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ARTICLE

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LC-MS/MS Quantification of Sulforaphane and Indole-3-carbinol Metabolites in Human Plasma and Urine after Dietary Intake of Selenium-Fortified Broccoli

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ABSTRACT: This study aimed at developing a sensitive LC-MS/MS method for the quantification of sulforaphane (SFN) and indole-3-carbinol metabolites in plasma and urine after dietary intake of regular and selenium-fertilized broccoli using stable isotope dilution analysis. In a three-armed, placebo-controlled, randomized human intervention study with 76 healthy volunteers, 200 g of regular (485 μg of total glucosinolates and <0.01 μg of selenium per gram fresh weight) or selenium-fertilized broccoli (589 μg of total glucosinolates and 0.25 μg of selenium per gram fresh weight) was administered daily for 4 weeks. Glucoraphanin and glucobrassicin metabolites quantified in plasma and urine were SFN−glutathione, SFN−cysteine, SFN−cysteinylglycine, SFN−acetylcysteine, and indole-3-carboxaldehyde, indole-3-carboxylic acid, and ascorbigen, respectively. Dietary intake of selenium-fertilized broccoli increased serum selenium concentration analyzed by means of atomic absorption spectroscopy by up to 25% ($p < 0.001$), but affected neither glucosinolate concentrations in broccoli nor their metabolite concentrations in plasma and urine compared to regular broccoli.

KEYWORDS: broccoli, glucosinolates, LC-MS/MS, glucobrassicin metabolites, glucoraphanin metabolites, sulforaphane, indole-3-carbinol, selenium

■ INTRODUCTION

Numerous human studies have shown an association between a diet rich in cruciferous vegetables (e.g., broccoli, cabbage, and Brussels sprouts) and a decreased risk for prostate cancer.[1,2] This chemopreventive activity of cruciferous vegetables is attributed to their contents in glucosinolates[3] and probably selenium (Se).[4]

Among the glucosinolates, glucoraphanin and glucobrassicin represent the quantitatively dominating groups in broccoli. After dietary intake, glucoraphanin and glucobrassicin undergo a myrosinase-catalyzed hydrolysis, yielding sulforaphane (SFN) and indole-3-carbinol (I3-C), respectively. Once absorbed in the gastrointestinal tract, SFN and I3-C can be further metabolized into a broad spectrum of metabolites.[5−7] SFN is primarily metabolized through the mercapturic acid pathway, whereby its glutathione conjugate (SFN−GSH) is formed intracellularly and is sequentially degraded to form cysteinylglycine (SFN−Cys-Gly), cysteine (SFN−Cys), and *N*-acetylcysteine (SFN−NAC) conjugates in the upper intestinal lumen.[5−7]

Glucobrassicin metabolites are also generated through the enzymatic activity of the myrosinase that releases indole-3-acetonitrile (I3-ACN) under acidic conditions.[8] At neutral pH, the myrosinase catalyzes the formation of I3-C via the unstable intermediate indole-3-methyl isothiocyanate.[9] I3-C might undergo oxidative metabolization, yielding the corresponding aldehyde and/or carboxylic acid (I3-CAL, I3-CA).[10] Numerous in vitro studies using the free compound I3-C also showed its oligomerization into dimers, linear and cyclic trimers, and higher oligomers at acidic pH values as they occur in the stomach.[9,11] Furthermore, I3-C has also been demonstrated to react with L-ascorbic acid to form ascorbigen (ASG) in vitro.[12]

Glucoraphanin metabolites in biological samples are commonly quantified by analytical methods based on HPLC coupled to UV or mass detection.[13−15] Recently, Egner et al. reported a very sensitive and well-validated method for quantification of SFN and its mercapturic acid pathway conjugates in urine samples using a stable isotope dilution assay (SIDA) after bolus administration of broccoli infusions containing 175 mg of glucoraphanin to healthy volunteers.[16]

Glucobrassicin metabolites after dietary intake have been quantified by means of less sensitive analytical methods that require higher doses for reliable quantification. In one of the previously reported studies, ASG was quantified in rat plasma after dietary administration of approximately 117 mg of ASG/kg of body weight for 7 days using HPLC-MS.[17] Reed et al.[18] administered amounts of 400−1200 mg of I3-C to human subjects and were able to quantify only one of the glucobrassicin metabolites, 3,3′-diindolylmethane (DIM), by means of HPLC-MS.[18] We aimed at elucidating whether other metabolites are formed in vivo after dietary intake of glucobrassicin-containing

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