Comparison of self-reported alcohol intake with the urinary excretion of 5-hydroxytryptophol:5-hydroxyindole-3-acetic acid, a biomarker of recent alcohol intake

Anja Kroke[1]*, Kerstin Klipstein-Grobusch[1], Kurt Hoffmann[1], Ines Terbeck[1], Heiner Boeing[1] and Anders Helander[2]

[1]*Department of Epidemiology, German Institute of Human Nutrition, Bergholz-Rehbruecke, Germany*
[2]*Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden*

Under-reporting of alcohol intake has been frequently reported. However, due to the lack of an objective reference method, e.g. a biomarker, information about the extent of under-reporting of alcohol intake obtained with dietary assessment instruments is not available. The objective of this study was to compare reported alcohol intake data derived from a 24 h recall with a biomarker of recent alcohol intake, the urinary excretion of 5-hydroxytryptophol (5-HTOL):5-hydroxyindole-3-acetic acid (5-HIAA). Embedded into the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study, Germany, a validation study that collected 24 h recall data and 24 h urine samples was conducted. Cohort study participants (*n* 107) volunteered to participate in this validation study. Among them were five subjects who reported no consumption of alcoholic beverages but had a 5-HTOL:5-HIAA ratio that indicated recent alcohol intake when the clinical cut-off point was taken as a judging criterion. After exclusion of these under-reporters, the Pearson's correlation coefficient between reported alcohol intake and the 5-HTOL:5-HIAA ratio was 0·92 ($P < 0·0001$). Except for low alcohol intake of $<0·1$ g/kg body mass, a significant increase in 5-HTOL:5-HIAA excretion was observed with increasing amounts of alcohol intake. In conclusion, the 5-HTOL:5-HIAA excretion ratio appears to be a valuable quantitative biomarker of recent alcohol consumption. Denial of alcohol intake can be detected, but for the quantification of under-reporting of alcohol intake 24 h reference data are not yet available. With these data at hand, however, 5-HTOL:5-HIAA could become a biomarker for validation purposes in nutritional epidemiology.

Alcohol: Biomarker: Epidemiology

While cardiovascular disease mortality was observed to be inversely related to moderate alcohol consumption (Bofetta & Garfinkel, 1990; Renaud *et al.* 1993; Grobbee *et al.* 1999) other chronic diseases, in particular specific types of cancer, liver cirrhosis and alcohol dependency, tended to increase as alcohol consumption increased (Anderson *et al.* 1993; Longnecker, 1995; Damström Thakker, 1998). This resulted in a strong debate about the recommendations regarding alcohol intake in general, and, if recommended at all, about the amounts to be recommended.

The main source of data on the relationship between alcohol intake and diseases are derived from epidemiological studies that applied alcohol intake assessment instruments based on self-reports. These assessment tools are designed to rank individuals according to their intake rather than to estimate absolute amounts of intake (Willett, 1998). Should these data be used to derive absolute values for intake recommendations, misjudgements with respect to the health effects of alcohol intake might occur. Under-reporting of dietary intake in general (Willett, 1998, Kroke *et al.* 1999), and alcohol intake in particular (Midanik, 1988; Høyer *et al.* 1995) are well-known phenomena. Even though under-reporting of alcohol intake is very likely, the actual extent as well as its distribution seem to be less clear. All available methods might be more or less affected, and validation of instruments has been mainly restricted to relative validations with other instruments based on self-reports as a reference method. Biomarkers as an 'objective' measure are therefore considered to be an appropriate reference method in validation studies, because here

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individual bias can be neglected and the errors of test and reference method are considered uncorrelated (Willett, 1998). So far, no biochemical markers for alcohol intake validation have been available, but are perceived to be an important tool in the evaluation of health effects of alcohol consumption (Feunekes et al. 1999).

In this present study we applied a sensitive biomarker of recent alcohol consumption, the value of the ratio of the serotonin (5-hydroxytryptamine) metabolites 5-hydroxytryptophol (5-HTOL):5-hydroxyindole-3-acetic acid (5-HIAA) in urine. The 5-HTOL:5-HIAA ratio increases appreciably during ethanol oxidation and will not normalise until several hours (about 5–15 h depending on dose) after ethanol is no longer measurable (Helander *et al.* 1996). This marker has been thoroughly evaluated in control subjects under experimental conditions, but also successfully applied clinically in therapeutic control of alcohol-dependent outpatients to detect any recent drinking, and in forensic medicine to test for post-mortem or post-sampling synthesis of ethanol (Voltaire *et al.* 1992; Voltaire Carlsson *et al.* 1993; Helander *et al.* 1994a, 1999; Helander & Jones, 1998).

As the 5-HTOL:5-HIAA ratio is a marker for recent alcohol consumption, it allows validation of self-reports on short-term alcohol consumption. In addition to studies using short-term assessment instruments (e.g. 24 h recalls), validation studies frequently apply repeated short-term assessments for the validation of other instruments. Information from validation studies with respect to alcohol consumption would be a first step in assessing both the data and the conclusions obtained from studies that relate alcohol consumption to health outcomes. In the present analysis, data on the alcohol consumption of 107 subjects derived from 24 h recalls were compared with this biomarker.

Materials and methods

Subjects for this study were selected from the validation study that was conducted during the baseline assessment of the European Prospective Investigation into Cancer and Nutrition (EPIC; Riboli, 1992; Riboli & Kaaks, 1997) at the study centre in Potsdam, Germany (Boeing *et al.* 1999). Details of the recruitment procedure for EPIC, and the recruitment and study design of the validation study, have been described in detail elsewhere (Boeing *et al.* 1999; Kroke *et al.* 1999).

In brief, subjects for the validation study were recruited from those participating in the EPIC-Potsdam study for which participants were recruited from a random population sample. From the 160 subjects initially recruited for the validation study, 134 completed this 1 year study and provided all necessary data. Informed consent was obtained for all parts of the study, and approval was given by the Ethical Committee of the State of Brandenburg, Germany.

24 h urine collection

From the 134 study subjects, a urine collection from 107 participants with a 24 h recall covering the sampling period was available for this analysis. For the remaining subjects, the day assessed by the 24 h recall did not cover the day of the urine collection. Para-aminobenzoic acid was administered to assess the completeness of urine samples (Bingham & Cummings, 1983). None of the samples eligible for this analysis had a *para*-aminobenzoic acid recovery $<85\,\%$ which would have indicated an incomplete urine collection (Bingham & Cummings, 1985; Bingham, 1994). The urine samples were processed within 48 h of sampling, centrifuged, and then stored at $-80°C$ until analysis.

Biomarker analysis

5-HTOL and 5-HIAA were analysed after transferring frozen 24 h urine subsamples to the Stockholm laboratory. 5-HTOL was determined with a specific GC–MS method, whereas 5-HIAA was measured by HPLC. Both measurements were performed according to methods previously described in detail (Helander *et al.* 1991; Voltaire *et al.* 1992). The within-day and between-day CV for quantification of 5-HTOL and 5-HIAA were 3-5 % and 7 % respectively (Helander *et al.* 1999).

Alcohol intake assessment

24 h recalls were obtained by one trained interviewer using a personal computer guided interview program (EPIC-Soft, German version) specifically developed for the EPIC study. Types and amounts of alcoholic beverages were recorded. Details of this program have been published previously (Voss *et al.* 1998; Slimani *et al.* 1999). Alcohol intake was calculated with alcohol content data from the German Food Code and Nutrient Database (BLS, version 2.2, 1998; Federal Institute for Health Protection of Consumers and Veterinary Medicine, Berlin, Germany) in g/d.

Body weight

Body weight was measured on a digital scale to the nearest 100 g, with the subjects wearing only light underwear, by trained and quality-monitored personnel (Klipstein-Grobusch *et al.* 1997). Based on previous experiences with the biomarker 5-HTOL:5-HIAA, it proved useful to express the ingested alcohol/kg body mass in order to improve the accuracy of measurement results by reducing a source of inter-individual error.

Data analysis

Both the alcohol intake data and the 5-HTOL:5-HIAA data were not normally distributed. Variables were then log-transformed and again tested for normality. Although the test for normality still rejected a normal distribution, the obtained empirical density was nearly symmetric and unimodal like a Gaussian density. Therefore, statistical analysis was performed on the basis of log-transformed data. Moreover, geometric means and standard deviations were preferred to the usual arithmetic means to take into account the log-normal form of the distribution.

Statistical significance of differences between categories of alcohol intake were determined with the *t* test using the log-transformed data which can be interpreted as
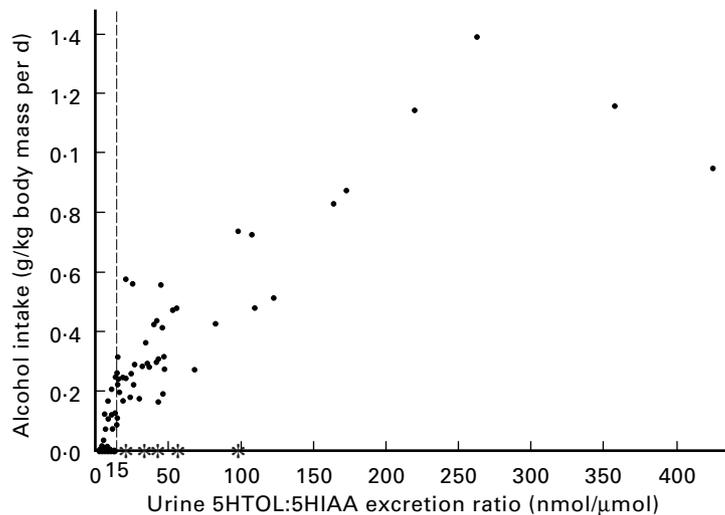
Fig. 1. Urinary 5-hydroxytryptophol (5-HTOL):5-hydroxyindole-3-acetic acid (5-HIAA) values *v.* alcohol intake derived from a 24 h recall, expressed as g alcohol/kg body mass per d. The clinical cut-off line of 15 nmol 5-HTOL/µmol 5-HIAA, used to indicate any alcohol intake. * Likely under-reporters (*n* 5) (EPIC-Potsdam Study, Germany, *n* 107.)

significant differences of the geometric means. Correlation between log-transformed variables was assessed with the Pearson's correlation coefficient, which can be interpreted as a measure of a proportional relationship in the original scale. Linear regression analysis was performed to describe the functional relationship between the test method and the reference method. *P* values are two-tailed, and values <0·05 were considered as significant. All statistical analyses were performed using SAS, version 6.12 (Statistical Analysis Systems Inc., Cary, NC, USA).

Results

The mean age of the study population was 57 years for men (median 59, range 40–67) and 52 years for women (median 55, range 35–66). In Fig. 1, reported alcohol intake (g/kg body mass per/d) was plotted against the urinary 5-HTOL:5-HIAA ratio. As previous experience with the 5-HTOL:5-HIAA marker have demonstrated that a 5-HTOL:5-HIAA value ≥15 nmol/µmol is indicative of recent alcohol consumption (Helander *et al.* 1994*b*, 1996), we have marked this cut-off line by a dotted line. Accordingly, five subjects were identified who did not report consumption of any alcoholic beverages but had 5-HTOL:5-HIAA values beyond the cut-off value (range 21–98 nmol/µmol). These subjects were considered as under-reporters and were excluded for certain analyses. Log-transformation of the data resulted in a reduced spread of the measurement values and a closer relationship between the alcohol intake and the 5-HTOL:5-HIAA data (Fig. 2). Descriptive statistics of the daily alcohol intake data, both in absolute amounts and as g alcohol/kg body mass, and mean urinary 5-HTOL:5-HIAA excretion are presented in Table 1.

Men had a significantly higher reported alcohol intake as compared with women, both in absolute amounts and in g/kg body mass per d. Corresponding to the higher intake of alcohol, men had a higher mean 5-HTOL:5-HIAA excretion than women. In total there were 30 subjects (twelve men, eighteen women) who reported no alcohol intake during the recalled day. As there is no difference in 5-HTOL:5-HIAA excretion between sexes (Helander *et al.* 1996), further analysis were not stratified by sex.

Overall, the 5-HTOL:5-HIAA excretion ratio increased from the lowest to the highest category of reported alcohol intake after exclusion of the previously identified under-reporters (Table 2); the mean 5-HTOL:5-HIAA excretion ratio in the group of those without reported alcohol intake decreased from 8·7 to 7·0 nmol/µmol when the under-reporters were excluded. Despite this exclusion, however, a significant difference between categories of reported alcohol intake and 5-HTOL:5-HIAA values could not be observed between the first and the second category of alcohol intake, that is, with reported alcohol intake <0·1 g/kg body mass. Between all following categories of alcohol intake statistically significant differences were observed. The Pearson's correlation coefficient between reported alcohol intake (g/kg body mass) and the 5-HTOL:5-HIAA excretion ratio increased from 0·85 to 0·92 after exclusion of the under-reporters. The explained variance of the linear regression model was 0·84 when the under-reporters were excluded (Fig. 2).

Discussion

The current literature on validity testing of self-reported alcohol intake data clearly states the need for objective reference measures (Grønbaek & Heitmann, 1996; Feunekes *et al.* 1999). However, appropriate biomarkers have been missing to date. In clinical work with alcohol-dependent patients, as well as in the area of alcohol testing for legal purposes, similar problems as in dietary
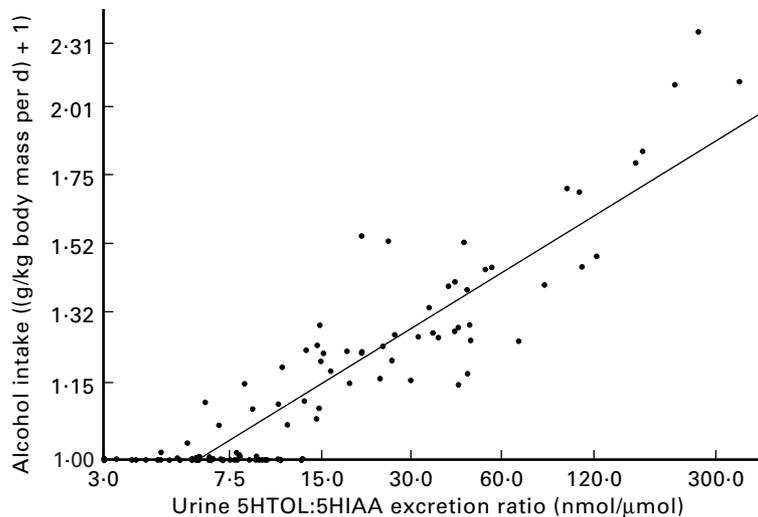
Fig. 2. Urinary 5-hydroxytryptophol (5-HTOL):5-hydroxyindole-3-acetic acid (5-HIAA) values (log-transformed) *v.* alcohol intake derived from a 24 h recall, expressed as g alcohol/kg body mass per d (log-transformed), and the regression line from the linear regression analysis after exclusion of five under-reporters. $y = 0.742x^{0.166} - 1$, $r^2 0.84$. (EPIC-Potsdam Study, Germany, n 102).

assessment, with discrepancies between reported and true alcohol intake exist. Researchers in this area, therefore, made strong efforts to identify biological markers other than direct alcohol measurements in samples of breath, blood, or urine due to several limitations of these measures (Helander & Jones, 1998). For these purposes, the urinary 5-HTOL:5-HIAA excretion ratio was proposed as a specific marker of recent alcohol consumption that proved to be detectable for longer after alcohol ingestion than ethanol measurements in blood, breath or urine (i.e. a higher sensitivity) (Helander *et al.* 1996), and responded in a dose-dependent manner to alcohol ingestion (Voltaire *et al.* 1992; Helander *et al.* 1996).

The use of the 5-HTOL:5-HIAA value as a marker for recent alcohol intake is based on the metabolic changes in

Table 1. Geometric mean and geometric standard deviation of alcohol intake (g/d and g/kg body mass/d) assessed with a 24 h recall, and geometric mean and standard deviation of the urinary 5-hydroxytryptophol (5-HTOL):5-hydroxyindole-3-acetic acid (5-HIAA) excretion ratio during the day covered by the 24 h recall, after exclusion of previously identified under-reporters (*n* 5) (EPIC-Potsdam Study, Germany, *n* 102)

(Geometric mean values, standard deviations and ranges for 102 subjects

	n	Geometric mean	Geometric SD	Range
Alcohol intake (g/d)				
All	102	5·7	5·0	0–91·4
Men	57	8·8	5·1	0–91·4
Women	45	3·3	4·1	0–54·3
Alcohol intake (g/kg body mass per d)				
All	102	1·18	1·23	0–1·39
Men	57	1·24	1·25	0–1·39
Women	45	1·11	1·18	0–0·95
Urine 5-HTOL:5-HIAA (nmol/μmol)				
All	102	16·0	3·1	3·0–424·1
Men	57	20·9	3·3	3·0–357·0
Women	45	11·5	2·6	3·0–424·1

the serotonin (5-hydroxytryptamine) metabolism induced by alcohol consumption. Serotonin is metabolised via 5-hyroxyindole-3-acetaldehyde to 5-HTOL and 5-HIAA, which are both excreted in urine. Alcohol intake induces a shift in the formation of 5-HIAA, the main metabolite of serotonin degradation, towards a higher production of 5-HTOL (Davis *et al.* 1967). This shift is on the one hand due to the competitive inhibition of the enzyme aldehyde dehydrogenase, which catalyses the formation of 5-HIAA from 5-hydroxyindole-3-acetaldehyde, by acetaldehyde, and on the other hand by an increased $NADH:NAD^+$ ratio, which also leads to increased 5-HTOL formation (Walsh, 1973; Svensson *et al.* 1999). These described changes are a fairly specific effect of alcohol. No other dietary constituent is known to influence the 5-HTOL: 5-HIAA ratio. Consumption of serotonin rich foods, such as bananas, leads to increased formation of both 5-HTOL and 5-HIAA, but the 5-HTOL:5-HIAA ratio remains unchanged. Except for alcohol intake, the only known cause of an increased urinary 5-HTOL:5-HIAA ratio is treatment with potent inhibitors of aldehyde dehydrogenase (so-called alcohol sensitising drugs) such as disulfiram (Antabuse®) or cyanamide (calcium carbimide; Dipsan®) (Beck *et al.* 1995). However, the change in the value resulting from taking aldehyde dehydrogenase inhibitors is less pronounced than after drinking alcohol. Moreover, disulfiram and cyanamide are not available over-the-counter, and the use of these drugs has not been reported by any of the participants in our study.

In the present study, we have applied this biomarker within the context of a validation study for a dietary assessment instrument (Kroke *et al.* 1999). In contrast to most previous studies with this biomarker (Voltaire Carlsson *et al.* 1993; Helander *et al.* 1996; Hiltunen *et al.* 1996), the study population comprised subjects from the general population with 'normal' drinking patterns. Our

Table 2. Urinary excretion of 5-hydroxytryptophol (5-HTOL):5-hydroxyindole-3-acetic acid (5-HIAA) by categories of reported alcohol intake assessed with a 24 h recall after exclusion of previously identified under-reporters (n 5), (EPIC-Potsdam Study, Germany)

(Geometric mean values, geometric standard deviations and ranges)

Reported alcohol intake (g/kg body mass per d)	n	Urine 5-HTOL:5-HIAA (nmol/μmol)			
		Geometric mean	Geometric SD	Range	P value*
0	30	7·0	1·4	3·0–13·2	
					0·770
>0–<0·1	21	6·7	1·5	3·0–14·8	
					<0·001
0·1–<0·2	13	16·3	1·9	6·4–46·2	
					0·017
0·2–<0·3	18	27·0	1·6	11·4–68·0	
					0·004
0·3–≤0·5	12	49·3	1·7	15·2–109·4	
					<0·001
>0·5	8	200·1	1·7	20·8–424·1	

* P values were derived from t tests of the logarithmically transformed data by testing the significance of difference between two adjacent categories.

observational data, which were assessed with a standard dietary assessment instrument to obtain information on alcohol intake, demonstrated a strong relationship between reported alcohol intake and urinary 5-HTOL:5-HIAA excretion after excluding those subjects identified as under-reporters (no reported alcohol intake but above the clinical cut-off point for 5-HTOL:5-HIAA excretion). However, significant differences between categories of alcohol intake in 5-HTOL:5-HIAA excretion were not observed at reported alcohol consumption levels <0·1 g alcohol/kg body mass per d (i.e. <7 g alcohol/d for a 70 kg subject). Several considerations might explain this observation. First, this very low amount might be close to the detection limit of the method. Second, inter-individual variation in 5-HTOL:5-HIAA excretion might exist due to certain diseases, or genetic factors (Hagan & Helander, 1997). Even though the response in urinary 5-HTOL:5-HIAA to a given amount of alcohol was highly reproducible within subjects, it showed appreciable variation between subjects also when administering the ethanol under strictly controlled experimental conditions (i.e. intravenous infusion) (Jones & Helander, 1999). Third, misreporting (e.g. under- or over-reporting) of alcohol intake might have occurred. As so far no reference values for 24 h urine samples are available to compare the observed with the expected value given the amount of alcohol consumed, and on which to judge the self-reports, no further conclusions can be drawn with respect to under-reporting from these data. To this end, an experimental study would be warranted to derive a regression line with its corresponding confidence limits.

An issue of critical concern in using this biomarker is the determination of the cut-off point beyond which alcohol consumption is to be assumed. Previous studies on various populations including alcohol-abstinent subjects resulted in the currently applied 5-HTOL:5-HIAA cut-off value of 15 nmol/μmol (Helander *et al.* 1994b). Beyond this threshold, the probability of recent alcohol ingestion was regarded as extremely high (P<0·0001) (Voltaire *et al.* 1992), and therefore the threshold was also applied in our present study. Although in this study 24 h urine was

collected, whereas previous studies used spot urine samples, the same cut-off value should be applicable because no difference in the 5-HTOL:5-HIAA excretion ratio has been demonstrated between samples collected during day- and night-time (Helander *et al.* 1992).

Even though the analysis of 5-HTOL is not routinely available so far, improvements in procedures will lead to a simplification of measurements and thereby to a wider distribution of this methodology. This study was able to show the feasibility of using routinely collected 24 h urine samples from a dietary validation study for the determination of this biomarker. In addition, as 24 h urine collections become more and more a standard tool in dietary assessment validation or calibration studies, biological specimens for the determination of the 5-HTOL:5-HIAA marker would be available in many studies. For the application of this biomarker in studies with long-term dietary assessment instruments, such as food-frequency questionnaires, this biomarker would only be useful if repeated measurements of a yet unknown number would be a good measure of usual alcohol consumption. Given the variation in drinking patterns this could not be feasible on an individual basis but might generate information on the population level about the validity of the obtained alcohol intake data. However, prior to the application of this biomarker for validation purposes, an experimental study is needed to determine reference values of 5-HTOL:5-HIAA in 24 h urine samples for a given amount of alcohol ingestion. With these data in hand, we conclude that the 5-HTOL:5-HIAA value could also prove a valuable biomarker for recent alcohol intake in dietary studies. Given the observed strong dose-dependent relationship between reported alcohol intake data and the urinary 5-HTOL:5-HIAA excretion, this biomarker could then help to solve the problem, as outlined in the introduction (pp. 621–622), of uncertainties in judging the alcohol–disease relationship in absolute terms.

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