Harnessing the Power of Cruciferous Vegetables: Developing a Biomarker for Brassica Vegetable Consumption Using Urinary 3,3′-Diindolylmethane

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Abstract

Glucobrassicin in Brassica vegetables gives rise to indole-3-carbinol (I3C), a compound with potent anticancer effects in preclinical models. We previously showed that the urinary metabolite 3,3′-diindolylmethane (DIM) could discriminate between volunteers fed high and low doses of Brassica vegetables. However, the quantitative relationship between glucobrassicin exposure and urinary DIM level is unclear. We conducted a clinical trial to examine the hypotheses that a range of glucobrassicin exposure from Brassica vegetables is reflected in urinary DIM and that this effect plateaus. Forty-five subjects consumed vegetables, a mixture of brussels sprouts and/or cabbage, at one of seven discrete dose levels of glucobrassicin ranging from 25 to 500 μmol, once daily for 2 consecutive days. All urine was collected for 24 hours after each vegetable-eating session. Urinary DIM was measured using our published liquid chromatography-electrospray ionization-tandem mass spectrometry-selected reaction monitoring (LC/ESI-MS/MS-SRM) method. Urinary DIM excretion increased predictably with increasing glucobrassicin dose and plateaued between 200 and 300 μmol of glucobrassicin. The association between glucobrassicin dose and urinary DIM was strong and positive (R² = 0.68). The majority of DIM was excreted in the first 12 hours after vegetable consumption. We conclude that urinary DIM is a reliable biomarker of glucobrassicin exposure and I3C uptake and that feeding glucobrassicin beyond 200 μmol did not consistently lead to more urinary DIM, suggesting a plateau in potential chemopreventive benefit.

Introduction

The anticancer effect of cruciferous vegetables is widely thought to be mediated by their glucosinolates (1), and epidemiologic evidence points to an association between high cruciferous vegetable consumption and decreased cancer risk (2), but the association is inconsistent, due in large part to a lack of objective measures of phytochemical exposure and uptake. Upon plant cell damage (i.e., chewing), inert glucosinolates are converted to indoles and isothiocyanates by myrosinase. Glucobrassicin, a predominant glucosinolate in many common Brassica vegetables (3, 4), is hydrolyzed to indole-3-carbinol (I3C, Fig. 1). In the acidic environment of the stomach, I3C readily undergoes acid condensation, primarily to 3,3′-diindolylmethane (DIM, Fig. 1; refs. 5–10). Both I3C and DIM possess strong effects in vitro and in vivo against cancers of the lung, colon, prostate, and breast (11–14). Mice gavaged with NNK and then treated with I3C or DIM developed significantly fewer lung tumors compared with controls (13, 15, 16). In a placebo-controlled clinical trial, I3C taken for 12 weeks resulted in regression of cervical intraepithelial neoplasia in approximately half of the 17 women randomized to either 200 or 400 mg daily compared with placebo (17). Other studies demonstrated that both I3C and DIM modulate estrogen metabolism in animals and humans to favor the formation of the antiproliferative estrogen 2-hydroxyestrone (2-OHE) over the proliferation-inducing 16α-hydroxyestrone (16-OHE; refs. 18–22); a higher 2-OHE:16-OHE ratio is associated with a decreased risk of estrogen-sensitive tumors such as breast cancer (23). Because of the rapid condensation of I3C to DIM and other oligomers, development of an in vivo biomarker to detect I3C in humans is not realistic (6, 7, 24). Alternatively, urine is a noninvasive and practical biospecimen ideal for use in large studies. We previously developed a novel method to quantify DIM in urine and showed that consuming vegetables with divergent glucobrassicin concentrations was consistently reflected in urinary DIM levels (25). To further define the utility of urinary DIM as a noninvasive biomarker of glucobrassicin exposure and I3C uptake from vegetables in humans, we conducted a clinical trial to determine the ability of urinary DIM to discriminate a wide range of glucobrassicin doses. We also sought to define the glucobrassicin dose at which this...
relationship plateaus, as observed with I3C and DIM administered as supplements (24, 26).

Materials and Methods

Study design

Healthy, nonsmoking, nonvegetarian adult subjects ages 18 to 60 years were recruited. Kidney and liver functions were required to be normal. Subjects taking H2 blockers, proton pump inhibitors, or calcium carbonate regularly and those recently treated with antibiotics were excluded. Questionnaires were administered to collect demographic information and histories of tobacco, medication, and alcohol use. At enrollment, subjects were randomly assigned to 1 of 7 doses of glucobrassicin—25, 50, 100, 200, 300, 400, or 500 μmol. Four subjects were recruited at each dose level. To determine interindividual variation in urinary DIM between glucobrassicin doses, an additional 6 subjects were recruited for 3 dose levels—50, 200, and 500 μmol. Subjects refrained from cruciferous vegetable consumption for a minimum of 5 days prior to the study intervention. Salads comprising fixed proportions of brussels sprouts and cabbage to attain the desired glucobrassicin dose (Table 1 in Supplementary Data) were freshly prepared with minimal chopping on the day of the study intervention. Subjects fasted 2 hours, provided a spot urine collection, and then consumed the assigned dose of study vegetables once daily for 2 consecutive days at the study center as quickly as possible. This was followed by another 2-hour fasting period. All urine was collected for 24 hours following each vegetable-eating session, divided into the following periods: 0–2, 2–6, 6–12, and 12–24 hours. This was done to inform whether briefer collection periods could be used in future studies. Urine volume was measured and aliquots stored at −20°C. Self-reported food diaries were kept throughout the study period and reviewed for obvious dietary intake of cruciferous vegetables. An overview of the trial design is shown in Figure 2. The protocol and consent form were approved by the Institutional Review Board at the University of Minnesota (Minneapolis, MN). All subjects provided informed consent.

Analysis of glucobrassicin concentration in the vegetables

"Blue Dynasty" cabbage and "Jade Cross" brussels sprouts were grown specifically for this study (see Supplementary Materials). Approximately 200 g, bulked from different heads of cabbage (n = 4) and brussels sprouts (n = 4), were taken for determination of glucobrassicin concentration at 1 time point shortly after harvest. Glucobrassicin concentration was analyzed using a published technique (25).

Urine sample preparation

Urine samples were prepared using a previously published technique (25). Analyses were blinded, but to minimize intraindividual variability, all samples from a given subject were prepared together in a single set.

LC-ESI-MS/MS-SRM

Analysis was performed as previously published (25), with slight modifications to increase sample throughput. Briefly, a TSQ

Figure 1.
Chemical structures of glucobrassicin, I3C, and DIM.

Figure 2.
Clinical trial schema. The study intervention consisted of consuming the assigned dose of brussels sprouts, cabbage, or a mixture for 2 consecutive days. Each vegetable-feeding session was followed by a 24-hour urine collection.
Table 1. Urinary DIM at baseline and after consuming escalating glucobrassicin doses from cabbage and brussels sprouts

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Glucobrassicin dose, µmol</th>
<th>Baseline DIM, pmol/mL</th>
<th>24-hour DIM, pmol/mL</th>
<th>Mean 24-h DIM ± SE, pmol/mL</th>
<th>DIM excreted in 12 h, %</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>&lt;LOD</td>
<td>7.91</td>
<td>1.84</td>
<td>4.88 ± 0.94</td>
<td>100</td>
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<tr>
<td>2</td>
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<td>7.27</td>
<td>4.70</td>
<td>5.99 ± 1.29</td>
<td>91.8</td>
</tr>
<tr>
<td>3</td>
<td>&lt;LOD</td>
<td>28.1</td>
<td>49.1</td>
<td>38.6 ± 10.5</td>
<td>92.2</td>
</tr>
<tr>
<td>4</td>
<td>1.24</td>
<td>8.83</td>
<td>3.83</td>
<td>6.33 ± 2.50</td>
<td>95.2</td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>3.19</td>
<td>12.7</td>
<td>7.95 ± 4.76</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>&lt;LOD</td>
<td>2.00</td>
<td>2.71</td>
<td>2.36 ± 0.36</td>
<td>60.0</td>
</tr>
<tr>
<td>7</td>
<td>&lt;LOD</td>
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<td>10.6</td>
<td>11.3 ± 0.7</td>
<td>95.0</td>
</tr>
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<td>ND</td>
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<td>15.6</td>
<td>15.2 ± 0.5</td>
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<td>&lt;LOD</td>
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<td>34.9 ± 19.8</td>
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<tr>
<td>10</td>
<td>0.82</td>
<td>13.8</td>
<td>22.1</td>
<td>18.0 ± 4.2</td>
<td>81.2</td>
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<td>43.3</td>
<td>24.6 ± 18.7</td>
<td>88.3</td>
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<td>4.12 ± 158</td>
<td>100</td>
</tr>
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<td>48.2 ± 1.0</td>
<td>97.6</td>
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<tr>
<td>14</td>
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<td>175</td>
<td>173 ± 2</td>
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<tr>
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<td>ND</td>
<td>122</td>
<td>127</td>
<td>125 ± 3</td>
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<td>16</td>
<td>2.45</td>
<td>35.4</td>
<td>66.2</td>
<td>50.8 ± 15.4</td>
<td>98.3</td>
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<td>17</td>
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<td>47.2</td>
<td>76.6 ± 29.4</td>
<td>96.9</td>
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<tr>
<td>18</td>
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<td>58.4</td>
<td>199 ± 141</td>
<td>99.2</td>
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<tr>
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<td>300</td>
<td>129</td>
<td>215 ± 86</td>
<td>96.5</td>
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<td>20</td>
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<td>1,060</td>
<td>1,050 ± 45</td>
<td>98.7</td>
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<td>26.5</td>
<td>37.8 ± 11.3</td>
<td>97.8</td>
</tr>
<tr>
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<td>&lt;LOD</td>
<td>126</td>
<td>193</td>
<td>160 ± 34</td>
<td>96.2</td>
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<tr>
<td>23</td>
<td>&lt;LOD</td>
<td>107</td>
<td>39.0</td>
<td>73.0 ± 34.0</td>
<td>98.2</td>
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<td>24</td>
<td>1.30</td>
<td>347</td>
<td>226</td>
<td>287 ± 61</td>
<td>94.8</td>
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<td>509</td>
<td>614</td>
<td>562 ± 53</td>
<td>97.8</td>
</tr>
<tr>
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<td>ND</td>
<td>502</td>
<td>335</td>
<td>419 ± 84</td>
<td>93.1</td>
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<tr>
<td>27</td>
<td>&lt;LOD</td>
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<td>24.7</td>
<td>20.7 ± 4.0</td>
<td>95.8</td>
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<td>28</td>
<td>300</td>
<td>&lt;LOD</td>
<td>517</td>
<td>554 ± 19</td>
<td>99.7</td>
</tr>
<tr>
<td>29</td>
<td>&lt;LOD</td>
<td>91.2</td>
<td>134</td>
<td>115 ± 21</td>
<td>97.5</td>
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<td>30</td>
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<td>64.9 a</td>
<td>106</td>
<td>85.5 ± 20.6</td>
<td>96.1</td>
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<td>&lt;LOD</td>
<td>457</td>
<td>106</td>
<td>282 ± 176</td>
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<tr>
<td>32</td>
<td>ND</td>
<td>738</td>
<td>957</td>
<td>848 ± 110</td>
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<td>33</td>
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<td>625</td>
<td>482</td>
<td>544 ± 72</td>
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<td>34</td>
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<td>76.3</td>
<td>159</td>
<td>118 ± 41</td>
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<tr>
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<td>&lt;LOD</td>
<td>151 b</td>
<td>406</td>
<td>279 ± 128</td>
<td>73.6</td>
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<tr>
<td>36</td>
<td>500</td>
<td>0.74</td>
<td>157</td>
<td>134 ± 46</td>
<td>82.3</td>
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<td>787</td>
<td>545 ± 243</td>
<td>99.1</td>
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<tr>
<td>38</td>
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<td>352 a</td>
<td>314 b</td>
<td>333 ± 19</td>
<td>97.9</td>
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<td>39</td>
<td>ND</td>
<td>2,260</td>
<td>2,100</td>
<td>2,180 ± 80</td>
<td>99.5</td>
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<tr>
<td>40</td>
<td>5.72</td>
<td>959</td>
<td>2,044</td>
<td>1,500 ± 543</td>
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<tr>
<td>41</td>
<td>ND</td>
<td>1,265</td>
<td>109 a</td>
<td>687 ± 578</td>
<td>100</td>
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<td>42</td>
<td>&lt;LOD</td>
<td>591 a</td>
<td>97 e</td>
<td>751 ± 160</td>
<td>99.4</td>
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<tr>
<td>43</td>
<td>&lt;LOD</td>
<td>193</td>
<td>74.9</td>
<td>92.0 ± 17.1</td>
<td>97.7</td>
</tr>
<tr>
<td>44</td>
<td>&lt;LOD</td>
<td>122 a</td>
<td>348 b</td>
<td>235 ± 113</td>
<td>100</td>
</tr>
<tr>
<td>45</td>
<td>&lt;LOD</td>
<td>180</td>
<td>108 e</td>
<td>144 ± 36</td>
<td>93.1</td>
</tr>
</tbody>
</table>

Abbreviations: LOD, level of detection; ND, not detectable.
aOriginally randomized to 500 µmol dose level but reassigned to 50 µmol dose level due to intolerance.
bMissed one void during the 12- to 24-hour collection period.
cOne void from 6- to 12-hour collection period inadvertently collected in the container allocated to the 12- to 24-hour collection period.
dDid not eat all of the assigned vegetables.

The Vantage instrument was used in addition to the TSQ Quantum Discovery Max instrument used previously. The TSQ Vantage was coupled to an Eksigent NanoLC Ultra (Eksigent Technologies) liquid chromatographic system; MS parameters on the TSQ Vantage were comparable to those previously published (25). The chromatographic method was shortened to decrease analysis time. Initial conditions were 40% 10 mmol/L NH₄OAc and 60% methanol; percent methanol was then increased to 70% over 15 minutes and held for 1 minute, then increased to 95% over 1 minute and held for 2 minutes, the system was then returned to initial conditions over 1 minute and allowed to reequilibrate for 10 minutes before injection of the next sample. Flow rate was held constant at 10 µL/min throughout the separation. The column was equipped with a KrudKatcher 0.5-µm prefilter (Phenomenex), which was exchanged after approximately 50 sample injections or when significant peak-broadening was
observed. Typical retention times for DIM and [3H]-DIM on the Vantage system were 14.7 and 14.8 minutes, respectively, and 16.6 and 16.5 on the Discovery. The detection limit of the assay was 0.4 pmol/mL.

Quality control
This laboratory method for quantifying urinary DIM has been previously validated (25). To ensure continued quality control, each set included a minimum of 2 water blanks, spiked with [3H]-DIM to monitor recovery, as well as 4 positive controls consisting of urine samples known to contain DIM, pooled from subjects who had consumed brussels sprouts. Spiked control samples were included in select sets to confirm reproducibility at the lower and upper ends of the observed DIM concentration range in study samples. Briefly, baseline urine samples from 9 subjects, which had been confirmed to contain no measurable DIM, were pooled and then spiked to yield final DIM concentrations of 35 or 0.90 pmol/mL or slightly over 2 times the detection limit of the assay. Samples with an apparent recovery below 5% were re-assayed.

Statistical analysis
The primary objective of this study was to characterize the relationship between glucobrassicin dose and urinary DIM, specifically to identify the maximum dose where the urinary DIM "response" levels off (plateau). The two 24-hour DIM measurements from each subject were averaged. Any DIM detected in the baseline spot urine sample prior to vegetable intake was subtracted from this value. The model used to determine the dose-response curve of glucobrassicin fed (dose) and urinary DIM (response) is based on the "median effect principle" of pharmacology (27) and is expressed as a 4-parameter logistic equation:

\[
\log(C/1-C) = \beta_1 + \beta_2 \times \log(\text{dose}),
\]

where \(C = (\text{DIM} - \beta_3)/(\beta_4 - \beta_3)\)

The parameters estimated by this model are \(\beta_1\) and \(\beta_2\), the intercept and slope of the linear portion of the sigmoidal curve, and \(\beta_3\) and \(\beta_4\), the minimum and maximum levels, respectively, of urinary DIM where the logistic curve flattens out at lower and higher doses. These parameters were estimated using the nonlinear (NLIN) procedure in SAS version 9.3 (SAS Institute Inc.). The dose that produces a 50% response (ED50) is calculated by the logarithmic scale and therefore the

\[
\text{ED}_{50} = \exp\left[\frac{1}{2}\left(\frac{1}{\beta_2}\right)\right]
\]

where IC is the intercept and ED is the estimated dose. The minimum, maximum DIM, and ED were reported with their 95% confidence intervals (CI). The minimum and maximum estimates were entered into the above equation to calculate \(C/1-C\), which results in a simple linear expression in the logarithmic scale and therefore the R-squared between glucobrassicin dose and urinary DIM was calculated.

All means are reported with their SE and/or 95% CIs unless otherwise noted.

Results

Subject characteristics and study compliance
Nineteen males and 26 females ranging in age from 19 to 56 years (mean, 31.5 ± 1.5 years) completed the study. The accrual goal was 46; one subject at the 50 µmol dose dropped out because of issues unrelated to the study and was not replaced. Thirty-five (78%) were Caucasian, 5 were Asian (11%), 2 were African-American (4%), and 3 (7%) reported more than one race. At the 500 µmol dose level, 2 subjects could not finish due to the taste of the raw brussels sprouts and were reassigned to the 50 µmol dose after a minimum 7-day washout period. In addition, subject 40 at the 500 µmol dose finished only 162.1 g (66.8%) of the brussels sprouts on day 1 and 210.5 g (86.7%) on day 2. Subject 43 at the 500 µmol dose finished 90.5 g (37.3%) on day 1 and 188.1 g (77.5%) on day 2. Subject 36 at the 500 µmol dose finished 128.1 g (52.3%) on day 1 and 184.8 g (76.1%) on day 2. At the 200 µmol dose level, one subject finished only 52% of the vegetables on day 1 and 75% of the vegetables on day 2 due to taste intolerance. As these salads were a mixture of cabbage and brussels sprouts, it was not possible to determine the exact amount of each consumed. Of the 352 total urine collection periods (8 collection periods per subject × 44 subjects), only 4 partial urine voids were missed. Subject 39 missed one void on day 2 during the 2- to 6-hour collection period. Subject 44 missed collecting 2 voids on day 2 during the 6- to 12-hour urine collection. Urine collection compliance was based on self-report. All subjects who consumed any study vegetables were included in the data analysis.

Glucobrassicin concentration
The cabbage (n = 4 samples) contained 33.5 ± 4.0 µmol per 100 grams food weight. The brussels sprouts (n = 4 samples) contained 206.0 ± 12.9 µmol per 100 grams food weight. Both are consistent with prior results (25).

Quality control
Interday precision was 8.7% and intraday precision was 3.9% (n = 68 across 12 sets). Measured DIM concentration (N = 14 samples, split between 3 sets) in the 35 pmol/mL spiked samples was 33.3 ± 2.5 (SD) pmol DIM/mL (CV, 7.5%) and in the 0.9 pmol/mL spiked samples was 0.87 ± 0.08 (SD) pmol DIM/mL (CV, 9.2%).

Dose-response between glucobrassicin exposure and urinary DIM excretion
Baseline urinary DIM levels, 24-hour DIM concentrations after consumption of the study vegetables, mean 24-hour DIM concentration, and percent DIM excreted in the first 12 hours of the 24-hour urine collection period are shown in Table 1. Very few subjects had DIM in the urine at baseline. On average, 95.1 ± 1.2% (95% CI, 92.7%–97.5%) on day 1 and 96.1 ± 0.8% (95% CI, 94.5%–97.7%) on day 2 of the total 24-hour DIM was excreted in the first 12 hours after vegetable consumption. In contrast, on average, only 76.9% (95% CI, 70.4%–83.4%) on day 1 and 80.4 (95% CI, 75.7%–83.1%) on day 2 of total 24-hour DIM was excreted in the first 6 hours. DIM excretion by collection period is shown in Table 2 (Supplementary Materials). As shown in Fig. 3,
DIM across all subjects was 0.68, indicating a strong association between dose and urinary DIM. Estimated parameters in the original scale (95% CI): Maximum DIM 421.5 pmol/mL (154.7–1148.4), minimum DIM 5.4 pmol/mL (0.7–44.3), EC50 90.2 pmol (29.1–151.3).

urinary DIM excretion plateaued between glucobrassicin doses of 200 and 300 µmol. The $R^2$ between measured DIM and predicted DIM across all subjects was 0.68, indicating a strong association between dose and urinary DIM. Excluding the 3 subjects at the 500 µmol dose who could not finish the brussels sprouts due to taste intolerance resulted in a very similar predictive value for the dose ($R^2 = 0.67$). We then analyzed the intraclass correlation (ICC) or how similar the two 24-hour urinary DIM levels from an individual compare to the similarity in other individuals. The ICC at the 3 expanded dose level cohorts is shown in Table 2. At 50 µmol, variability in 24-hour urinary DIM levels appears to stem from both within an individual and between individuals. At the 200 and 500 µmol dose levels, most of the variability is coming from between individuals rather than within an individual.

Discussion

We showed, for the first time in humans, that urinary DIM accurately reflects exposure to a wide range of glucobrassicin concentrations in vegetables. Ours is also the first study to demonstrate that urinary DIM excretion plateaus after eating vegetables. Similar pharmacokinetic profiles were observed when I3C or DIM was administered to humans as supplements (24, 26).

Interestingly, the dose of glucobrassicin at which the plateau in urinary DIM occurs after vegetable consumption is strikingly lower than when I3C or DIM are taken as supplements. The predominant acid condensation heterodimer of I3C, has poor bioavailability as a supplement due to its poor water solubility, although an absorption-enhanced form is available (28). The factors that influence the relative bioavailability of I3C or DIM after food consumption and the major route of their excretion in humans remain largely uncharacterized, although gastric pH influences the relative abundance of oligomers derived from I3C, whereby more acidic conditions favor the formation of higher order oligomers (5). For this reason, participants in our study were asked to fast and were not on medications that might affect gastric pH. However, our approach of using urinary DIM to quantify I3C uptake potentially bypasses this issue and can mitigate other sources of variation in glucobrassicin/I3C exposure (despite individuals eating identical amounts of glucobrassicin) that occur prior to gastric absorption, such as cultivating, growing conditions, and preparation method (3, 29).

Furthermore, DIM appears to be a good surrogate biomarker, as I3C itself is rapidly hydrolyzed and is therefore quickly undetectable in vivo, whereas DIM is quite stable (10, 24). Importantly, our data support the notion that the cancer-preventive properties that might be derived from cruciferous vegetable consumption may not require a large quantity of vegetables nor high-dose supplements, which certainly has practical implications (30). The optimal dose and duration of vegetable consumption remain to be worked out.

Our results indicate that DIM is excreted in the urine rapidly after vegetable consumption, consistent with pharmacokinetic studies done after single- and multiple-dose I3C (24) and single-dose DIM (26) and consistent with our prior study (25). This implies that daily consumption may be necessary to maximize phytochemical exposure. In addition, our data suggest that a full 24-hour urine collection may not be necessary to capture the magnitude of I3C exposure.

Consistent with prior studies (15, 31), we observed interindividual variability in 24-hour urinary DIM levels at each glucobrassicin dose level. However, the correlation between measured and predicted DIM remained very strong. At moderate to higher doses (200 and 500 µmol glucobrassicin), we did not see significant variability between the two 24-hour urinary DIM measurements from an individual subject. In other words, the majority of the variability within a dose level occurs between subjects, not within a subject. Variability at the 50 µmol dose level was high, likely due to the very small amount of vegetables fed at this dose level. These data suggest that a single urine collection after consuming a known dose of glucobrassicin, in amounts that would reasonably be consumed in a meal is fairly reliable. In addition, the interindividual variability we observed may reflect the relative benefit an individual derives from consuming glucobrassicin from vegetables, responsive not only to how much glucobrassicin was consumed but also to variations in I3C uptake and DIM metabolism, many of which are not characterized. We will explore this hypothesis in future studies.

In conclusion, the amount of DIM excreted in the urine correlates with the amount of glucobrassicin consumed from vegetables, making it an easily accessible, noninvasive biomarker of glucobrassicin exposure and I3C uptake. Feeding glucobrassicin beyond 200 µmol, or about 100 g of raw brussels sprouts, did not consistently lead to more urinary DIM. This represents a first step in defining a biologically relevant dose of Brassica vegetable consumption based on glucobrassicin. Our work sets the stage for objectively quantifying I3C uptake from the diet in epidemiologic studies, overcoming major limitations of observational studies. Furthermore, we can now correlate I3C uptake in glucobrassicin-based vegetable feeding interventions with outcomes in chemoprevention studies.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions

Figure 3.
Dose curve between glucobrassicin dose (25–500 µmol) and urinary DIM. Bars represent SD. Glucobrassicin dose ranged from 25 to 500 µmol. Estimated parameters in the original scale (95% CI): Maximum DIM 421.5 pmol/mL (154.7–1148.4), minimum DIM 5.4 pmol/mL (0.7–44.3), EC50 90.2 pmol (29.1–151.3).
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): N. Fujioka, B.W. Ransoms, S.C. Carmella, A. Roper-Batker, D.K. Haksuani, V.A. Fritz, C. Rohwer, S.S. Hecht

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): N. Fujioka, P. Upadhyaya, B.R. Lindgren, C. Rohwer

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): N. Fujioka, B.W. Ransoms

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References


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