Effects of Watercress Consumption on Metabolism of a Tobacco-specific Lung Carcinogen in Smokers


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Abstract

Epidemiological studies indicate that vegetable consumption protects against lung cancer in humans, but the protective constituents have not been identified. Phenethyl isothiocyanate (PEITC), which is released upon chewing of watercress (nasturtium officinale), is a chemopreventive agent against lung cancer induced by the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in rats and mice. PEITC inhibits the carcinogenicity of NNK by inhibiting its metabolic activation and thereby increasing the levels of detoxified metabolites excreted in urine. In this study, our goal was to determine whether watercress consumption would modify NNK metabolism in smokers. Eleven smokers maintained constant smoking habits and avoided cruciferous vegetables and other sources of isothiocyanates throughout the study. They donated 24-h urine samples on 3 consecutive days (baseline period). One to 3 days later, they consumed 2 ounces (56.8 g) of watercress at each meal for 3 days and donated 24-h urine samples on each of these days (watercress consumption period). One and 2 weeks later, they again donated 24-h urine samples on 2–3 consecutive days (follow-up periods). The samples were analyzed for two metabolites of NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNAL) and 4-(methylnitrosamino)-1-(3-pyridyl)but-1-yl]-β-O-α-glucosiduronic acid (NNAL-Gluc). NNAL-Gluc, a metabolite of NNAL, is believed to be a detoxification product of NNK. The urine samples were also analyzed for PEITC-NAC, a metabolite of PEITC. Minimum exposure to PEITC during the watercress consumption period averaged 19–38 mg/day. Seven of the 11 subjects had increased levels of urinary NNAL plus NNAL-Gluc on days 2 and 3 of the watercress consumption period, compared to the baseline period. Overall, the increase in urinary NNAL plus NNAL-Gluc in this period was significant [mean ± SD 0.924 ± 1.12 nmol/24 h (33.5%); P < 0.01]. Urinary levels of NNAL plus NNAL-Gluc returned to near baseline levels in the follow-up periods.

Introduction

NNK, a potent pulmonary carcinogen in rodents, is believed to be one of the causes of lung cancer in smokers (1, 2). The total dose of NNK required to produce lung tumors in rats is similar to its total uptake during a lifetime of smoking (3, 4). The structure of NNK is illustrated in Fig. 1. NNK requires metabolic activation by α-hydroxylation to express its carcinogenic activity (1, 2, 5–7). The metabolic activation of NNK is catalyzed by cytochrome P-450 enzymes in rodents and humans (8–12). Other enzymes may also be involved. The metabolic activation of NNK generates reactive intermediates that form a variety of DNA adducts that are involved in carcinogenesis (1, 2, 5–7). In rodents and humans, NNK is also extensively converted to its carbonyl reduction product (NNAL), which, like NNK, is a strong pulmonary carcinogen (4, 12–15). NNAL undergoes metabolic activation by α-hydroxylation, producing the same DNA adducts as does NNK (16). NNAL also can be conjugated to NNAL-Gluc, which is believed to be a detoxified metabolite of NNAL and NNK. NNAL and NNAL-Gluc are excreted in the urine of rodents, primates, and humans exposed to NNK (4, 17, 18). If the α-hydroxylation pathways and other oxidative metabolic pathways of NNK and NNAL are inhibited, the result will be increased excretion of NNAL and NNAL-Gluc in urine.

A number of epidemiological studies have shown that vegetable consumption protects against lung cancer in smokers (19). Experimental studies have demonstrated that compounds found in vegetables can prevent tumor induction (20, 21). One such chemopreventive agent is PEITC, which inhibits NNK pulmonary carcinogenesis in rats and mice (22–25). The mechanism of inhibition has been studied in detail. The results of these investigations have clearly shown that PEITC decreases the metabolic activation of NNK by inhibiting cytochrome P-450 enzymes involved in its α-hydroxylation (11, 23, 24).

The abbreviations used are: NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNAL-Gluc, 4-(methylnitrosamino)-1-(3-pyridyl)but-1-yl]-β-O-α-glucosiduronic acid; PEITC, phenethyl isothiocyanate; PEITC-NAC, N-acetyl-S-(N-phenethylthiocarbamoyl)-cysteine.

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1 This study was supported by National Cancer Institute Grant CA-46535. Cottinine and creatinine analyses were carried out in the American Health Foundation Clinical Biochemistry Facility, supported in part by National Cancer Institute Cancer Center Support Grant CA-17613.

2 To whom requests for reprints should be addressed, at American Health Foundation, 1 Dana Road, Valhalla, NY 10595.
demonstrated that chronic dietary administration of PEITC to administered in the diet (23). In ongoing studies, we have coside conjugate and catalyzes its hydrolysis. Thus, upon chew-
disrupted, and myrosinase comes into contact with the thioglu-
ble is chewed or otherwise macerated, cellular structure is cellubarly from the thioglucoside conjugate. When the vegeta-
moprevention of lung cancer in addicted smokers.

PEITC appears to be a highly promising compound for che-
tant increase in urinary excretion of NNAL plus NNAL-Gluc.4

Decreases in NNK metabolic activation and lung tu-
cancer in smokers. Number of days between periods varied. See “Materials and Methods” for details.

Materials and Methods

Subjects. Protocols were reviewed and approved by the Amer-
ican Health Foundation Institutional Review Board for protec-
tion of human subjects. All subjects signed a consent form before participation. All subjects were healthy smokers.

Protocol. The critical parameter in this study was total NNK
plus NNAL-Gluc in urine. This varies among individuals de-
pending on the number of cigarettes smoked and on metabolic
differences (31). Therefore, each subject served as his/her own
control during the study.

The protocol is summarized in Fig. 2. In the “baseline period,” 24-h urine samples were collected beginning with the first morning void on each of the 3 watercress consumption days and on the subsequent day. Eight of the 11 subjects also collected 24-h urine samples in “follow-up period 1.” which was 3 consecutive
days, 1 week after the watercress consumption period. Five of the subjects collected 24-h urine samples in “follow-up period 2,” which was 3 consecutive
days, 2 weeks after the watercress consumption period.

The protocol was carried out in 3 segments: March 1994
for subjects 5 and 6; August 1994 for subjects 1–4; and Feb-
ruary to March 1995 for subjects 7–11. Subjects 5 and 6
collected urine only during the baseline and watercress con-
sumption periods because this was an initial test to determine
whether watercress consumption might have long-lasting ef-
fects, as suggested by the data for subjects 2 and 3 in follow-up
period 1.

Each subject was asked to smoke a constant number of
cigarettes per day ± 2 throughout the entire study. They re-
corded the number of cigarettes smoked on each day of the
study. Subjects were asked not to eat cruciferous vegetables
during the entire study, with the exception of watercress on the
prescribed days. Cruciferous vegetables excluded were broc-
coli, cabbage (including cole slaw and sauerkraut), cauliflower,
brussels sprouts, radishes, turnips, arugula, mustard greens, and
collard greens. Also excluded were other potential sources of
isothiocyanates including mustard, salad dressing (except oil
and vinegar), and mayonnaise. Permissible vegetables included
potatoes, corn, peas, lettuce, cucumbers, carrots, peppers,
beans, asparagus, tomatoes, onions, beets, celery, squash, and
spinach. All subjects kept a record of everything eaten during the
study.

Collection and Analysis of Urine. Twenty-four-h urine sam-
plies were collected in amber bottles containing ammonium
sulfamate (4). NNAL and NNAL-Gluc were determined by gas
chromatography with detection by a Thermal Energy Analyzer,
a nitrosamine-selective chemiluminescence detector (31). PEITC-NAC was analyzed as described by Chung et al. (30).

Cotinine was determined by radioimmunoassay using antisera
produced in rabbits (32). Creatinine was measured with a Kodak Ektachem 500 clinical chemistry analyzer.

Statistical Methods. Data are expressed as mean ± SD unless
otherwise stated. The effects of watercress consumption on
urinary metabolites were analyzed by a paired t test, and cor-

\[ \text{Fig. 1. Overview of NNK metabolism. For details, see Ref. 2, 3, and 5–7.} \]

\[ \text{Fig. 2. Protocol for examining the effects of watercress consumption on NNK} \]

metabolism in smokers. Number of days between periods varied. See “Materials and Methods” for details.

![Diagram](image)
of day 3. The corresponding minimum amounts of PEITC on day 2 from 36.5 to 141 mg/24h, and on day 3 from 10.4 to 19.0 (9.5).

Characteristics of the study subjects and their urinary levels of PEITC-NAC during the watercress consumption period

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age</th>
<th>PEITC-NAC in urine (minimum amount of PEITC ingested in mg/24 h)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>34</td>
<td>36.8 (18.4)</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>27</td>
<td>15.7 (7.85)</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>26</td>
<td>21.8 (10.9)</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>24</td>
<td>19.3 (9.64)</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>26</td>
<td>90.8 (45.4)</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>26</td>
<td>27.6 (13.8)</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>30</td>
<td>25.6 (12.8)</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>25</td>
<td>75.1 (37.6)</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>39</td>
<td>46.3 (23.1)</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>48</td>
<td>39.4 (19.7)</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>27</td>
<td>21.8 (10.9)</td>
</tr>
</tbody>
</table>

Mean ± SD 30.2 ± 7.4 38.2 ± 24.2 73.1 ± 33.3 75.3 ± 57.7 28.9 ± 25.3

* To convert to mmol/24 h, divide by 326 (PEITC-NAC) or 163 (PEITC).

ND, not determined.

Urinary NNAL plus NNAL-Gluc and cotinine in smokers who consumed watercress

<table>
<thead>
<tr>
<th>Subject</th>
<th>Cigarettes per day</th>
<th>Cotinine (μmol/24 h ± SD)*</th>
<th>NNAL plus NNAL-Gluc [μmol/24 h; mean ± SD or mean (individual values)]</th>
<th>Difference [μmol/24 h (% change)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Period 1 (watercress consumption) Period 2 (Follow-up 1)* Period 3 (Follow-up 2)*</td>
<td>Period 2 minus period 1 Period 3 minus period 1 Period 4 minus period 1</td>
</tr>
<tr>
<td>1</td>
<td>12.2 ± 2.2</td>
<td>49.4 ± 14.5</td>
<td>6.40 ± 2.10 3.80 (10.4, 6.21) NDf ND 1.90 (29.7) ND ND</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>16.7 ± 1.1</td>
<td>51.3 ± 7.9</td>
<td>5.80 ± 0.20 8.18 (7.57, 8.78) 9.92 (9.96, 10.4) NDf ND 2.38 (41.0) 3.52 (55.0) ND ND</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>16.8 ± 1.1</td>
<td>37.5 ± 9.9</td>
<td>3.79 ± 1.08 4.66 (4.83, 4.48) 6.66 ± 1.44 NDf ND 0.87 (23.0) 4.33 (75.7) ND ND</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>15.0 ± 1.1</td>
<td>13.6 ± 4.14</td>
<td>4.18 ± 0.85 3.72 (3.66, 3.78) 2.30 (2.70, 1.89) NDf ND -0.46 (11.0) -1.88 (45.0) ND ND</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>15.8 ± 0.4</td>
<td>51.4 ± 6.65</td>
<td>4.01 ± 0.46 5.64 (5.29, 6.00) NDf ND 1.63 (46.0) ND ND</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>9.5 ± 1.2</td>
<td>31.6 ± 7.61</td>
<td>0.799 ± 0.12 1.22 (1.16, 1.27) NDf ND 0.42 (52.7) ND ND</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>14.2 ± 1.1</td>
<td>23.6 ± 7.90</td>
<td>2.26 ± 0.50 1.26 (1.32, 1.20) 2.12 (1.48, 2.76) 0.913 ± 0.401 NDf ND</td>
<td>-1.00 (44.2) -0.14 (6.2) -1.53 (59.6)</td>
</tr>
<tr>
<td>8</td>
<td>13.6 ± 0.7</td>
<td>29.3 ± 7.90</td>
<td>1.21 ± 0.44 2.69 (2.84, 2.54) 1.60 ± 0.29 1.43 ± 0.141 NDf ND 1.48 (22.2) 0.39 (32.2) 0.22 (18.2)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>19.1 ± 1.7</td>
<td>32.5 ± 5.49</td>
<td>3.25 ± 0.74 5.57 (5.46, 5.68) 3.32 ± 0.74 3.71 ± 0.547 NDf ND 2.32 (74.1) 0.07 (2.2) 0.46 (14.2)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>8.0 ± 1.4</td>
<td>17.4 ± 6.59</td>
<td>3.62 ± 1.72 3.99 (3.99, 4.99) 2.46 ± 0.77 2.56 ± 0.214 NDf ND 0.37 (10.2) -1.16 (32.0) -1.06 (29.3)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>15.9 ± 1.0</td>
<td>14.6 ± 2.57</td>
<td>0.812 ± 0.11 1.06 (0.852, 1.27) 0.791 ± 0.118 1.07 ± 0.111 NDf ND 0.25 (30.5) -0.02 (2.6) 0.26 (31.8)</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SD 14.8 ± 3.4 32.1 ± 14.2 3.28 ± 1.88 4.21 ± 2.58 3.64 ± 3.08 1.94 ± 1.18 0.924 ± 1.12* 0.456 ± 1.85 (9.9 -0.294 ± 0.753 (33.5 ± 43.2) ± 41.6 (4.9 ± 38.2) |

* Eleven smokers maintained constant smoking habits and avoided cruciferous vegetables, except during the watercress consumption period. Twenty-four-h urine samples were collected as illustrated in Fig. 2. See "Materials and Methods" for details.

b On 3 baseline days, 3 watercress days, and 3 follow-up days. To convert to mg cotinine/24 h, multiply by 0.176.

Twenty-four-h urine samples from days 2 and 3 of the watercress consumption period (see Fig. 2).

Significant compared to 0. P < 0.01 (paired t test).

Results

Characteristics of the study subjects and their urinary levels of a PEITC metabolite, PEITC-NAC, are summarized in Table 1. Watercress proved to be a good source of PEITC, confirming previous studies (28–30). On day 1 of watercress consumption, PEITC-NAC levels in urine ranged from 15.7 to 90.8 mg/24h, on day 2 from 36.5 to 141 mg/24h, and on day 3 from 10.4 to 19.0 mg/24h. Most of the PEITC-NAC was excreted by the end of day 3. The corresponding minimum amounts of PEITC ingested on each day are also shown in Table 1. The actual amount ingested cannot be determined without knowing the extent of metabolism of PEITC to PEITC-NAC, which has been estimated to range from 30 to 67% in humans (30).

Diet records indicated that all subjects complied with the protocol and avoided cruciferous vegetables or other sources of isothiocyanates during the study, except on the watercress consumption days. The urine of 7 subjects was analyzed for PEITC-NAC during the baseline and follow-up periods; PEITC-NAC was not detected.

Nine of the 11 subjects smoked between 10 and 20 cigarettes per day, as summarized in Table 2. Numbers of cigarettes smoked per day remained relatively constant, as requested in the protocol. Levels of NNAL plus NNAL-Gluc during the baseline period were also relatively constant for each smoker (mean coefficient of variation ± SD = 24.2 ± 11.3%).
Mean levels of urinary NNAL plus NNAL-Gluc in the baseline period, on days 2 and 3 of the watercress consumption period, and in the follow-up periods, are summarized in Table 2. In 7 of the 11 smokers, excretion of NNAL plus NNAL-Gluc increased on the second 2 days of the watercress consumption period compared to the baseline period. The amount was considered to have increased if the percentage increase was greater than 24.2%, the mean coefficient of variation in the baseline period. The percentage increases in these seven subjects ranged from 29.7 to 122% (mean ± SD = 55.8 ± 32.9%). Three subjects exhibited no change according to this criterion, and one had a decrease of 44.2%. The mean ± SD increase in NNAL plus NNAL-Gluc for all 11 subjects was 0.924 ± 1.12 nmol/24 h, which was significant ($P < 0.01$). In follow-up period 1, levels of NNAL plus NNAL-Gluc were determined in 8 of 11 subjects. They were higher than in the baseline period in 3 of the subjects, lower in 2, and unchanged in 3. The overall difference between follow-up 1 and baseline periods was 0.456 ± 1.85 nmol/24 h, which was not significant. Changes in NNAL plus NNAL-Gluc excretion in each subject in the watercress consumption period and follow-up period 1 compared to baseline are summarized in Fig. 3. Levels of NNAL plus NNAL-Gluc were determined in 5 subjects in follow-up period 2. Compared to baseline, they increased in 1, decreased in 2, and did not change in 2. The overall change compared to baseline was not significant. The composite percentage changes in the watercress consumption and follow-up periods are summarized in Fig. 4.

The NNAL plus NNAL-Gluc data were also expressed per mg creatinine, and the same calculations as described above were performed (Table 3). The mean ± SD increase on the second 2 days of the watercress consumption period compared to baseline was 0.454 ± 0.684 pmol/mg creatinine, which was significant ($P < 0.05$). The difference between follow-up period 1 and baseline was not significant. In follow-up period 2, there was a significant decrease in NNAL plus NNAL-Gluc compared to baseline ($P < 0.05$).

Although the maximum effects of watercress consumption on levels of NNAL plus NNAL-Gluc in urine were seen on days 2 and 3 of the watercress consumption period, the mean increase in levels of these metabolites on all 3 days of watercress consumption were still significant compared to the baseline period (mean increase, 0.626 ± 0.982 nmol/24 h; $P = 0.03$). Levels of NNAL plus NNAL-Gluc were also measured in the 24-h urine samples of 5 subjects on the day after the watercress consumption period. In 3 of the 5 subjects, the amount decreased compared to the preceding 2 days, by 34.9 ± 7.5%, whereas it was unchanged in the other 2 (data not shown).

The percentage change from baseline in urinary levels of NNAL plus NNAL-Gluc on days 2 and 3 of the watercress consumption period was examined with respect to total urinary PEITC-NAC during days 1–3 of the watercress consumption period for each subject. These data are summarized in Fig. 5, which shows that these two variables correlated ($r = 0.62$; $P = 0.04$).

Insight on the potential effect of watercress consumption on UDP-glucuronosyl transferase activity can be obtained by considering levels of NNAL and NNAL-Gluc separately. These data for the first 3 periods of the study are summarized in Table 4. Six of the 11 subjects showed an increase in free NNAL on days 2 and 3 of the watercress consumption period compared to baseline, and 5 of these 6 individuals also had increases in
Twenty-four-h urine samples from 3 consecutive days.

5. Consideration of Table S indicates that the NNAL-Gluc:NNAL ratio should have increased in two subjects (subjects 2 and 3) on days 2 and 3 of the watercress consumption period compared to baseline levels (Fig. 4). Urinary NNAL plus NNAL-Gluc levels decreased after the watercress consumption period. Changes in urinary NNAL plus NNAL-Gluc correlated with PEITC intake, as measured by PEITC-NAC levels in urine (Fig. 5). These data provide strong evidence that PEITC inhibits metabolic oxidation of NNK in smokers.

The major contribution to increased urinary NNAL plus NNAL-Gluc was made by NNAL-Gluc because this is the more abundant of these two NNK metabolites. As shown in Tables 4 and 5, the 7 subjects with increased levels of NNAL-Gluc on days 2 and 3 of the watercress consumption period were the same 7 who had increased levels of NNAL plus NNAL-Gluc. The increase in urinary NNAL plus NNAL-Gluc on days 2 and 3 of the watercress consumption period (mean ± SD, 0.769 ± 0.968 nmol/24 h) was significant compared to baseline (P < 0.02).

**Discussion**

The results of this study support our hypothesis that watercress consumption increases urinary excretion of NNAL plus NNAL-Gluc in smokers. The mean increase in urinary NNAL plus NNAL-Gluc was significant in the watercress consumption period compared to baseline levels (Fig. 4). Urinary NNAL plus NNAL-Gluc levels decreased after the watercress consumption period. Changes in urinary NNAL plus NNAL-Gluc correlated with PEITC intake, as measured by PEITC-NAC levels in urine (Fig. 5). These data provide strong evidence that PEITC inhibits metabolic oxidation of NNK in smokers.

Studies in rats and mice have demonstrated that administration of PEITC results in inhibition of hepatic and pulmonary NNK α-hydroxylation and NNK-N-oxide formation (11, 23, 26–27). As shown in Fig. 1, this should result in accumulation and increased urinary excretion of NNAL and NNAL-Gluc. This was observed in a recently completed carcinogenicity

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**Table 3** Urinary NNAL plus NNAL-Gluc in smokers who consumed watercress

<table>
<thead>
<tr>
<th>Subject</th>
<th>Period 1 (baseline)</th>
<th>Period 2 (watercress consumption)</th>
<th>Period 3 (follow-up 1)</th>
<th>Period 4 (follow-up 2)</th>
<th>Difference (pmol/mg creatinine (% change))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.23 ± 0.15</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.55 (36.6)  ND ND</td>
</tr>
<tr>
<td>2</td>
<td>3.62 ± 0.23</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.60 (16.6)  1.55 (42.8) ND ND</td>
</tr>
<tr>
<td>3</td>
<td>3.69 ± 0.70</td>
<td>ND</td>
<td>7.43 ± 0.43</td>
<td>ND</td>
<td>0.79 (21.4)  3.74 (101) ND ND</td>
</tr>
<tr>
<td>4</td>
<td>3.83 ± 0.62</td>
<td>3.31 (3.21, 3.41)</td>
<td>3.08 (3.37, 2.78)</td>
<td>ND</td>
<td>-0.52 (-13.6) -0.75 (-19.6) ND ND</td>
</tr>
<tr>
<td>5</td>
<td>2.84 ± 0.31</td>
<td>3.42 (3.06, 3.78)</td>
<td>ND</td>
<td>ND</td>
<td>0.58 (20.4)  ND ND</td>
</tr>
<tr>
<td>6</td>
<td>0.914 ± 0.121</td>
<td>1.33 (1.23, 1.44)</td>
<td>ND</td>
<td>ND</td>
<td>0.42 (45.5)  ND ND</td>
</tr>
<tr>
<td>7</td>
<td>1.65 ± 0.09</td>
<td>1.06 (1.34, 0.77)</td>
<td>0.937 ± 0.580</td>
<td>0.632 ± 0.150</td>
<td>-0.59 (-35.8) -0.71 (-43.2) -1.02 (-61.8)</td>
</tr>
<tr>
<td>8</td>
<td>2.88 ± 0.26</td>
<td>2.14 (1.85, 2.54)</td>
<td>1.27 ± 0.12</td>
<td>1.29 ± 0.09</td>
<td>0.89 (58.6)  0.20 (13.2) -0.23 (-15.1)</td>
</tr>
<tr>
<td>9</td>
<td>3.45 ± 0.43</td>
<td>3.73 (5.92, 5.54)</td>
<td>4.38 ± 0.08</td>
<td>3.99 ± 0.25</td>
<td>1.28 (28.8)  -0.07 (-1.6) -0.46 (-10.3)</td>
</tr>
<tr>
<td>10</td>
<td>2.04 ± 0.57</td>
<td>2.09 (1.90, 2.28)</td>
<td>1.34 ± 0.09</td>
<td>1.49 ± 0.18</td>
<td>0.05 (2.5)  -0.70 (-34.3) -0.55 (-30.0)</td>
</tr>
<tr>
<td>11</td>
<td>0.599 ± 0.186</td>
<td>0.547 (0.417, 0.676)</td>
<td>0.392 ± 0.060</td>
<td>0.474 ± 0.040</td>
<td>-0.052 (-8.7) -0.207 (-35.0) -0.125 (-21.7)</td>
</tr>
</tbody>
</table>

Mean ± SD 2.67 ± 1.37 3.13 ± 1.81 3.06 ± 2.44 1.57 ± 1.42 0.454 ± 0.684b 0.381 ± 1.55 -0.478 ± 0.347b (15.7 ± 27.7) (11.6 ± 47.4) (-27.8 ± 20.4)

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* Eleven smokers maintained constant smoking habits and avoided cruciferous vegetables except during the watercress consumption period. Twenty-four-h urine samples were collected as illustrated in Fig. 2. See "Materials and Methods" for details.

* Twenty-four-h urine samples from 3 consecutive days.

* Twenty-four-h urine samples from days 2 and 3 of the watercress consumption period (see Fig. 2).

* Twenty-four-h urine samples from 2–3 consecutive days.

* Not determined.

* Significant compared to 0; P < 0.05 (paired t test).

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[Fig. 5. Relationship between the percentage of change from baseline of urinary NNAL plus NNAL-Gluc on days 2 and 3 of the watercress consumption period and total urinary PEITC-NAC on days 1–3 of the watercress consumption period for the 11 smokers.]

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days 2 and 3 of watercress consumption, whereas for subject 9, the corresponding figures were 3.5 and 5.9. In the other 5 subjects with increased levels of NNAL plus NNAL-Gluc, there was no increase in ratio between baseline and days 2 and 3 of the watercress consumption period. Collectively, these data indicate that the major effect leading to increased NNAL plus NNAL-Gluc was an increase in the amount of available NNAL due to inhibition of another metabolic pathway. However, in two cases, UDP-glucuronosyl transferase activity may also have been induced.

The major contribution to increased urinary NNAL plus NNAL-Gluc was made by NNAL-Gluc because this is the more abundant of these two NNK metabolites. As shown in Tables 4 and 5, the 7 subjects with increased levels of NNAL-Gluc on days 2 and 3 of the watercress consumption period were the same 7 who had increased levels of NNAL plus NNAL-Gluc. The increase in urinary NNAL plus NNAL-Gluc on days 2 and 3 of the watercress consumption period (mean ± SD, 0.769 ± 0.968 nmol/24 h) was significant compared to baseline (P < 0.02).

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**Discussion**

The results of this study support our hypothesis that watercress consumption increases urinary excretion of NNAL plus NNAL-Gluc in smokers. The mean increase in urinary NNAL plus NNAL-Gluc was significant in the watercress consumption period compared to baseline levels (Fig. 4). Urinary NNAL plus NNAL-Gluc levels decreased after the watercress consumption period. Changes in urinary NNAL plus NNAL-Gluc correlated with PEITC intake, as measured by PEITC-NAC levels in urine (Fig. 5). These data provide strong evidence that PEITC inhibits metabolic oxidation of NNK in smokers.

Studies in rats and mice have demonstrated that administration of PEITC results in inhibition of hepatic and pulmonary NNK α-hydroxylation and NNK-N-oxide formation (11, 23, 26–27). As shown in Fig. 1, this should result in accumulation and increased urinary excretion of NNAL and NNAL-Gluc. This was observed in a recently completed carcinogenicity
Twenty-four-h urine samples from 2-3 consecutive days. Because technology to assess levels of NNAL plus NNAL-Gluc were elevated in the PEITC-treated rats compared to control animals. The amounts of NNAL measured at two intervals during that study. The amounts of NNAL plus NNAL-Gluc were elevated in the PEITC-treated rats compared to control animals. Because technology to assess levels of \( \alpha \)-hydroxylation and pyridine-N-oxidation metabolites of NNK in the urine of smokers is not yet available, we do not know whether one or both pathways are being inhibited by watercress in the present study; based on studies in rodents, however, it is likely that both \( \alpha \)-hydroxylation and pyridine-N-oxidation have resulted from inhibition of other pathways, leading to increased levels of NNAL available for conjugation. Alternatively, that PEITC may be one of the protective compounds. Despite the strong epidemiological evidence that vegetable consumption inhibits lung cancer, we are aware of no previous reports on the effects of vegetable consumption on lung carcinogen metabolism in humans. In related work, one study demonstrated that consumption of brussels sprouts and cabbage increased the metabolism of phenacetin in humans, whereas two others showed that consumption of brussels sprouts resulted in elevated levels of \( \alpha \)-class glutathione S-transferase (37).

An additional mechanism to explain increased levels of urinary NNAL-Gluc after watercress consumption would be induction of UDP-glucuronosyltransferase enzymes by PEITC or another constituent of watercress. In rats, PEITC treatment results in a small induction of hepatic UDP-glucuronosyltransferase (27). However, consideration of the data in Tables 4 and 5 indicates that in most smokers in our study, the major effect resulted from inhibition of other pathways, leading to increased levels of NNAL available for conjugation. Alternatively, experiments in rodents have shown that decreased \( \alpha \)-hydroxylation is the major mechanism by which PEITC inhibits lung cancer induction by NNK (11, 23, 26-27). If similar mechanisms occur in humans, as this study indicates, PEITC may be an effective chemopreventive agent against NNK in smokers. Several epidemiological studies have shown that vegetable consumption protects against lung cancer in smokers, but the constituents of vegetables responsible for the protective effect have not been identified (19, 20). It is unlikely that any one constituent of vegetables is responsible. Our results suggest

<table>
<thead>
<tr>
<th>Subject</th>
<th>Period 1 (baseline; mean ± SD)</th>
<th>Period 2 [wattercress consumption; mean ± SD or (individual values)]</th>
<th>Period 3 (Folow-up; mean ± SD or (individual values))</th>
<th>Difference [nmol/24 h (% change)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Period 2 minus period 1</td>
<td>Period 3 minus period 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>1.74 ± 0.78</td>
<td>2.49 (3.16, 1.82)</td>
<td>ND</td>
<td>0.75 (43.1)</td>
</tr>
<tr>
<td>2</td>
<td>1.34 ± 0.076</td>
<td>1.31 (0.994, 1.63)</td>
<td>1.75 (1.67, 1.83)</td>
<td>-0.03 (-2.2)</td>
</tr>
<tr>
<td>3</td>
<td>0.871 ± 0.228</td>
<td>1.20 (1.34, 1.05)</td>
<td>1.57 ± 0.344</td>
<td>0.329 (37.8)</td>
</tr>
<tr>
<td>4</td>
<td>0.925 ± 0.213</td>
<td>0.751 (0.832, 0.669)</td>
<td>0.559 ± 0.20</td>
<td>-0.174 (-23.2)</td>
</tr>
<tr>
<td>5</td>
<td>0.745 ± 0.105</td>
<td>1.28 (1.12, 1.43)</td>
<td>ND</td>
<td>0.553 (71.8)</td>
</tr>
<tr>
<td>6</td>
<td>0.221 ± 0.031</td>
<td>0.358 (0.342, 0.373)</td>
<td>ND</td>
<td>0.137 (62.0)</td>
</tr>
<tr>
<td>7</td>
<td>0.413 ± 0.111</td>
<td>0.138 (0.83, 0.193)</td>
<td>0.404 (0.184, 0.623)</td>
<td>-0.275 (-66.6)</td>
</tr>
<tr>
<td>8</td>
<td>0.356 ± 0.218</td>
<td>0.621 (0.609, 0.633)</td>
<td>0.318 ± 0.070</td>
<td>0.265 (74.4)</td>
</tr>
<tr>
<td>9</td>
<td>0.720 ± 0.226</td>
<td>0.805 (0.753, 0.886)</td>
<td>0.631 ± 0.111</td>
<td>0.085 (11.8)</td>
</tr>
<tr>
<td>10</td>
<td>0.646 ± 0.363</td>
<td>0.595 (0.519, 0.670)</td>
<td>0.480 ± 0.200</td>
<td>-0.051 (-7.8)</td>
</tr>
<tr>
<td>11</td>
<td>0.119 ± 0.042</td>
<td>0.206 (0.136, 0.278)</td>
<td>0.112 ± 0.041</td>
<td>0.087 (73.1)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.736 ± 0.482</td>
<td>0.887 ± 0.670</td>
<td>0.728 ± 0.598</td>
<td>0.151 ± 0.303</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>0.058 ± 0.344</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.66 ± 1.34</td>
<td>5.79 (7.19, 4.39)</td>
<td>ND</td>
<td>1.13 (24.2)</td>
</tr>
<tr>
<td>2</td>
<td>4.46 ± 0.18</td>
<td>6.87 (6.58, 7.15)</td>
<td>8.17 (7.79, 8.55)</td>
<td>2.41 (53.9)</td>
</tr>
<tr>
<td>3</td>
<td>2.92 ± 0.87</td>
<td>3.46 (3.44, 3.43)</td>
<td>5.08 ± 1.13</td>
<td>0.54 (18.4)</td>
</tr>
<tr>
<td>4</td>
<td>3.26 ± 0.71</td>
<td>2.97 (2.83, 3.11)</td>
<td>1.86 ± 0.26</td>
<td>-0.29 (-8.9)</td>
</tr>
<tr>
<td>5</td>
<td>3.27 ± 0.36</td>
<td>4.37 (4.17, 4.57)</td>
<td>ND</td>
<td>1.10 (33.6)</td>
</tr>
<tr>
<td>6</td>
<td>0.578 ± 0.096</td>
<td>0.858 (0.784, 0.932)</td>
<td>ND</td>
<td>0.280 (48.4)</td>
</tr>
<tr>
<td>7</td>
<td>1.852 ± 0.395</td>
<td>1.12 (1.13, 1.12)</td>
<td>1.72 (1.30, 2.13)</td>
<td>-0.332 (-39.5)</td>
</tr>
<tr>
<td>8</td>
<td>0.860 ± 0.261</td>
<td>2.07 (2.23, 1.90)</td>
<td>1.28 ± 0.28</td>
<td>1.21 (114)</td>
</tr>
<tr>
<td>9</td>
<td>2.53 ± 0.518</td>
<td>4.75 (4.70, 7.80)</td>
<td>2.69 ± 0.63</td>
<td>2.22 (87.7)</td>
</tr>
<tr>
<td>10</td>
<td>2.97 ± 1.36</td>
<td>3.40 (2.47, 4.32)</td>
<td>1.98 ± 0.60</td>
<td>0.43 (14.5)</td>
</tr>
<tr>
<td>11</td>
<td>0.693 ± 0.087</td>
<td>0.858 (0.722, 0.994)</td>
<td>0.678 ± 0.132</td>
<td>0.165 (23.8)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2.55 ± 1.42</td>
<td>3.32 ± 2.01</td>
<td>2.93 ± 2.49</td>
<td>0.769 ± 0.968</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.489 ± 1.676</td>
</tr>
</tbody>
</table>

\(^a\) See "Materials and Methods," and footnote a of Table 2 for details of protocol.

\(^b\) Twenty-four-h urine samples from 3 consecutive days.

\(^c\) Twenty-four-h urine samples from days 2 and 3 of the watercress consumption period (see Fig. 2).

\(^d\) Twenty-four-h urine samples from 2-3 consecutive days.

\(^*\) Not determined.

\(^\dagger\) Significant compared to 0; \( P < 0.02.\)
Thus, the effects of PEITC and other constituents of watercress decreased in 1 subject. The effects that we observed are most crease, ranging from 29.7 to 122% on days 2 and 3. Levels of consumption period were observed in only 7 of the subjects, "

These percentages are the means of the coefficients of variation for these values for all subjects during the baseline period. -, decrease. NC, no change.

PEITC or another watercress constituent could induce carbonyl reductase activity or affect the rate of urinary excretion of NNK and its metabolites.

Ongoing studies of the effects of PEITC on NNK metabolism in the patas monkey indicate that the inhibition of oxidative metabolism is transitory and dependent on the presence of PEITC. Changes in urinary NNK metabolite levels consistent with those described here were observed, but the effect decreased 24 h after PEITC administration. The results of the present study also indicate that the effects of PEITC are transient in some smokers because levels of NNAL plus NNAL-Gluc decreased on the day after the watercress consumption period and in the follow-up periods. However, in two smokers, levels of NNAL plus NNAL-Gluc were higher in follow-up period 1 than in the watercress consumption period. This requires further study.

The greatest increases in levels of urinary NNAL plus NNAL-Gluc were seen on days 2 and 3 of the watercress consumption period. This can be attributed to the experimental design. On day 1 of the watercress consumption period, urine collection began with the first morning void and thus contained NNK metabolites from smoking on the previous day. Therefore, the effect of watercress consumption on day 1 was diluted, although the overall increase in NNAL plus NNAL-Gluc in the watercress consumption period was still significant.

Although the aggregate data indicate a predictable effect of watercress consumption on NNK metabolism in smokers, increases of NNAL plus NNAL-Gluc during the watercress consumption period were observed in only 7 of the subjects, and there was considerable variation in the extent of the increase, ranging from 29.7 to 122% on days 2 and 3. Levels of NNAL plus NNAL-Gluc in 3 subjects did not change in the watercress consumption period, whereas they were markedly decreased in 1 subject. The effects that we observed are most likely due to inhibition by PEITC of human cytochromes P-450, including but not restricted to cytochrome P450 1A2 (12, 38). Levels of cytochrome P450 enzymes will vary greatly among individuals, as will the metabolism of NNK (12, 13). Thus, the effects of PEITC and other constituents of watercress will also be different in each individual. If chemopreventive agents such as PEITC are to be used in intervention studies in smokers, it will be advisable to use biomarkers to select those smokers who are likely to be responsive.

In rats, the daily dose of PEITC that inhibited lung cancer induction by NNK was approximately 40 mg/kg body weight (240 μmol/kg) administered in the diet (23). NNK was given by s.c. injection at a daily dose of 1.76 mg/kg (8.5 μmol/kg). However, only about 0.3% (0.005 mg/kg or 0.025 μmol/kg) reaches the lung. The PEITC:NNK molar ratio was approximately 9600, using the value for NNK dose in lung. In this study, the mean minimum amount of PEITC ingested on days 2 and 3 of the watercress consumption period was 37 mg/day, or approximately 0.5 mg/kg body weight (3 μmol/kg). This dose was only 1/80th as great as that used in the rat chemoprevention study. However, the daily dose of NNK was also much lower than in the rat study. Considering that urinary NNAL plus NNAL-Gluc may represent 50% of the dose of NNK, the mean daily dose of NNK would be approximately 6.4 nmol (0.085 nmol/kg), using the NNAL plus NNAL-Gluc values from the baseline period. Thus, the PEITC:NNK molar ratio in our study was approximately 35,000 (about 4 times as great as in the rat study). Although differences in routes of administration and possibly metabolism of PEITC and NNK in rats versus smokers preclude further comparisons, the concordance between the rat and human data are encouraging with respect to further development of PEITC as a chemopreventive agent.

Chemoprevention may be one way to decrease lung cancer risk in smokers who are addicted to nicotine and cannot stop smoking. The results of this study provide human data that bolster the growing body of evidence supporting further development of PEITC as a chemopreventive agent against lung cancer in smokers. Nevertheless, the potential beneficial effects of inhibition of oxidative metabolism of NNK may be offset by the increased levels of NNAL, which is a potent carcinogen. Moreover, the complexity of tobacco smoke must be kept in mind when considering chemoprevention strategies. The major lung carcinogens in tobacco smoke appear to be NNK and polynuclear aromatic hydrocarbons such as benzo[a]pyrene (39). Tobacco smoke also contains toxic agents, cocarcinogens, and tumor promoters. Whereas PEITC is a good inhibitor of NNK-induced lung carcinogenesis in animals, and the present results indicate that it also inhibits NNK metabolic activation in humans, it is not an effective inhibitor of lung tumor induction by benzo[a]pyrene (40, 41). Furthermore, suppressing agents that could reverse some of the damage done by tobacco smoke carcinogens and other toxic agents are needed, and PEITC has no activity of this type (42). It is likely that specifically designed mixtures of chemopreventive agents will be necessary for inhibition of lung cancer development in smokers.

Acknowledgments
We thank the participants for conscientiously following the protocol.

References
Watercress and a Tobacco-specific Lung Carcinogen


Effects of watercress consumption on metabolism of a tobacco-specific lung carcinogen in smokers.
