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Bioavailability and Kinetics of Sulforaphane in Humans after Consumption of Cooked versus Raw Broccoli

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The aim of this study was to determine the bioavailability and kinetics of the supposed anticarcinogen sulforaphane, the hydrolysis product of glucoraphanin, from raw and cooked broccoli. Eight men consumed 200 g of crushed broccoli, raw or cooked, with a warm meal in a randomized, free-living, open cross-over trial. Higher amounts of sulforaphane were found in the blood and urine when broccoli was eaten raw (bioavailability of 37%) versus cooked (3.4%, $p = 0.002$). Absorption of sulforaphane was delayed when cooked broccoli was consumed (peak plasma time = 6 h) versus raw broccoli (1.6 h, $p = 0.001$). Excretion half-lives were comparable, 2.6 and 2.4 h on average, for raw and cooked broccoli, respectively ($p = 0.5$). This study gives complete kinetic data and shows that consumption of raw broccoli results in faster absorption, higher bioavailability, and higher peak plasma amounts of sulforaphane, compared to cooked broccoli.

KEYWORDS: Broccoli; bioavailability; kinetics; sulforaphane

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*). In particular, cruciferous vegetables, for example, cabbages, kale, broccoli, Brussels sprouts, radish, mustard, and cress, are expected to be beneficial for human health (*2, 3*). Cruciferous vegetables contain glucosinolates, which are not present in other vegetables. These phytochemicals might therefore be responsible for the protecting effect (*4*). Broccoli contains high amounts of glucoraphanin, which, upon chewing, is enzymatically hydrolyzed by myrosinase into the corresponding isothiocyanate, sulforaphane, and other breakdown products (*5*). Myrosinase is present in all cruciferous vegetables. Glucosinolates that are not hydrolyzed can be degraded by thioglucosidase activity of microbes present in the human gut (*6*), but are not likely to be absorbed intact. Isothiocyanates that are absorbed will be conjugated to glutathione, further metabolized to mercapturic acids, and subsequently excreted in the urine. Mercapturic acids present in urine reflect the uptake of isothiocyanates and, thus, the intake of glucosinolates from cruciferous vegetables (*7, 8*). Sulforaphane has been shown to have anticarcinogenic properties in animal experiments. The proposed mechanism of action is by inhibition of carcinogen-activating phase 1 biotransformation enzymes, induction of phase 2 detoxification enzymes, anti-inflammation, and induction of apoptosis (*9*).

In this study we demonstrate the differences in bioavailability and kinetics of the isothiocyanate sulforaphane after the consumption of raw versus cooked broccoli. Eight healthy men consumed broccoli in a cross-over design study. Sulforaphane in raw and glucoraphanin in cooked broccoli were determined, and metabolites were measured in urine (sulforaphane mercapturic acid) and blood (sulforaphane conjugates).

MATERIALS AND METHODS

Subjects. Apparently healthy, adult (aged 18−60 years) male volunteers were recruited from the Utrecht−Zeist area (The Netherlands). Among the inclusion criteria were a regular (Dutch) food pattern; good health as determined by medical history, physical examination, and clinical laboratory analysis of blood and urine; and a body mass index (BMI) between 19 and 27. Ten volunteers were recruited, and eight subjects completed the entire intervention period. One subject was missing at day 1 and could not be followed up. Another subject was eliminated from the study because of illness that was not related to the study. Characteristics of the remaining eight volunteers were as follows: age, 34 ± 13 years; BMI, 25.0 ± 1.8 kg/m^2 (average ± SD). Genomic DNA was extracted from whole blood using the QIAamp 96 DNA isolation blood kit (Qiagen, Inc.), diluted to a concentration of 20 ng/μL, and stored at 4 °C until analysis. A multiplex PCR was performed to determine the presence or absence of the GSTM1 and GSTT1 genes simultaneously, and the GSTP1 polymorphism A313G was determined. The *N*-acetyltransferase 2 (NAT2) gene was amplified primarily, followed by three nested PCRs to amplify the regions with possible point mutations, T341C, G590A, and A803G + G857A combined.

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Table 1. Urinary Sulforaphane Mercapturic Acid Pharmacokinetic Secondary Parameter Summary after a Single Dose of 200 g of Broccoli, either Raw or Cooked, Was Consumed[a]

		bioavailability		peak time		excretion half-life		genotype			
	creatinine	(F, %)		(t_{max}, h)		($t_{1/2}$, h)			GST		
person no.	clearance (mL/m²/min)	raw	cooked	raw	cooked	raw	cooked	NAT2	M1	P1	T1
1	85	21	3.0	5.0	6.0	2.1	1.3	slow	null	null	
2	81	62	1.7	3.0	5.0	2.4	2.8				null
3	68	37	5.8	4.5	5.5	2.5	1.6	slow	null		
4	76	21	3.3	4.0	5.5	2.5	2.7				
5	81	37	3.1	4.5	4.0	2.6	2.4				
6	66	69	3.2	5.0	6.0	2.4	3.0				
7	46	30	2.4	5.5	7.5	3.1	1.7				
8	68	20	3.2	7.5	7.0	3.3	4.0		null		
av	71	37	3.4	4.9	5.8	2.6	2.4				
SD	12	19	1.0	1.3	1.1	0.4	0.9				
p, t test		0.002		0.03		0.5					

[a] Calculated from excreted sulforaphane mercapturic acid in urine.

Each subject gave written informed consent after being informed about the study. The study was performed according to ICH (International Conference on Harmonization of Technical Requirements of Registration of Pharmaceuticals for Human Use) guidelines for Good Clinical Practice and was approved by an external Medical Ethical Committee. The study was conducted at the Department of Physiological Sciences of TNO Nutrition and Food Research, Zeist, The Netherlands.

Study Design. Volunteers consumed 200 g of crushed broccoli, raw or microwave cooked, together with a warm meal on two separate days, in the morning, with 1 day of wash out between in a randomized, free-living, open cross-over trial. Volunteers were not fasted before consumption of this meal. The warm meal consisted of a fixed amount of a meat burger and mashed potatoes (days 3 and 5). Volunteers refrained from eating crucifers on the days before treatment (days 1, 2, and 4).

Diets. Broccoli was obtained from a local greengrocer's shop (Zeist, The Netherlands) on day 2 and stored at 4 °C. On days 3 and 5, 1 kg of raw broccoli was crushed with a blender and incubated at room temperature for 2 h prior to serving to the volunteers. One kilogram of broccoli was microwave cooked at 1000 W, crushed with a blender, and immediately served. Samples were taken on both days and frozen at −20 °C for later analysis of glucosinolates and isothiocyanates.

Urine and Blood Collection. Urine samples were collected during 24 h in one flask on days 2 and 4, and spot urine samples were collected during 24 h in separate flasks on days 3 and 5. All urine samples were kept refrigerated and were frozen on the same day. On both treatment days, blood samples were collected from five of the eight subjects, at 13 time points during 12 h, in vacutainer tubes with EDTA as anticoagulant. Whole blood was divided into portions; remaining whole blood was centrifuged to obtain plasma, and all samples were stored frozen on the same day.

Chemical Analyses. *Glucosinolates* were determined according to a modified method (*8*). Briefly, glucosinolates were extracted, trapped on solid phase extraction columns, and desulfated. The corresponding desulfoglucosinolates were eluted with water, measured using high-performance liquid chromatography (HPLC) coupled to a diode array detector (DAD), and determined using relative response factors. *Isothiocyanates* were conjugated with 2-mercaptoethanol and determined by HPLC-DAD (*8*). *Isothiocyanate mercapturic acids* in urine were measured as described before (*10*). Briefly, internal standard was added to urine, and the samples were purified on solid phase extraction columns. Eluates were analyzed using reversed phase HPLC coupled to an LCQ ion trap mass spectrometer (MS/MS, Finnigan-Thermo Quest, Breda, The Netherlands). 4-Methylsulfinylbutyl isothiocyanate mercapturic acid was prepared as described (*11*). *Sulforaphane conjugates* in plasma were measured with reversed phase HPLC-MS/MS. Phenyl isothiocyanate was added as internal standard, and the sample was added to *n*-butanethiol diluted in methanol and incubated at 50 °C for 2 h. The *n*-butanethiol conjugates of sulforaphane and phenyl isothiocyanate were measured on a Sciex-API3000 triple-quadrupole mass spectrometer (Applied Biosystems, Breda, The Netherlands). *Creatinine* was measured in urine to verify the collection of urine and in plasma and urine combined to calculate creatinine clearance. Creatinine was measured enzymatically on a Hitachi 911. *Liver damage markers* in plasma of five volunteers, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), γ-glutamyltransferase (γ-GT) and albumin, were measured enzymatically on a Hitachi 911.

Statistical and Pharmacokinetic Analysis. Plasma concentrations of sulforaphane conjugates and urinary excretion of sulforaphane mercapturic acid were fitted to a one-compartmental model with the assumption of first-order absorption and excretion kinetics. The elimination rate constant (k) was calculated from the natural logarithm of plasma amounts of sulforaphane conjugates plotted versus time and expressed as $t_{1/2}$ (0.693/k). The absorption rate constant (k_a) was calculated using the intercept and slope of the same plot with the method of residuals. Using the trapezoid method, the area under the plasma concentration versus time curve (extrapolated to infinite) was calculated. The lag time was determined graphically from the curve of plasma amounts of sulforaphane conjugates plotted versus time. The bioavailability, F, was estimated by dividing the cumulative amount of sulforaphane mercapturic acid excreted in urine in 24 h by the consumed amount of glucoraphanin for cooked broccoli or sulforaphane for raw broccoli. The excretion rate constant (k_e) was calculated from the natural logarithm of absolute amounts of excreted sulforaphane mercapturic acid per hour plotted versus time and expressed as $t_{1/2}$ ($0.693/k_e$). The statistical significance of the difference between the kinetic parameters, after consumption of raw versus cooked broccoli, was calculated with a two-sided, paired Student's t test.

RESULTS

Subjects. Liver function markers (enzymes) in plasma were with one exception all within the normal ranges. There were no significant differences in ASAT, ALAT, γ-GT, and albumin levels (p = 0.3, 0.7, 0.4, and 0.6, respectively) between both days of intervention as determined with a two-sided, paired Student's t test. On average 11.3 (range, 9−16) portions of urine/24 h were produced at both days of intervention with amounts of 0.1−0.5 L. Two subjects were slow acetylators as determined with NAT2 genotyping; all other subjects were heterozygous in NAT2 (**Table 1**). Furthermore, three subjects lacked GST M1 and one subject lacked GST P1 as well. Another polymorphism was found in one subject who lacked GST T1.

Diets. The glucoraphanin content of the cooked broccoli serving (200 g) was 61.4 μmol on average for both days of intervention (319 and 296 μmol/kg, respectively). Cooked broccoli also contained glucoiberin (27 μmol/kg) and glucobrassicins (508 μmol/kg in total). The raw broccoli serving (200

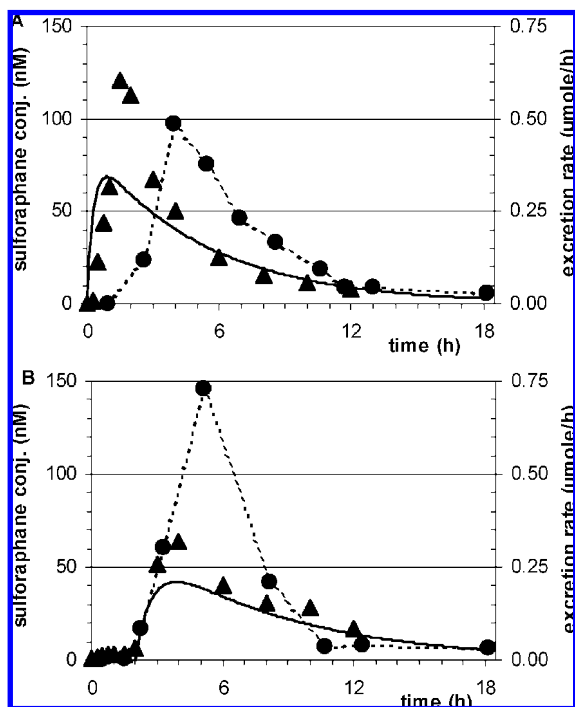

Figure 1. Typical uptake and excretion curves from volunteer 3: amount of sulforaphane conjugates in blood (▲, data; —, kinetic fit) and excretion of its mercapturic acid in urine (● with dashed line, data) after consumption of 200 g of broccoli that was crushed raw (**A**) or cooked and crushed (**B**).

g) contained 9.92 μmol of sulforaphane on average, 48 and 51 μmol/kg for both days of intervention, respectively. No other isothiocyanates were detected.

Chemical Analyses. Cumulative creatinine concentration in urine correlated with time (R^2 = 0.993−0.9996) for all volunteers, confirming the collection of all urine. There was no significant ($p = 0.3$) difference in creatinine levels between both days of intervention as determined with a two-sided, paired Student's *t* test, and corrected creatinine clearance was on average 124 (range, 80−147) mL/min/1.73 m^2, indicating a healthy excretion (**Table 1**).

Kinetics. Typical results for the absorption and excretion curves are depicted in **Figure 1**. The results of mercapturic acid excretion and of plasma sulforaphane conjugate kinetic parameters are depicted in **Tables 1** and **2**, respectively. When calculated using the amount of excreted sulforaphane mercapturic acid, the bioavailability of sulforaphane from raw broccoli (F = 37%) is 11 times higher than that from cooked broccoli (F = 3.4%), which is statistically significant ($p = 0.002$). The same difference is apparent from the amounts of sulforaphane conjugates in blood, for which a corrected difference in AUC of 10.2 (times) can be calculated. When raw broccoli was consumed, the AUC was 1.7 times higher than when cooked broccoli was consumed, and the content of glucoraphanin in cooked broccoli (61.4 μmol) is 6 times higher than the content of sulforaphane in raw broccoli (9.92 μmol). The peak plasma time is significantly different, 1.6 h when raw broccoli is consumed versus 6 h for cooked broccoli. The peak plasma concentration of sulforaphane is 20 times higher when raw broccoli is consumed, compared to cooked broccoli, relative to the intake. The variation in absorption rate between volunteers is large; the relative standard deviations (RSD) were 61 and 71% for raw and cooked broccoli, respectively. The variation in AUC is, however, only 8% (RSD) for raw broccoli and 49% for cooked broccoli. The elimination of sulforaphane from blood is slower compared to the excretion in urine alone; elimination (excretion) half-lives are 3.8 (2.6) and 4.6 (2.4) h for raw and cooked broccoli, respectively.

DISCUSSION

The kinetics of isothiocyanate absorption and excretion have been described in several publications. In this study we used LC-MS techniques to increase sensitivity and, mostly, specificity compared to earlier studies. In addition, the accuracy of the data gave us the possibility to study the kinetics of sulforaphane from raw and cooked broccoli in detail, for example, the parameters AUC and t_{max}. The concentrations of sulforaphane conjugates in blood and of sulforaphane-derived mercapturic acid in urine were used for calculation of the kinetics.

Intake. The sulforaphane content of raw broccoli was lower than the glucoraphanin content of cooked broccoli, 9.92 and 61.4 μmol, respectively. It seems that the conversion from glucosinolate to isothiocyanate was incomplete or that another reaction occurred. No glucoraphanin (<10 μmol/kg) was detected after crushing and incubation, indicating that glucoraphanin was completely converted. The pH of the crushed raw broccoli was 7, at which, in theory, isothiocyanates are the only metabolites from glucosinolates. However, broccoli might contain additional enzymes that inhibit the conversion or convert glucoraphanin into other hydrolytic products such as thiocyanate and nitrile (*12*). These products have a different bioactivity from sulforaphane and are not known to induce phase II enzymes. Nitriles are formed by epithiospecifier protein (ESP), and the formation of nitriles can be reduced by thermal treatment to inactivate ESP (*13*).

Kinetics. The mercapturic acid pathway is the major route of elimination of isothiocyanates. Minor routes of excretion could be defecation, exhalation, and perspiration. From the ingested amount of glucoraphanin and sulforaphane from cooked and raw broccoli, 3.4 and 37% were recovered in urine as sulforaphane mercapturic acid, respectively. This is comparable with a similar study in which these values for bioavailability were 10 and 32%, respectively (*14*). Possible explanations for these low recoveries are that glucoraphanin is not entirely converted into sulforaphane or that sulforaphane is not entirely absorbed from the gut. It might also be the case that it is excreted via other routes or it is metabolized into non-isothiocyanate metabolites, which is confirmed by Combourieu et al. (*15*). They found that besides the slow and incomplete conversion of glucoraphanin into sulforaphane in the colon, sulforaphane is further degraded into its amine. Indeed, several studies show that less than 100% recovery was obtained for dosed glucosinolates or isothiocyanates. For example, pure benzyl isothiocyanate from garden cress administered to humans was 54% recovered as mercapturic acid in urine (*16*), and phenylethyl isothiocyanate from watercress, after chewing, was 47% recovered as mercapturic acid (*17*). The recovery of isothiocyanates from 350 g of cooked watercress was 1.2−7.3% and from 150 g of uncooked watercress was 17.2−77.7% (*18*). Cumulative excretion of dithiocarbamates after consumption of homogenates of boiled broccoli sprouts was 12%, and after myrosinase treatment of the homogenate, the cumulative excretion was 80% (*19*). In a recent study, allyl isothiocyanate from mustard was 68% recovered as mercapturic acid in urine. Sinigrin recoveries from cooked and raw cabbage were 15 and 37%, respectively (*20*). However, in another study we showed that after the consumption of several raw cruciferous vegetables, on average, 100% of the ingested isothiocyanate was recovered in urine as mercapturic acid (*8*). From that study it seems that the vegetable

Table 2. Plasma Sulforaphane Conjugate Pharmacokinetic Secondary Parameter Summary after a Single Dose of 200 g of Broccoli, either Raw or Cooked, Was Consumed[a]

	peak concn (C_{max}, nM)		peak time (t_{max}, h)		lag time (t, h)		$AUC_{0-\infty}$[b] (nM·h)		absorption rate constant (k_a)		elimination half-life ($t_{1/2}$, h)	
person no.	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked
1	160	19.0	1	6	0	4	535	147	5.9	1.3	3.7	4.3
2	77.2	17.9	3	8	0	2	438	195	1.1	0.2	2.8	4.0
3	121	63.6	1.5	4	0	2	470	485	3.4	1.3	3.7	4.6
4	75.6	22.7	1.5	6	0	1.5	510	233	2.9	0.3	4.0	4.2
5	78.5	31.1	0.75	6	0	1	523	371	7.3	0.4	5.0	6.0
av	103	31	1.6	6	0	2.1	495	286	4.1	0.7	3.8	4.6
SD	37	19	0.9	1.4	0	1.1	40	139	2.5	0.5	0.8	0.8
p, t test	0.01		0.001		0.01		0.04		0.03		0.01	

[a] Calculated from sulforaphane conjugate amounts measured in blood. [b] $AUC_{0-\infty}$ is the sum of AUC_{0-12} (measured amounts) and $AUC_{12-\infty}$ (extrapolated from $t = 12$).

matrix is an important factor determining the bioavailability. In general, the bioavailability of isothiocyanates is higher from condiments such as crushed horseradish than from vegetables such as cabbage.

Glutathione conjugates of sulforaphane are the means of transport of this bioactive substance through the body. After ingestion of glucoraphanin from cooked broccoli and sulforaphane from raw broccoli, peak concentrations of sulforaphane conjugates in blood were maximal at, on average, 6 and 1.6 h, respectively. This difference in peak time indicates that the absorption of sulforaphane after the consumption of cooked broccoli was delayed, on average, by approximately 4.5 h. Furthermore, if glucoraphanin was fully hydrolyzed into sulforaphane by myrosinase in crushed raw broccoli, the amount of bioactive sulforaphane conjugates in blood would be 10, instead of only 1.7, times higher than after the consumption of cooked broccoli. When the production of healthier cruciferous vegetables is attempted, it is therefore important to not only selectively increase glucoraphanin but also to control the cofactors responsible for incomplete conversion of the glucosinolate into the isothiocyanate, as already stated by Faulkner et al. (*12*). The elimination half-lives of sulforaphane from blood after the consumption of cooked and raw broccoli were 4.6 and 3.8 h, respectively. These values are higher than expected from the excretion half-lives of sulforaphane mercapturic acid in urine, 2.4 and 2.6 h, respectively, probably because of a prolonged absorption from the gut, in particular after the consumption of cooked broccoli.

Individuals who are slow acetylators as determined with NAT2, and/or lack GST M1, T1, and/or P1, do not seem to eliminate or excrete sulforaphane more slowly or have higher AUC or F values than other individuals. In another trial with broccoli, comparing GSTM1-null subjects with GSTM1-positive subjects, three parameters (AUC, k_e, and F) were slightly, but statistically significantly, higher (*21*). Therefore, a difference in kinetics could be expected, but the group size in our study was too small to perform a statistical test.

In conclusion, higher amounts of sulforaphane conjugates in blood and sulforaphane-derived mercapturic acid in urine were found when broccoli was eaten raw (bioavailability of 37% on average) versus cooked (3.4%, $p = 0.002$). In future research, care should be taken that glucoraphanin is not hydrolyzed into other metabolites when broccoli is crushed. Absorption of sulforaphane was delayed when cooked broccoli was consumed, and the peak plasma time was 6 h on average versus 1.6 h for raw broccoli ($p = 0.001$). Excretion half-lives were comparable, 2.6 and 2.4 h on average, for raw and cooked broccoli, respectively ($p = 0.5$). Consumption of raw broccoli resulted in faster absorption, higher bioavailability, and higher peak plasma amounts of sulforaphane, compared to cooked broccoli.

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