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File: ■ Broccoli (*Brassica oleracea* var. *italica*, Brassicaceae)
■ Sulforaphane Bioavailability
■ Myrosinase

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RE: Increased Sulforaphane Bioavailability from Broccoli Preparations with Myrosinase

Fahey JW, Holtzclaw WD, Wehage SL, Wade KL, Stephenson KK, Talalay P. Sulforaphane bioavailability from glucoraphanin-rich broccoli: control by active endogenous myrosinase. *PLoS One*. 2015;10(11):e0140963. doi: 10.1371/journal.pone.0140963.

Many of the health benefits associated with cruciferous vegetables are attributed to glucosinolates. Although the glucosinolate glucoraphanin (GR), found in broccoli (*Brassica oleracea* var. *italica*, Brassicaceae), is inert, this compound can be converted into the bioactive compound sulforaphane by the microflora of the gastrointestinal tract or the endogenous plant enzyme myrosinase. The aim of this clinical study was to evaluate if the addition of myrosinase to different broccoli preparations containing GR increased the bioavailability of sulforaphane.

GR and active myrosinase are found in both broccoli seeds and sprouts. The broccoli products used in this study included broccoli sprout extracts (BSE) and broccoli seed powder (BSdP) (prepared by Johns Hopkins University; Baltimore, Maryland); broccoli seed extracts (BSdE) (Brassica Protection Products LLC; Baltimore, Maryland); and freeze-dried broccoli sprouts (FDBS) (grown under contract by Hanover Foods Corp.; Sunsprout; Ridgely, Maryland). The GR content of the broccoli products was determined using high-performance liquid chromatography (HPLC). Myrosinase activity was determined spectrophotometrically.

Subjects were instructed not to consume any cruciferous vegetables or products 3 days prior to the study and 24 hours after dosing. After an overnight fast, baseline urine samples were collected and then the broccoli preparations were consumed. Afterwards, the subjects collected their urine for 24 hours.

Subjects consumed BSE either mixed with water (≤1 g; 50 mL x 2), in clear capsules without excipients (50 µmol GR), or in opaque green gel-caps with inert excipients (69 or 230 µmol of GR) (encapsulated by Xymogen; Orlando, Florida). Similarly, BSdE and BSdP were consumed in gel-caps (69 or 230 µmol of GR and 100 µmol of GR, respectively). FDBS were consumed as standard capsules; acid-resistant gel-caps (100 µmol GR); or prehydrolyzed in 50% water, 46.5% pineapple (*Ananas comosus*, Bromeliaceae) juice (Dole; Westlake Village, California), and 3.5% lime (*Citrus* × *aurantifolia*, Rutaceae) juice (Safeway; Pleasanton, California) (50, 100, and 200 µmol of GR). All subjects (17 females and 5 males;

mean age, 51.3 years; 9 African-American and 13 white) included in the study were healthy. Only 5 of these subjects (4 females and 1 male; mean age, 53.8 years; 1 African-American and 4 white) were included for extended studies.

Urinary dithiocarbamate (DTC) excretion levels were used to measure sulforaphane bioavailability from the broccoli preparations. DTC was quantified from all the urine samples. Compliance also was assessed.

The bioavailability of GR varied among and within the subjects. Baseline conversion efficiency of a 50-µmol dose of GR-rich BSE, lacking myrosinase, had DTC levels ranging from 3.7 to 6.9 µmol (mean, 4.7 µmol or 9.4% of dose). Comparison of the bioavailability of BSE to that of a commercially prepared GR-rich supplement indicated they were equally bioavailable (10.4% and 10.3%, respectively; P=0.2340). It was also found that higher doses were slightly, but not significantly, higher in bioavailability than the lower doses for the commercial BSdE (9.7% and 11.2%, P=0.412) and non-commercial BSE (8.2% and 12.5%, P=0.0956) supplements.

FDBS, in which the glucosinolates had been hydrolyzed to isothiocyanates by myrosinase (50, 100, and 200 µmol of GR), resulted in urinary DTCs that ranged from 13.8 to 30.1 µmol (mean, 20.5 µmol or 41.0% of dose). FDBS in gel-cap form produced similar results. The amount of 24-hr urine DTC was also similar for FDBS consumed in standard capsules with myrosinase that dissolved in the stomach (range, 31.3 to 42.8 µmol; mean, 35.1% of dose), compared to acid-resistant capsules that dissolved in the gastrointestinal tract (range, 21.7 to 37.7 µmol; mean, 32.7% of dose). Evaluation of the bioavailability of BSdP, which had active myrosinase, indicated the mean conversion to DTC urinary metabolites was 36.1% of the dose. Compliance was considered 100% for the dosing studies and overall considered good.

The authors found that regardless of the matrix used, the presence of active myrosinase led to the greatest enhancement of sulforaphane bioavailability from the broccoli preparations. These results are consistent with the results of a previous study.[1] FDBS mixed with a diluted juice mixture that had endogenous myrosinase was found to be the most effective at increasing sulforaphane bioavailability. The authors indicate this is over 4-fold greater than the DTC recovery found for BSE without myrosinase, but only marginally better than other preparations with myrosinase. Future studies may want to focus on optimizing broccoli preparations that produce less variability among subjects in order to obtain predictable results. This study was partially funded by Brassica Protection Products LLC, and the supplements were provided free of charge by the manufacturer, Xymogen.

—Laura M. Bystrom, PhD

Reference

[1]Shapiro TA, Fahey JW, Dinkova-Kostova AT, et al. Safety, tolerance, and metabolism of broccoli sprout glucosinolates and isothiocyanates: a clinical phase I study. *Nutr Cancer*. 2006;55(1):53-62.

Referenced article can be accessed at
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0140963.