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Urinary Excretion of Sulfate and Glucuronate Conjugates in a Free Living Population of Adult Males

Peter S. Murano, Vernice Y. Robichaud, and Ryland E. Webb

Department of Human Nutrition and Foods, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, 24061, USA

On a daily basis people come in contact with hundreds of chemical substances in countless combinations. Exposure may be due to: ingestion of foods, beverages, and pharmaceuticals; by inhalation, or through skin contact. Exposure occurs in all routine situations, be they domestic, occupational, or recreational. While some of these substances are life-sustaining and many are benign, others are of no known functional value. Such nonnutrient, nonbeneficial compounds are termed xenobiotics (Mason et al. 1965). Upon absorption into the body, the fate of xenobiotic compounds varies. Excretion by way of the urine, perspiration, or expired air is possible for those which are water soluble. Lipophilic xenobiotics, on the other hand, must be metabolized by special enzymes present in the liver and other tissue. Williams (1959) proposed these enzymatic reactions to be of two types; phase I and phase II reactions. The kinds of reactions which characterize phase I include oxidations, reductions, and hydrolyses. During phase I reactions, xenobiotics are converted to metabolites that can serve as substrates for phase II enzymes. Phase II enzymatic reactions include acetylations, amino acid conjugations, and sulfations. The products of phase II reactions are called conjugates. Conjugation reactions involve the combination of an endogenous conjugating agent with a foreign compound or metabolite, under the influence of a transferase enzyme specific for the conjugating agent. Conjugations serve to prevent toxic accumulations of xenobiotics in the body by increasing the water solubility and excretion potential of these compounds. Conjugation with glucuronic acid and sulfate are two of the major pathways of detoxication in the body, and as such are life-sustaining processes.

Much work has been done in the past couple of decades developing methods to isolate and characterize specific conjugate species obtained from biological fluid samples (Tomasic 1978). Other research has focused on the pharmacokinetic aspects of drug conjugations. Clinical investigations have been conducted to correlate the concentrations of conjugates found in blood or urine to various diseases (Dutton 1980). Given the importance of glucuronidation and sulfation in detoxication, relatively little has been reported in the literature regarding the normal total daily excretion of conjugates of glucuronates and sulfates in humans.

Send reprint requests to Ryland E. Webb at the above address

The primary objective of this study was to determine the daily urinary excretion of the conjugates in a free living population of adult males. The measured levels of urinary conjugates of sulfate and of glucuronic acid in each subject were compared. Additionally, the potential influence of several dietary and nondietary factors upon conjugate excretion was evaluated using the statistical F-test.

MATERIALS AND METHODS

The daily urinary excretion of conjugates of glucuronates and sulfates (glucuronic acid and sulfate conjugated to a substrate compound, respectively) in a free living population of 40 adult male volunteers was measured. The subjects were students attending Virginia Polytechnic Institute and State University and were caucasian with a mean age of 20.2 years (range 18-34). None of the subjects were obese or vegetarians. Relative health was self-described as excellent.

A modified naphthoresorcinol method (Mazzuchin et al. 1971) was used to quantify conjugated glucuronic acid, and atomic absorption of barium chloride-precipitated sulfate (Lundquist et al. 1980) was used to quantify conjugated sulfate. Forty subjects were randomly selected from a sample of 135 free living adult male volunteers and three consecutive 24 hr urine voidings were analyzed for each subject.

For the glucuronide assay, 1.0 ml of boiled and filtered urine was incubated with 1.9 ml acetate buffer, pH 5.6, 1.0 ml of distilled water and 1.0 ml glucose oxidase enzyme, under continuous aeration at 37 C for 45 minutes. Samples were diluted to 100 ml with distilled water and three 1.5 ml aliquots were subjected to the naphthoresorcinol treatment. Glucuronate conjugates were extracted with ethyl acetate and the absorbance read at 564 nm on a Bausch and Lomb Spectronic 20 spectrophotometer.

For the sulfate assay, 1.0 ml of freeze-dried urine was heated at 100 C for 24 hours with 1.5 ml fuming nitric acid to hydrolyze bound sulfate. After cooling, the sample was reheated for 6 hr at 350 C to remove nitric acid. The nitric acid-free residue was removed and barium chloride was used to precipitate the sulfate. The barium sulfate pellet was washed and ultimately barium was determined using a Perkin Elmer atomic absorption spectrophotometer equipped with a barium hollow-cathode ray tube. Conjugated sulfate was calculated as the difference between acid hydrolyzed and nonhydrolyzed urine sulfate.

RESULTS AND DISCUSSION

As shown in Table 1, the mean excretion of conjugates of glucuronic acid for the 40 subjects was 0.848 mmole/24 hr and ranged for 0.199 to 2.674 mmole/24 hr. The between-subject (inter-) variation was 48.2% while the within-subject (intra-) variation was 29.2% (Table 2).

The mean excretion of sulfoconjugates (Table 1) for the 40 subjects was 7.65 mmole/24 hr and ranged from 0.12 to 26.42 mmole/24 hr. The between-subject variation was 69.8% and the within-subject variability was 57.4% (Table 2).

Intra-individual variation is often erroneously considered to be negligible as compared with inter-individual variation. Indeed, in our study, we did observe large coefficients of variation for the intra-individual parameter. The large inter-individual variation was expected due to uncontrolled genetic and environmental influences on the subjects.

It is interesting to note that in comparing the levels of urinary conjugates between these two competing pathways that the level of sulfoconjugates was 9-fold greater than the level of urinary glucuronides (the 40 free-living males in this study excreted a mean value of 7.65 mmole sulfoconjugate per day and 0.848 mmole glucuronic acid conjugate per day).

Caldwell and coworkers (1982) reported a shift to the utilization of the glucuronidation pathway when sulfate is limiting. In our study, this effect was seen above the 90th percentile of glucuronic acid conjugate excretion (n=12). This was equivalent to 1.343 mmoles and above of glucuronic acid conjugate excreted per day. The Spearman correlation coefficient was -0.61, indicative of a moderately strong correlation between decreasing sulfoconjugate excretion with increasing glucuronic acid conjugate excretion. This finding supports the notion that with decreased sulfation, there is increased glucuronidation. In contrast no correlation was observed (r=-0.09) between increased sulfoconjugate excretion above the 90th percentile (n=12) and decreased glucuronide excretion.

The urinary excretion of the conjugates was compared to xenobiotic exposure/ingestion information obtained from the study participants via questionnaire. Specific parameters examined were charbroiled food intake, alcohol, caffeine, tobacco and marijuana use and incidence of cancer in the subject's family. None of these parameters were significantly correlated with the excretion of either conjugate.

The large variability in the conjugate excretions probably masked any effects of diet, environment, or genetics upon the data obtained. On the other hand, the reason for the large variation may be a consequence of dynamic interactions among suspected or established dietary factors superimposed upon multiple genetic and environmental differences between subjects. Further research in this area is, therefore, warranted in order to clearly establish the relationship between these interactions. From the results obtained in this study it is concluded that (1) large intra- and inter-individual differences exist in the urinary excretion of both sulfate and glucuronate conjugates, (2) sulfation is quantitatively the dominant pathway of detoxication for the subjects, (3) with limited sulfation glucuronidation increases and, yet, with limited glucuronidation there is not increased sulfation, and (4) xenobiotic exposure does no significantly affect the urinary excretion of either conjugate.

Table 1. Urinary excretion of conjugates from a randomly-selected group of 40 adult male volunteers

Conjugate Type	3-day excretion (mmole/24 hr) Mean	SD	Range
Glucuronate	0.848	0.409	(0.199-2.674)
Sulfate	7.650	5.340	(0.120-26.42)

Table 2. Between-subject and within-subject variation in urinary excretion of the conjugates

Conjugate Type	Variation Inter	CV[b]	Intra	CV[b]
Glucuronate	0.409	48.2	0.248	29.2
Sulfate	5.34	69.8	4.39	57.4

n=40
Inter- and intra-variations reported as standard deviations.
[b]CV - coefficient of variation (%).

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