

# Hydrolysis of Glucosinolates to Isothiocyanates after Ingestion of Raw or Microwaved Cabbage by Human Volunteers

Gabrielle Rouzaud, Sheila A. Young, and Alan J. Duncan

Macaulay Institute, Craigiebuckler, Aberdeen, Scotland, United Kingdom

## Abstract

Cabbage contains the glucosinolate sinigrin, which is hydrolyzed by myrosinase to allyl isothiocyanate. Isothiocyanates are thought to inhibit the development of cancer cells by a number of mechanisms. The effect of cooking cabbage on isothiocyanate production from glucosinolates during and after their ingestion was examined in human subjects. Each of 12 healthy human volunteers consumed three meals, at 48-h intervals, containing either raw cabbage, cooked cabbage, or mustard according to a cross-over design. At each meal, watercress juice, which is rich in phenethyl isothiocyanate, was also consumed to allow individual and temporal variation in postabsorptive isothiocyanate recovery to be measured. Volunteers recorded the time and volume of each urination for 24 h after each meal. Samples of each urination were analyzed for *N*-acetyl cysteine conjugates of isothiocyanates as a measure of entry of isothiocyanates into the peripheral circulation. Excretion of isothiocyanates was rapid and substantial after ingestion of mustard, a source of preformed allyl isothiocyanate. After raw cabbage consumption, allyl isothiocyanate was again rapidly excreted, although to a lesser extent than when mustard was consumed. On the cooked cabbage treatment, excretion of allyl isothiocyanate was considerably less than for raw cabbage, and the excretion was delayed. The results indicate that isothiocyanate production is more extensive after consumption of raw vegetables but that isothiocyanates still arise, albeit to a lesser degree, when cooked vegetables are consumed. The lag in excretion on the cooked cabbage treatment suggests that the colon microflora catalyze glucosinolate hydrolysis in this case.

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**Note:** Gabrielle Rouzaud is currently in the Food and Microbial Sciences Unit, School of Food Biosciences, Whiteknights, University of Reading, United Kingdom.

**Requests for reprints:** Alan Duncan, Macaulay Institute, Craigiebuckler, Aberdeen AB15 8QH, United Kingdom. Fax: 01224-311556; E-mail: a.duncan@macaulay.ac.uk.

## Introduction

Epidemiological studies indicate that consumption of brassica vegetables is associated with a reduced incidence of cancers at a number of sites including the lung, stomach, colon, and rectum (1). Among compounds present in brassica vegetables, which are thought to contribute to this phenomenon, are a group of thioglucosides, the glucosinolates. Glucosinolates are accompanied in brassica vegetables by the enzyme myrosinase, a thioglucosidase (E.C. 3.2.3.1) that catalyzes the hydrolysis of glucosinolates to a range of breakdown products after cellular damage (2). The processes of ingestion and digestion of brassica vegetables lead, therefore, to glucosinolate hydrolysis. Myrosinase activity has also been demonstrated in the human gut microflora (3, 4). Glucosinolate hydrolysis can lead to a range of breakdown products, depending on the conditions under which hydrolysis occurs and the structure of the parent glucosinolate. Myrosinase catalyzes cleavage of the glucose group, leading to production of an unstable aglycone intermediate. This unstable intermediate then spontaneously degrades to one of a number of products depending on factors such as pH and the presence of various cofactors. For example, in the case of sinigrin (Fig. 1), low pH tends to favor production of allyl cyanide, whereas under conditions of neutral and alkaline pH, allyl isothiocyanate is the dominant breakdown product (5). Furthermore, in the additional presence of epithiospecifier protein, 1-cyano-2,3-epithiopropane can arise (6).

The isothiocyanates are an important group of breakdown products of glucosinolates and have been shown to have anticarcinogenic effects. Isothiocyanates appear to act at a number of points in the tumor development process. Isothiocyanates act as blocking agents by modifying the metabolism of carcinogenic compounds through their influence on biotransformation enzymes. The action of isothiocyanates is generally to enhance the activity of phase II enzymes (7, 8), with some evidence that they also inhibit phase I enzymes (9). This dual action is thought to reduce the production of electrophilic intermediates with carcinogenic activity and to enhance the detoxification and clearance of carcinogens. Isothiocyanates also act as suppressing agents during the promotion phase of the neoplastic process. Recent work has shown that isothiocyanates act on signal transduction pathways within the cell and induce apoptosis and inhibit cell growth (10, 11).

Although the evidence for an anticarcinogenic role for isothiocyanates is growing, our understanding of the extent to which these compounds are released after brassica vegetable consumption is less well developed. Glucosinolate hydrolysis in the gut may lead to production of a range of breakdown products in addition to the isothiocyanates. The yields of different groups of metabolites including isothiocyanates, nitriles and epithioalkane nitriles, and the factors that may affect these yields after glucosinolate ingestion are not well understood. The primary method used to assess isothiocyanate production *in vivo* has been to quantify excretion of their urinary metab-

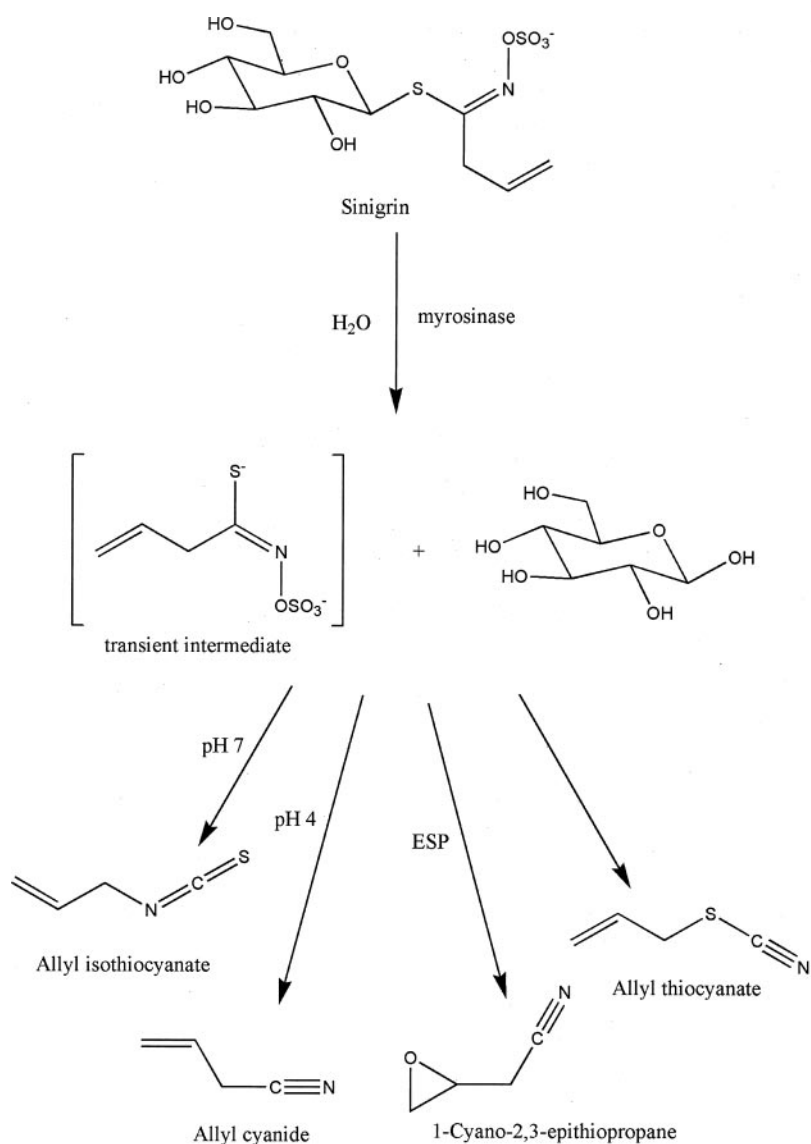


Fig. 1. Scheme showing hydrolysis of sinigrin under the action of myrosinase and factors that may influence the outcome of hydrolysis.

olites. The main excretory route for isothiocyanates in humans is the mercapturic acid pathway with urinary excretion of *N*-acetyl cysteine conjugates of isothiocyanates (12). This provides a convenient means of assessing the release of isothiocyanates after ingestion of intact glucosinolates. A number of recent studies have adopted this approach and demonstrated significant isothiocyanate release after consumption of vegetables such as cauliflower (13), watercress (14), and broccoli (15).

Cooking of brassica vegetables causes myrosinase in the plant to be denatured with potential consequences for glucosinolate hydrolysis during and after vegetable consumption (16). The absence of an active source of myrosinase may reduce the extent to which glucosinolates are hydrolyzed in the digestive tract. However, recent research has demonstrated myrosinase activity in the gut microflora (3), and this may lead to glucosinolate hydrolysis after ingestion, even when vegetables are cooked. The aim of the study reported here was to assess the relative influence of plant and microbial myrosinase activity on the extent and timing of release of isothiocyanates *in vivo*.

## Materials and Methods

### Healthy Volunteers

Twelve healthy, adult subjects (nine males and three females) were recruited for the study using an e-mail advertisement within the research institution. The volunteers were all nonsmokers. Volunteers were briefed on the background to the experiment and asked to sign a consent form. The briefing included information on the procedures for collecting and storing urine samples and on the practicalities of measuring total urine output. All volunteers were science professionals and understood the importance of accurate recording of data. Volunteers were given a list of restricted foods that they were asked to refrain from eating for 48 h before the first experimental meal and throughout the study. The restricted list included the common brassica vegetables and condiments known to include glucosinolates or isothiocyanates. The experiment was approved by the Grampian Research Ethics Committee of the Grampian University Hospitals National Health Service Trust.

### Study Design

The experiment involved consumption of three experimental meals on the Monday, Wednesday, and Friday lunchtimes of a single week. The study was conducted according to a cross-over design with three meal-types that were offered in turn. The allocation of treatments to days was balanced so that a third of the volunteers received each treatment on each experimental day. The order of offering treatments was also balanced so that each treatment followed each other treatment an equal number of times. The meal types consisted of a standard portion of chicken fricassee and rice with a different brassica component according to treatment. The treatments consisted of a portion of 150 g of microwaved cabbage (Cooked treatment), a portion of 150 g of raw cabbage (Raw treatment), and a portion of 5 g of ready-made English mustard (Mustard treatment) as a source of preformed allyl isothiocyanate. Each volunteer was offered each of the meal types in turn.

Each experimental meal also included a 100-ml autolysate of fresh watercress as a source of preformed phenethyl isothiocyanate. The inclusion of the watercress autolysate was to quantify postabsorptive recovery of isothiocyanates and to assess interindividual and temporal variation in isothiocyanate biotransformation.

### Urine Collection

Volunteers were asked to collect one urine sample just before each experimental meal and then to collect 20-ml samples of each urination for the subsequent 24 h, noting the time of urination on each occasion. In addition, volunteers were issued with a data sheet, jug, and measuring cylinder and asked to record the volume of each urination. Samples were stored in sealed sample bottles (Sterilin, Staffordshire, United Kingdom) in a cool bag containing cool blocks until 24 h after the meal, at which time they were stored at  $-20^{\circ}\text{C}$  until analysis.

### Preparation of Meals

White cabbage is a good source of prop-2-enyl glucosinolate, commonly called sinigrin. Sinigrin is the precursor of allyl isothiocyanate and was chosen as the model glucosinolate in this study. The brassica component of each meal was prepared during the 30 min immediately preceding each meal. Cabbage (Dutch white cabbage) was purchased in a single batch from a local vegetable wholesaler (Knowles Fruit Merchants, Aberdeen, United Kingdom) and stored in a refrigerator until preparation. Cabbage was cut into longitudinal wedges to form a series of 300-g segments, each of which contained equivalent amounts of stem and leaf. Each segment was again divided longitudinally, and the two halves were treated identically. One half was weighed and offered to volunteers, whereas the other was set aside and then flash-frozen in liquid nitrogen while volunteers were consuming their experimental meal. For the Raw treatment, a 150-g segment of cabbage was roughly chopped and served with approximately 30 g of natural yogurt as a dressing. For the Cooked treatment, a 150-g segment of cabbage was roughly chopped, placed in a microwave dish with 15 ml of tap water, covered in pierced kitchen film, and cooked for 8 min at full power in an 850 W domestic microwave oven. For the Mustard treatment, 5 g of ready-made English mustard (Coleman's, Norwich, United Kingdom) were weighed to two decimal places and offered to volunteers in a small dish. English mustard is available in powder form or as a ready-made product. The ready-made form was included as a treatment because it represents a relatively stable and rich source of

preformed allyl isothiocyanate. For the watercress juice, one batch of organic watercress was purchased from a local supermarket (Sainsbury's Aberdeen, United Kingdom) and homogenized in a domestic liquidizer before the experiment. One hundred g of watercress were mixed with 240 ml of tap water and homogenized in a domestic food blender for 1 min before being decanted into a common vessel covered in plastic film. This process was repeated 15 times to produce a single batch of homogenate, which was then rapidly weighed in 50-ml aliquots into polypropylene sample bottles and sealed. Sealed bottles were allowed to incubate at room temperature for 60 min before being placed in a water bath at  $80^{\circ}\text{C}$  for 5 min. This procedure had been tested previously and found to result in complete hydrolysis of phenethyl glucosinolate to phenethyl isothiocyanate during the incubation step and complete inactivation of myrosinase without excessive loss of phenethyl isothiocyanate during the heat treatment step. Sample bottles were stored at  $-20^{\circ}\text{C}$  and thawed for use just before each experimental meal. A sample of thawed watercress juice and mustard was reserved for isothiocyanate analysis at each experimental meal. Each volunteer was offered the contents of two sample bottles of watercress autolysate of known weight in a plastic cup. All volunteers complied completely with the requirements of the experimental meals. Residues of cabbage, mustard, and watercress after each experimental meal were negligible.

### Chemical Analysis

**Brassica Vegetables and Condiments.** The segments of cabbage that had been frozen previously for analysis were freeze-dried, finely ground, and analyzed for individual glucosinolates by high-performance liquid chromatography (17) using 1 g freeze-dried vegetable/duplicate and benzyl glucosinolate (Merck, Poole, United Kingdom) as the internal standard. Isothiocyanate concentrations were measured in mustard and in watercress homogenates by gas chromatography. For the watercress samples, 100  $\mu\text{l}$  of 100 mM benzyl isothiocyanate (Sigma/Aldrich, Poole, United Kingdom) in absolute ethanol were added, as an internal standard, to 10 ml of watercress homogenate in a thick-walled screw-cap test tube. The homogenate was extracted twice with 10 ml of dichloromethane using low-speed centrifugation to separate the layers between extractions. The pooled dichloromethane extracts were concentrated to 1 ml under a stream of air before being injected onto a gas chromatography. The column used was a BPX5 capillary column (SGE, Melbourne, Australia; length, 25 m; internal diameter, 0.52  $\mu\text{m}$ ) with a temperature program starting at  $70^{\circ}\text{C}$  and rising linearly to  $120^{\circ}\text{C}$  over 17 min. Detection was by flame ionization detector. Allyl isothiocyanate concentrations in mustard were measured in the same way, except that pure mustard was diluted 10-fold in deionized water before extraction. Response factors were determined by adding 100  $\mu\text{l}$  of 100 mM phenethyl isothiocyanate and 100  $\mu\text{l}$  of 100 mM allyl isothiocyanate (Sigma/Aldrich) in absolute ethanol to 10 ml of deionized water and subjecting these standards to the same extraction and chromatographic procedures used for unknown samples.

Myrosinase activity was determined by measuring degradation of sinigrin by UV spectroscopy (15).

***N*-Acetyl-Cysteine Conjugates in Urine.** Each isothiocyanate yields a separate *N*-acetyl-cysteine conjugate, also known as a mercapturic acid, in urine. *N*-Acetyl cysteine conjugates were analyzed by modification of methods developed for analysis of rat urine (18). Four ml urine/duplicate were analyzed. *N*-Acetyl-S-(*N*-butylthiocarbonyl)-L-cysteine was synthesized

Table 1 Effect of treatment on the amount of sinigrin, allyl isothiocyanate, and phenethyl isothiocyanate ingested and on the amounts excreted as urinary mercapturic acids

	Mustard		Raw cabbage		Cooked cabbage	
	Mean	SE	Mean	SE	Mean	SE
Sinigrin ingested ( $\mu\text{mol}$ )			82.0	8.64	75.5	3.80
Allyl isothiocyanate ingested ( $\mu\text{mol}$ )	54.0	0.96				
Allyl isothiocyanate excreted ( $\mu\text{mol}$ )	36.5	1.36	29.4	3.59	11.5	1.96
Proportion of allyl isothiocyanate excreted	0.68	0.031	0.37	0.033	0.15	0.027
Phenethyl isothiocyanate ingested ( $\mu\text{mol}$ )	16.1	0.29	16.3	0.14	16.1	0.15
Phenethyl isothiocyanate excreted ( $\mu\text{mol}$ )	14.2	0.88	15.0	0.86	13.0	1.13
Proportion of phenethyl isothiocyanate excreted	0.88	0.054	0.93	0.057	0.80	0.068

as its dicyclohexylamine salt (19) and used as an internal standard. *N*-Acetyl-*S*-(*N*-allylthiocarbamoyl)-*L*-cysteine and *N*-acetyl-*S*-(*N*-phenethylthiocarbamoyl)-*L*-cysteine, which are the mercapturic acids of allyl isothiocyanate and phenethyl isothiocyanate, respectively, were synthesized as their dicyclohexylamine salts for determination of response factors. Dicyclohexylamine salts were synthesized to allow preparation of a crystalline solid, allowing known amounts of mercapturic acids to be weighed when preparing standard solutions. High-performance liquid chromatography separation was conducted on an Agilent 1100 instrument with a Lichrosphere RP-18 column (Merck) using a solvent gradient of 30% (v/v) acetonitrile in water changing linearly to 70% (v/v) acetonitrile in water over 20 min. Detection was by UV absorbance at 240 nm.

### Statistical Analysis

Excretion data were summarized for analysis in two ways. Firstly, the amount of *N*-acetyl cysteine conjugates excreted over 24 h as a molar proportion of the amount of precursor ingested (glucosinolate in the case of cabbage and isothiocyanate in the case of watercress and mustard) was calculated. Volunteers (*V*) were treated as blocks to allow treatment effects to be assessed within individuals. Terms in the ANOVA model were meal (*M*; Monday, Wednesday, or Friday) followed by treatment (*T*; Cooked, Raw, or Mustard). The ANOVA model structure was therefore  $V + M + T$ . We then used the fitted ANOVA model to study the linear contrast between the factor levels Cooked and Raw of the treatment factor, thus enabling a direct comparison. Twelve volunteers and three treatments meant that the number of observations was 36 per compound.

The second method of summarizing data was to divide *N*-acetyl cysteine conjugate excretion into blocks of time. The amounts excreted over 0–6 h, 6–12 h, and 12–24 h were calculated and expressed as proportions of ingested precursors as described above. This allowed the time course of excretion to be compared. The time periods for summarizing excretion data were selected to allow comparison of equivalent data across treatments. The frequency of urination by volunteers was variable, and although shorter time intervals could have been used, this would have led to increasing numbers of missing values in the data set, compromising the statistical analysis. The ANOVA was set up to allow meal (*M*) and treatment (*T*) effects to be assessed within volunteers (*V*) and interactions with time (*H*) to be assessed between volunteers. The ANOVA model structure was  $(V/M/H) + (M^*H) + (T^*H)$ . A linear contrast was again used to allow the raw and cooked cabbage treatments to be directly compared. Twelve volunteers, three treatments, and three times meant that the number of observations was 108 per compound.

The statistical package Genstat 5 (20) was used for all statistical analysis.

### Results

**Precursors Ingested.** The amounts of sinigrin consumed on the raw cabbage and cooked cabbage treatments were similar (approximately 80  $\mu\text{mol}/\text{meal}$ ), whereas the amount of allyl isothiocyanate consumed on the mustard treatment was lower (54  $\mu\text{mol}$ ; Table 1). The amounts of phenethyl isothiocyanate consumed in the watercress juice were consistent across treatment at approximately 16  $\mu\text{mol}/\text{meal}$  (Table 1). Myrosinase activity of cabbage consumed on the cooked cabbage treatment (mean  $\pm$  SE,  $0.107 \pm 0.019$   $\mu\text{mol}$  sinigrin hydrolyzed/g dry matter cabbage/min;  $n = 12$ ) was substantially lower ( $P < 0.001$ ) than the raw cabbage treatment (mean  $\pm$  SE,  $0.496 \pm 0.051$   $\mu\text{mol}$  sinigrin hydrolyzed/g dry matter cabbage/min;  $n = 12$ ), although some myrosinase activity was retained even when cabbage was subjected to microwave treatment.

**Metabolites Excreted.** Urinary excretion of allyl isothiocyanate as its *N*-acetyl cysteine conjugate was substantial when mustard was consumed. Urinary output as a proportion of allyl isothiocyanate ingested was 0.68 (Table 1). The output of the *N*-acetyl cysteine conjugate of allyl isothiocyanate was lower ( $P < 0.001$ ) after ingestion of raw and cooked cabbage than for the meal including mustard, reflecting incomplete hydrolysis of sinigrin to its allyl isothiocyanate breakdown product. Urinary output of the *N*-acetyl cysteine conjugate of allyl isothiocyanate was higher when cabbage was ingested raw than when cabbage was cooked before ingestion (Fig. 2;  $P < 0.001$ ). When expressed as a proportion of sinigrin ingested, the excreted proportions were 0.37 (SE, 0.033;  $n = 12$ ) for raw cabbage and 0.15 (SE, 0.027;  $n = 12$ ) for cooked cabbage (Table 1;  $P < 0.001$ ). Urinary excretion of phenethyl isothiocyanate as its *N*-acetyl cysteine conjugate was consistent across treatments (Table 1; Figs. 2 and 3). A mean proportion of 0.87 (SE, 0.036;  $n = 36$ ) of phenethyl isothiocyanate ingested was excreted as its *N*-acetyl conjugate in urine, and this proportion was not significantly influenced by treatment ( $P > 0.05$ ).

The time course of excretion of allyl isothiocyanate as its *N*-acetyl cysteine conjugate was also different in the cooked and raw cabbage treatments. Cooking of cabbage resulted in a delayed excretion of the *N*-acetyl cysteine conjugate of allyl isothiocyanate. Considering the mean amounts of metabolites excreted over consecutive time periods, the time course of excretion after ingestion of raw cabbage followed a similar pattern, at a lower level, to that of mustard (Fig. 2). When cooked cabbage was ingested, there was a lag phase in excretion, with more conjugate being excreted between 6 and 12 h than between 0 and 6 h. The delay was also evident in the

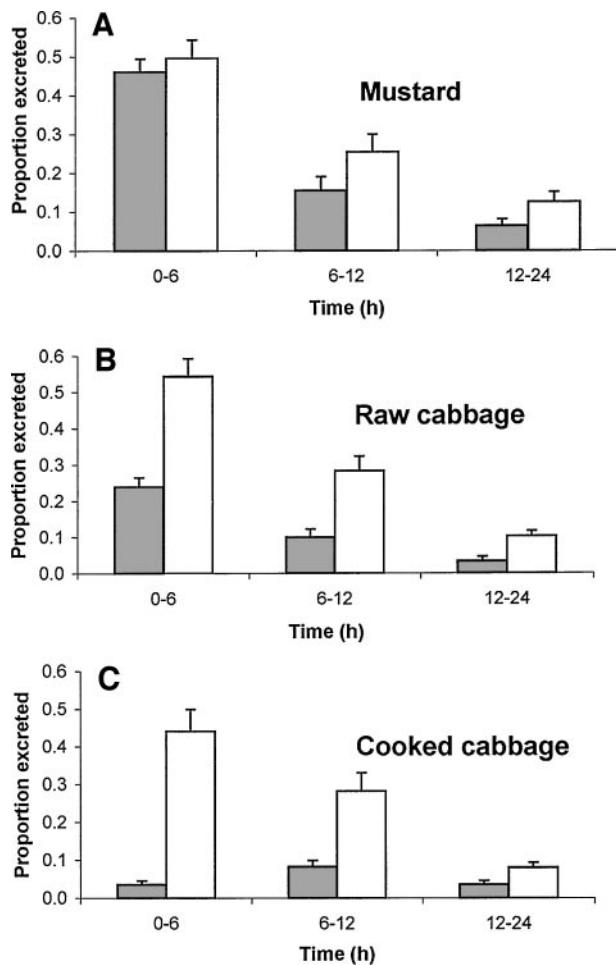


Fig. 2. Mean proportion of ingested precursors excreted as urinary *N*-acetyl cysteine conjugates of allyl isothiocyanate (■) and phenethyl isothiocyanate (□) over time after a meal including (A) mustard, (B) raw cabbage, or (C) cooked cabbage. Watercress juice containing phenethyl isothiocyanate was ingested with each meal.

cumulative excretion curves of allyl mercapturic acid on the cooked cabbage treatment. In the case of volunteers consuming cooked cabbage, excretion rate of allyl mercapturic acid peaked between 9 and 12 h compared with a peak occurring between 3 and 6 h for the raw cabbage and mustard treatments (Fig. 3). The different temporal excretion pattern of the allyl isothiocyanate conjugate after ingestion of cooked and raw cabbage was reflected in a significant time by treatment interaction when the model included a contrast to compare the cooked and raw treatments ( $P < 0.001$  for time by contrast interaction). The time course of excretion for phenethyl isothiocyanate was not significantly affected by treatment ( $P = 0.747$  for time by contrast interaction).

## Discussion

The measurement of excretory products of isothiocyanates has proved a useful tool for assessing the metabolism of glucosinolates. A number of studies in rats and humans have used urinary markers to estimate isothiocyanate release *in vivo* after consumption of a range of crucifers including mustard (21), cauliflower (13), watercress (14), and broccoli (22). The use of

urinary metabolites of isothiocyanates has also been suggested as a means of assessing brassica vegetable consumption in epidemiological studies (23). In this study we examined the effect of cooking cabbage in a domestic microwave cooker on the extent and timing of release of allyl isothiocyanate from sinigrin. Our main findings were (a) that cooking cabbage caused a reduction in the extent of isothiocyanate release and (b) that isothiocyanate release after consumption of cooked cabbage was delayed by approximately 6 h. These findings are in general agreement with a recent study in which sulforaphane release from broccoli was compared after consumption, by human volunteers, of steamed and raw vegetables (22). The anticarcinogenic properties of sulforaphane have been attributed to its action as a potent phase 2 enzyme inducer (24), although enhancement of apoptosis may also be involved (11). Studies on the anticarcinogenic action of allyl isothiocyanate suggest that the mechanism of action is broadly similar, although in this case, enhancement of apoptosis may play a more prominent role (25). Both glucosinolates appear to break down

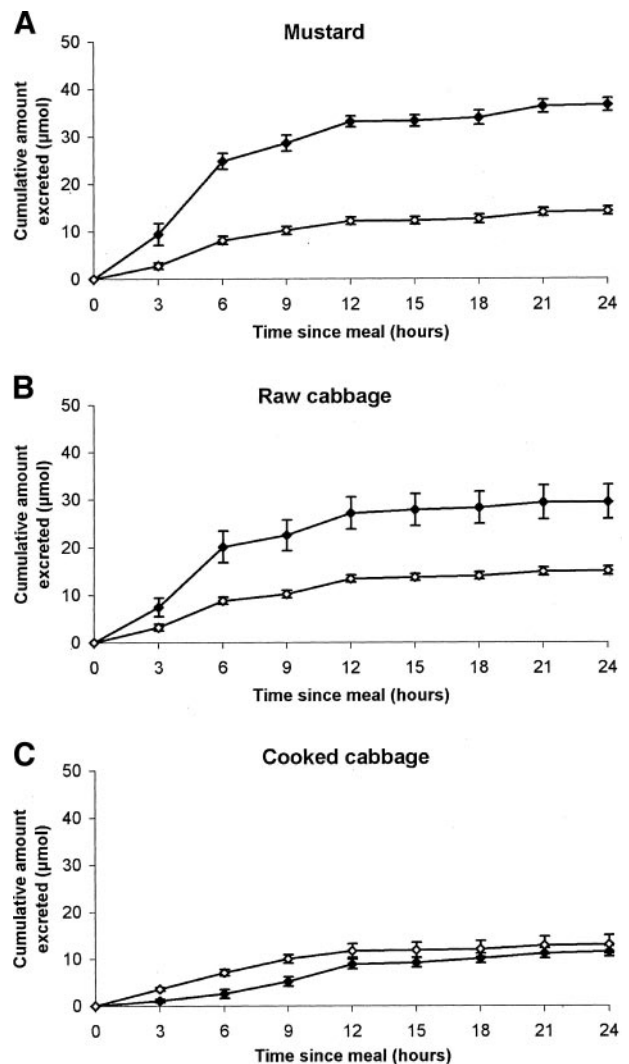


Fig. 3. Cumulative urinary output of *N*-acetyl cysteine conjugates of allyl isothiocyanate (♦) and phenethyl isothiocyanate (◇) over time after a meal including (A) mustard, (B) raw cabbage, or (C) cooked cabbage. Watercress juice containing phenethyl isothiocyanate was ingested with each meal.

in similar ways in the digestive tract, and the ready availability of sinigrin may make it a suitable candidate for additional studies on the general principles of glucosinolate hydrolysis in the digestive tract.

The reduction in isothiocyanate release in cooked cabbage observed in our study was presumably due to the inactivation of plant myrosinase. This would have allowed intact glucosinolates to pass through the upper digestive tract unchanged. The delayed release of isothiocyanates after consumption of cooked cabbage suggested that hydrolysis then occurred under the action of the colon microflora when dietary glucosinolates reached the colon. The reason for the lower overall level of isothiocyanate production may be that the overall activity of myrosinase in the gut microflora is lower than that of plant myrosinase. Furthermore, hydrolysis of glucosinolates in the colon may yield other products in addition to isothiocyanates such as nitriles. Also, isothiocyanates produced in the colon may be subject to further degradation by the microflora (26), reducing the amount available for absorption. *In vitro* studies using nuclear magnetic resonance suggest amines as potential products after degradation of allyl isothiocyanate by a human fecal microflora (27). Although the metabolism and disposition of isothiocyanates and their conjugates have been well studied, little is known about how the rate and site of isothiocyanate release influence their anticarcinogenic efficacy. The current study has demonstrated that isothiocyanate release in the intestinal tract is strongly influenced by vegetable cooking. What is unclear is the relative anticarcinogenic benefits of an extensive and abrupt production of isothiocyanates in the upper digestive tract or a lower rate of production at a site further down the digestive tract. Although raw cabbage gave a higher overall yield of isothiocyanates, it may be that a gradual release of isothiocyanates in the colon after consumption of cooked vegetables has direct effects on colonocytes and biotransformation enzymes at this site such as glutathione *S*-transferases (28). Isothiocyanates released in the large bowel may contribute greatly to the activation of detoxification enzymes in the gastrointestinal compartment (29, 30).

Measurement of isothiocyanate products in previous studies has been most commonly achieved by measuring the cyclocondensation products of isothiocyanates (dithiocarbamates; Ref. 31). This technique allows measurement of total isothiocyanate excretion but is a nonspecific method that does not yield information on the presence of specific isothiocyanates. Our own study differed from many previous experiments in that we measured the release of one specific isothiocyanate, allyl isothiocyanate, derived from a plant matrix where different glucosinolates were present. We also included a treatment where the preformed isothiocyanate was administered directly to subjects, allowing the extent of recovery of this compound as its *N*-acetyl cysteine conjugate in urine to be determined. We were thus able to make an estimate of isothiocyanate release in the gut that took account of postabsorptive losses. The use of a specific analytical method for the *N*-acetyl cysteine conjugates of individual isothiocyanates also allowed us to simultaneously study the excretion of phenethyl isothiocyanate that was absent from white cabbage. This approach allowed interindividual and temporal variation in isothiocyanate excretion to be assessed. The recoveries of isothiocyanates as *N*-acetyl cysteine conjugates in our study were higher than those reported in early studies (12) but relatively consistent between individual subjects and over time. Only a weak relationship between the cumulative excretion of allyl isothiocyanate and phenethyl isothiocyanate was observed ( $r^2 = 0.34$ ). The correlation was weaker than in our previous work with rats (13, 26), mainly

because overall variation in urinary recovery of isothiocyanates was lower in this experiment with humans than in our previous work with rats. In previous studies with rats (13, 26), we have used homologous isothiocyanates as internal standards to correct for temporal and interindividual variation in postabsorptive isothiocyanate recovery. In the previous work, we found that isothiocyanate recovery varied widely between individuals and occasions (between 10% and 65% of administered allyl isothiocyanate recovered) but that recovery of different isothiocyanates covaried within an individual [ $r^2 = 94%$  for correlation between urinary allyl mercapturic acid output and urinary butyl mercapturic acid output after an oral dose of 50  $\mu\text{mol}$  to rats (13)]. In the current study with humans, interindividual variation was less pronounced (60–90% of administered allyl isothiocyanate recovered) and less predictable ( $r^2 = 34%$  for correlation between urinary allyl mercapturic acid output and urinary phenethyl mercapturic acid output after ingestion of isothiocyanates in vegetable matrices by humans). The lower interindividual variation together with the lower predictive power of phenethyl isothiocyanate as an internal standard meant that correction of allyl isothiocyanate recoveries using phenethyl isothiocyanate as an internal standard was not justified in the current experiment. The use of standard compounds that follow similar metabolic routes as compounds whose metabolism is being studied does, however, offer a useful methodology in studies of this kind. The similar extent and time course of excretion of phenethyl isothiocyanate regardless of meal type (Fig. 3) provided some reassurance in the current experiment that the composition of the meal itself did not greatly influence postabsorptive metabolism of isothiocyanates and that the observed effects therefore related to preabsorptive differences in metabolic fate. The rather weak relationship between allyl isothiocyanate and phenethyl isothiocyanate recoveries in the current experiment partly relate to the low overall spread of the data but also perhaps relate to the fact that isothiocyanates were presented in different food matrices; the rate of release and absorption of isothiocyanates from mustard and our watercress slurry may have differed slightly on the different occasions on which they were ingested.

In conclusion, our results indicate that consumption of raw vegetables leads to greater absorption of anticarcinogenic isothiocyanates than when vegetables are subjected to microwave cooking. The colon microflora appear to be able to catalyze glucosinolate hydrolysis when vegetables are cooked. Although the extent of isothiocyanate availability is reduced in this situation, the effect of the altered site of release on the relative protective effects against tumor formation at different sites remains unknown.

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