

Total Vitamin C (ascorbic acid and dehydroascorbic acid) was measured in supernatant after plasma precipitation, by isocratic reversed-phase HPLC with an electrochemical detector with amperometric detection (Bioanalytical Systems, Inc., West Lafayette, IN). A reversed-phase gradient HPLC method and a C30-carotenoid column (3 μ m, 150 x 4.6 mm, YMC, Wilmington, NC) was used for the simultaneous determination of carotenoids (lutein; zeaxanthin; beta-cryptoxanthin; alpha-carotene; cis-, trans- and beta-carotene isomers; and lycopene) and vitamin E (alpha- and gamma-tocopherol) in plasma. Echinenone and alpha-tocopheryl acetate were added to plasma as internal standards for carotenoids and tocopherols, respectively, prior to their extraction with chloroform: methanol and hexane. A photodiode array detector was programmed to monitor carotenoids at 450 nm, and tocopherols at 292 nm. The compounds were quantified by determining peak areas in the HPLC chromatograms calibrated against known amounts of standards. The values were corrected for recovery of the added internal standards.