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Paracetamol metabolism in two ethnically different Spanish populations

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SUMMARY

The 24 h urinary excretion of paracetamol and its metabolites following a single oral dose of 1.5 g was compared in two ethnically different Spanish populations: 39 volunteers from the Basque country and 32 from Alicante. The urinary concentrations of unchanged paracetamol and its glucuronide, sulphate, cysteine, and mercapturic acid conjugates were determined by high-performance liquid chromatography. Statistically significant differences in the urinary excretion of unchanged paracetamol and the fractional urinary recovery of each conjugate between subjects from Alicante and subjects from the Basque country were not found. In both populations, an inverse relationship between glucuronide and sulphate conjugation following a bimodal frequency distribution pattern was found. In contrast to paracetamol oxidation, intersubject variation in paracetamol conjugation was negligible. The urinary excretion of unchanged paracetamol was higher in smokers than in nonsmokers. As compared with other studies, the urinary excretion of oxidation-derived paracetamol metabolites in both Spanish populations was intermediate and significantly different than that found in Caucasians from Scotland and West Africans (Ghana). This may determine a susceptibility to paracetamol hepatotoxicity following overdosage in the Spanish population.

INTRODUCTION

Paracetamol (N-acetyl-*p*-aminophenol) is a widely used nonprescription antipyretic and analgesic. It is relatively safe when used at therapeutic doses, but large overdoses are associated with potentially fatal liver necrosis (1). A major route of paracetamol metabolism involves conjugation with glucuronic and sulphuric acid, producing nontoxic glucuronide and sulphate conjugates. A small proportion is metabolised by the hepatic cytochrome P450 system to a highly reactive intermediate metabolite, possibly N-acetyl-*p*-benzo-quinoneimine, which is rendered nontoxic by conjugation with glutathione and later excreted as cysteine and mercapturic acid conjugates. Hepatic toxicity has been related to the production of this toxic intermediate that binds covalently to cellular macromolecules in the presence of depletion of glutathione stores (2).

Environmental inequalities and underlying genetic factors have been shown to contribute to interindividual variations in the metabolism of paracetamol (3–6). It has been recognized that sulphate conjugation of

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paracetamol is a saturable pathway in man (7) that depends on the availability of inorganic sulphate in blood. Moreover, ethnic differences in the fractional recovery of oxidation pathway-derived metabolites were documented by Critchley et al. (8). These authors found that the mean combined recovery of the mercapturic acid and cysteine conjugates of paracetamol in white Caucasians was about twice that observed in Ghanaian and Kenyan Africans.

The aim of this study was to compare paracetamol metabolism in two ethnically different Spanish populations: Basques and subjects from Alicante.

MATERIALS AND METHODS

Subjects

A total of 79 healthy medical students, 29 men and 42 women with a mean (SD) age of 21.2 (1.7) years, volunteered to participate in this investigation. 32 subjects, with a mean age of 21.5 years, were born in the Autonomous Community of Valencia and recruited at the University of Alicante in Alicante, a city of Spain in the southeast on the Mediterranean. The remaining 47 subjects, with a mean age of 20.9 years, were born in the Basque country (three generations) and recruited in Bilbao, a city of northern Spain on an inlet of the Bay of Biscay. All subjects were nonalcoholics (daily alcohol intake < 30 g) and were not taking medication at least within 2 weeks of the beginning of the study. The volunteers were healthy as judged by medical history, physical examination and laboratory screening (haematological, liver and renal function tests).

Treatment and sampling

Following an overnight fast, the subjects provided a blank urine specimen and then received a single oral dose of 1.5 g of paracetamol which was swallowed with a glass of water. Urine was collected for the next 24 h and, during this period, the subjects took no other drugs or ethanol. Urine samples were collected in containers to which 250 µl of a solution of 4 mg/ml of D-saccharolactone had been added in order to inhibit β-glucuronidase of bacterial origin. Urine aliquots were stored frozen at –80°C before analysis. To confirm that the total volume of urine excreted in 24 h has been collected, all samples were analyzed for creatinine concentration by the alkaline picrate (Jaffe) method. No sample was excluded for this reason in the group of subjects from Alicante; however, incomplete urine collections was the reason for exclusion in 8 subjects from the Basque country.

Assays

The concentrations of unchanged paracetamol and its glucuronide, sulphate, cysteine, and mercapturic acid conjugates in urine were determined by high-performance liquid chromatography using a modification of a method previously described by our group (9). A Beckman liquid chromatograph (San Ramon, CA, USA) equipped with a solvent delivery system with two model 110B pumps, a model 166 UV photometric detector, and a model 210A injector with 20 µl sample loop was used. Separation was performed at ambient temperature on a C_{18} (Ultrasphere ODS) 250 × 4.6 mm I.D. (5 µm particle size, Beckman). The mobile phase consisted of an aqueous solution of 0.01 M tetrabutylammonium chloride (TBA) and 0.01 M tris(hydroxymethyl)-aminomethane (Tris), with the pH adjusted to 5.0 with phosphoric acid to which 30% methanol (v/v) was added. Methanol was used as organic solvent. The gradient elution started with 0% methanol. After a delay of 0.5 min, the concentration of methanol was increased linearly to 64% over 7.5 min. The column was returned to the initial conditions. The flow speed was 1.5 ml/min and the variable-wavelength detector was set at 254 nm. Paracetamol, theophylline (internal standard), and TBA were obtained from Sigma Chemical Co., (St Louis, MO, USA); Tris and methanol were purchased from Merck (Darmstadt, Germany); and paracetamol glucuronide, sulphate, cysteine, and mercapturic acid conjugates were generously donated by Winthrop Laboratories Production Division (Fawdon, Newcastle upon Tyne, UK). Urine samples were thawed, centrifuged at 11,000 *g* for 3 min, and diluted (1:4) in distilled water. A 100 µg/ml aqueous solution of theophylline was added to the samples at a ratio of 1:1.

Calibration curves were prepared using known amounts of aqueous solutions of paracetamol and its 4 metabolites. Urine concentrations of paracetamol and its metabolites were obtained in µg/ml, transformed into equivalents of paracetamol, and expressed as percentage of the total paracetamol recovered. The sum of glucuronide and sulphate conjugates were considered indicative of the overall paracetamol conjugation, whereas the sum of cysteine and mercapturic acid metabolites were considered indicative of the overall paracetamol oxidation. The ratio glucuronide to sulphate conjugates was also calculated.

Statistics

The Wilcoxon's rank sum test and the linear regression analysis were applied for statistical analysis of study data. The Student's t test was used for the comparison of our results with those reported by Critchley et al. (8). Statistical significance was set at $P < 0.05$.

RESULTS

Details of the 71 subjects included in the study are given in Table I. The two populations were similar with regard to mean age, mean weight, percentage of smokers and mean volume of urine collected in 24 h.

Statistically significant differences in the urinary excretion of unchanged paracetamol and the fractional urinary recovery of each conjugate between subjects from Alicante and subjects from the Basque country were not found (Table II). When compared with the Caucasians (Scotland) of the study of Critchley et al. (8), both populations in our study showed significantly different values for the 24 h urinary excretion of paracetamol and its metabolites, in particular significantly lower urinary excretion of metabolites of the oxidation pathway (mercapturic acid and cysteine conjugates). However, when compared with West Africans (Ghana) of the study of Critchley et al. (8), both subjects from Alicante and from the Basque country showed significantly different values for all parameters, except for the glucuronide and sulphate conjugates. The Spanish populations showed significantly higher urinary excretion of metabolites of the oxidation pathway than Ghanaians (Table II).

Table I : Characteristics of volunteers.

	Subjects from Alicante	*Subjects from the Basque country*
Sample size	32	39
Sex, male/female	14/18	15/24
Weight (kg)	62.8 (10.1)	62.1 (9.2)
Age (years)	21.5 (1.8)	20.9 (1.6)
Smokers	28%	23%
Urine volume (l)	1.18 (0.56)	1.42 (0.43)

Values within ( ) are SD.

Table II : Percentage of fractional recovery of paracetamol and its metabolites. Comparison among two Spanish populations and those reported by Critchley et al. (8).

	Total recovery	*Unchanged paracetamol*	*Glucuronide*	*Sulphate*	*Cysteine*	*Mercapturic acid*	*Total conjugation*	*Total oxidation*
Caucasians (n = 111)								
Mean	86	5.7	53.8	31.2	4.3	5.0	85.0	9.3
SD	15	2.6	6.2	4.7	1.6	1.9		
CV	17.4	45.6	11.5	15.1	37.2	38.0		
West Africans (n = 67)								
Mean	76[a]	4.6[a]	58.2[b]	32.0	2.9[b]	2.3[b]	90.2	5.2
SD	13	2.0	8.7	7.7	1.5	1.3		
CV	17.1	43.5	14.9	24.1	51.7	56.5		
From Alicante (n = 32)								
Mean	92.2[a,c]	3.0[b,c]	56.2[a]	33.3[a]	3.7[a,c]	3.8[b,c]	89.5	7.5
SD	7.9	1.2	6.5	6.2	1.0	0.9	2.0	1.6
CV	8.6	38.9	11.5	18.6	27.2	23.7	2.2	21.9
Basques (n = 39)								
Mean	84.4[c,d]	4.1[b,d]	57.8[a]	31.3	3.5[a,c]	3.2[b,c]	89.1	6.7
SD	7.4	1.7	7.8	6.5	0.9	0.9	2.6	1.6
CV	8.8	41.4	13.5	20.9	27.0	29.4	2.9	24.2

Statistically significant differences between Caucasians ([a]$P < 0.05$, [b]$P < 0.0005$), West Africans ([c]$P < 0.0005$) and subjects from Alicante ([d]$P < 0.05$); SD, standard deviation; CV, coefficient of variation.

Table III : Urinary paracetamol glucuronide to sulphate ratio (G/S), range and correlation coefficients (r) between the two conjugates.

	Mean G/S	*(range)*	*G to S r*
Caucasians (n = 111)[a]	1.67	(0.83–3.45)	–0.73
West Africans (n = 67)[a]	1.72	(0.71–4.00)	–0.92
Chinese (n = 24)[b]	1.66		–0.93
Indians (n = 24)[b]	2.45		–0.96
Subjects from Alicante (n = 32)	1.78	(1.06–3.35)	–0.95
Basques (n = 39)	1.97	(0.75–3.87)	–0.95

[a]Reference (8); [b]Reference (10).

The mean glucuronide to sulphate ratio found in our study was similar to that reported in other populations (8,10,11). In all cases, an inverse correlation between glucuronide and sulphate metabolites was observed (Table III).

In both Spanish populations, the study variables followed an approximately symmetrical frequency distribution, with the exception of glucuronide and sulphate conjugates and glucuronide to sulphate ratio that showed a bimodal pattern (Figs 1–3). When these parameters were compared according to gender, no statistically significant differences were found.

When smokers and nonsmokers in the group of subjects from the Basque country were compared, statistically significant differences in the fractional recovery of unchanged paracetamol, glucuronide and sulphate conjugates, and glucuronide to sulphate ratio were found. Concentrations of paracetamol were lower and showed a narrower distribution in smokers than in nonsmokers (Fig. 4). Smokers showed a higher concentration of the glucuronide conjugate and a lower concentration of the sulphate conjugate. According to the frequency distribution of the glucuronide to sulphate ratio (Fig. 3), the group of smokers was found in

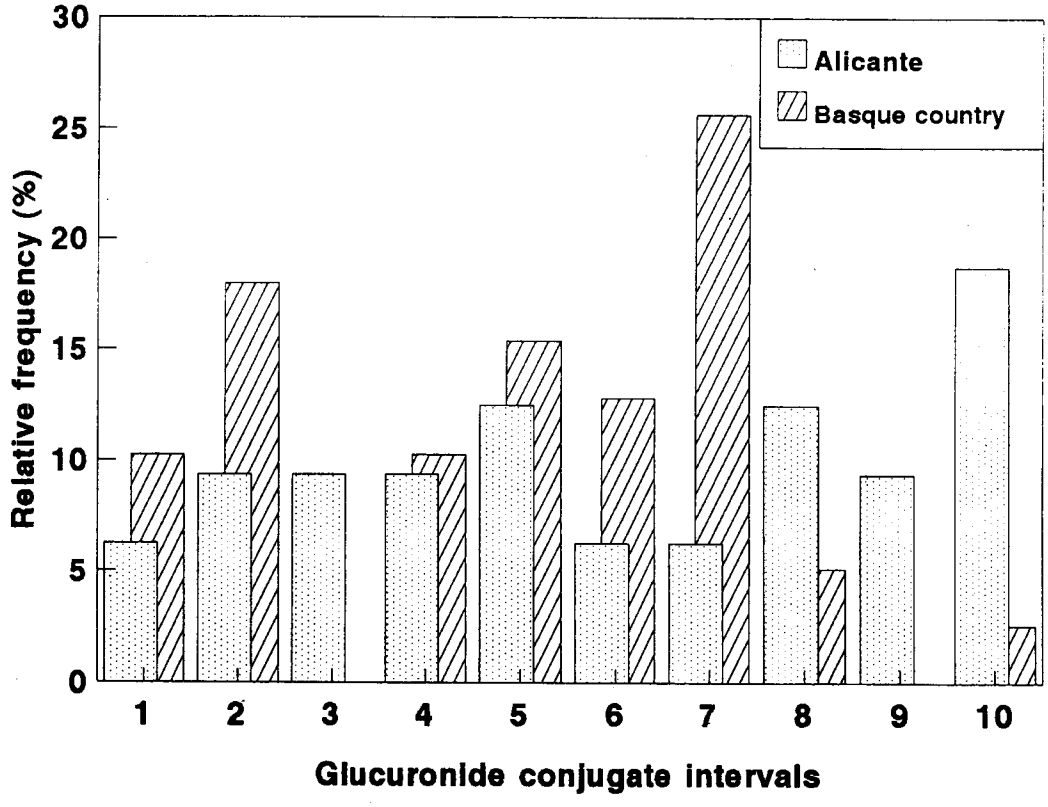

Fig. 1 : Frequency histogram of paracetamol glucuronide in volunteers from Alicante and volunteers from the Basque country. Intervals begin at 45% with 2% increments among subjects from Alicante and 3% increments among Basques.

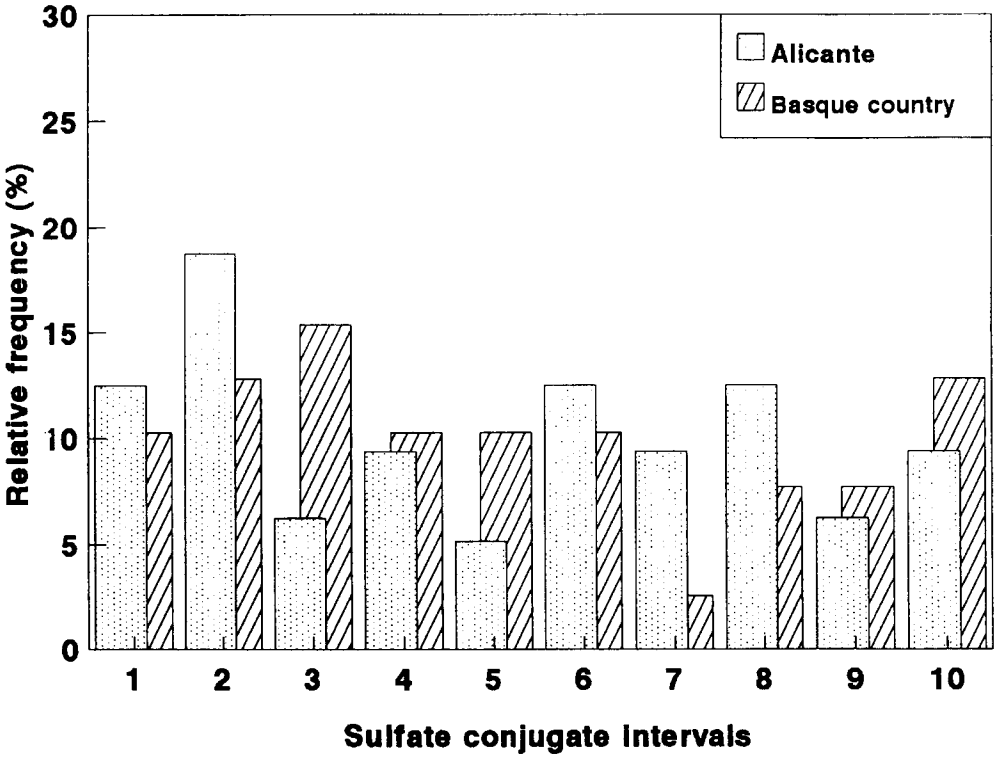

Fig. 2 : Frequency histogram of paracetamol sulphate in volunteers from Alicante and volunteers from the Basque country. Intervals begin at 24% with 2% increments among subjects from Alicante and at 22% with equal increments among Basques.

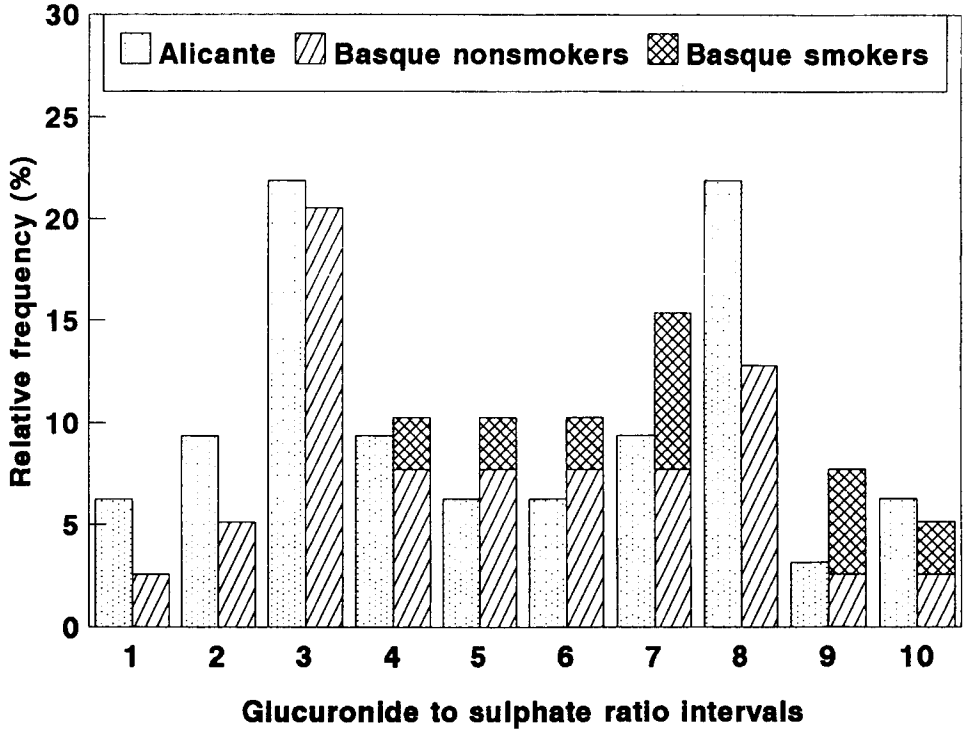

Fig. 3 : Frequency histogram of glucuronide to sulphate ratio in volunteers from Alicante and smokers and nonsmokers from the Basque country. Intervals begin at 0.90 with 0.18 increments among subjects from Alicante and at 0.70 with 0.25 increments among Basques.

the region in which glucuronide conjugation was 2-fold in respect to sulphate conjugation, whereas nonsmokers were uniformly distributed throughout. This finding was also observed in the group of volunteers from Alicante, although statistical significance was not reached (Fig. 4).

DISCUSSION

The series of healthy volunteers in this study were selected with the purpose of comparing the metabolism of paracetamol in two relatively accessible and genetically different populations (12). Moreover, it is well known that the Basque population has a low genetic heterogeneity (12).

There were no ethnic differences between subjects from Alicante and Basques in the pattern of paracetamol metabolism, which is in agreement with results reported by other authors in other series of patients (7,8,13–16). The conjugation pathway showed a very low intersubject variability in both population groups. Although there was a greater variability when paracetamol glucuronide and sulphate conjugates were considered separately, since these metabolites are inversely related to each other, paracetamol conjugation remained a constant parameter. However, according to the glucuronide to sulphate ratio, two types of subjects were identified in both study populations, i.e. those in whom glucuronide conjugation was 2-fold greater in respect to sulphate conjugation, and those in whom both conjugates were excreted in the same proportion. This bimodal pattern was also documented in the studies of Critchley et al. (8), Lee et al. (10), and Patel et al. (11). No ethnic differences have been found in the sulphate conjugation of paracetamol, indicating that sulphate conjugation of paracetamol is a saturable pathway at doses usually used in clinical studies (about 1,500 mg) (14). Phenol sulphotransferase is present in all human tissues in at least two forms: thermolabile and the thermostable. It has been shown that paracetamol is a substrate for both forms of phenol sulphotransferases and that the urinary excretion of paracetamol sulphate correlates with both thermolabile and thermostable phenol sulphotransferase activity in platelets (10). A bimodal variability in platelet thermolabile and thermostable phenol sulphotransferase activity may be consistent with our findings. Different authors (4,5) have shown that interindividual variation in paracetamol metabolism is predominantly due to environmental rather than genetic factors. However, given that platelet thermolabile and thermostable phenol sulphotransferase activity is at least in part controlled by inheritance, the role of genetic factors in the

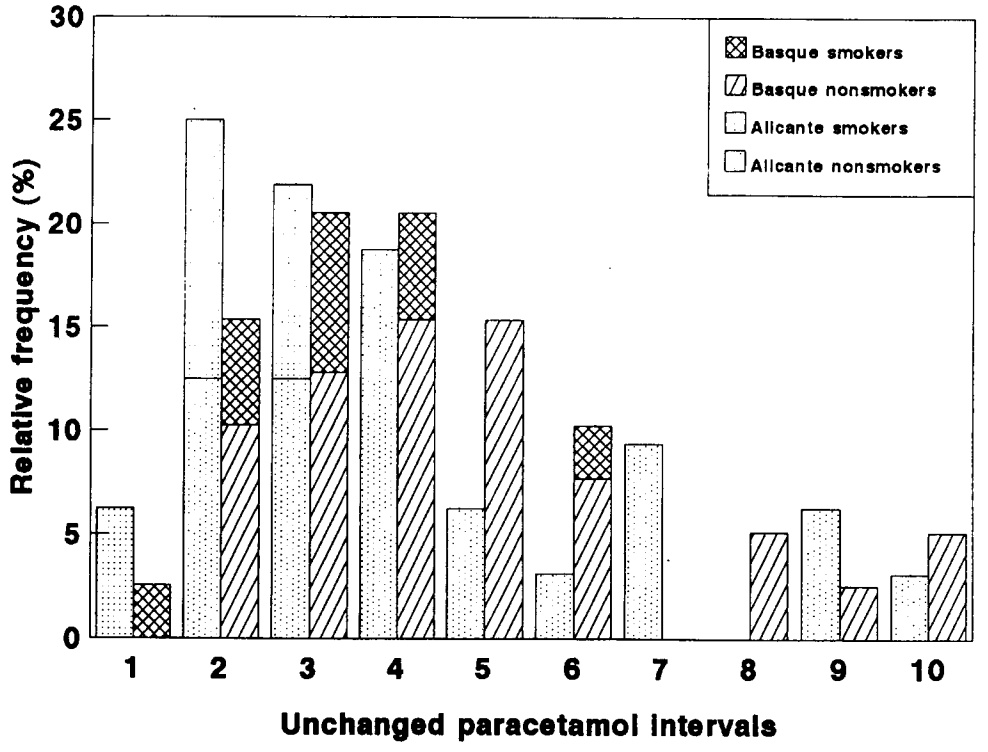

Fig. 4: Frequency histogram of unchanged paracetamol among smokers and nonsmokers from Alicante and from the Basque country. Intervals begin at 1.25% with 0.50% increments among subjects from Alicante and at 0.70% among Basques.

regulation of paracetamol metabolism cannot be excluded. The heterogeneity of paracetamol glucuronidation in subjects with Gilbert's disease (an autosomal dominant inherited disorder) also supports the influence of genetic factors in the metabolism of this drug (17,18).

The same proportion of cysteine and mercapturic acid conjugates were excreted. These paracetamol metabolites appear to be well correlated so that there is a parallelism between increases and decreases of both conjugates. The oxidation pathway of paracetamol shows a greater intersubject variation than the conjugation pathway. This finding may have important implications for individual susceptibility to paracetamol hepatotoxicity.

The importance of smoking has remained controversial so far (4,8,19,20). In some studies, glucuronidation has been shown to be enhanced in smokers, while in others, cigarette smoking did not appear to influence the pattern of paracetamol metabolism. In this study, the urinary excretion of unchanged paracetamol was higher in smokers as compared with nonsmokers either among subjects from Alicante or Basques. In subjects from the Basque country, the excretion of the glucuronide conjugate was significantly higher among smokers which, in turn, determined a slight decrease in the fractional excretion of the sulphate conjugate and shifting of the glucuronide to sulphate ratio to higher levels that reached statistical significance. This may indicate an enhancement of the glucuronidation pathway of paracetamol in smokers.

When our results are compared with those reported by other authors (8,10), it is noteworthy that the overall paracetamol conjugation to oxidation ratio was 12 in the Spanish populations, 9 in Caucasians from Scotland (8), 15 in Chinese and Indians (10), and 17 in West Africans (8). This is due to a higher recovery of oxidation-derived conjugates among Caucasians from Scotland and a lower recovery of these conjugates among West Africans and Orientals. Accordingly, it may be suggested that the Spanish population may present an intermediate susceptibility to hepatic toxicity after paracetamol overdose.

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