Dear Sir:

I read with interest the article by Loke et al (1) published in the October issue of the Journal. In their study they show that the dietary flavonoids quercetin and (−)-epicatechin increase plasma and urinary concentrations of nitric oxide (NO) reaction products. More specifically, the concentrations of nitrite and S-nitrosothiols increased in plasma, whereas nitrate concentrations increased in urine. These stable NO reaction products are commonly used as markers of endogenous NO formation. Consequently, the authors suggest that stimulation of endothelial NO synthase by the flavonoids is the underlying mechanism. Although I have little doubt that flavonoids can increase endogenous NO generation and bioavailability, I was concerned about the actual concentrations of NO reaction products reported in the study. Loke et al reported plasma nitrite concentrations of ~4 μM, and these increased to >5 μM after ingestion of flavonoids. It seems clear from a great number of recent studies (some of which are cited by Loke et al) that fasting circulating nitrite concentrations are in the 50–500 nM range, ie, 10–100-fold lower (2–7). I wonder if the authors have confirmed these high concentrations by using another method, eg, the chemiluminescence technique that they used for S-nitrosothiols in the present study. This highly sensitive method is also commonly used for nitrate measurements (8). It was also surprising that the observed increases in urinary nitrate were not paralleled by increases in circulating nitrate. Moreover, in the Discussion, the authors state that normal plasma concentrations of S-nitrosothiols are 7 μM, but at the same time they now report concentrations of 8 nM, ie, 1000-fold lower. Finally, although the authors attempted to control basal intake of flavonoids during the study periods, I could find no information on restriction in dietary intakes of nitrate and nitrite—the actual markers measured in this study. Diet has a profound impact on circulating nitrate and nitrite, and these stable NO reaction products are commonly used as markers of endogenous NO formation (2). The reported value of 7 nM in 10 healthy subjects. Another group that used a similar method showed an increase in plasma SNO of 3 nM after cocoa intake; unfortunately, only changes were reported and not the actual baseline values (2). The reported value of 7 μM for plasma SNO was based on the photolytic cleavage of the S-NO bond. Photolysis has been shown to be nonspecific for SNOs and also causes S-nitrosoylation in biological tissues and fluids: implications for the fate of NO in vivo. FASEB J 2002:16:1773–85.


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REFERENCES


Letters to the Editor

Reply to JO Lundberg

Dear Sir:

Dr Lundberg is correct to point out that diet can have a profound effect on circulating nitrate and nitrite concentrations. Our study was designed to investigate the effect of particular flavonoids on nitric oxide (NO) status in healthy subjects. Although subjects were asked to follow a flavonoid-restricted diet for 48 h before treatment, they were not fasted before each treatment but asked to consume a standard breakfast before attending the clinic for blood sampling. Therefore, the values for nitrate and nitrite that we quote are not fasting values.

Our values (8 nM) for plasma S-nitrosothiols (SNO) are similar to those found by Marley et al (1) whose chemiluminescence-based assay we followed (1). They found a mean concentration of total SNO of 28 ± 7 nM in 10 healthy subjects. Another group that used a similar method showed an increase in plasma SNO of 3 nM after cocoa intake; unfortunately, only changes were reported and not the actual baseline values (2). The reported value of 7 μM for plasma SNO was based on the photolytic cleavage of the S-NO bond. Photolysis has been shown to be nonspecific for SNOs and also causes NO release from compounds such as nitrite, nitrosoamines, and dinitrosyliron complexes, thus potentially giving an overestimation of true SNO concentration (3). For measurement of plasma nitrate and nitrite, we chose a gas chromatography mass spectrometry–based method