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Association between Consumption of Cruciferous Vegetables and Condiments and Excretion in Urine of Isothiocyanate Mercapturic Acids

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A high intake of cruciferous vegetables is associated with a reduced risk of cancer and cardiovascular diseases. This protective effect has been linked to isothiocyanates, enzymatic hydrolysis products of glucosinolates. In this study, the metabolic fate of glucosinolates and isothiocyanates after ingestion of 19 different cruciferous vegetables was studied in three male subjects. After the consumption of 13 cruciferous vegetables (glucosinolate content, 0.01−0.94 mmol/kg) and six condiments (isothiocyanate content, 0.06−49.3 mmol/kg), eight different isothiocyanate mercapturic acids were determined in urine samples. Excretion levels after the consumption of raw vegetables and condiments were higher (bioavailability, 8.2−113%) as compared to cooked vegetables (bioavailability, 1.8−43%), but the excretion rate was similar ($t_{1/2}$ = 2.1−3.9 h). Isothiocyanates in urine remain longer at a nonzero level after the consumption of glucosinolates from cooked vegetables, as compared to raw vegetables and condiments, and maximal levels in urine were reached about 4 h later. Isothiocyanate mercapturic acids can be used as a biomarker to reflect the active dose of isothiocyanates absorbed.

KEYWORDS: Cruciferous vegetables; glucosinolates; HPLC-MS analysis; kinetics; biomarker

INTRODUCTION

Epidemiological studies indicate that the consumption of fruits and vegetables is associated with a reduced risk of degenerative diseases such as cancer and cardiovascular diseases (*1*, *2*). In particular, cruciferous vegetables, e.g., cabbages, kale, broccoli, Brussels sprouts, radish, mustard, and cress, are possibly beneficial for human health (*3*, *4*). Because cruciferous vegetables differ from other vegetables in that they contain glucosinolates, these phytochemicals might be responsible for the health-promoting effect (*5*, *6*). Cruciferous vegetables can be differentiated by the variety (**Table 1**) and amount of glucosinolates (*7*). One vegetable generally contains high amounts of 2−5 different glucosinolates (**Table 2**). In all cruciferous plants together, some 120 different glucosinolates have been identified. There is a wide variation in the contents of glucosinolates in cruciferous vegetables, and it is clear that processing, storage, and cooking affect these components. For example, broccoli is rich in 3-methylsulfinylpropyl glucosinolate (glucoiberin), whereas Brussels sprouts are rich in allyl and 2-hydroxy-3-butenyl glucosinolate (progoitrin). Garden and watercress, however, are rich in arylaliphatic glucosinolates (*8*). Unfortunately, there are no reliable tables of compositional data, which makes it difficult to conduct studies in populations (*9*). Therefore, we present an overview of the presence of glucosinolates in commonly consumed vegetables and condiments (**Table 2**).

Glucosinolates remain intact within the plant until the vegetable is processed, e.g., cutting or chewing. These processes release the enzyme myrosinase, which hydrolyzes glucosinolates into isothiocyanates and other breakdown products (**Figure 1**) (*8*, *10*). Glucosinolates that are not hydrolyzed by myrosinase can be degraded, to a lesser degree, by microbes present in the human gut (*11*) but are likely not absorbed intact. After absorption, isothiocyanates are conjugated to glutathione and excreted into the urine as their corresponding mercapturic acids (**Figure 1**) as demonstrated in rats (*12*), guinea pigs, rabbits (*13*), and humans (*14*). Mercapturic acids excreted in urine reflect the uptake of isothiocyanates and thus the intake of glucosinolates, which are present in cruciferous vegetables (*15*).

To give more insight in the health effect of isothiocyanates, we determined the kinetic parameters for the individual isothiocyanates and the difference in bioavailability of isothiocyanates from raw and cooked vegetables. In literature, the sum of isothiocyanate conjugates is analyzed; therefore, no distinction can be made between different isothiocyanates from the same vegetable source. We developed methods for the analysis of individual isothiocyanates in milled, raw vegetables and condi-

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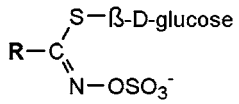
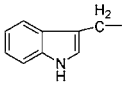
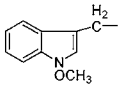
Table 1. Glucosinolates That Are Present in a Wide Range of Vegetables and Condiments



MW[a]	R =	rrf[b]	name	main dietary source
371.5	H_3C-	1.00	glucocapparin	capers
397.5	$H_2C{=}CHCH_2$-	1.00	sinigrin	cabbage, mustard
411.5	$H_2C{=}CH(CH_2)_2$-	1.11	gluconapin	Chinese cabbage
425.5	$H_2C{=}CH(CH_2)_3$-	1.15	glucobrassicanapin	Chinese cabbage
427	$H_2C{=}CHC(OH)HCH_2$-	1.09	progoitrin	Brussels sprouts, kale
441	$H_2C{=}CHCH_2C(OH)HCH_2$-	1.00	gluconapoleiferin	cabbage
461.6	$H_3CS(=O)(CH_2)_3$-	1.07	glucoiberin	broccoli
459.6	$H_3CS(CH_2)_4$-	1.00	glucoerucin	rutabaga, turnip
475.6	$H_3CS(=O)(CH_2)_4$-	1.07	glucoraphanin	cabbage, cauliflower
589.6	$H_3CS(=O)(CH_2)_5$-	1.07	glucoalyssin	paksoi
463.5	$pHOC_6H_4CH_2$-	1.00	glucosinalbin	mustard
447.5	$C_6H_5CH_2$-	0.95	glucotropaeolin	garden cress
461.5	$C_6H_5(CH_2)_2$-	0.95	gluconasturtiin	water cress
486		0.29	glucobrassicin /	all cruciferous
516		0.20	neoglucobrassicin[c]	vegetables

[a] The molecular weight of each glucosinolate is calculated as its potassium salt in g/mol. [b] The relative response factor at 229 nm in water:acetonitrile (v:v, 9:1), relative to sinigrin (*17*). [c] Brassicins are 3-indolylmethyl compounds.

Table 2. Principal Glucosinolates Occurring in Commonly Consumed Cruciferous Vegetables[a]

	mmol glucosinolate/kg fresh weight (average range)				
vegetable	sinigrin	gluconapin	glucobrassicanapin	progoitrin	glucoiberin
broccoli		0.03 (0–0.06)		0.25 (0.20–0.31)	0.74 (0.01–3.3)
cauliflower	0.28 (0.01–1.6)				0.34 (0.01–3.3)
red cabbage	0.12 (0.02–0.26)	0.20 (0.05–0.30)		0.10 (0.04–0.14)	0.16 (0.05–0.31)
kohlrabi		0.11			0.19
Brussels sprouts	0.86 (0.04–3.9)	0.34 (0.01–2.2)		0.63 (0.01–3.0)	0.44 (0–1.5)
white cabbage	0.44 (0.04–1.6)	0.13		0.10	0.58 (0.05–2.8)
kale	0.97 (0.63–2.0)	0.21 (0–0.38)		0.70 (0.17–1.3)	0.12 (0–0.50)
Chinese cabbage		0.12 (0–0.59)	0.20 (0.03–0.62)	0.08 (0–0.44)	
rutabage/swede				0.63 (0.09–1.5)	
collards	0.21 (0.13–0.29)				0.39 (0.08–0.69)
savoy cabbage	0.53 (0.01–1.6)			0.07 (0–0.30)	0.11 (0.15–2.8)
turnip tops		0.61 (0.01–2.9)	0.43 (0.01–1.5)	0.52 (0–1.0)	

	glucoerucin	gluco-raphanin	gluco-alyssin	gluco-nasturtiin	indolyl glucosinolates
broccoli		0.81 (0.29–1.9)			0.67 (0.23–1.18)
cauliflower		0.64 (0.02–1.9)			0.59 (0.14–1.92)
red cabbage	0.02 (0.01–0.07)	0.55 (0.32–0.82)			0.73 (0.32–1.0)
kohlrabi					0.26
Brussels sprouts		0.08 (0–0.23)			2.31 (0.45–5.04)
white cabbage		0.1 (0–0.29)			0.5 (0.09–2.0)
kale					1.1 (0.67–1.7)
Chinese cabbage			0.13 (0.01–0.51)	0.15 (0.05–0.67)	0.41 (0.19–1.1)
rutabage/swede	0.20 (0.01–0.89)		0.08 (0–0.22)	0.14 (0.01–1.0)	0.42 (0.14–1.1)
collards					0.50 (0.44–0.70)
savoy cabbage					1.2 (0.7–2.0)
turnip tops	0.10 (0.01–0.29)			0.39 (0.02–1.2)	0.50 (0.12–1.1)

[a] Adapted from refs *8*, *28*, and *29*. Condiments: Garden cress; gluconasturtiin and glucotropaeolin present, horseradish; sinigrin (65–70) and gluconasturtiin (8.8–15), black mustard seed; sinigrin (39–175), brown mustard seed; sinigrin (1–43) and gluconapin (23–163), water cress; and gluconasturtiin present.

ments and for the analysis of individual isothiocyanate mercapturic acids in urine. The effect of cooking on the bioavailability and excretion kinetics is determined for raw and cooked vegetables. In the current study, we want to demonstrate a clear

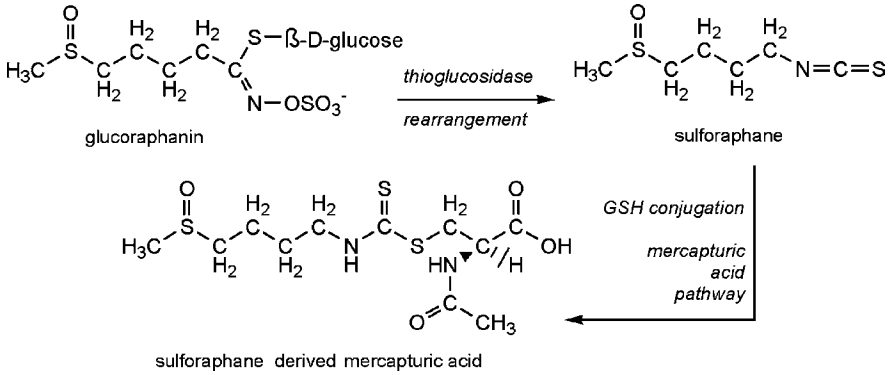


Figure 1. Example of the metabolism of glucosinolates; glucoraphanin is enzymatically hydrolyzed into sulforaphane, which is conjugated to glutathione (GSH) and further metabolized to sulforaphane mercapturic acid.

relation between the intake of glucosinolates and isothiocyanates from cruciferous vegetables and the excretion in urine of their corresponding mercapturic acids.

Spot urine samples of three healthy volunteers were collected for 24 h after the consumption of 13 different cruciferous vegetables and six different cruciferous condiments. Seven of these vegetables were cooked, and six different vegetables and six different condiments were eaten raw. Vegetables and condiments were analyzed for their glucosinolate and isothiocyanate contents, and the urine samples were analyzed for individual mercapturic acids. Absorption and excretion kinetics and the bioavailability were calculated.

MATERIALS AND METHODS

Subjects. Three apparently healthy, male subjects (31 ± 1 years, body mass index of 21 ± 2 kg/m^2) were recruited from the Utrecht area (The Netherlands). Each subject gave written informed consent after being informed about the study, both verbally and in writing.

Experimental Design. Thirteen different vegetables and six different condiments were freshly obtained from a local grocer. Over a period of 9 months, each study substance was ingested on separate days at 12.30 p.m. as part of a complete warm meal (for amounts, see **Table 3**). The composition and amount of this meal were the same for each subject. No cruciferous vegetables were eaten for at least 1 day before each intervention day to make sure that all metabolites from glucosinolates and isothiocyanates were cleared from the body.

Food Preparation. Vegetables that were consumed raw were freshly cut and served immediately. Cooking procedures were as follows. Brussels sprouts were cooked in boiling water for 10 min, white cabbage was stir fried for 10 min, sauerkraut was boiled for 20 min and subsequently heated in an oven for 10 min at 175 °C, green cabbage was stir fried for 20 min, curly kale was microwave cooked for 15 min at 1000 W, Chinese cabbage was stir fried for 10 min, and rutabaga was cooked in boiling water for 15 min.

Sampling Procedure. Shortly before serving, a representative sample of all vegetables (after cooking, where applicable) and condiments (raw) was taken for analysis of glucosinolates and isothiocyanates and was quickly frozen at < −18 °C. Spot urine samples were separately collected for 24 h in clear polyethylene flasks for analysis of isothiocyanate mercapturic acids. Shortly before consumption of the vegetable, a time zero urine sample was collected. After consumption of the vegetable, urine samples were collected at will and as often as possible. All samples were refrigerated immediately after collection, and aliquot samples were stored frozen at < −18 °C.

Chemicals. Water was demineralized using an ELGA Option 7 Plus water purifier (Salm en Kipp, Breukelen, The Netherlands). Methanol, acetonitrile, and dichloromethane for (solid phase) extraction and chromatography were high-performance liquid chromatography (HPLC) grade. Sinigrin monohydrate, aryl sulfate sulfohydrolase (thioglucosidase), and 2-mercaptoethanol were from Sigma (Sigma-Aldrich, Zwijndrecht, The Netherlands). Methyl, allyl (2-propenyl), phenyl, benzyl, and 2-phenylethyl isothiocyanate were purchased from Across Organics. All other chemicals were from Merck (Merck KGaA, Darmstadt, Germany) and of analytical grade. Erucin, sulforaphane, butenyl, and pentenyl isothiocyanate were prepared as described (*16*). Ibervirin, iberin, and erysolin were correspondingly prepared; methylthiopropylamine was converted into ibervirin using dipiridylthionocarbonyl, and ibervirin was subsequently oxidized into iberin using *m*-chloroperbenzoic acid (MCPBA). Erysolin was prepared from sulforaphane by overnight oxidation using MCPBA. Methyl, allyl, 3-butenyl, 4-pentenyl, 4-methylthiobutyl, 4-methylsulfinylbutyl, phenyl, benzyl, and 2-phenylethyl isothiocyanate mercapturic acid were prepared as described (*16*).

Analysis of Glucosinolates in Vegetables. Glucosinolates were measured in extracts of vegetables after desulfation. Two different vegetable sample preparation protocols were used as follows: (A) Samples were snap frozen in liquid nitrogen, freeze-dried, and milled, or (B) samples were snap frozen and milled in liquid nitrogen. A previous study showed only a slight difference in glucosinolate levels between these two different sample preparation protocols. Protocol A yielded lower levels of brassicins than protocol B, but because levels of aliphatic and aromatic glucosinolates were not affected significantly, both protocols were used simultaneously. Samples were kept strictly frozen before extraction to prevent any myrosinase activity.

Glucosinolates were extracted using boiling methanol−water and trapped on quaternary amine solid phase extraction (SPE) columns (Mallinckrodt Baker B. V., Deventer, The Netherlands). Subsequently, glucosinolates were desulfated using aryl sulfate sulfohydrolase, and the corresponding desulfoglucosinolates were eluted with water. Desulfoglucosinolates were separated on a Supelco Discovery C18 5 μm column (2.1 mm × 10 + 150 mm) with a water−acetonitrile (0.1% formic acid) gradient. Quantification was performed using diode array detection, and the relative response factors are shown in **Table 1** (*17*). Sinigrin was used as an external standard. The linearity of analysis ranged from 0.5 to 500 μM desulfosinigrin, and the valid range of analysis was 0.01−9.9 mmol/kg sinigrin.

Analysis of Isothiocyanates in Condiments. The condiments mustard, horseradish, garden cress, water cress, rocket, and capers were eaten uncooked and were only analyzed for their isothiocyanate content because glucosinolates were already converted into isothiocyanates or would easily be converted upon thorough chewing. From garden cress, water cress, rocket, and capers, only minimal sample material was available for freeze drying; these samples could then easily defrost leading to loss of glucosinolates. Mustard and horseradish are products of crushing the seeds, respectively, the root, of a plant, and this crushing leads to complete conversion of glucosinolates to isothiocyanates.

Isothiocyanates were measured in extracts of condiments after conjugation to mercaptoethanol. Samples (2−5 g) were milled using an ultra-turrax after the addition of an equal volume of water. Phenyl isothiocyanate (internal standard) and phosphate buffer (pH 7.0) were added before incubation with additional thioglucosidase. Isothiocyanates were extracted twice with 10 mL of dichloromethane. From the combined extracts, 10 mL was cleaned on Supelco Envi-Carb SPE columns and 1 mL of the eluent was pipetted into an HPLC crimpcap vial. To the vial, reagent was added (0.5 mL of 20 mM triethylamine

Table 3. Isothiocyanate Levels in Six Condiments and Glucosinolate Levels in 13 Vegetables that Were Used for Treatment with Its Biomarker Found in Urine

cruciferous vegetable, consumed amount, and period (*Genus*)	isothiocyanates[a]	mmol/kg	found ($n = 3$)[b]
capers, 10 g raw, August (*Capparis spinosa*)	methyl	0.34	3
garden cress, 7 g raw, August (*Lepidium sativum* L.)	benzyl	49.3	3
horseradish, 20 g raw, February (*Armoracia rusticana* G., M. & S.)	allyl	1.88	3
	phenylethyl	1.10	3
mustard, 9 g raw, June (*Brassica juncea* Coss.)	allyl	0.83	3
rocket, 14 g raw, August (*Eruca sativa* Mill.)	erucin	0.06	3
	sulforaphane	0.75	3
	2-OH-4-pentenyl[c]	2.77	NI
water cress, 10 g raw, September (*Nasturtium officinale* R.Br.)	phenylethyl	7.72	3
	unknown	±0.4	ND
	glucosinolates[a]		
radish, 50 g raw, June (*Raphanus sativus* L.)	glucoraphenin	0.93	ND
	gluconasturtiin	0.02	0
	indole	0.05	NI
broccoli, 162 g raw, June (*Brassica oleracea* L. *italica*)	glucoiberin	0.04	ND
	glucoerucin	<0.01	3, NE
	glucoraphanin	0.32	3
	indole	0.22	NI
cauliflower, 102 g raw, August (*B. oleracea* L. *botrytis cauliflora*)	glucoiberin	0.11	ND
	sinigrin	0.09	3
	glucoraphanin	0.02	3
	indole	0.18	NI
red cabbage, 100 g raw, August (*B. oleracea* L. *capitata* f. *rubra* Thell.)	glucoiberin	0.03	ND
	sinigrin	0.03	3
	glucoerucin	<0.01	3, NE
	glucoraphanin	0.08	3
	gluconapin	0.06	3
	indole	0.09	NI
pak choi, 75 g raw, October (*Brassica campestris* L. *chinensis*)	glucoallysin	0.09	ND
	gluconapin	0.52	3
	glucobrassicanapin	0.44	3
	gluconasturtiin	0.03	3
	indole	0.21	NI
kohl rabi, 100 g raw, February (*Brassica oleracea* L. *gongylodes*)	glucoerucin	0.01	3
	glucoraphanin	0.01	3
Brussels sprouts, 200 g cooked, August (*Brassica oleracea* L. *gemmifera*)	glucoiberin	0.23	ND
	progoitrin	0.24	NI
	sinigrin	0.67	3
	glucoraphanin	0.07	3
	gluconapin	0.44	3
	indole	0.39	NI
white cabbage, 200 g cooked, January (*B. oleracea* L. *capitata* f. *alba* DC.)	glucoiberin	0.42	ND
	progoitrin	0.09	NI
	sinigrin	0.59	3
	glucoerucin	<0.01	3, NE
	glucoraphanin	0.05	3
	gluconapin	0.10	3
	indole	0.26	NI
sauerkraut, 135 g cooked, September (*B. oleracea* L. *capitata* f. *alba*)	sinigrin/allyl isothiocyanate	<0.01	3, NE
green cabbage, 177 g cooked, January (*Brassica oleracea* L. *acephala*)	glucoiberin	0.86	ND
	progoitrin	0.05	NI
	sinigrin	0.94	3
	glucoraphanin	0.02	3
	gluconapoleiferin	0.07	ND
	indole	0.70	NI
(curly) kale, 107 g cooked, October (*Brassica oleracea* L. *acephala* var. *sabellica*)	glucoiberin	0.15	ND
	progoitrin	0.04	NI
	sinigrin	0.02	3
	glucoraphanin	0.02	3
	indole	0.07	NI
Chinese cabbage, 200 g cooked, October (*Brassica chinensis* L.)	progoitrin	0.06	NI
	glucoraphanin	0.02	3
	glucoallysin	0.09	ND
	gluconapin	0.03	3
	glucobrassicanapin	0.07	3
	gluconasturtiin	0.03	3
	indole	0.16	NI
rutabaga, 220 g cooked, January (*Brassica napus* L. *napobrassica*)	glucoiberin	0.01	ND
	progoitrin	0.13	NI
	glucoerucin	0.02	3
	gluconapoleiferin	0.03	ND
	glucoallysin	0.04	ND
	gluconasturtiin	0.11	3
	indole	0.17	NI

[a] Condiments were analyzed for their isothiocyanate content because glucosinolates were already converted into isothiocyanates or would easily be converted upon thorough chewing. [b] Isothiocyanate mercapturic acid was found in 24 h urine of all volunteers; NI, no isothiocyanate was formed after hydrolysis of the glucosinolate; ND, not determined; and NE, not expected. [c] This isothiocyanate was tentatively assigned 2-hydroxy-4-pentenyl.

and 200 mM 2-mercaptoethanol in dichloromethane), and the vial was capped and incubated at 30 °C for 60 min. Vials were decapped and dried under a stream of nitrogen, and the residue was redissolved in 1 mL of acetonitrile:water 1:9 (v:v). Mercaptoethanol conjugates of isothiocyanates were separated on a Supelco Discovery C18 5 μm column (2.1 mm × 10 mm + 150 mm) with a water−acetonitrile gradient. Peak identification was performed using the retention times, and the UV spectra were obtained with diode array detection. Quantification was performed at 271 nm with external standard calibration with phenyl isothiocyanate as the internal standard. The performance of the method was accepted, and characteristics were as follows. The linearity of analysis ranged from 0.1 to 100 μM, corresponding to a limit of detection of 0.01 mmol/kg fresh vegetable. The recovery of 0.25 μmol of nine standards, added to 2 g of horseradish before extraction, was 94−103%.

Analysis of Isothiocyanate Mercapturic Acids in Urine. Individual isothiocyanate mercapturic acids were determined in urine as described (*18*). Briefly, internal standard solution was added to urine, and the samples were extracted on SPE columns and analyzed on an HPLC-MS/MS system (LCQ ion-trap with electrospray ionization, Finnigan MAT). A widely used method of analysis measured the total of isothiocyanates and conjugates by cyclocondensation with a reagent, which gave a sum result instead of results for individual isothiocyanates (*19*, *20*). Because of differences in biological activity between structurally different isothiocyanates, we measured individual isothiocyanates in condiments and individual isothiocyanate mercapturic acids in urine.

Statistical and Pharmacokinetic Analysis. Results of urinary excretion were fitted to a one-compartmental model with the assumption of first-order isothiocyanate absorption and first-order isothiocyanate mercapturic acid excretion kinetics. For raw vegetables and condiments, the initial dose (D_0) was taken as the amount of isothiocyanates, while for cooked vegetables, D_0 was assumed to be the amount of glucosinolates. The bioavailability, F, was estimated by dividing the cumulative amount of a single isothiocyanate mercapturic acid excreted in 24 h by the consumed amount of isothiocyanate or glucosinolate.

For each isothiocyanate mercapturic acid, curves were fitted and visually checked for abnormalities. Excretion data were converted to their natural logarithm, the average excretion rate was plotted against the average time, the midpoint of the urine collection period, linearity was visually inspected, and the slope (excretion rate constant, k_e) was calculated by linear regression. Glycosilated compounds such as glucosinolates were generally poorly absorbed from the gut; glucosinolates from cooked vegetables first had to be converted in the colon into isothiocyanates. This will result in a delayed (lag time) and slower absorption; therefore, for cooked vegetables, a first-order kinetic model cannot be used and the absorption rate constant (k_a) was not calculated. The k_a of isothiocyanates from raw vegetables was estimated from the intercept (i) using the formula: $e^i = [(k_e \times k_a \times F \times D_0)/(k_a - k_e)]$ (*21*). From the analysis of metabolites in urine, as performed in this study, only a rough estimation of the absorption rate constant (k_a) can be made. Because the lag time cannot be calculated from urinary excretion data, the estimation of k_a included the lag time, which will result in a lower apparent k_a. The data point of maximal excretion rate equaled the time of maximal concentration in urine (t_{max}); t_{MAX} was not calculated from the fitted curve.

RESULTS

Vegetables and Condiments. Amounts of glucosinolates and isothiocyanates found in the consumed 13 vegetables and six condiments are presented in **Table 3**. The vegetables radish, broccoli, cauliflower, red cabbage, pak choi, and kohl rabi were eaten raw, and glucosinolate levels ranged from 0.02 to 0.93 mmol/kg. In the vegetables Brussels sprouts, white cabbage, sauerkraut, green cabbage, kale, Chinese cabbage, and rutabaga, which were eaten cooked, the glucosinolate levels after cooking ranged from 0.01 to 0.94 mmol/kg. The condiments mustard, horseradish, garden cress, water cress, rocket, and capers were eaten uncooked, and amounts of isothiocyanates varied from 0.06 to 49.3 mmol/kg. A typical chromatogram of the analysis of isothiocyanates in horseradish and of a mixture of isothiocyanates is shown in **Figure 2**.

Mercapturic Acids. Three volunteers consumed 13 different cruciferous vegetables and six different condiments, and their urine was collected in portions. Mercapturic acids of the following eight isothiocyanates were measured in these urine portions: methyl, allyl, butenyl, pentenyl, benzyl, phenylethyl isothiocyanate, erucin, and sulforaphane. Not all possible mercapturic acids were analyzed, e.g., the mercapturic acids from sulforaphene (radish) and iberin (broccoli and cabbages) were not determined in urine since reference compounds were not available. After consumption of the vegetables and condiments, 1−4 mercapturic acids per vegetable or condiment were found (**Table 3**). **Table 3** shows the relation between intake of isothiocyanates or glucosinolates and mercapturic acid excretion. Hydroxy alkenyl glucosinolates, like progoitrin, and indole glucosinolates, like glucobrassicin, are not hydrolyzed into (stable) isothiocyanates, and metabolites were therefore not measured in urine. Isothiocyanate mercapturic acids were analyzed in each individual spot urine sample, and results were plotted as depicted in **Figure 3**. In urine samples collected shortly before consumption of the vegetable, no isothiocyanate mercapturic acids were found. Each subject produced a sufficient number of spot urine samples to construct reliable excretion curves. Subject 1 produced on average [± standard deviation (SD)] 3.5 ± 0.8 urine portions per 24 h; subject 2, 5.2 ± 0.9, and subject 3, 5.5 ± 1.8. In urine samples that were collected from 24 to 48 h after ingestion, only a subsequent 5% of isothiocyanate mercapturic acids was recovered, so these samples were discarded.

Kinetics and Bioavailability. The calculated k_a from vegetables and condiments that were consumed raw was, on average, 2.0−3.0 h^{-1}. The excretion rate constant (k_e) ranged from 0.18 to 0.33 h^{-1} ($t_{1/2}$ = 2.1−3.9 h) for both raw and cooked vegetables (**Table 4**), indicating that the same route of excretion was followed.

In **Figure 3**, the average excretion rates of two different isothiocyanate mercapturic acids in urine after the consumption of four different vegetables by subjects 1, 2, and 3 are plotted against the average time (midpoint of the collection period). Sulforaphane mercapturic acid was excreted after consumption of cooked Chinese cabbage and raw broccoli, and allyl isothiocyanate mercapturic acid was excreted after consumption of cooked Brussels sprouts and mustard. As shown in **Figure 3D**,**B**, respectively, the average t_{MAX} of allyl isothiocyanate from mustard and of sinigrin derived allyl isothiocyanate from Brussels sprouts was 3.1 and 8.5 h, respectively. With Brussels sprouts, only the glucosinolate sinigrin is ingested, while with mustard only the allyl isothiocyanate is ingested. After consumption of raw vegetables, isothiocyanate mercapturic acids appeared fast in urine (average t_{MAX} = 4 h) with high excretion levels (average F = 8.2−113%). Because of metabolic reduction of conjugates of sulforaphane into conjugates of erucin, F values of up to 464% were found. For cooked vegetables, isothiocyanate mercapturic acids were excreted several hours after consumption (average t_{MAX} = 6 h) and the concentration in urine was lower (average F = 1.8−43%) than for raw vegetables.

The concentration of isothiocyanates in urine was increased for a longer time after the consumption of cooked vegetables, as compared to raw vegetables and condiments, because of prolonged absorption (**Figure 3**). The total amount of isothio-

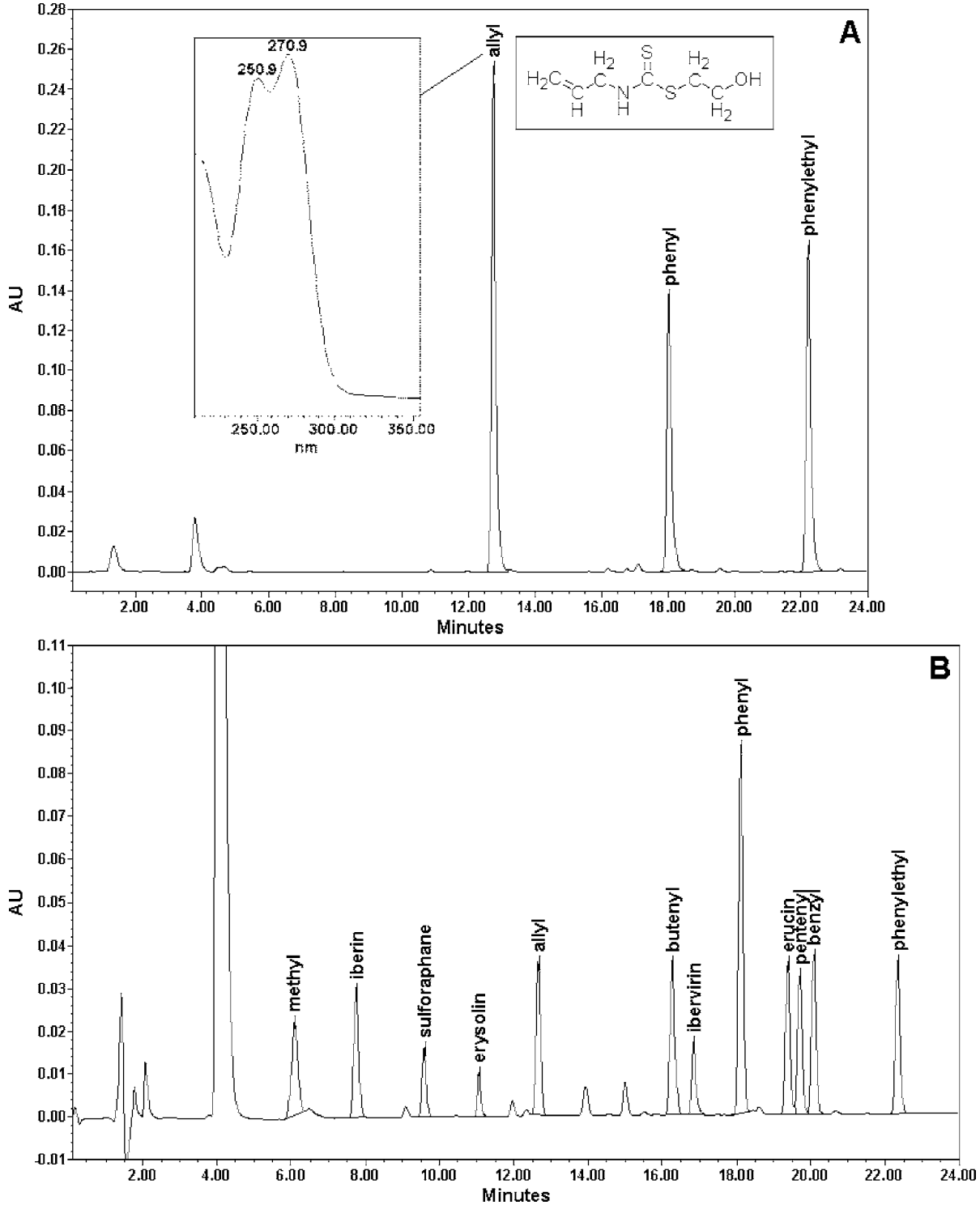


Figure 2. Typical chromatogram of a dichloromethane extract of horseradish containing (2-mercaptoethanol conjugates of) allyl and phenylethyl isothiocyanate. Insert: UV spectrum and structural formula of S-(N-2-propenylthiocarbamoyl)-2-mercaptoethanol, the 2-mercaptoethanol conjugate of allyl isothiocyanate (**A**). Phenyl isothiocyanate was used as the internal standard. A typical chromatogram of 2-mercaptoethanol conjugates of a standard mixture of isothiocyanates (10 μM, **B**).

cyanate conjugates, however, was lower after the consumption of cooked vegetables, as compared to raw vegetables and condiments. The bioavailability of allyl isothiocyanate from mustard is similar for all three subjects and is over 100% (**Table 4**).

DISCUSSION

This study shows that isothiocyanate mercapturic acids in urine reflect the dose of glucosinolates and isothiocyanates absorbed after a meal containing cruciferous vegetables (n = 3). After the consumption of 13 different vegetables and six condiments on separate days, a selection of eight isothiocyanate mercapturic acids was found in the urine portions collected for 24 h. The results of this study show that it is possible to make a distinction between cabbages by analyzing the glucosinolate pattern since each crucifer contains a number of glucosinolates with a different side chain and varying amount. Our results show

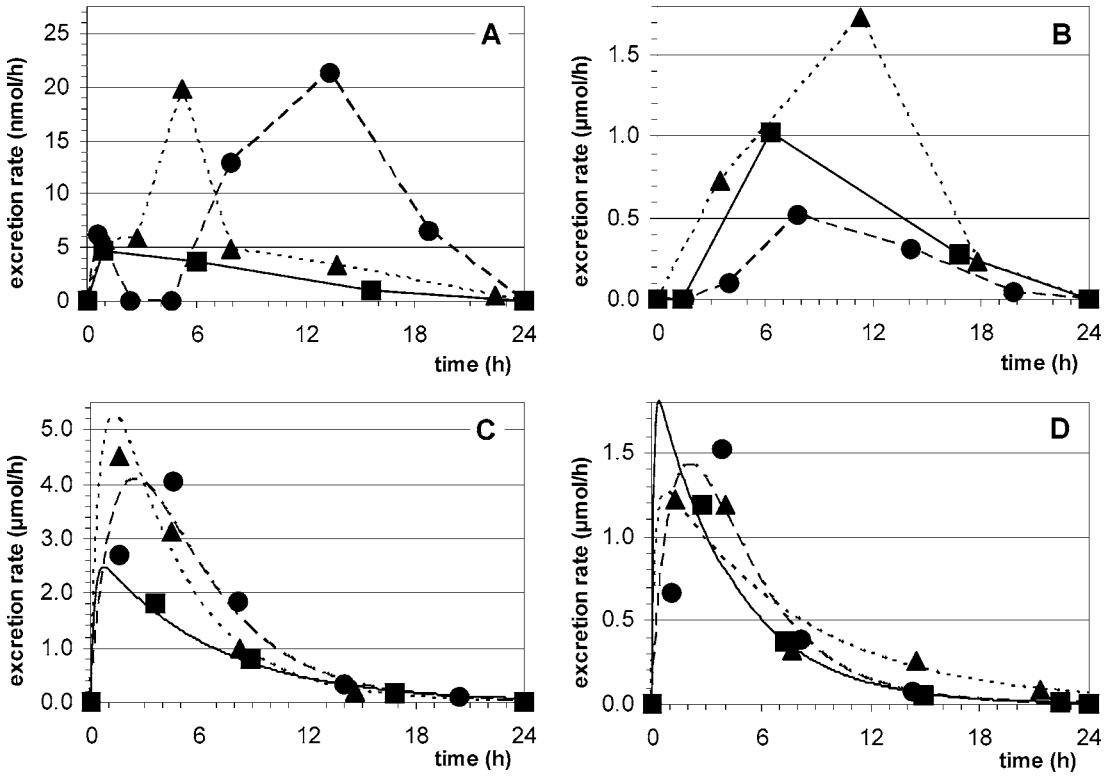


Figure 3. Average excretion rates for persons 1 (■), 2 (●), and 3 (▲) for sulforaphane mercapturic acid after consumption of 200 g of cooked Chinese cabbage (**A**, raw data in nmol/h) and 200 g of cooked Brussels sprouts (**B**, raw data in μmol/h) and for allyl isothiocyanate mercapturic acid after consumption of 200 g of raw broccoli (**C**, fitted curve) and 9 g of mustard (**D**, fitted curve).

that isothiocyanate mercapturic acids in urine reflect the pattern of side chains and amounts of glucosinolates present in the vegetable or condiment that was consumed. We confirmed the finding that condiments contain less different glucosinolates but in higher concentrations than vegetables. All mercapturic acids that were detected were derived from isothiocyanates that were expected from the consumed crucifer, with some minor inconsistencies that will be discussed later. Mercapturic acids cannot easily be validated as a biomarker of intake of a specific cruciferous vegetable because of the variation in glucosinolate content in different vegetable subspecies. Nevertheless, this study clearly shows that mercapturic acids can be used as a marker of the active dose of isothiocyanates that is absorbed.

Bioavailability. In this study, we show that there are substantial differences in bioavailability, absorption, and excretion kinetics between glucosinolates from cooked vegetables and isothiocyanates from raw vegetables and condiments (**Table 4**). The amount of isothiocyanates absorbed from cooked vegetables was 2−6 times lower than from (thoroughly chewed) raw vegetables. This difference cannot be attributed to loss of glucosinolates due to cooking since the bioavailability was calculated relative to the content of glucosinolates present in cooked vegetables. The bioavailability of isothiocyanates from condiments was almost 100%. The only exceptions were benzyl isothiocyanate from garden cress and to a lesser extent phenylethyl isothiocyanate from water cress, which seem difficult to liberate from the plant cells by chewing since the bioavailability was on average 14 and 50%, respectively. Butenyl and pentenyl isothiocyanate from raw pak choi were also poorly absorbed; the bioavailability was 8% on average. The absorption of butenyl and pentenyl isothiocyanate from cooked Chinese cabbage was 4 and 2% on average, respectively, which is low as expected for cooked vegetables. The overall average bioavailability for raw vegetables was 61% (range, 8.2−113%) and for cooked vegetables was 10% (range, 1.8−43%). This indicates that from raw crucifers complete uptake of isothiocyanates is possible and that, apparently, after cooking only 10% is bioavailable. Conaway et al. (*22*) showed that after the consumption of fresh and cooked broccoli, 32 and 10% of the dose of isothiocyanates were excreted in 24 h urine, respectively. They (*22*) measured metabolites in the urine by cyclocondensation with 1,2-benzenedithiol. This is a generic method, which results in a sumparameter for all isothiocyanates so kinetic results for individual isothiocyanates could not be calculated. The bioavailability values were comparable to our results.

Hydrolysis of glucosinolates to isothiocyanates is dependent on the enzyme myrosinase, which is inactivated3 upon cooking of the vegetable. The thioglucosidase enzyme, which is present in the colon, is less effective. When eating crucifers, the intake thus consists of glucosinolates as well as isothiocyanates. The variation in bioavailability between the three volunteers can partly be explained by the difference in intensity of chewing (see, for example, garden and water cress) and by the difference in colonic microflora. The difference in activity of the colonic microflora between subjects could result in interindividual variation where one subject could metabolize three times more glucosinolates into isothiocyanates than another subject (*11*). The colonic microflora could also have a negative effect on the proportion of isothiocyanates available for absorption, as demonstrated by Rouzaud et al. (*23*). They found that in rats harboring a whole human fecal flora, as compared to germfree rats, the administered dose of benzyl glucosinolate was completely converted into nonisothiocyanate metabolites.

Metabolism. Isothiocyanates are conjugated in a phase II reaction to glutahione, catalyzed by glutathione S-transferase, followed by conversion to the mercapturic acid. Phase II biotransformation is a fast reaction; therefore, phase I biotrans-

Table 4. Kinetics of Isothiocyanates from Raw and Cooked Crucifers, Measured as Their Derived Isothiocyanate Mercapturic Acids in Spot Urine Samples from Three Human Volunteers

	excreted mercapturic acid	average (SD)			
		k_a (h^{-1})	k_e (h^{-1})	t_{MAX} (h)	F (%)
		raw cruciferous vegetables			
mustard	allyl	5.2 (4.7)	0.21 (0.08)	3.1 (0.6)	123 (14)
horseradish	allyl	1.3 (0.4)	0.31 (0.13)	2.7 (0.8)	96 (8.3)
	phenylethyl	1.1 (0.5)	0.28 (0.15)	2.7 (0.8)	95 (6.6)
garden cress	benzyl	0.8 (0.5)	0.22 (0.07)	5.3 (1.6)	14 (7.8)
water cress	phenylethyl	1.2 (1.2)	0.27 (0.07)	4.6 (2.5)	50 (23)
rocket	erucin	2.5 (2.0)	0.24 (0.08)	4.1 (2.0)	
	sulforaphane	2.0 (1.3)	0.19 (0.04)	4.1 (2.0)	72
	combined[b]				94 (28)
capers	methyl	1.2 (0.7)	0.25 (0.06)	4.8 (3.7)	71 (41)
radish	none measured				
broccoli	erucin[c]			3.7 (0.8)	29 (11)
	sulforaphane	2.4 (2.2)	0.22 (0.05)	3.7 (0.8)	50 (13)
cauliflower	allyl	2.6 (1.7)	0.21 (0.01)	2.9 (1.4)	113 (44)
	erucin[c]			2.9 (1.4)	43 (40)
	sulforaphane	2.1 (0.8)	0.23 (0.07)	2.9 (1.4)	81 (44)
red cabbage	allyl	1.7 (0.5)	0.20 (0.07)	3.6 (1.1)	46 (25
	butenyl	1.7 (0.9)	0.28 (0.06)	3.6 (1.1)	24 (7.5)
	erucin[c]			4.4 (1.4)	25 (2.1)
	sulforaphane	2.2 (1.7)	0.24 (0.04)	3.6 (1.1)	71 (11)
pak choi	butenyl	2.0 (0.4)	0.27 (0.05)	2.2 (0.2)	8.2 (3.1)
	pentenyl	1.5 (0.3)	0.33 (0.07)	2.2 (0.2)	8.2 (3.1)
kohl rabi	erucin	0.5 (0.3)	0.22 (0.06)	3.7 (0.6)	66 (29)
	sulforaphane	1.6 (1.7)	0.18 (0.01)	3.7 (0.6)	108 (65)
		cooked cruciferous vegetables			
Brussels sprouts	allyl	*a*	0.26 (0.11)	8.5 (2.6)	9.3 (5.8)
	butenyl		0.28 (0.14)	8.5 (2.6)	3.3 (2.3)
	sulforaphane		0.23 (0.07)	8.5 (2.6)	5.2 (2.3)
white cabbage	allyl		0.31 (0.14)	5.2 (0.8)	12 (9.0)
	butenyl		0.29 (0.10)	5.2 (0.8)	12 (8.5)
	erucin[c]			5.2 (0.8)	16 (16)
	sulforaphane		0.30 (0.13)	5.2 (0.8)	21 (13)
sauerkraut	allyl (F in μmol)		0.25 (0.06)	3.8 (0.5)	0.8 (0.3)
green cabbage	allyl		0.27 (0.06)	7.4 (2.0)	7.3 (6.7)
	sulforaphane		0.20 (0.06)	7.4 (2.0)	4.3 (2.4)
(curly) kale	allyl		0.33 (0.04)	6.8 (1.6)	43 (44)
	sulforaphane		0.29 (0.11)	6.8 (1.6)	18 (19)
Chinese cabbage	butenyl		0.19 (0.02)	5.3 (0.7)	4.2 (4.1)
	pentenyl		0.23 (0.01)	5.3 (0.7)	1.8 (1.6)
	sulforaphane		0.18 (0.05)	5.3 (0.7)	3.6 (2.2)
	phenylethyl		0.25 (0.05)	5.8 (0.5)	4.1 (1.5)
rutabaga	erucin		0.19 (0.05)	7.8 (1.1)	5.2 (1.0)
	phenylethyl		0.18 (0.03)	7.8 (1.1)	1.8 (0.2)

[a] Absorption rate constants for isothiocyanates derived from glucosinolates in cooked vegetables were not calculated from urine data since these data do not follow first-order absorption kinetics. Furthermore, the conversion of glucosinolates into isothiocyanates in the colon results in a lag time in absorption. [b] Excretions of erucin and sulforaphane mercapturic acids were combined because of metabolic interconversion. [c] Erucin was not taken in (<0.01 mmol/kg), but erucin mercapturic acid was excreted because of metabolic conversion from sulforaphane. Erucin mercapturic acid is expressed as % of the dose of glucoraphanin.

formation was not expected, and the metabolic rate constant (k_m) was assumed to be zero. The cellular enzyme β-lyase converts a mercapturic acid into a free thiol compound by cleavage of the *N*-acetyl-cysteine group, leaving the sulfur. β-Lyase activity is present in liver and kidney but is not a logical step in metabolism and was not determined. Oxidation of sulfur in, e.g., erucin-derived mercapturic acid could occur but was not expected since conjugation to glutathione is fast.

Interestingly, mercapturic acids from both erucin and sulforaphane were found after consumption of rocket, broccoli, and red and white cabbage although broccoli and red and white cabbage did not contain glucoerucin (<0.01 mmol/kg). These results show that the reduction of sulfinyl, changing sulforaphane into erucin, takes place. This is also confirmed in rats by Kassahun et al. (*24*) who showed that 12% of a dose of sulforaphane was excreted as erucin instead of sulforaphane mercapturic acid. Oxidation of sulfur in erucin also takes place, since after administration of erucin, 67% of the dose was excreted as sulforaphane mercapturic acid (*24*).

Kinetics. In our study, in which vegetables were eaten during lunch, the lag time of the appearance of isothiocyanate mercapturic acid in urine was 4 h on average. Isothiocyanate conjugates in the body reach higher levels but are excreted faster after consumption of raw vegetables and condiments. The results show that levels of isothiocyanate conjugates in the body were longer at nonzero levels after cooked vegetables were consumed.

Ye et al. (*25*) described the pharmacokinetics of broccoli sprout isothiocyanates in humans also using a cyclocondensation sumparameter assay (described in bioavailability). They reported that an average of 58% of the ingested dose of isothiocyanates was excreted in urine collected for 8 h with an elimination half-life of 1.8 h (*25*). The difference between our study and the study of Ye et al. is that we found somewhat longer elimination half-lives, 2−4 h on average. Shapiro et al. (*26*) showed that the excreted cyclocondensation product was related to the glucosinolate/isothiocyanate profiles administered. Furthermore, consumption of graded doses of isothiocyanates resulted in the rapid excretion of 42% of urinary conjugates with first-order kinetics with an excretion rate constant of approximately 0.42 h^{-1}, which is faster than the excretion rate that we found, 0.18−0.33 h^{-1}. Moreover, the conversion of glucosinolates was negligible after bowel microflora were reduced (*26*). Further studies of Shapiro et al. (*27*) showed an even higher bioavailability, measured by excreted isothiocyanate conjugates in 72 h urine. From broccoli sprouts that were treated with myrosinase, 80% of the dose of isothiocyanates was excreted, and 12% of the dose of glucosinolates from cooked broccoli sprouts was excreted as isothiocyanate conjugates. They (*27*) also conducted dose−response experiments, but no further kinetic data were obtained.

In conclusion, isothiocyanate mercapturic acids excreted in urine reflect the intake of glucosinolates from cooked vegetables and of isothiocyanates from raw vegetables and condiments. Interindividual variation occurs and might be explained by differences in the extent of chewing, absorption, conversion, colonic degradation, and/or metabolism. Urinary isothiocyanate mercapturic acids can be used as a biomarker to reflect the active dose of isothiocyanates absorbed.

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