COLLEGE OF PUBLIC HEALTH AND HUMAN SCIENCES

Plasma metabolites altered by sulforaphane in humans

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I. Introduction

- Consuming cruciferous vegetables such as broccoli sprouts, kale, Brussels sprouts, and bok choy is associated with several health benefits, including a decreased risk of certain cancers. [1]
- Cruciferous vegetables contain the glucosinolate glucoraphanin (GFN).
- Cutting, chopping, or chewing these vegetables releases GFN and the enzyme myrosinase, which transforms GFN into sulforaphane (SFN). [2]
- SFN has many demonstrated health benefits in cell and animal models, including anticarcinogenic, antioxidant, and antibacterial properties. [3]
- SFN has been shown to target biological pathways leading to:
  - apoptosis and the excretion of carcinogens
  - cell cycle arrest
  - eradication of *H. pylori* infections[4,5]
- In humans, SFN is metabolized to yield four bioactive metabolites via the mercapturic acid pathway (Fig. 1).
- Specific SFN metabolites may be responsible for some of the health benefits of consuming SFN.
- In humans, SFN metabolites peak in the plasma at 3 hours following consumption and are mostly excreted by 24 hours.[6]

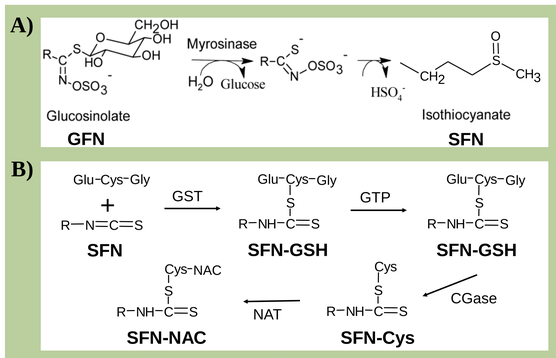


Figure 1. Formation and metabolism of SFN. (A) Hydrolysis of GFN to SFN via myrosinase. (B) Metabolism of SFN via the mercapturic acid pathway. GFN, glucoraphanin; SFN, sulforaphane; GST, Glutathione-*S*-transferase; GTP, γ-Glutamyltranspeptidase; CGase, Cysteinylglycinase; NAT, *N*-acetyltransferase.

II. Study Goal

Identify plasma metabolites altered in humans following sulforaphane consumption using novel, unbiased metabolomic technology.

III. Significance

Understanding mechanisms responsible for SFN's health benefits is critical for developing dietary strategies using SFN to promote health and prevent disease. This work will provide information for directing further human studies to understand mechanisms by which SFN and cruciferous vegetable consumption promote health.

IV. Design

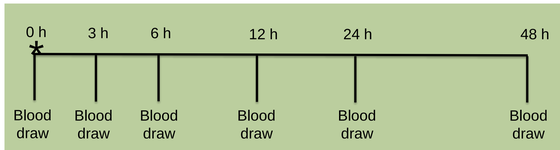


Figure 2. Human study timeline. Healthy adults (*n*=10), ages 20-60 years, consumed a single dose of 200 µmol SFN equivalents from fresh broccoli sprouts. Subjects provided blood samples at time 0, 3, 6, 12, 24, & 48 hours after sprout consumption to determine what changes occur early and what changes occur later.
✶ = time of sprout consumption

V. Methods

The metabolome of plasma samples from each time point was analyzed by HPLC tandem mass spectrometry.

Plasma Processing for Metabolomic Analysis:
- Plasma collected from Histopaque separation procedure[7] & stored at -80ºC.
- Metabolites extracted from plasma by addition of 150 µl ice-cold 50:50 (v:v) methanol:ethanol.
- Samples centrifuged for 15 minutes & stored at -80ºC until analysis.
- Quality control (QC) samples prepared by combining 15 µl of each sample into a single vial.

Metabolomic Analysis:
- High-performance liquid chromatography (HPLC) information/settings:
  - HPLC Column: Inertsil ® Phenyl-3 5 µM, 4.6 x 150 mm (GL Sciences, Inc., Tokyo, Japan)
  - Cooling temperature: 15ºC
  - Solvents: Methanol + 0.1% (v/v) formic acid (FA) (organic), water + 0.1% (v/v) FA (aqueous)
  - Flow rate: 0.4 ml/min
  - Injection volume: 10 µl plasma

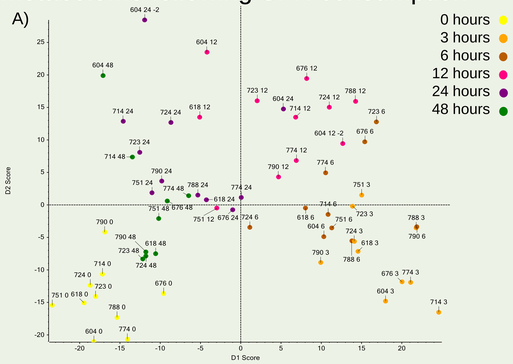
V. Methods (continued)

- MS information/settings:
  - Positive ion mode
- AB SciexTripleTOF™ 5600 mass spectrometer
- Peak intensity threshold for MS/MS experiments: 100
- Collision energies & mass ranges measured:
  - MS only: 10 eV (mass range: 70-1000 m/z)
  - MS/MS: 40 eV (mass range: 40-1000 m/z)

Metabolite identification:
- Student's t tests were conducted to compare levels of detected features between time 0 & other time points (3, 6, 12, 24, & 48 hours)
- Fragment spectra for altered features (p<0.05) meeting intensity threshold for MS/MS analysis were compared to MS/MS spectra in metabolomic databases: 1) Metlin: Metabolite and Tandem MS Database, and 2) Human Metabolome Database (HMDB).
- Criteria for candidate metabolite identification:
  - Endogenous in origin
  - MS/MS data (i.e., fragment spectra) available in database
  - Mass tolerances of ≤ 5ppm (Metlin) and 0.01 ppm (HMDB)
  - Matching m/z ratios of MS/MS fragments between database & experimentally derived spectra

VI. Results

Alterations detected in human plasma metabolome following SFN consumption



B)

Time point	# of features altered
3-48 hours, early sustained	52
48 hours, late transient	28

Figure 3. Changes in human plasma metabolome were detected following SFN consumption from broccoli sprouts. A) PCA-DA plot showing separation of time points based on all features detected in subjects at each time point. B) Number of features significantly altered ($p<0.05$, fold change > 2) over time after consuming sprouts ($n=10$). PCA-DA, principal components analysis – discriminant analysis.

VI. Results (continued)

Creatinine possibly increased 48 hours after SFN consumption

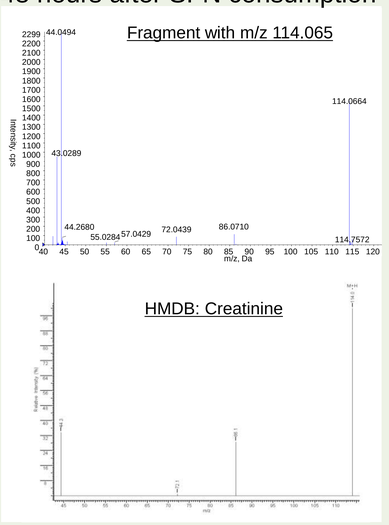


Figure 4. Fragment spectra from MS/MS experiments. Fragment spectra for A) feature with mass-to-charge ratio of 114.065 from study data, and B) creatinine from Human Metabolome Database (HMDB) showing matching m/z values (relative intensity varies from one machine to another).

VII. Discussion

Biochemical pathways affected may involve creatinine
- To our knowledge, SFN has not previously been reported to alter plasma creatinine levels in humans.
- Creatinine plays a large role in muscle metabolism.
- High creatinine levels may indicate decreased risk of type 2 diabetes.[8]
- Molecular pathways involving creatinine may signify a novel mechanism of SFN in promoting human health.

VIII. Summary and Conclusions

- **The plasma metabolome was altered in human subjects following SFN consumption from broccoli sprouts.**
  - Altered features were observed in the plasma at each time point following sprout consumption.
  - Some alterations were transient, while others were sustained through multiple time points.
- **Biochemical pathways affected by consumption of broccoli sprouts may involve creatinine.**
  - Fragment spectra of a feature significantly altered at 48 hours following sprout consumption was visually matched to the fragment spectra of creatinine from the database.
- **Future directions:**
  - Additional experiments are needed to validate the feature's identity.
  - More work is needed to understand the full potential of SFN in human health.

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Acknowledgements:
This work was supported by NIH grants CA090890, CA122906, NIEHS grant P30 ES000210. We gratefully acknowledge technical assistance from Lauren Atwell, Dr. Carmen Wong, Dr. Laura Beaver, Karin Hardin and Jeff Morré. Broccoli sprouts were provided by Sprouters NW, Inc.

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