Alcohol Consumption, Alcohol Dehydrogenase 3 Polymorphism, and Colorectal Adenomas[1]

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Abstract

Alcohol is a probable risk factor with regard to colorectal neoplasm and is metabolized to the carcinogen acetaldehyde by the genetically polymorphic alcohol dehydrogenase 3 (ADH3) enzyme. We evaluated whether the association between alcohol and colorectal adenomas is modified by *ADH3* polymorphism. We recruited 433 cases with adenomatous polyps and 436 polyp-free controls among Caucasians undergoing endoscopy between 1995 and 2000. Frequency and amount of habitual alcohol consumption were assessed by beverage type, using a validated self-administered food frequency questionnaire. All participants provided blood for genotyping of *ADH3*. Multivariate analyses adjusting for gender, age, and indication for endoscopy showed that alcohol increased the risk of colorectal adenomas among women [odds ratio (OR), 1.8; 95% confidence interval (CI), 1.0–3.2, ≥10 *versus* <1 drink/week]. Among men, the risk of adenomas was increased only for those consuming > 21 drinks/week (OR, 1.8; 95% CI, 0.9–3.8, compared with men drinking < 1 drink/week). Among subjects in the highest tertile of alcohol consumption, those with the *ADH3*1/*1* genotype were at higher risk (OR, 1.8; 95% CI, 1.0–3.1) than those with other *ADH3* genotypes (OR, 1.2; 95% CI, 0.7–1.9) when compared with those in the lowest tertile with *ADH3*1/*2* or *ADH3*2/*2* genotypes. In conclusion, our findings are consistent with results of other studies, suggesting that alcohol consumption elevates the risk of adenomatous colorectal polyps. *ADH3* polymorphism may modify the association between alcohol consumption and colorectal adenomas.

Introduction

The majority of colorectal cancers is thought to arise from adenomas (1, 2). Alcohol consumption is a probable risk factor with respect to colorectal adenomas (2) and colorectal cancer (3).

This increased risk might be caused by ethanol via cocarcinogenesis, induction of DNA hypomethylation, or tumor promotion (4) but might also result from its metabolite acetaldehyde, which was qualified as a probable carcinogen to humans (5).

Acetaldehyde is mainly produced from ethanol via oxidation by the ADH[3] enzyme (6) and is subsequently detoxified into acetate by ALDH. Ethanol metabolism predominates in the liver, but ADH is also expressed in other tissues, including the colon (7, 8). ADH isoenzymes primarily involved in ethanol oxidation consist of subunits encoded by *ADH2* and *ADH3* genes (6). In contrast to *ADH2* (9–11), the *ADH3* gene is highly polymorphic in Caucasians. Of the two allelic variants, *ADH3*1* is associated with higher enzyme activity than *ADH3*2* and occurs in Caucasians at frequencies of 55–63%. Polymorphism in the *ALDH* gene is extremely rare in Caucasians (12, 13).

To our knowledge, thus far, no studies with regard to the role of *ADH3* genotype in alcohol-associated colorectal neoplasm have been published. Risk of oral cancer was five times higher among heavy drinkers (>57 drinks/week) with the *ADH3*1/*1* genotype than in heavy drinkers with other *ADH3* genotypes (14). Among alcoholics, the risk of laryngeal cancer, but not of oropharyngeal cancer, was increased only in combination with the *ADH3*1/*1* genotype (15). Similarly, the risk of premenopausal breast cancer was increased only in women consuming > 6.5 alcoholic drinks/month with the *ADH3*1/*1* genotype (16). However, other studies investigating oral (17, 18), breast (19), and head and neck (20) cancers did not indicate that *ADH3* polymorphism plays a major role in the association between alcohol and cancer.

ADH3 polymorphism might be relevant to alcohol-associated colorectal carcinogenesis. Acetaldehyde might accumulate in the colon epithelium (6) where ADH activity is present (8), whereas ALDH activity is relatively low (21). If the rate of acetaldehyde accumulation and the duration of exposure to acetaldehyde depend on *ADH3* genotype, the association between alcohol consumption and adenomas in colon and probably also rectum would probably be strongest in subjects with the *ADH3*1/*1* genotype. Because acetaldehyde accumulation increases with alcohol consumption, the role of *ADH3* polymorphism might be most relevant in heavy drinkers.

In this study, among Dutch Caucasians, we evaluated whether alcohol and *ADH3* genotype interplay in the etiology

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[3] The abbreviations used are: ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; OR, odds ratio; CI, confidence interval.

of adenomatous colorectal polyps. We also investigated the effect of *ADH3* genotype among those with highest alcohol consumption.

Materials and Methods

Population. Cases and controls were recruited among those undergoing endoscopy in the outpatient clinics of eight hospitals in the Netherlands between June 1997 and June 2000. Medical ethical committees of all participating hospitals and of Wageningen University approved the study protocol.

Potential participants were recruited at the time of endoscopy by the endoscopy staff (47%) or were selected at regular intervals using endoscopy reports of all patients who had undergone endoscopy in the previous 3 months, and these were invited by mail (53%). Eligible subjects were Dutch speaking, Caucasian, aged 18–75 years at the time of endoscopy, had no hereditary forms of colorectal cancer (*i.e.*, hereditary nonpolyposis colorectal cancer, familial adenomatous polyposis, and other hereditary syndromes), chronic inflammatory bowel disease, history of colorectal cancer or (partial) bowel resection, or serious disabling morbidity. After obtaining informed consent, blood samples were drawn for DNA analysis, and dietary and lifestyle questionnaires were administered. Overall response was 54%.

We defined cases as those having had at least one histologically confirmed colorectal adenomatous polyp in their life; controls had no history of any type of polyps. Complete colonoscopy was conducted on 82% of the cases and 60% of the controls; in 7% of cases and 17% of controls, complete visualization of the colon was achieved by sigmoidoscopy followed by colon X-ray.

Among cases, main indications for endoscopy were routine check-up for adenoma recurrence (37%), anal bleeding (27%), and large bowel complaints (15%), whereas controls mainly underwent endoscopy because of large bowel complaints (38%), defecation problems (21%), or anal bleeding (17%).

Medical files were checked for additional information with regard to medical history and information on polyp recurrence, size, localization, histology, and the number of excised polyps.

In retrospect, based on information from questionnaires and medical files, we excluded 170 participants who did not meet the eligibility criteria, mainly because of nonadenomatous or unknown types of polyps (59%). In addition, we used complete information of 64 subjects (40 cases and 24 controls, all having undergone complete visualization of their colon) meeting our criteria, recruited between December 1995 and June 1997, from a preceding study on somatic mutations in colorectal adenomas conducted in one of eight hospitals (22). The recruitment procedures as well as the questionnaires used in this preceding study were essentially the same as those described here for the main study population. This increased the study population from 861 to 925 subjects.

Questionnaires. All invited subjects received a short questionnaire inquiring about important characteristics such as age, gender, alcohol consumption, education level, and smoking habits. Approximately one-third of the subjects who did not want to participate in the study completed this short questionnaire. Although they were older, they did not differ from the participants with respect to gender, education level, smoking habits, and alcohol consumption.

Participants additionally received dietary and lifestyle questionnaires and were requested to complete these with regard to habits in the year previous to their last endoscopy or complaints.

To assess dietary habits, we used a standardized and validated semiquantitative food frequency questionnaire, described in detail by Ocké *et al.* (23). By means of this questionnaire, consumption of alcoholic beverages was assessed with respect to beer, white wine, red wine, ports, and liquors separately. Subjects could choose to report average consumption in glasses/day, week, month, or year. Reproducibility of alcohol consumption as assessed by this questionnaire was high for both males and females ($r = 0.91$) as was its relative validity ($r = 0.74$ for males, $r = 0.87$ for females compared with the means of 12 24-h recalls; Ref. 23). Intakes of total energy and of various nutrients and ethanol were calculated by using a computerized version of the Dutch food composition table. A Dutch alcoholic consumption contains ~10 g of ethanol. Nutrients, except ethanol, were adjusted for total energy intake using the residual regression method (24).

Laboratory Analyses. Blood samples were stored at −20°C. DNA was isolated from 200 μl of frozen whole blood, using the QIAamp blood kit (Qiagen, Inc.), diluted to a concentration of ~20 ng/μl, and stored at 4°C until analyzed.

We used a PCR-RFLP method for determination of *ADH3* genotype. A 145-bp fragment of exon 8 of the *ADH3* gene was amplified using primers described by Groppi *et al.* (Ref. 25; 5′-GCTTTAAGAGTAAATATTCTGTCCCC-3′ and 5′-AATCTACCTCTTTCCAGAGC-3′). To check for DNA cross-contamination, one in eight samples contained no DNA but contained water instead.

For RFLP analysis, *Ssp*I digested the *ADH3*1* allele into fragments of 67, 63, and 15 bp and the *ADH3*2* allele into fragments of 130 and 15 bp. DNA fragments were separated on an ethidium bromide stained agarose gel (4%) and visualized under UV light.

To control the specificity of *ADH3* genotyping, a random sample of primary PCR products was digested by *Nla*III, cleaving the *ADH1* and *ADH2* genes that are closely related to *ADH3* but leaving *ADH3* intact (25). Laboratory personnel was blinded to case-control status. DNA of 10 participants was not available, and it was not possible to genotype another 8 samples (<1%) for *ADH3*.

Data Analysis. Subjects with incomplete dietary data ($n = 38$) were excluded, as were the 18 subjects of whom *ADH3* genotype was not assessed. The analyses thus included 869 subjects: 433 cases and 436 controls. We studied total alcohol consumption in glasses/week, calculated by summing the separately reported intakes of beer, wines, ports, and spirits, as well as total ethanol intake in grams/day from all dietary sources, including small amounts from sauces, puddings, chocolates, and low-alcohol beer. Alcohol consumption was divided in tertiles based on the distribution in the total study population. To additionally evaluate the effect of a combination of *ADH3* polymorphism with high alcohol consumption, we defined high alcohol consumption as the consumption of >3 drinks/day because this amount exceeds the recommended daily maximum for both men and women. *ADH3*1/*1* was considered as the high-risk genotype and compared with the combination of *ADH3*1/*2* and *ADH3*2/*2* genotypes.

The analyses were conducted using Statistical Analysis Software (SAS version 6.12, SAS Institute, Cary, NC). All tests of statistical significance were two-sided. To test for linear trend, we modeled the tertile of alcohol intake as a continuous variable in the logistic regression model in which each tertile was assigned its median value. Logistic regression models were

Table 1 Characteristics of the study population by gender

	Women ($N = 471$)		Men ($N = 398$)	
	Cases ($n = 196$)	Controls ($n = 275$)	Cases ($n = 237$)	Controls ($n = 161$)
Age (yr), mean ± SD	58.6 ± 10.7	49.5 ± 15.0[a]	59.3 ± 10.6	51.9 ± 12.2[a]
Genotype, %				
*ADH3*1/*1*	35.2	36.7	36.3	34.2
*ADH3*1/*2*	48.0	47.6	44.7	46.6
*ADH3*2/*2*	16.8	15.6	19.0	19.3
Alcohol intake, median (25^{th}, 75^{th} percentile)				
Ethanol (g/day)	3.9 (0.3; 14.6)	1.5 (0.1; 8.7)[a]	14.2 (4.3; 30.4)	14.5 (3.5; 26.5)
Alcoholic consumptions/wk	2.5 (0.2; 10.0)	1.0 (0; 6.0)[a]	10.0 (2.3; 21.1)	9.3 (2.5; 18.0)
Beer	0 (0; 0)	0 (0; 0.1)	0.5 (0; 7.0)	2.0 (0; 7.0)
Wine (red, white, rosé)	0.5 (0; 4.0)	0.2 (0; 2.7)	0.5 (0; 5.2)	0.7 (0; 4.0)
Spirits (incl port, sherry etc)	0.1 (0; 1.1)	0 (0; 0.5)[a]	0.9 (0; 6.5)	0.5 (0; 4.0)
Dietary habits, median (25^{th}, 75^{th} percentile)				
Total energy (KJ/day)	7,684 (6,539; 8,979)	7,440 (6,419; 8,978)	9,293 (7,942; 10,951)	10,239 (8,435; 11,623)[a]
Vegetables (g/day)	122 (98, 154)	114 (90, 144)[a]	110 (86, 140)	112 (83, 137)
Fruit (pieces/day)	1.5 (1.0, 2.3)	1.0 (0.6, 2.0)[a]	1.0 (0.4, 2.0)	1.0 (0.4, 2.0)
Red meat (g/day)	57 (28, 77)	48 (26, 73)	74 (48, 91)	73 (42, 90)
Coffee (cups/day)	4.0 (2.5; 5)	4.0 (2; 6)	4.0 (3; 6)	5.0 (3; 6)
Folic acid (μg/day)	187 (162; 221)	175 (153; 204)[a]	204 (171; 245)	216 (187; 252)[a]
Retinol (μg/day)	554 (392; 785)	499 (346; 744)	736 (524; 1223)	838 (517; 1189)
Medical history, %				
Dietary changes because of bowel complaints	18.9	33.8[a]	17.3	19.3
Family history of colorectal cancer	26.5	19.3	20.3	19.3
Previous diagnosis of adenomas	45.9	n.a.[b]	40.1	n.a.
Complaints-related endoscopy indication	46.9	78.2[a]	55.7	74.5[a]
Other characteristics				
Body mass index (kg/m^2), mean ± SD	25.8 ± 4.5	25.1 ± 4.5	26.3 ± 3.3	25.9 ± 3.1
Cigarette smoking, % never smokers	45.9	52.7	32.1	46.6[a]
High physical activity, %	35.7	34.6	20.3	26.7
Low education level, %	44.4	33.1	30.0	26.1

[a] Significantly different from cases: $P < 0.05$ (Fisher's exact test for categorical variables, *t* test for age, and body mass index, and Wilcoxon's rank test for dietary variables).
[b] n.a., not applicable.

used to calculate ORs and 95% CIs. Factors selected as possible confounders were age, gender, body mass index, indication for endoscopy, center, cigarette smoking, physical activity, family history of colorectal cancer, education level, use of nonsteroidal anti-inflammatory drugs, total energy intake, consumption of vegetables, fruit, total meat and red meat, and nutrients related to these food groups. Variables related to colorectal adenomatous polyps as well as to exposure at $P < 0.5$ (26) were separately entered as covariates in the regression models. None of these changed the OR for alcohol consumption by >10%. However, we included age and indication for endoscopy (complaints-related, screening, and other/unknown) in the multivariate models to control for potential confounding. Analyses of the total population were additionally adjusted for gender. Gender-stratified analyses were conducted because of (*a*) different male-female ratios between cases and controls, (*b*) gender-specific patterns of alcohol consumption, and (*c*) gender-specific differences in alcohol vulnerability (27).

To evaluate the possible interplay between *ADH3* genotype and alcohol consumption, the group with a combination *ADH3*1/*2* or *ADH3*2/*2* genotypes and low-alcohol consumption (lowest tertile) served as reference category.

As different risk factors may operate in different stages of carcinogenesis, we conducted case-case analyses for adenoma recurrence (primary *versus* recurrent), size (<1 cm *versus* ≥1 cm), localization (proximal *versus* distal) and number (multiple *versus* single) of polyps.

To check whether former adenomas among cases or undetected right-sided polyps in controls could have biased our results, we repeated all analyses after restriction of our study population to cases with first diagnosis of adenomas not longer than 1 year ago ($n = 299$) and controls with complete visualization of the colon ($n = 334$).

Results

The case group contained more men than the control group (55 *versus* 37%). Table 1 shows characteristics of the study population for cases and controls stratified by gender. Among women and men, cases were significantly older than controls, and less often underwent endoscopy because of bowel complaints. Among women, cases had a higher intake of alcohol (especially of spirits and fortified wines), vegetables, fruits, and folic acid and had changed their diet less frequently because of bowel complaints compared with controls. Among men, cases more frequently (had) smoked and consumed less energy and folic acid than controls. There were no differences with respect to *ADH3* genotype. The distribution of *ADH3* was in Hardy-Weinberg equilibrium.

Alcohol intake from all sources ranged from 0 to 10 glasses/day. In the control group, the median alcohol consumption among men was 9 consumptions/week, whereas the median consumption of alcohol among women was only 1 glass/week. Among cases, median alcohol intake was 10 drinks/week among men and 2.5 drinks/week among women.

Alcohol intake correlated positively with energy intake and education level. Also, alcohol consumption was related to

Table 2 Association between alcohol consumption and risk of adenomatous colorectal polyps

		Alcohol consumption (drinks/week)[a]			*P* for trend
		<1	1–10	≥10	
All	*N* (cases/controls)	122/163	139/153	172/120	
	Gender and age adjusted OR (95% CI)	1 (REF)[b]	1.11 (0.78–1.58)	1.54 (1.06–2.24)	0.02
	Multivariate OR (95% CI)[c]	1 (REF)	1.00 (0.68–1.46)	1.38 (0.93–2.07)	0.07
Women	*N* (cases/controls)	76/135	69/99	51/41	
	Age adjusted OR (95% CI)	1 (REF)	1.17 (0.75–1.82)	2.19 (1.30–3.68)	0.003
	Multivariate OR (95% CI)[c]	1 (REF)	0.99 (0.61–1.60)	1.81 (1.02–3.21)	0.04
Men	*N* (cases/controls)	46/28	70/54	121/79	
	Age adjusted OR (95% CI)	1 (REF)	0.93 (0.50–1.73)	1.07 (0.60–1.91)	0.63
	Multivariate OR (95% CI)[c]	1 (REF)	0.96 (0.50–1.85)	1.12 (0.61–2.05)	0.57
		Alcohol consumption (drinks/week)[a]			
		<1	1–20	≥21	
Men	*N*	47/29	130/104	60/28	
	Age adjusted OR	1(REF)	0.86(0.49–1.51)	1.67(0.85–3.30)	0.04
	Multivariate OR[c]	1(REF)	0.90(0.50–1.63)	1.77(0.86–3.64)	0.04

[a] One alcoholic consumption contains ~10 g of ethanol.
[b] REF, reference group.
[c] Adjusted for sex, age, and indication for endoscopy (complaints related, screening, other/unknown).

Table 3 Risk of adenomatous colorectal polyps as a result of alcohol consumption and *ADH3* genotype[a]

			Alcohol consumption (drinks/week)[b]		
			<1	1–10	≥10
All	*ADH3*1/*2, *2/*2*	*N* (cases/controls)	72/96	99/105	107/79
		OR (95% CI)	1 (REF)[c]	0.97 (0.60–1.56)	1.15 (0.70–1.90)
	*ADH3*1/*1*	*N* (cases/controls)	50/67	40/48	65/41
		OR (95% CI)	0.94 (0.53–1.64)	0.99 (0.54–1.79)	1.76 (1.00–3.11)
Women	*ADH3*1/*2, *2/*2*	*N* (cases/controls)	41/77	51/68	35/29
		OR (95% CI)	1 (REF)	0.94 (0.51–1.74)	1.38 (0.66–2.87)
	*ADH3*1/*1*	*N* (cases/controls)	35/58	18/31	16/12
		OR (95% CI)	0.90 (0.46–1.73)	0.92 (0.42–2.05)	2.61 (1.05–6.50)
Men	*ADH3*1/*2, *2/*2*	*N* (cases/controls)	31/19	48/37	72/50
		OR (95% CI)	1 (REF)	1.03 (0.47–2.27)	1.05 (0.50–2.20)
	*ADH3*1/*1*	*N* (cases/controls)	15/9	22/17	49/29
		OR (95% CI)	1.31 (0.41–4.16)	1.06 (0.42–2.69)	1.50 (0.67–3.34)

[a] Adjusted for sex, age, and indication for endoscopy (complaints related, screening, other/unknown).
[b] One alcoholic consumption contains ~10 g of ethanol.
[c] REF, reference group.

(history of) cigarette smoking and family history of colorectal cancer (data not shown).

In Table 2, risk estimates for the association between the number of drinks/week (in tertiles) and colorectal adenomas are shown. Overall, alcohol intake was weakly but not statistically significantly related to colorectal adenomas. Among women, alcohol consumption significantly increased the risk of colorectal adenomas. When consumers of >21 alcoholic consumptions/week were compared with consumers of <1 glass/week, alcohol appeared to also be a risk factor for men. We did not find increased risks for women consuming > 21 glasses/week, probably because this category only contained 10 cases and 12 controls (data not shown). When using sex-specific tertiles (<4.5, 4.5–15.9, and ≥16 drinks/week for men and <0.2, 0.2–3.9, and ≥4.0 drinks/week for women), results were comparable with those presented in Table 2 (i.e., OR for the third compared with the first tertile, 1.1; 95% CI, 0.6–1.9 and OR, 1.5; 95% CI, 0.9–2.6 for men and women, respectively).

The analysis of alcohol intake from all sources in grams/day, which was slightly different from the daily alcohol intake in grams acquired from alcoholic beverages only, yielded ORs comparable with those presented in Table 2. No specific type of beverage was responsible for the increased risk of colorectal adenomas (data not shown). Case-case analyses did not indicate that alcohol consumption was related to specific adenoma characteristics such as location, size, type, and number of adenomas (data not shown).

After exclusion of cases who had previously been diagnosed with adenomas and of controls whose proximal colon was not examined, results remained similar (OR and 95% CI for women in the highest tertile of alcohol consumption, 2.2 and 1.1–4.2 and for men, 1.0 and 0.5–2.0). After exclusion of those who underwent endoscopy because of large bowel complaints, the gender and age adjusted OR inflated moderately among women (OR, 1.9; 95% CI, 1.0–3.4) but not among men (OR, 1.1; 95%, CI 0.5–2.1).

In Table 3, we show risk estimates for the combined associations of alcohol consumption and *ADH3* genotype with colorectal adenomas. The association between alcohol and adenomas was not markedly influenced by *ADH3* polymorphism, although the risk of adenomas was highest among subjects who had the *ADH3*1/*1* genotype and were in the upper tertile of alcohol consumption (Table 3). When comparing consumers of >21 alcoholic drinks/week to those consuming <1 drink/week, the risk increased most markedly for men with the *ADH3*1/*1* genotype (OR, 2.8; 95% CI, 1.0–8.3) and less so for men with different genotypes (OR, 1.6; 95% CI, 0.7–4.1). However, the interaction term was not statistically significant ($P = 0.4$). All ORs (sex-specific, age adjusted, and multivariate) for interaction varied between 0.95 and 1.05 and were nonsignificant.

Discussion

In this first study on alcohol consumption and *ADH3* genotype in the epidemiology of colorectal adenomas, we observed that alcohol consumption increased the risk of colorectal polyps most markedly among women and that this association may be influenced by *ADH3* genotype.

We recruited both cases and controls among those undergoing endoscopy. In the Netherlands, endoscopies are not routinely conducted for screening purposes like in the United States. Consequently, in our study, endoscopies were mainly conducted for bowel pain, anal bleeding, or defecation problems (64%). These complaints may influence dietary patterns. Our study population might also be more health conscious than the general population. This implies that our findings cannot easily be extrapolated to the general population. However, alcohol consumption in our control group was similar to the habitual alcohol consumption we assessed using the same questionnaire in a random sample ($n = 1935$) from the general population inhabiting the same regions as the controls.[4]

Of those invited, ~54% were willing to participate. Selection bias might have occurred if habitual alcohol consumption influenced the probabilities of being invited or of participating. It is not likely that alcohol consumption influenced the chance of being invited because habitual alcohol consumption was unknown at the time of the selection for almost all subjects (>95%). Moreover, participants did not differ in alcohol consumption from those who refused to participate but who completed the short questionnaire.

The control group consisted of significantly more women, possibly because women are more likely than men to undergo endoscopy for major bowel complaints such as irritable bowel syndrome, which was found to be more prevalent in Dutch women than in men (28). Also, cases were older than controls. Gender and age differences between cases and controls were also observed in other case-control studies on colorectal adenomas (29–31). Cases with history of adenomas might be overrepresented in our study population because these had a higher probability of being invited, and this might have introduced bias. We therefore included indication for endoscopy in our multivariate model. Exclusion of those with a history of adenomas yielded essentially the same results.

Inclusion of controls with bowel complaints probably did not lead to important overestimation of the true associations between alcohol and adenomas, although bowel complaints occurred more often in the control group and were associated to lower alcohol consumption in women. ORs only marginally inflated after exclusion of those undergoing endoscopy because of bowel complaints. We expect no misclassification by inclusion of controls with incomplete visualization of the colon (22%). In theory, these could have proximal adenomas, leading to bias toward the null. However, exclusion of controls with incomplete colonoscopy did not change our results.

Recall bias might have occurred because most cases and controls were aware of their status at the time of completion of the questionnaires. If alcohol would have been known as a risk factor for polyps, cases might have reported lower or higher intake than their true intake of alcohol. However, alcohol consumption is generally believed to increase the risk of several cancers but probably not of colorectal adenomas. Indeed, none of those previously being diagnosed with adenomas indicated to have changed alcohol consumption because of colorectal adenomas.

We assessed alcohol consumption by a food frequency questionnaire. Although a validation study of our questionnaire showed that habitual alcohol intake might be systematically underestimated, especially by men, subjects were appropriately ranked with regard to alcohol consumption (23). Ideally, per beverage type, both the frequency and the number of drinks/occasion should be noted (32). We had no information with respect to drinking patterns or on drinking habits over the years. Recent drinking habits might well reflect those in the past, as in a Dutch cohort, alcohol consumption patterns were found to be relatively stable, especially among men (33). The same was concluded from a follow-up study among British male doctors (34).

Allele frequencies of *ADH3*1* and *ADH3*2* among controls were 59 and 41%, respectively, which is similar to frequencies reported from other Caucasian populations (10, 11, 16, 19). We correctly amplified *ADH3* and not *ADH1* or *ADH2* in all samples (as checked by digestion of a random sample of PCR products with the restriction enzyme *Nla*III). Use of an internal control in RFLP analysis proved that all digestions were successful.

Our finding that alcohol consumption increases the risk of adenomatous colorectal polyps corresponds with the results of most previously conducted studies (29–31, 35–40). Among women, the risk of adenomas increased with consumption of ≥10 alcoholic beverages/week, whereas among men, the risk was increased only with consumption of >21 alcoholic beverages/week. It is difficult to compare these results with those obtained in other studies because different cutoff points are used and gender-specific results are not always presented. In general, like in our study population, men consume more alcohol than women do, and the range of alcohol consumption is wider in men than in women (33, 41). A possible explanation of our results is that the threshold for an effect of alcohol on adenomas could be higher in men than in women. Women are more vulnerable to alcohol than men, mainly because of a lower rate of first-pass ethanol metabolism in the stomach (27, 42). Because blood ethanol levels are higher in women than in men at equal consumption and ethanol reaches colonic epithelium via the blood circulation, this might imply that at equal intake of alcohol, the colonic epithelium of women is exposed to higher levels of ethanol and acetaldehyde than that of men. However, the differences we found between men and women might also reflect sex-specific differences in alcohol consumption. Among women, 23% were not exposed to alcohol and 19% consumed ≥10 drinks/week, whereas among men, only 11% were nondrinkers and >50% consumed ≥10 drinks/week. Possibly, the difference in adenoma risk is much greater be-

[4] E. W. Tiemersma, D. W. Voskuil, M. Kriege, E. Kampman, unpublished data.

tween no and low exposure to alcohol than the difference between low and medium exposure to alcohol.

Alcohol did not specifically increase adenoma recurrence and other adenoma characteristics. To our knowledge, only Boutron *et al.* (43) found that alcohol consumption specifically increased risk of large adenomas.

We did not find specific types of beverages to be responsible for the observed increase in risk, which is in line with the conclusion of the World Cancer Research Fund expert committee reading that "the effect generally seems to be related to total ethanol intake, irrespective of the type of drink" (44).

The effect of ethanol is probably cocarcinogenic rather than carcinogenic (4, 45). In contrast, its major metabolite acetaldehyde is a probable carcinogen and was found to form adducts, induce DNA cross-links, chromosomal aberrations, and sister chromatid exchanges *in vitro* and inhibit DNA repair enzymes (5). Therefore, we hypothesized that polymorphism of the *ADH3* gene, encoding the principal enzyme oxidizing ethanol to acetaldehyde, would play a role in the association between alcohol and adenomas. We found stronger associations between alcohol consumption and colorectal adenomas in carriers of the *ADH3*1/*1* genotype than in those with other *ADH3* genotypes. However, interaction terms for *ADH3* genotype and alcohol consumption were not statistically significant, possibly because modest gene-environment interactions can only be studied in populations with several thousands of subjects (46). An alternative explanation is that the role of *ADH3* genetic polymorphism might be obscured by ADH production (47) or acetaldehyde production by intestinal microflora (21). Because of these effects, *ADH3* genotype might be especially important among heavy drinkers. In contradiction to our expectations, the potential role of *ADH3* polymorphism did not become more pronounced with high alcohol consumption, *i.e.,* >21 drinks/week. However, our study population only included very few heavy drinkers or alcoholics, and this may have influenced our results. Alternatively, ADH-catalyzed oxidation of ethanol might be less important because other enzymatic systems are upgraded in heavy drinkers (48).

Thus far, *ADH3* polymorphism has not been considered in studies on colorectal adenomas. Other studies with regard to the role of *ADH3* polymorphism in the association between alcohol and neoplasm concerned oropharyngeal, laryngeal, head and neck, and breast cancer (14–20). Three of these seven studies indicated that drinkers with the *ADH3*1/*1* genotype are at higher risk of neoplasm than those carrying *ADH3*1/*2* and *ADH3*2/*2* genotypes (14–16).

We conclude that alcohol consumption elevates the risk of adenomatous colorectal polyps. Although we found no indications for a statistically significant effect modification by *ADH3*, we found that high alcohol consumption most markedly increased the risk of colorectal adenomas in subjects with the *ADH3*1/*1* genotype. These findings need confirmation in larger studies. Our hypothesis that the influence of *ADH3* genotype becomes relevant at high ethanol concentrations should preferably be tested in a large population with higher alcohol consumption. Moreover, exposure of the human colon to ethanol and acetaldehyde and effects of this, as well as the role and impact of ADH synthesis by gastrointestinal bacteria, need to be studied in more detail.

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