6. [PubMed abstract]
28. Hecht SS. Approaches to cancer prevention based on an understanding of N-nitrosamine carcinogenesis. Proc Soc Exp Biol Med 1997;216:181-91. [PubMed abstract]
29. Zhang S, Hunter DJ, Forman MR, Rosner BA, Speizer FE, Colditz GA, et al. Dietary carotenoids and vitamins A, C, and E and risk of breast cancer. J Natl Cancer Inst 1999;91:547-56. [PubMed abstract]
30. Kushi LH, Fee RM, Sellers TA, Zheng W, Folsom AR. Intake of vitamins A, C, and E and postmenopausal breast cancer. The Iowa Women's Health Study. Am J Epidemiol 1996;144:165-74. [PubMed abstract]
31. Levine M, Wang Y, Padayatty SJ, Morrow J. A new recommended dietary allowance of vitamin C for healthy young women. Proc Natl Acad Sci U S A 2001;98:9842-6. [PubMed abstract]
32. Hercberg S, Galan P, Preziosi P, Bertrais S, Mennen L, Malvy D, et al. The SU.VI.MAX Study: a randomized, placebo-controlled trial of the health effects of antioxidant vitamins and minerals. Arch Intern Med 2004;164:2335-42. [PubMed abstract]
33. Galan P, Briançon S, Favier A, Bertrais S, Preziosi P, Faure H, et al. Antioxidant status and risk of cancer in the SU.VI.MAX study: is the effect of supplementation dependent on baseline levels? Br J Nutr 2005;94:125-32. [PubMed abstract]
34. Gaziano JM, Glynn RJ, Christen WG, Kurth T, Belanger C, MacFadyen J, et al. Vitamins E and C in the prevention of prostate and total cancer in men: the Physicians' Health Study II randomized controlled trial. JAMA 2009;301:52-62. [PubMed abstract]

35. Lin J, Cook NR, Albert C, Zaharris E, Gaziano JM, Van Denburgh M, et al. Vitamins C and E and beta carotene supplementation and cancer risk: a randomized controlled trial. J Natl Cancer Inst 2009;101:14-23. [PubMed abstract]
36. Taylor PR, Li B, Dawsey SM, Li JY, Yang CS, Guo W, et al. Prevention of esophageal cancer: the nutrition intervention trials in Linxian, China. Linxian Nutrition Intervention Trials Study Group. Cancer Res 1994;54(7 Suppl):2029s-31s. [PubMed abstract]
37. Qiao YL, Dawsey SM, Kamangar F, Fan JH, Abnet CC, Sun XD, et al. Total and cancer mortality after supplementation with vitamins and minerals: follow-up of the Linxian General Population Nutrition Intervention Trial. J Natl Cancer Inst 2009;101:507-18. [PubMed abstract]
38. Bjelakovic G, Nikolova D, Simonetti RG, Gluud C. Antioxidant supplements for preventing gastrointestinal cancers. Cochrane Database Syst Rev 2008;(3):CD004183. [PubMed abstract]
39. Coulter I, Hardy M, Shekelle P, Udani J, Spar M, Oda K, et al. Effect of the supplemental use of antioxidants vitamin C, vitamin E, and coenzyme Q10 for the prevention and treatment of cancer. Evidence Report/Technology Assessment Number 75. AHRQ Publication No. 04-E003. Rockville, MD: Agency for Healthcare Research and Quality, 2003. [PubMed abstract]
40. Padayatty SJ, Levine M. Vitamins C and E and the prevention of preeclampsia. N Engl J Med 2006;355:1065. [PubMed abstract]
41. Padayatty SJ, Levine M. Antioxidant supplements and cardiovascular disease in men. JAMA 2009;301:1336. [PubMed abstract]
42. Cameron E, Campbell A. The orthomolecular treatment of cancer. II. Clinical trial of high-dose ascorbic acid supplements in advanced human cancer. Chem Biol Interact 1974;9:285-315. [PubMed abstract]
43. Cameron E, Pauling L. Supplemental ascorbate in the supportive treatment of cancer: prolongation of survival times in terminal human cancer. Proc Natl Acad Sci U S A 1976;73:3685-9. [PubMed abstract]
44. Moertel CG, Fleming TR, Creagan ET, Rubin J, O'Connell MJ, Ames MM. High-dose vitamin C versus placebo in the treatment of patients with advanced cancer who have had no prior chemotherapy. A randomized double-blind comparison. N Engl J Med 1985;312:137-41. [PubMed abstract]
45. Bruno EJ Jr, Ziegenfuss TN, Landis J. Vitamin C: research update. Curr Sports Med Rep 2006;5:177-81. [PubMed abstract]
46. Padayatty SJ, Riordan HD, Hewitt SM, Katz A, Hoffer LJ, Levine M. Intravenously administered vitamin C as cancer therapy: three cases. CMAJ 2006;174:937-42. [PubMed abstract]
47. Hoffer LJ, Levine M, Assouline S, Melnychuk D, Padayatty SJ, Rosadiuk K, et al. Phase I clinical trial of i.v. ascorbic acid in advanced malignancy. Ann Oncol 2008;19:1969-74. [PubMed abstract]
48. Chen Q, Espey MG, Sun AY, Pooput C, Kirk KL, Krishna MC, et al. Pharmacologic doses of ascorbate act as a prooxidant and decrease growth of aggressive tumor xenografts in mice. Proc Natl Acad Sci U S A 2008;105:11105-9. [PubMed abstract]
49. Chen Q, Espey MG, Krishna MC, Mitchell JB, Corpe CP, Buettner GR, et al. Pharmacologic ascorbic acid concentrations selectively kill cancer cells: action as a pro-drug to deliver hydrogen peroxide to tissues. Proc Natl Acad Sci U S A 2005;102:13604-9. [PubMed abstract]
50. Chen Q, Espey MG, Sun AY, Lee JH, Krishna MC, Shacter E, et al. Ascorbate in pharmacologic concentrations selectively generates ascorbate radical and hydrogen peroxide in extracellular fluid in vivo. Proc Natl Acad Sci U S A 2007;104:8749-54. [PubMed abstract]
51. Levine M, Espey MG, Chen Q. Losing and finding a way at C: new promise for pharmacologic ascorbate in cancer treatment. Free Radic Biol Med 2009;47:27-9. [PubMed abstract]
52. Seifried HE, Anderson DE, Sorkin BC, Costello RB. Free radicals: the pros and cons of antioxidants. Executive summary report. J Nutr 2004;134:3143S-63S. [PubMed abstract]
53. Natural Medicines Comprehensive Database. Vitamin C.

54. Ye Z, Song H. Antioxidant vitamins intake and the risk of coronary heart disease: meta-analysis of cohort studies. Eur J Cardiovasc Prev Rehabil 2008;15:26-34. [PubMed abstract]
55. Willcox BJ, Curb JD, Rodriguez BL. Antioxidants in cardiovascular health and disease: key lessons from epidemiologic studies. Am J Cardiol 2008;101:75D-86D. [PubMed abstract]
56. Honarbakhsh S, Schachter M. Vitamins and cardiovascular disease. Br J Nutr 2008:1-19. [PubMed abstract]
57. Osganian SK, Stampfer MJ, Rimm E, Spiegelman D, Hu FB, Manson JE, et al. Vitamin C and risk of coronary heart disease in women. J Am Coll Cardiol 2003;42:246-52. [PubMed abstract]
58. Lee DH, Folsom AR, Harnack L, Halliwell B, Jacobs DR Jr. Does supplemental vitamin C increase cardiovascular disease risk in women with diabetes? Am J Clin Nutr 2004;80:1194-200. [PubMed abstract]
59. Myint PK, Luben RN, Welch AA, Bingham SA, Wareham NJ, Khaw KT. Plasma vitamin C concentrations predict risk of incident stroke over 10 y in 20 649 participants of the European Prospective Investigation into Cancer Norfolk prospective population study. Am J Clin Nutr 2008;87:64-9. [PubMed abstract]
60. Muntwyler J, Hennekens CH, Manson JE, Buring JE, Gaziano JM. Vitamin supplement use in a low-risk population of US male physicians and subsequent cardiovascular mortality. Arch Intern Med 2002;162:1472-6. [PubMed abstract]
61. Knekt P, Ritz J, Pereira MA, O'Reilly EJ, Augustsson K, Fraser GE, et al. Antioxidant vitamins and coronary heart disease risk: a pooled analysis of 9 cohorts. Am J Clin Nutr 2004;80:1508-20. [PubMed abstract]
62. Cook NR, Albert CM, Gaziano JM, Zaharris E, MacFadyen J, Danielson E, et al. A randomized factorial trial of vitamins C and E and beta carotene in the secondary prevention of cardiovascular events in women: results from the Women's Antioxidant Cardiovascular Study. Arch Intern Med 2007;167:1610-8. [PubMed abstract]
63. Sesso HD, Buring JE, Christen WG, Kurth T, Belanger C, MacFadyen J, et al. Vitamins E and C in the prevention of cardiovascular disease in men: the Physicians' Health Study II randomized controlled trial. JAMA 2008;300:2123-33. [PubMed abstract]
64. Waters DD, Alderman EL, Hsia J, Howard BV, Cobb FR, Rogers WJ, et al. Effects of hormone replacement therapy and antioxidant vitamin supplements on coronary atherosclerosis in postmenopausal women: a randomized controlled trial. JAMA 2002;288:2432-40. [PubMed abstract]
65. Bleys J, Miller ER 3rd, Pastor-Barriuso R, Appel LJ, Guallar E. Vitamin-mineral supplementation and the progression of atherosclerosis: a meta-analysis of randomized controlled trials. Am J Clin Nutr 2006;84:880-7. [PubMed abstract]
66. Shekelle P, Morton S, Hardy M. Effect of supplemental antioxidants vitamin C, vitamin E, and coenzyme Q10 for the prevention and treatment of cardiovascular disease. Evidence Report/Technology Assessment No. 83 AHRQ Publication No. 03-E043. Rockville, MD: Agency for Healthcare Research and Quality, 2003. [PubMed abstract]
67. van Leeuwen R, Boekhoorn S, Vingerling JR, Witteman JC, Klaver CC, Hofman A, et al. Dietary intake of antioxidants and risk of age-related macular degeneration. JAMA 2005;294:3101-7. [PubMed abstract]
68. Evans J. Primary prevention of age related macular degeneration. BMJ 2007;335:729. [PubMed abstract]
69. Chong EW, Wong TY, Kreis AJ, Simpson JA, Guymer RH. Dietary antioxidants and primary prevention of age related macular degeneration: systematic review and meta-analysis. BMJ 2007;335:755. [PubMed abstract]
70. Evans JR. Antioxidant vitamin and mineral supplements for slowing the progression of age-related macular degeneration. Cochrane Database Syst Rev 2006;(2):CD000254. [PubMed abstract]
71. Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8.

Arch Ophthalmol 2001;119:1417-36. [PubMed abstract]
72. Yoshida M, Takashima Y, Inoue M, Iwasaki M, Otani T, Sasaki S; JPHC Study Group. Prospective study showing that dietary vitamin C reduced the risk of age-related cataracts in a middle-aged Japanese population. Eur J Nutr 2007;46:118-24. [PubMed abstract]
73. Rautiainen S, Lindblad BE, Morgenstern R, Wolk A. Vitamin C supplements and the risk of age-related cataract: a population-based prospective cohort study in women. Am J Clin Nutr. 2010 Feb;91(2):487-93. [PubMed abstract]
74. Sperduto RD, Hu TS, Milton RC, Zhao JL, Everett DF, Cheng QF, et al. The Linxian cataract studies. Two nutrition intervention trials. Arch Ophthalmol 1993;111:1246-53. [PubMed abstract]
75. Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E and beta carotene for age-related cataract and vision loss: AREDS report no. 9. Arch Ophthalmol 2001;119:1439-52. [PubMed abstract]
76. Pauling L. The significance of the evidence about ascorbic acid and the common cold. Proc Natl Acad Sci U S A 1971;68:2678-81. [PubMed abstract]
77. Douglas RM, Hemilä H. Vitamin C for preventing and treating the common cold. PLoS Med 2005;2:e168. [PubMed abstract]
78. Douglas RM, Hemilä H, Chalker E, Treacy B. Vitamin C for preventing and treating the common cold. Cochrane Database Syst Rev 2007;(3):CD000980. [PubMed abstract]
79. Wintergerst ES, Maggini S, Hornig DH. Immune-enhancing role of vitamin C and zinc and effect on clinical conditions. Ann Nutr Metab 2006;50:85-94. [PubMed abstract]
80. Hemilä H. The role of vitamin C in the treatment of the common cold. Am Fam Physician 2007;76:1111, 1115. [PubMed abstract]
81. Johnston CS. The antihistamine action of ascorbic acid. Subcell Biochem 1996;25:189-213. [PubMed abstract]
82. Curhan GC, Willett WC, Rimm EB, Stampfer MJ. A prospective study of the intake of vitamins C and B6, and the risk of kidney stones in men. J Urol 1996;155:1847-51. [PubMed abstract]
83. Curhan GC, Willett WC, Speizer FE, Stampfer MJ. Intake of vitamins B6 and C and the risk of kidney stones in women. J Am Soc Nephrol 1999;10:840-5. [PubMed abstract]

*****