Antibodies against 5-Hydroxymethyl-2′-deoxyuridine Are Associated with Lifestyle Factors and *GSTM1* Genotype: A Report from the Malmö Diet and Cancer Cohort[1]

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Abstract

Plasma autoantibodies (aAbs) against the oxidized DNA base derivative 5-hydroxymethyl-2′-deoxyuridine (5-HMdU) are potential biomarkers of cancer risk and oxidative stress. We examined their association with a number of cancer risk factors: smoking, alcohol habits, body fatness, and absence of the glutathione *S*-transferases M1 and T1 (GSTM1 and GSTT1) in a sample from the population-based Malmö Diet and Cancer cohort (Sweden).

This was a cross-sectional study of 264 men and 280 women, 46–67 years of age. Anti-5-HMdU aAb concentration was determined by an ELISA. Data on tobacco exposure were collected through a questionnaire. Alcohol consumption was estimated by a modified diet history method. Body fatness was assessed by a bioimpedance method. The absence or presence of genes coding for GSTM1 and GSTT1 was determined in granulocyte DNA by a multiplex PCR technique.

aAb titers were significantly greater in those with high alcohol consumption. Current smokers lacking GSTM1, particularly men, had greater aAb titers compared with nonsmokers or persons expressing GSTM1. Body fatness was inversely associated with antibody titers in men. *GSTT1* genotype was not associated with aAb titers. Overall, women had higher aAb titers than men. Adjustment for potential confounders (history of chronic diseases, anti-inflammatory medication, and season of blood sampling) did not change the results.

Our study shows that a high alcohol consumption, smoking in combination with lack of GSTM1, and low body fatness (in men) is associated with high titers of anti-5-HMdU aAbs in this population.

Introduction

ROS[3] are normal byproducts of cellular respiration. However, the amounts of ROS in the body are elevated in inflammatory conditions and by tobacco smoking. Also, exposure to many carcinogens and tumor promoters lead to ROS production. ROS in excess of defense systems, *i.e.*, oxidative stress, may damage DNA, proteins, and cellular membranes, and impair DNA repair. Thus, oxidative stress could contribute to cancer, and has been associated with many other diseases and conditions (1–3).

Typical immediate results of oxidative attacks on DNA include the formation of 5-HMdU (4), an oxidized DNA base derivative. It appears that the presence of 5-HMdU in DNA stimulates the production of specific IgM-class aAbs (5). Thus, the titers of these antibodies may constitute a marker of oxidative DNA damage and a biological response to that damage. Anti-5-HMdU aAbs have been shown to be elevated in sera of women who developed cancer of the breast, colon, or rectum within 6 years after the donation of blood samples analyzed in that study (6). The anti-5-HMdU aAb titers in persons who later developed cancer appeared to be increased to a much greater extent than IgM antibodies in general (6). In the same study, antibody titers were also higher in women with a strong family history of breast cancer, with benign breast disease, or with benign gastrointestinal disease, than in controls. Furthermore, the levels of these aAbs were elevated in persons with chronic inflammatory diseases, such as systemic lupus erythematosus and ulcerative colitis (5, 7), which may be predisposing to cancer, whereas they were lowered in patients on systemic anti-inflammatory therapy (5). There is also evidence of increased titers in persons with a history of occupational heavy metal exposures (8). The intraindividual titer variation has been shown to be small (6, 9, 10). Thus, anti-5-HMdU aAb appears to be a promising candidate for a marker of cancer risk associated with oxidative stress.

Genetic factors may influence the redox balance of the body, because several enzyme systems that protect against oxidative stress are at least partly under genetic control. Two of these are the GSTs M1 and T1, which catalyze the conjugation

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[3] The abbreviations used are: ROS, reactive oxygen species; aAb, autoantibody; 5-HMdU, 5-hydroxymethyl-2′-deoxyuridine; GST, glutathione *S*-transferase; MDC, Malmö Diet and Cancer; BF, body fat; TPS, Tween 20 containing PBS; GLM, General Linear Model; CI, confidence interval.

of reactive electrophiles with glutathione (11, 12). About 50% of the Caucasian population are homozygous for the *GSTM1∗0*-allele, leading to total absence of GSTM1. Similarly, GSTT1 absence is found in ~20% of the same population (13). Absence of the GSTs has been associated with an increased risk of many forms of cancer, including lung cancer in smokers (12, 14). Furthermore, absence of GSTM1 in smokers has been associated with greater DNA damage than when GSTM1 is present (15, 16).

By studying the association between biological markers of oxidative stress and potential risk factors of cancer and oxidative stress in relation to enzymatic variations, it should be possible to achieve a better understanding of how environmental and genetic factors contribute to the occurrence of oxidative stress. Thus, the aim of this study was to compare titers of anti-5-HMdU aAbs in groups defined in terms of tobacco exposure, alcohol habits, degree of body fatness, and *GSTM1* and *GSTT1* genotypes in a middle-aged urban population of men and women residing in Malmö, Sweden. A secondary aim was to explore whether the associations between aAb titers and the other factors differed by *GSTM1* or *GSTT1* genotype.

Materials and Methods

Study Population

The MDC study is a prospective, population-based cohort study set in Malmö, the third largest city in Sweden. At the time when the present study was planned (1993), the background population consisted of all men and women born between 1926 and 1945, and living in Malmö (n = 53,325 in 1991; Ref. 17). This population was identified through the Swedish national population registries. From the background population, a random sample of 1000 persons was selected. Because recruitment to the MDC cohort had started in 1991, some individuals from the sample had already joined the study. The other subjects were invited by mail. Several attempts were made to reach each nonresponder by telephone. The only exclusion criterion was inadequate Swedish language skills. Five hundred and forty-four subjects (264 men and 280 women) completed the parts of the baseline examination relevant to this study (questionnaire and blood sampling). The data were collected during 1991–1994. The Ethics Committee at Lund University has approved the design of the MDC study.

Data Collection

The study subjects visited the MDC study center twice. At the first visit project staff provided information on the background and aim of the project, and detailed instructions about the lifestyle questionnaire and the other procedures of the study, including dietary data. Blood samples from nonfasting subjects were drawn and processed. At the second visit, the lifestyle questionnaires were checked for incomplete answers, and a dietary interview was performed.

Questionnaire Data

A structured multiple-choice questionnaire collected information on smoking status, alcohol habits, and other factors. The subjects defined themselves as being regular, occasional, former, or never-smokers. In this study, we combined the regular and occasional smokers into a current smoker category. The current smokers recorded the amount of tobacco smoked per day (cigarettes, cigars, or grams of pipe tobacco). The total amount was calculated and expressed in grams. Five occasional smokers did not give information on amount of smoking. They were coded as smoking 5 cigarettes/day, which was the median amount smoked by the other occasional smokers in the sample. The current smokers were initially divided into light (≤10 g tobacco/day) and heavy (>10 g tobacco/day) smokers.

Alcohol intake was mainly assessed from the 7-day registration of the modified diet history method (18, 19). Those subjects who reported zero consumption during the registration, and who in the questionnaire indicated no alcohol consumption during the previous 30 days, were categorized as zero consumers. The other subjects were categorized according to an assumption of biological risk (20). Category ranges were for men/women as follows: <20 g/<15 g alcohol/day (low), 20–40 g/15–30 g (medium), and >40 g/>30 g (high). Thus, the information on alcohol consumption was collapsed into a four-category variable. The relative validity of alcohol consumption (unadjusted for energy intake) in the MDC study was 0.76 for men and 0.82 in women (19).

Information on birth country, education, socioeconomic status, and physical activity was collected by the same questionnaire, as described previously (21).

Information on cancer diagnoses (if any) of the subjects before baseline examination was obtained by record linkage with the Swedish Cancer Registry and the Southern Swedish Regional Cancer Registry. The diagnoses include squamous cell carcinomas but not basal cell carcinomas. Information on regular use of antidiabetic, antilipaemic, or anti-inflammatory (nonsteroidal anti-inflammatory drugs, acetylsalicylic acid, or corticosteroids) medication was taken from the questionnaire. History of rheumatoid arthritis, chronic inflammatory bowel disease, and diabetes mellitus was collected by the questionnaire.

Bioimpedance Analysis

Bioelectric impedance analysis was used for estimating body composition according to procedures provided by the manufacturer (BIA 103; RJL-Systems, Detroit, MI; single-frequency analyzer). The algorithm used to estimate BF from impedance was supplied by the manufacturer, but the results were highly correlated to results obtained with published algorithms (22). Estimated BF was used to calculate %BF. We chose %BF rather than body mass index as the measure of body fatness, because body mass index does not discriminate between muscle mass and fat mass.

Blood Sample Analyses

The blood samples were processed and separated within 1 h of drawing, as described previously (23). The plasma and granulocyte samples were stored at −80°C until analysis. The quality of the stored samples was satisfactory, as described elsewhere (24).

Analysis of Sera for the Presence of anti-HMdU aAbs. Coded plasma samples from study subjects were assayed by ELISA as described previously (5). Briefly, wells of half of the 96-well plates were coated with 10 μg/ml HMdU-BSA conjugate [2 μg of antigen (20 pmol HMdU)/well (200 μl)] and the other half with 10 μg/ml mock-BSA conjugate, sealed with a plastic tape, and incubated for 3 days at 4°C. After emptying, wells were washed three times with TPS and then blocked with 10 μg/ml BSA for 24 h.

Antigen-coated wells were incubated (37°C for 2 h) with human plasma diluted initially 1×10^4-fold with PBS containing 0.1% of BSA. After incubation with the diluted plasma, wells were washed three times with TPS, treated with a 1:1000

Table 1 Geometric mean concentration of anti-5-HMdU aAbs (A_{492}/μl plasma) by alcohol habits, smoking habits, *GST* genotypes, and body fatness in middle-aged men (n = 264) and women (n = 280), Malmö, Sweden

Analysis of variance or t-test, except where otherwise noted.

Variable	Men	95% CI	*n*	Women	95% CI	*n*
Age group						
46–50	11.3	8.7–14.7	49	20.9	16.2–26.9	55
51–55	7.6	6.0–9.6	56	13.3	10.7–16.6	67
56–60	9.0	7.3–11.0	64	14.1	11.7–17.0	67
61–65	7.2	6.1–8.5	71	12.3	10.1–15.0	72
66–67	7.4	5.0–11.0	24	17.6	11.3–27.3	19
P for linear trend, continuous	0.015		264	0.042		280
Alcohol habits[a]						
None	9.9	7.2–13.7	25	12.5	8.8–17.9	36
Low	7.7	6.9–8.7	162	15.0	13.3–17.0	194
Medium	8.5	6.6–10.9	58	13.4	10.4–17.2	38
High[b]	15.3	9.1–25.8	17	29.4	19.7–44.0	10
P	0.009		262	0.046		278
Smoking status						
Never-smokers	8.6	7.2–10.4	76	13.8	12.1–15.8	133
Ex-smokers	8.2	7.0–9.6	94	15.2	12.3–18.7	75
Smokers, 1–10 cigarettes	9.9	7.5–13.0	49	17.0	11.7–24.8	31
Smokers, >10 cigarettes	6.9	5.2–9.1	45	15.4	11.2–21.2	41
P	0.22		264	0.64		280
GSTM1						
Yes	8.1	6.8–9.6	106	14.4	12.3–17.0	122
No	8.6	7.5–9.8	155	15.0	13.1–17.2	156
P	0.58		261	0.72		278
GSTT1						
Yes	8.2	7.4–9.2	231	14.9	13.3–16.8	230
No	9.5	7.0–13.0	30	14.0	11.0–17.8	48
P	0.38		261	0.64		278
Percentage BF (quartiles)						
1 (lowest)	10.7	8.8–13.0	64	16.4	13.6–19.7	70
2	8.5	6.6–10.9	65	15.7	13.0–18.9	70
3	7.7	6.3–9.4	66	13.3	10.8–16.4	71
4 (highest)	7.1	5.9–8.5	65	13.7	10.7–17.6	69
P for linear trend, continuous	0.007		260	0.09		280
Overall mean	8.4	7.5–9.3	264	14.7	13.3–16.3	280

[a] See Methods for cut-off values.
[b] Men: Mean intake 66 g/d (range 40–162). Women: Mean intake 40 g/d (range 33–58).

goat antihuman IgM (Sigma Chemical Co., St. Louis, MO) having horseradish peroxidase covalently coupled to the antibody, incubated 1 h, and washed with TPS again. *O*-Phenylenediamine was used as a substrate for H_2O_2 oxidation mediated by horseradish peroxidase bound to the wells through the antihuman IgM antibody. The development of a yellow color (stabilized within 0.5 h at room temperature), measured at acid pH at 492 nm in an ELISA plate reader (Anthos 2000), is proportional in its intensity to the amount of human aAb bound to the coated plates. The results are presented as net mean aAb titers calculated as A_{492}/μl undiluted plasma with 95% CIs, after subtracting nonspecific binding to mock-BSA-coated wells (5–10% of the total binding to HMdU-BSA-coated wells) analyzed at the same plasma dilutions on the same plates. Only plasma dilutions giving A_{492} values within the linear region of the response of the ELISA plate reader were used in the calculation of mean aAb titers. The expression of anti-HMdU aAb levels as A_{492}/μl undiluted plasma incorporates dilution factors presented commonly as antibody titers, and for this reason, A_{492}/μl undiluted plasma is referred to as aAb titer. Such expression also allows for more facile tabular and graphic presentation of results, as well as for comparisons among different populations.

Coded samples were analyzed at least three separate times on different plates, with each section of the plate also containing three negative and three positive controls. This assay is not only sensitive but also very reproducible with a coefficient of variation for repeated measurements during the course of 1 year of 3.1% (10, 25). Use of positive controls on each microtiter plate provided plate factors, which were used to control for batch effects. Plate factors were calculated as the ratio between the net (after subtracting nonspecific binding to mock-BSA-coated wells) expected standard and experimental values. The standard values were based on determinations of the positive control (in triplicates) on numerous separate plates, whereas experimental values were those obtained on the same plates as other (unknown) analyzed samples. Use of plate factors permits obtaining reliable data on many samples analyzed over long periods of time. Only after completion of the biochemical determinations were samples decoded and statistically analyzed. The anti-5-HMdU antibody analyses were performed during 1996–1997.

Analysis of *GST* Genotype. DNA from granulocytes was extracted and analyzed for *GST* genotype by Professional Genetics Laboratory AB (Uppsala, Sweden) using a multiplex PCR method, modified from Brockmöller *et al.* (26) and Pemble *et al.* (27).

Table 2 Geometric mean concentrations of anti-5-HMdU aAbs (A_{492}/μl plasma) by selected background factors in middle-aged men ($n = 264$) and women ($n = 280$), Malmö, Sweden

Analysis of variance or *t* test.

Variable	Men	95% CI	*n*	Women	95% CI	*n*
History of chronic disease[a]						
No	8.6	7.7–9.7	222	14.7	13.2–16.4	234
Yes	7.1	5.7–8.8	42	14.7	10.9–19.8	46
P	0.18		264	1.00		280
Regular use of anti-inflammatory drugs						
No	8.3	7.5–9.3	243	14.5	13.1–16.2	264
Yes	8.6	6.6–11.2	21	18.1	10.5–31.1	16
P	0.87		264	0.33		280
Season of blood sampling						
Winter (Dec–Feb)	7.7	5.6–10.6	30	16.3	12.3–21.7	38
Spring (Mar–May)	9.3	7.7–11.1	83	16.0	13.7–18.7	85
Summer (Jun–Aug)	7.9	6.7–9.4	88	10.8	8.7–13.4	76
Fall (Sep–Nov)	8.1	6.5–10.2	63	17.2	14.0–21.0	81
P	0.64		264	0.004		280

[a] Includes history of cancer, rheumatoid arthritis, chronic inflammatory bowel disease, diabetes mellitus, or two signs of the metabolic syndrome (see Methods).

Variable Selection

Explanatory Variables. The explanatory variables were age (age groups were used in the Tables for illustrative purposes), alcohol habits (nondrinkers, low-, medium-, and high-consumers), smoking status (never-smokers, ex-smokers, light smokers, and heavy smokers), %BF, *GSTM1* genotype, and *GSTT1* genotype.

Background Variables. We selected a number of background variables to try to clarify the associations between the hypothesized factors and aAb titers. A number of chronic diseases have been associated with altered levels of anti-5-HMdU aAbs, including rheumatoid arthritis, systemic lupus erythematosus, and several forms of cancer (5–7, 28). Because each of these patient groups was small in this study, we created a combined disease variable. Persons who stated having rheumatoid arthritis, chronic inflammatory bowel disease, or a history of cancer (past or current) were defined as having a chronic disease. We also attempted to include persons who might have diabetes or the metabolic syndrome, because insulin resistance and abnormal glucose metabolism have been associated with several indices of oxidative stress, even in nondiabetic individuals (29). Thus, we included in our disease definition those who reported having diabetes mellitus or used antidiabetic drugs. We also included nondiabetic persons with waist circumferences above 93 cm (36.6 inches; men) and 79 cm (31.1 inches; women) who also used lipid-lowering drugs. This latter part of the definition was taken from the criteria of the European Group for the Study of Insulin Resistance, developed for use in epidemiological studies (30). We have not strictly adhered to the European Group for the Study of Insulin Resistance definition, because we did not assess insulin resistance or fasting hyperinsulinaemia.

Regular use of anti-inflammatory medication might alter aAb titers and was, therefore, included among the background variables.

Finally, season was included, because dietary intake, which could alter oxidative balance significantly, may vary substantially across seasons.

Statistical Methods

Primary Analyses. Men and women were analyzed separately throughout. We examined the differences (or linear trends) in antibody titers between groups based on age, tobacco exposure, alcohol habits, the absence or presence of the *GSTM1* and *GSTT1* genes, %BF, history of certain diseases (as defined above), regular use of anti-inflammatory drugs, and season of examination. The ANOVA or *t* test procedures were used. The distributions of antibody titers were skewed positively; they were therefore ln-transformed in all of the analyses.

We performed multivariate analyses (GLM procedure) to take into account the possibility of covariation among variables. 5-HMdU aAb was the dependent variable, and age, tobacco exposure, alcohol habits, %BF, and *GSTM1* and *GSTT1* genotypes were explanatory variables. The bivariate analyses revealed what appeared to be threshold values in the distribution of aAb titers by categories of alcohol habits and age. Therefore, several categories of these variables were merged, creating two dichotomous variables. Similarly, the four smoking categories were merged into two to increase statistical power and decrease the effects of misclassification between light and heavy smokers. Data from 257 men and 277 women were available for multivariate analysis. Adjusted geometric means were calculated by the Estimated Marginal Means option of the GLM procedure.

All of the statistical tests were two-sided and were performed with SPSS for Windows, version 10.0.7 (SPSS Inc., Chicago, IL). Ps ≤ 0.05 were considered significant.

Exploratory Analyses. A number of potential interactions were also evaluated, namely all of the two-way interactions between *GSTM1* and *GSTT1* genotypes and the other explanatory variables, plus an alcohol/smoking interaction term (10 terms for each sex). Each interaction term was added individually to the multivariate model. They were considered eligible for additional analyses if the P was <0.20. These remaining interaction terms were entered simultaneously into the multivariate model, and removed, one at a time, if the P associated with them was >0.10 (backward stepwise procedure).

To assess confounding, we also evaluated a number of other variables in the multivariate analyses. These were selected as follows. Each potentially confounding variable (history of disease, regular use of anti-inflammatory drugs, and season) was entered individually into the multivariate model, together with the explanatory variables and the selected interaction terms. Potential confounders were selected by the same inclusion/exclusion criteria as the interaction terms (see above).

Table 3 Adjusted geometric mean anti-5-HMdU aAb titers by age, smoking/GSTM1 interaction, alcohol/smoking interaction, and body fatness in middle-aged men (n = 257), Malmö, Sweden

All of the variables were adjusted for each other (GLM procedure).

Variable	n	Adjusted mean	95% CI	n	Adjusted mean	95% CI
Age						
46–50	49	12.9	9.8–17.1			
51–67	208	9.8	8.0–12.1			
P for difference		0.034				
Smoking status						
Nonsmokers	63[a]	10.1[a]	7.0–14.6[a]	104[b]	8.3[b]	11.1–16.4[b]
Current smokers	42[a]	11.9[a]	8.6–16.4[a]	48[b]	16.2[b]	11.8–22.1[b]
P for interaction				0.018		
Smoking status						
Nonsmokers	160[c]	9.6[c]	8.3–11.1[c]	7[d]	8.8[d]	4.8–16.0[d]
Current smokers	80[c]	7.8[c]	6.4–9.5[c]	10[d]	24.7[d]	15.0–40.6[d]
P for interaction				0.003		
Percentage BF (quartiles)						
1 (lowest)	64	13.2	10.0–17.2			
2	65	11.4	8.7–14.9			
3	64	10.7	8.1–14.0			
4 (highest)	64	9.4	7.1–12.6			
P for linear trend, continuous		0.017				
Adjusted R^2 (explanatory value)	0.108[e]					

[a] GSTM: Yes.
[b] GSTM: No.
[c] Alcohol habits: none-medium.
[d] Alcohol habits: high.
[e] 257.

Results

Primary Analyses

Bivariate Results. The study participants are described in Tables 1 and 2, which also list their anti-5-HMdU antibody titers stratified by various background factors. We noted that women had higher aAb titers than men overall (geometric means 14.7 *versus* 8.4; $P < 0.001$). There was a negative linear association between aAb titers and age, both in men and women. aAb titers were higher at a "high" alcohol intake (>40 g alcohol/day in men and >30 g/day in women) than at lower intakes in both men and women.

There were no significant differences in aAb titers by amount of smoking, nor were there any differences between those with and those without GSTM1 or GSTT1 (Table 1). There was an inverse association between degree of body fatness and aAb titer in men ($P = 0.007$). We observed a similar association in women, but it was not statistically significant ($P = 0.09$).

There were no significant differences by history of serious diseases (Table 2). We noted a seasonal difference in women: the titers in summer were significantly lower than in the other seasons ($P = 0.004$), but there was no difference in men. There were no statistically significant differences in aAb titers by education, socioeconomic status, cohabitation status, and leisure time physical activity (data not shown).

Basic Multivariate Models. The basic multivariate analyses featured the same variables as were listed in Table 1, but some of the variable categories were merged, as described in the "Statistical Methods and Design" section. This analysis mostly confirmed the bivariate results, indicating independent associations between aAb titers and age, and alcohol habits, in men and women, and %BF in men (data not shown).

Exploratory Analyses

Evaluation of Interactions. The interaction between smoking status and alcohol habits, plus all of the two-way interaction terms between *GSTM1* and *GSTT1*, and the other explanatory variables, were evaluated, one at a time, with adjustment for all of the explanatory variables. Those eligible for additional evaluation were among men smoking status/*GSTM1* ($P = 0.018$), alcohol habits/*GSTT1* ($P = 0.18$), and smoking status/alcohol habits ($P = 0.002$). In women, the only eligible interaction was smoking status/*GSTM1* ($P = 0.068$). In a second step, these interactions were all added to the basic multivariate models. They were then removed, one at a time, if the *P*s associated with them were >0.10. The resulting models are shown in Tables 3 and 4. Because there was no significant difference in aAb titers by *GSTT1* status, either alone or in combination with other variables, we decided to exclude *GSTT1* status from additional analysis.

Comprehensive Models. Table 3 shows the male multivariate model with added interaction terms. This analysis revealed two statistically significant interactions in men: one between smoking and *GSTM1* genotype, and one between smoking and alcohol habits. In male smokers expressing GSTM1, the antibody titers were similar regardless of smoking status, but in *GSTM1*-negative men, the titers were higher among smokers compared with nonsmokers (P for interaction = 0.018). Heavily drinking smokers had higher titers than all of the other combinations of smoking and drinking (P for interaction = 0.003). As noted above, there was no interaction between alcohol habits and *GSTM1* status. Furthermore, there was no significant three-way interaction among alcohol habits, *GSTM1* status, and smoking status (data not shown).

Table 4 shows the corresponding female model. This model yet again confirmed the bivariate results. However,

Table 4 Adjusted geometric mean anti-5-HMdU aAb titers by age, alcohol habits, smoking/GSTM1 interaction, and body fatness in middle-aged women (n = 277), Malmö, Sweden

All of the variables were adjusted for each other (GLM procedure).

Variable	n	Adjusted mean	95% CI	n	Adjusted mean	95% CI
Age group						
46–50	55	27.8	19.8–38.9			
51–67	222	18.3	13.8–24.3			
P		0.002				
Alcohol habits[a]						
None-medium	267	16.7	14.5–19.3			
High	10	30.4	17.7–52.2			
P		0.034				
Smoking status						
Nonsmokers	87[b]	22.6[b]	16.2–31.5[b]	119[c]	21.1[c]	15.5–28.6[c]
Current smokers	34[b]	19.4[b]	13.1–28.7[b]	37[c]	27.9[c]	19.5–40.1[c]
P for interaction		0.070				
Percentage BF (quartiles)						
1 (lowest)	70	23.4	17.0–32.3			
2	69	24.7	17.6–34.6			
3	70	21.5	15.2–30.4			
4 (highest)	68	21.1	15.0–29.6			
P for linear trend, continuous		0.24				
Adjusted R^2 (explanatory value)	0.056[d]					

[a] See Methods for cut-off values.
[b] GSTM1: Yes.
[c] GSTM1: No.
[d] 277.

similarly to the male model, it also suggested an interaction between smoking and *GSTM1* status (P = 0.070), with the highest titers once again occurring among current smokers who did not express GSTM1. We observed no significant interaction between smoking status and alcohol habits in women.

Evaluation of Possible Confounders. As described above, we evaluated potential confounding factors by entering the variables from Table 2 one at a time into the models described in Tables 3 and 4. The only variable of reasonable statistical significance was season in women (P = 0.003).

When season was added to the female model (data not shown), the bivariate results (Table 2) were confirmed: women who were examined during the summer had significantly lower aAb titers than women examined during spring or fall. Apart from this, the final model was very similar to the model shown in Table 4. The adjusted R^2 value for the female model increased from 0.056 to 0.083. This indicates that season was independently associated with aAb titers.

Discussion

This population-based study of middle-age people showed that high alcohol consumption was associated with higher titers of anti-5-HMdU aAbs in both men and women. Current smoking was associated with higher aAb titers in subjects lacking GSTM1. In men, the high aAb titers associated with high alcohol consumption appeared in current smokers only. Body fatness was associated with lower aAb titers, particularly in men. *GSTT1* genotype was not associated with aAb titers.

The lack of association between smoking status and aAb titers may appear surprising, but is in agreement with two previous studies (9, 31). However, the study by Hu *et al.* (31) was small, and the degree of exposure to tobacco was high in the study by Mooney *et al.* (Ref. 9; no subject smoked <20 cigarettes/day). In our study, the exposure to tobacco was low: only around half of the current smokers smoked >10 g of tobacco, corresponding to 10 cigarettes/day. Thus, none of the three studies may have been optimally suited to study the smoking association. However, the power of this study should have been sufficient to detect even minor differences between the groups. Furthermore, it is common for studies of markers of oxidative stress not to show associations between smoking and the markers (*e.g.* Ref. 29 and references therein).

Antibody titers were greater in men and women with high alcohol consumption than in persons with lower consumption. This is in accordance with studies in animals and humans, indicating that ethanol induces oxidative stress, particularly in the liver (32), although it would perhaps be premature to claim a role for ethanol in oxidative stress in other parts of the body. In the study by Hu *et al.* (31), aAb titers at baseline were *lower* in persons with consumption above the median consumption of 21 g/day (0.75 ounces/day). However, that result was probably because almost all of the males in that study were in the high-consumer group, and it is clear from the data published in their report that men had lower titers than women overall (P = 0.004, Mann-Whitney test).

This is the first study to show an association between anti-5-HMdU aAb titers and body fatness: antibody titers were lower in obese men than in lean men. This is consistent with an impaired immune response in obesity (33, 34), but alterations in DNA damage and/or repair rates in obesity are other possibilities (35).

Women had higher aAb titers than men overall; this was also true regardless of the other studied factors. This observation is in agreement with the recent work on heavy smokers by Mooney *et al.* (9). The reason for this difference is unknown, but may be related to a difference in immune responsiveness to 5-HMdU between men and women.

We observed the highest aAb titers in the youngest age groups of both men and women. Prior studies and unpublished data from the authors have shown an age-dependent decline in

titers in patients with inflammatory diseases (5), in healthy female controls (including some with family history of cancer or benign breast disease; Ref. 6), and in nonsmoking women.[4] Mooney *et al.* (9) noted higher titers in women <50 years than in women >50 years old and suggested that it was because of these women being premenopausal (9). It was not possible to test this hypothesis properly in this study, because no women in the present sample were younger than 46 years old, and should, thus, probably be regarded either as post- or peri-menopausal. Our findings would rather suggest an age-related mechanism, because the results were similar in both men and women (Tables 3 and 4). Furthermore, the results were the same in subjects with and without history of disease (data not shown).

It was shown previously that a generalized activation of the immune system (*e.g.*, inflammatory diseases) might elevate aAb titers to some extent (5, 7), but subsequent research showed that anti-5-HMdU aAbs were elevated to a much larger extent than were total IgM titers in persons at risk of breast cancer (6). Furthermore, in the present study, adjustment for subjects reporting chronic inflammatory bowel disease, diabetes, rheumatoid arthritis, or having a history of cancer and/or signs of the metabolic syndrome did not alter the results. Thus, these aAbs are not likely to be a generalized marker of immune function, although they could be a marker of immune responsiveness to oxidative stress. It may also be noted that levels of 5-HMdU have been shown to be increased significantly in WBC DNA of women at risk of breast cancer and of women with breast cancer (36, 37).

The present study showed no obvious effect of chronic diseases on aAb titers, which is in contrast to previous studies (5–7). Although there is only little evidence that these conditions will influence aAb titers in the same way (if at all), we still attempted to capture at least some of the expected variation by creating the combined disease variable, because all groups of subjects with a history of a certain condition were too small to evaluate. Hence, this variable may be a blunt instrument by design. Furthermore, and perhaps more importantly, we do not know in what state and stage of disease the person was in at the time of the blood sampling.

Each individual was only sampled once in the present study. If the within-individual variation in aAb titers had been substantial compared with the between-individual variation, it would have meant low power to detect any present associations. However, the within-individual variation has been shown previously to be low, particularly in relation to the between-individual variation (6, 10, 25, 38). Thus, repeated aAb measurements would probably not have altered the results.

One could question the specificity of the aAb assay, because similar antigens could cross-react with anti-HMdU aAbs. This was shown to be the case, to some extent, with BSA-coupled thymidine (5). However, it is less likely that 5-hydroxy-2′-deoxyuridine, if formed, would persist in DNA, because uracil glycosylase is such an efficient and ubiquitous enzyme, and would remove it from the DNA.

Men and women who smoked and who did not express GSTM1 had greater aAb titers than *GSTM1*-negative nonsmokers. In men, these titers were also greater than in *GSTM1*-positive subjects, regardless of smoking status. This provides some support to the hypothesis that GSTM1 protects against the oxidative effects of smoking (15, 39–41).

Because of the small numbers of participants who did not express GSTT1, we cannot entirely exclude an effect of *GSTT1* genotype. However, there is little in these data that suggests an important role of GSTT1 in determining titer of anti-5-HMdU aAbs.

Heavy drinking was associated with increased aAb titers in both men and women, although those titers appeared to be limited to current smokers among men. This interaction was difficult to evaluate among women, because of small numbers of subjects. However, the available data did not suggest the presence of this interaction in women. Instead, our findings suggest that alcohol drinking itself might be able to increase aAb titers. In contrast, the increased aAb titers in current smokers was only evident in *GSTM1*-negative subjects. If these associations were causal, they would imply that alcohol and tobacco use different pathways for increasing aAb titers.

Although the results were rather similar in men and women, they should be interpreted with caution, both because of the many statistical tests we report in the exploratory analyses, and because of the small numbers of subjects in certain categories, *e.g.*, high alcohol consumption. This was also true of the interactions among GSTM1 status, smoking, and alcohol habits. On the other hand, the results appeared fairly robust. For example, very similar results were obtained by nonparametric tests (data not shown).

Considering previous observations in the MDC study cohort, we believe it is possible that many of the observed associations, particularly those among aAbs and smoking, body fatness, age, and season, may be confounded by diet, *e.g.*, vegetable and fruit consumption (21). An analysis of the associations between dietary factors and anti-HMdU aAbs in the present population is in progress.

Although the results should be interpreted with caution, this study shows that high alcohol consumption, and smoking in combination with lack of GSTM1, is associated with greater titers of anti-5-HMdU aAbs in both men and women in this middle-aged, Scandinavian, general population. Furthermore, body fatness is associated with lower titers, at least in men.

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