

Alcohol consumption and type 2 diabetes: influence of genetic variation in alcohol dehydrogenase

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Abstract

Objective: To investigate whether a polymorphism in the alcohol dehydrogenase 1c (*ADH1C*) gene modifies the association between alcohol consumption and type 2 diabetes.

Research Design and Methods: In nested case-control studies of 640 women with incident diabetes and 1000 controls from the Nurses' Health Study, and 383 men with incident diabetes and 382 controls from the Health Professionals Follow-up Study, we determined associations between the *ADH1C* polymorphism, alcohol consumption and diabetes risk.

Results: Moderate to heavier alcohol consumption (>5 g/day for women and >10 g/day for men) was associated with a decreased risk of diabetes among women (odds ratio (OR): 0.45; 95%-CI: 0.33-0.63), but not men (OR: 1.08; 0.67-1.75). *ADH1C* genotype modified the relation between alcohol consumption and diabetes for women ($p_{\text{interaction}} = 0.02$). The number of *ADH1C*2* alleles, related to a slower rate of ethanol oxidation, attenuated the lower risk of diabetes among women consuming ≥ 5 g alcohol/day ($p_{\text{trend}} = 0.002$). These results were not significant among men. Results were similar in pooled analyses ($p_{\text{interaction}} = 0.02$) with OR's for diabetes among moderate drinkers of 0.44 (0.21-0.94) among *ADH1C*1* homozygotes, 0.65 (0.39-1.06) for heterozygotes and 0.78 (0.50-1.22) for *ADH1C*2* homozygotes compared to *ADH1C*1* homozygote abstainers ($p_{\text{trend}} = 0.02$).

Conclusions: *ADH1C* genotype modifies the association between alcohol consumption and diabetes. The *ADH1C*2* allele, related to a slower oxidation rate, attenuates the lower diabetes risk among moderate to heavier drinkers. This suggests that the association between alcohol consumption and diabetes may be causal but mediated by downstream metabolites such as acetate rather than ethanol itself.

Moderate alcohol consumption is associated with a decreased risk of type 2 diabetes compared to abstinence (1;2). Moreover, randomized controlled trials have shown that moderate drinking improves insulin sensitivity (3;4), suggesting that this relation may be causal and due to the effect of ethanol or its metabolites.

Ethanol, when consumed in moderation, is oxidized to acetaldehyde and acetate by alcohol dehydrogenase (*ADH*) and aldehyde dehydrogenase (*ALDH*) (5). Four genes of the *ADH* family, coding for class I (*ADH1A-C*) and class II (*ADH2*) enzymes, are involved in hepatic metabolism of ethanol (6). At the *ADH1B* locus, a functional polymorphism occurs exhibiting a 30-40-fold difference in V_{\max} for ethanol oxidation, but the variant allele is rare among Caucasians (< 5%) (7).

At the *ADH1C* locus, two polymorphisms occur at amino acids 271 and 349, that are in nearly complete linkage disequilibrium (8;9). The 271Arg/349Ile allele is frequently designated as $\gamma 1$ or *ADH1C*1* and the 271Gln-349Val as $\gamma 2$ or *ADH1C*2* (10). The $\gamma 1$ subunit has a 2.5-fold higher V_{\max} for ethanol oxidation than $\gamma 2$, and has a prevalence of 50-60% among Caucasian populations (7;10). Although the extent to which this polymorphism affects blood alcohol concentrations remains unclear (11), slower ethanol oxidation is thought to increase hepatic exposure to ethanol. Thus, this polymorphism could modify the association of alcohol consumption with disease risk, but with an effect anticipated only among drinkers. Indeed, such an interaction between alcohol consumption and *ADH1C* genotype has been observed in some, but not all (8;12), studies of coronary heart disease and levels of HDL cholesterol (13-15).

Whether this interaction is also present for type 2 diabetes has not been studied to date. The finding that *ADH1C* genotype modifies the association of alcohol intake with diabetes would support the hypothesis that the association is causal,

because genotypes are distributed randomly and hence mimic the random assignment of alcohol exposure that would occur in a randomized trial (sometimes referred to as mendelian randomization) (16;17). Therefore, we investigated the interaction between *ADH1C* and alcohol consumption on risk for type 2 diabetes in nested case-control studies from the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS). Moderate drinking has previously been associated with lower risk of diabetes in the overall populations of both cohorts (18-21).

Research Design and Methods

Study Participants

The NHS began in 1976, when 121,700 female nurses aged 30 to 55 years responded to a questionnaire of health-related information. Questionnaires have been administered biennially to update health information and identify new cases of disease. During 1989-1990, 32,826 women free of diagnosed diabetes, coronary heart disease, stroke, or cancer provided blood samples. Women providing blood samples had higher prevalence of obesity and family history of diabetes and a lower prevalence of current smoking, but were otherwise similar to women not providing blood. By 2000, 678 of these women had a confirmed diagnosis of type 2 diabetes. Controls providing blood samples were matched to diabetes cases by year of birth, date of blood draw, race, and fasting status at blood draw. From 1990 until 1996, 2 controls were matched to each case based on the above factors. One of the two control subjects was also matched according to body-mass index (BMI) within 1 kg/m². After 1996, one control was matched to each case based on the same characteristics, and another control was matched on these characteristics and BMI to each of the cases in the top decile of the BMI distribution. Non-Caucasian women were excluded, leaving 640 cases and 1000 controls for analysis. The selection

of cases and controls for both studies is depicted in table 1.

The HPFS began in 1986, when 51,529 male health professionals 40 to 75 years of age completed the initial questionnaire. Biennial follow-up has mirrored the NHS. During 1993-1994, 18,225 participants of the HPFS provided a blood sample. Characteristics of participants providing blood samples were similar to other HPFS participants. By 2002, 431 incident cases of type 2 diabetes, free from cardiovascular disease or cancer (except nonmelanoma skin cancer) at baseline, were confirmed. One control was matched per case by year of birth, month of blood collection, and fasting status at blood draw using the risk-set sampling strategy (22). We excluded non-Caucasian men, leaving 383 cases and 382 controls for analysis.

Subjects provided written informed consent, and the studies were approved by the institutional review board of Partners HealthCare System, Boston, Mass.

Ascertainment of Diabetes

Incident cases of type 2 diabetes were identified by self-report and confirmed by a validated supplementary questionnaire detailing symptoms, diagnostic laboratory test results, and diabetes treatment. The diagnosis was confirmed if participants reported at least 1 of the following on the questionnaire: treatment with either insulin or an oral hypoglycemic agent, at least 1 classic symptom of diabetes (for instance, polyuria, polydipsia, weight loss) plus elevated plasma glucose level, or an elevated plasma glucose level on at least on 2 occasions in the absence of symptoms. Elevated plasma glucose was defined as at least 140 mg/dL (≥ 7.8 mmol/L) fasting, or at least 200 mg/dL (≥ 11.1 mmol/L) nonfasting, or at least 200 mg/dL (≥ 11.1 mmol/L) at ≥ 2 hours after an oral glucose tolerance test for cases diagnosed before 1998; for cases diagnosed in 1998 and later, the fasting plasma glucose threshold was lowered to ≥ 126 mg/dL (≥ 7.0 mmol/L) (23). The validity of self-reported diabetes

has been confirmed with medical record review in a sample (18;24).

Assessment of alcohol consumption

We assessed average alcohol consumption within a semi-quantitative food-frequency questionnaire (25) including separate items for beer, white wine, red wine and liquor. We specified standard portions as a glass, bottle, or can of beer; a 4-ounce glass of wine; and a shot of liquor. For each beverage participants were asked to estimate their average consumption over the past year. We calculated ethanol intake by multiplying the frequency of consumption of each beverage by the alcohol content of the specified portion size (12.8 g for beer, 11.0 g for wine, and 14.0 g for liquor) and summing across beverages (26). We used alcohol consumption reported on the food frequency questionnaire in 1990 for women and 1994 for men and replaced information with data from 1986 onwards in case of missing data for alcohol consumption. In 1988, men and women also reported the number of days per week that they typically drank any form of alcohol.

We previously assessed the validity of alcohol consumption estimated with the food-frequency questionnaire against intake from two 1-week dietary records collected approximately 6 months apart among 173 women and 136 men residing in eastern Massachusetts; the Spearman correlation coefficient between these 2 measures was 0.90 for women and 0.86 for men (27).

Assessment of lifestyle factors

Lifestyle factors were assessed using questionnaires, including smoking, body weight, physical activity, family history of diabetes, menopausal status, and use or nonuse of postmenopausal hormone therapy. Reported weights have been shown to correlate well with measured weights ($r=0.96$) (28) and the assessment of physical activity was previously validated (29). We obtained energy intake, glycemic load, coffee consumption and energy-adjusted intakes of saturated fat, *trans* fatty acids,

polyunsaturated fatty acids, and dietary fiber from the semi-quantitative food frequency questionnaire (30).

Laboratory procedures

All samples were genotyped using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA), in 384-well format. The PCR amplifications were carried out on a minimum of 5-20ng DNA using 1 X TaqMan® universal PCR master mix (No Amp-erase UNG). Amplification conditions on a AB 9700 dual plate thermal cycle (Applied Biosystems, Foster City, CA) were as follows: 1 cycle of 95°C for 10min, followed by 50 cycles of 92°C for 15s and 60°C for 1 min. TaqMan® primers and probes were designed using the Primer Express® Oligo Design software v2.0 (ABI PRISM) and are available on request. Genotyping was successful in 94% of women and 96% of men (table 1). Both for the NHS and HPFS 21 quality control sets were genotyped with no discordant pairs detected.

Biomarkers related to diabetes were assessed only among women. Insulin levels were measured using a double antibody system with less than 0.2% cross-reactivity between insulin and its precursors (Linco Research, St Louis, Mo). Hemoglobin A_{1c} was measured by immunoassay (Roche Diagnostics, Indianapolis, Ind). The coefficients of variation were 3.5% to 11.7% for fasting insulin and 1.9% to 3.0% for hemoglobin A_{1c} (HbA_{1c}).

Statistical analysis

We used a chi-square test to determine whether *ADHIC* genotypes were in Hardy-Weinberg equilibrium. Logistic regression adjusted for matching factors was used to estimate odds ratios (and 95% confidence intervals) for type 2 diabetes based on alcohol consumption and *ADHIC* genotypes (*ADHIC**1/*1, *ADHIC**1/*2, *ADHIC**2/*2). We simultaneously adjusted for BMI (quintiles) and for smoking (never-, former- or current smokers of 1-14, 15-34 or

≥35 cigarettes/day), family history of diabetes in a first degree relative (present or not), physical activity (5 categories), postmenopausal hormone therapy (premenopausal, never-, past- or current user), energy intake, coffee consumption and energy-adjusted intakes of saturated fat, *trans* fatty acids, polyunsaturated fatty acids, glycemic load and dietary fiber (each in quintiles for women and tertiles for men).

We first examined independent associations of alcohol consumption and *ADHIC* genotype with type 2 diabetes risk. Second, their interaction on type 2 diabetes risk (the primary test of our hypothesis) was assessed, and we examined the main effect of *ADHIC* genotype within strata of alcohol consumption (0, <5 and ≥ 5 g/day for women and 0, <10 and ≥10 g/day for men). Interaction terms of alcohol consumption (modeled on the log-scale to maximize model fit) multiplied by *ADHIC* genotype (modeled as the number of variant alleles) were tested. Finally, we examined the joint association of alcohol consumption and *ADHIC* genotype with risk of type 2 diabetes. To combine men and women, beta-coefficients of the interaction term were pooled using the DerSimonian and Laird random-effects model (31).

We also assessed whether *ADHIC* genotype modified the relation between alcohol consumption and fasting insulin and HbA_{1c} among controls, using analysis of variance adjusted for covariates described for conditional logistic regression above. For these analyses, fasting insulin was log-transformed due to deviation from homogeneity of variance.

We performed sensitivity analyses using cutoffs of 7 gram/day for women and 14 gram/day for men, drinking frequency (0 days/week, 1-4 days/week and ≥5 days/week) rather than average quantity, linear modeling instead of categorical analysis, a restricted subset of women with HbA_{1c} concentrations ≤6.5%, excluding heavier drinkers (>30 g/day for women and >40 g/day for men), and using conditional logistic regression, all of which gave similar

results. Analyses were performed using SAS statistical package, version 8.2 (SAS Institute, Cary, North Carolina) and Intercooled STATA 9.0 (STATA Corporation, College Station, Texas, USA).

Results

Baseline characteristics and allele frequencies

Baseline characteristics of the cases and controls from NHS and HPFS are shown in table 2. Women who developed type 2 diabetes tended to have higher BMI, fasting insulin, and HbA_{1c} than controls. Alcohol consumption and physical activity were lower among women developing type 2 diabetes compared to matched controls. Men who developed type 2 diabetes had higher BMI and lower physical activity than controls.

The frequencies of the *ADH1C*2* allele among controls were 41% among women and 40% among men. The distribution of *ADH1C* genotype was in Hardy-Weinberg equilibrium in the total population of NHS ($p=0.09$) and HPFS ($p=0.72$) and among controls from NHS ($p=0.41$) and the HPFS ($p=0.38$). **We assessed differences in alcohol consumption between genotypes within strata of alcohol consumption. Among women, *ADH1C*1* homozygotes consumed 2.2 gm/day, heterozygotes 2.1 and *ADH1C*2* homozygotes 2.1 gm/day ($p=0.78$) in the stratum of light drinkers. In the stratum of moderate drinkers, *ADH1C*1* homozygotes consumed 16.2 gm/day, heterozygotes 14.1 and *ADH1C*2* homozygotes 14.4 gm/day ($p=0.32$). Among men, *ADH1C*1* homozygotes consumed 4.5 gm/day, heterozygotes 4.5 and *ADH1C*2* homozygotes 4.0 gm/day ($p=0.35$) among light drinkers. In the stratum of moderate drinkers, *ADH1C*1* homozygotes consumed 25.9 gm/day, heterozygotes 26.3 and *ADH1C*2* homozygotes 25.7 gm/day ($p=0.97$). The lifestyle factors included in our multivariate models were not associated with *ADH1C* genotype among women**

($p=0.13$ to $p=0.95$) or men ($p=0.06$ to $p=0.92$) as well (on-line supplementary table).

Main effects of alcohol consumption and ADH1C genotype

We first examined the main effects of alcohol consumption and *ADH1C* genotype. Alcohol consumption was inversely associated ($p<0.001$) with risk of type 2 diabetes within this nested case-control population from the NHS, with a multivariate-adjusted odds ratio of 0.45 (0.33- 0.63) among women consuming ≥ 5 g alcohol/day compared to abstainers as previously reported for the entire NHS (19;20). Despite the previously reported inverse association between alcohol consumption and risk of type 2 diabetes in the entire HPFS cohort (18), alcohol consumption was not associated with risk of type 2 diabetes in this nested case-control study (odds ratio 1.08 (95%-CI: 0.67- 1.75) for intake of ≥ 10 g alcohol/day versus abstinence).

ADH1C genotype per se was not associated with risk of type 2 diabetes among men or women (table 3). Adjusting for alcohol consumption did not alter these results.

Interaction between alcohol consumption and ADH1C genotype

We next assessed the main effect of *ADH1C* genotype within strata of alcohol consumption. *ADH1C* genotype significantly modified the association between alcohol consumption and type 2 diabetes among women ($p_{\text{interaction}}=0.02$) (table 4). The *ADH1C*2* allele attenuated the decreased risk of type 2 diabetes in a dose-dependent manner among consumers of ≥ 5 g alcohol/day, while *ADH1C* genotype was not associated with risk of type 2 diabetes among abstainers and light drinkers.

Among men, the overall interaction between alcohol consumption and *ADH1C* genotype was not significant ($p_{\text{interaction}}=0.41$). As among women, we

observed a higher type 2 diabetes risk associated with the *2 allele among men consuming ≥ 10 g/day, but this trend was not significant.

Because we found no heterogeneity in the interaction between alcohol consumption and genotype among women and men ($p_{\text{homogeneity}} = 0.46$), estimates for women and men were pooled. We observed a significant ($p_{\text{interaction}} = 0.02$) interaction between alcohol consumption and *ADH1C* genotype in the pooled analysis (figure 1). Moderate to heavier alcohol consumption was associated with a decreased risk of type 2 diabetes. However, because the *2 allele tended to attenuate the lower risk associated with moderate drinking, the risk among moderate drinkers homozygous for the *2 allele was nearly identical to that among abstainers homozygous for the *1 allele.

Mediating biomarkers

We also assessed whether *ADH1C* genotype modified the relation between alcohol consumption and markers of type 2 diabetes among control participants. Alcohol consumption was inversely associated with fasting insulin (beta-coefficient \pm se: -0.017 ± 0.010), with concentrations of 8.2 ± 0.41 , 8.0 ± 0.41 and 6.9 ± 0.39 among non-drinkers and consumers of 0.1- 4.9 and ≥ 5.0 g/day respectively. *ADH1C* genotype was not associated with fasting insulin among abstainers ($p_{\text{trend}} = 0.81$) and light drinkers ($p_{\text{trend}} = 0.89$). Among consumers of ≥ 5 g alcohol/day, homozygotes for *ADH1C**2/*2 (8.3 ± 1.4 $\mu\text{U/mL}$) tended to have higher concentrations of fasting insulin than homozygotes *ADH1C**1/*1 (7.0 ± 0.7 $\mu\text{U/mL}$) and heterozygotes (5.8 ± 0.6 $\mu\text{U/mL}$), but the interaction between alcohol consumption and *ADH1C* genotype was not significant ($p_{\text{interaction}} = 0.86$).

No association between alcohol consumption and HbA_{1c} was observed, nor was an interaction between alcohol consumption and *ADH1C* genotype (data not shown).

Discussion

The primary function of *ADH1C* is ethanol oxidation. A common polymorphism of this gene is associated with a slower rate of oxidation and may increase exposure to ethanol. Therefore we hypothesized that *ADH1C* genotype would modify the association between alcohol consumption and type 2 diabetes risk. In support of this hypothesis and of a causal relationship between alcohol consumption and type 2 diabetes, an interaction between *ADH1C* genotype and alcohol consumption for type 2 diabetes was observed among women and in the pooled analysis of both studies.

A key finding of this study was that the *ADH1C**2 allele, related to slower ethanol oxidation, was associated with a dose-dependent attenuation in the lower diabetes risk among moderate to heavier drinkers, while no associations between *ADH1C* genotype and diabetes were observed for those consuming less or no alcohol. This interaction is in the opposite direction from previous observations for cardiovascular disease (13), where the benefit from moderate alcohol consumption is mainly due to increased HDL cholesterol concentrations. Ethanol itself directly increases HDL cholesterol in a dose-dependent manner (32). Thus, slower gastric and hepatic metabolism of ethanol would lead to increased HDL cholesterol concentrations and reduced cardiovascular risk as observed (13). In contrast, we observed a lower risk of diabetes with moderate drinking in this study, but this was attenuated (rather than accentuated) by alleles conferring slower oxidation (Figure 1).

This disparity between our findings for type 2 diabetes and those for cardiovascular disease suggest different mechanisms underlying the associations with moderate drinking. Ethanol oxidation produces measurable downstream metabolites such as acetaldehyde and acetate (33), that could affect risk of type 2 diabetes themselves instead of ethanol. Slower

ethanol oxidation would tend to produce lower concentrations of these metabolites.

Perhaps the strongest data relating ethanol metabolites to type 2 diabetes risk involve acetate and its effects on peripheral tissue (34). Acetate, the end-product of ethanol oxidation, is oxidized to acetyl-CoA by acetyl-CoA synthetase (35;36) primarily in peripheral tissues such as muscle (37). Acetyl-CoA enters the Krebs cycle, stimulating oxidative phosphorylation (38). Several studies have shown that impaired mitochondrial oxidative phosphorylation is associated with insulin resistance (39) and type 2 diabetes (40;41). In addition, alcohol consumption via acetate acutely affects energy expenditure and fat oxidation (7;42). Moreover, acetate itself decreases lipolysis and thereby free fatty acid concentrations (7;43). Indeed, in human studies, acetic acid and acetate acutely decrease free fatty acid concentrations (44) and improve insulin sensitivity (45).

Studies directly manipulating ADH activity confirm decreased acetate concentrations that may support our hypothesis. Sarkola et al. showed that 4-methylpyrazole, an ADH inhibitor, decreased production of acetate in men and women after ingestion of 0.4 g/kg of alcohol (33). Although the effect of genotype on blood alcohol and acetate concentrations remains uncertain, *ADH1C*2* genotype has been associated with decreased production of salivary acetaldehyde as expected (46).

The association of *ADH1C* genotype per se with risk of type 2 diabetes was not significant. This reflects the fact that abstainers or light drinkers, in whom we hypothesized and observed no association of genotype with risk, comprised a high proportion of participants in both studies (75% of women and 64% of men). In other populations with larger proportions of frequent drinkers, we hypothesize that genotype itself would be associated with risk.

Strengths of our study include its prospective design and detailed assessment of alcohol consumption, lifestyle factors and

diet. Nonetheless, several limitations of this study need to be addressed. First, our study population consists of relatively light drinkers with limited variation in alcohol consumption. We could therefore not explore the effect of *ADH1C* genotype on the association between alcohol consumption and type 2 diabetes among heavier drinkers. However, it is possible that genetic variation in *ADH1C* would have less impact among heavier drinkers because at higher concentrations, ethanol is also metabolized through ADH4, CYP2E4, and other enzymes with higher K_m values for alcohol.

Second, we could not replicate the inverse association between alcohol consumption and type 2 diabetes observed in the entire HPFS cohort in the smaller nested case-control study. We have previously shown a strong inverse association in the full HPFS cohort of men. When we examined the entire cohort of men from 1994 (as opposed to 1986, the original baseline of the study) onward, we observed an inverse association between alcohol intake and risk of diabetes similar to our previous studies, suggesting that results in this smaller case-control study that also dates from 1994 may be due to chance. The interaction, however, tended to be qualitatively similar among both men and women.

Third, the *ADH1C Arg271Gln* polymorphism was not assessed in this study, but, since linkage disequilibrium is nearly complete, similar associations for both polymorphisms with coronary heart disease have been shown (8). Finally, the *ADH1C* polymorphisms are in linkage disequilibrium with the *ADH1B*47His* polymorphism (47), although the latter SNP is quite rare in Caucasian populations. However, regardless of which ADH SNP is truly responsible for this interaction, the finding that genetic variation in ADH enzymes significantly modifies the alcohol-DM relationship provides important evidence that this relationship may be causal.

In conclusion, this study indicates that *ADH1C* genotype modifies the association between alcohol consumption and type 2 diabetes. Among moderate to heavier drinkers, the *ADH1C*2* allele, related to a slower rate of ethanol oxidation, was associated with a dose-dependent attenuation in the lower risk of type 2 diabetes among moderate to heavier drinkers. This suggests that the association between alcohol consumption and type 2 diabetes may be causal but due to downstream metabolites such as acetate rather than ethanol itself.

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Author Contributions:

Dr. Beulens had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Table 1: Flow-chart of selection of cases and controls in the Nurses' Health Study and Health Professionals Follow-up Study

Year	Nurses' Health Study	Health Professionals Follow-up Study
1990	Blood draw (n= 32, 826)	
1992		
1994		Blood draw (n= 18, 225)
1996	Incident cases diabetes identified between 1990 and 1996 (n= 397) Matching to 2 controls (n= 793)	
1998 ¹	Incident cases diabetes identified between 1996 and 1998 (n= 189) Matching to 1 control (n= 189) 2 controls in top decile BMI (n= 17)	
2000 ²	Incident cases diabetes identified between 1998 and 2000 (n= 138) Matching to 1 control (n= 138) 2 controls in top decile BMI (n= 3)	Incident cases diabetes identified between 1994 and 2000 (n= 427) Matching to 1 control (n= 431)
EXCLUSIONS		
	Missing ADH1C (n= 116)	Missing ADH1C (n= 32)
	Missing alcohol consumption (n= 15)	Missing alcohol consumption (n= 9)
	Non-caucasians (n=93)	Non-caucasians (n= 52)
	Cases (n= 640)	Cases (n= 383)
	Controls (n= 1000)	Controls (n= 382)

Table 2: Characteristics of incident cases of type 2 diabetes and matched controls.

	Women		Men	
	Controls	Cases	Controls	Cases
n	1000	640	382	383
Age (years)	57.0	57.0	60.4	60.6
BMI (kg/m ²)‡	27.3*	30.5	25.8*	29.0
Physical activity (Mets/week)	15.1*	12.4	36.6*	27.0
Alcohol consumption (g/day)	5.2*	2.9	11.5	10.7
Smoking (%)				
Never	46.7	42.2	49.2*	38.4
Past	42.0	44.2	46.1*	56.4
Current	10.8	13.6	3.7	4.7
Family history of diabetes (%)	21.2*	44.8	16.5*	26.6
Menopausal status (%)	79.5	82.0	NA	NA
Nutrients†:				
Energy (kcal)	1780	1828	2135	2130
Saturated fatty acids (g)	21.3*	22.5	22.3*	23.3
Trans fatty acids (g)	3.1	3.2	3.0	3.2
Polyunsaturated fatty acids (g)	11.9	12.2	12.2	12.5
Fiber (g)	19.9	20.2	22.3	21.4
Glycemic load	117.8	121.1	134.6*	130.6
Coffee consumption (cups/day)	2.2	2.0	2.1	1.9
Biomarkers:				
HbA1c (%)	5.6*	6.4	-	-
Insulin (μU/mL)	9.6*	13.5	-	-

* p < 0.05 cases versus controls.

† All nutrients are energy-adjusted except energy.

‡ Cases in the top decile of the BMI distribution were matched to an additional control on BMI.

Table 3: *ADHIC* polymorphism and risk of type 2 diabetes among 640 cases and 1000 controls from the Nurses Health Study and 383 cases and 382 controls from the Health Professionals Follow-up Study*

	<i>ADHIC</i> *1/*1	<i>ADHIC</i> *1/*2	<i>ADHIC</i> *2/*2	P-value linear trend
Cases (n/ %)	376/ 36.8	469/ 45.8	178/ 17.4	
Controls (n/ %)	479/ 34.7	665/ 48.1	238/ 17.2	
Matched, BMI-adjusted	1.0	0.93 (0.77- 1.12)	1.00 (0.78- 1.29)	0.83
Matched, multivariate adjusted†	1.0	0.96 (0.79- 1.18)	1.05 (0.80- 1.36)	0.87
Matched, alcohol- & multivariate adjusted†	1.0	0.97 (0.79- 1.19)	1.11 (0.85- 1.45)	0.59

* Results obtained from pooling the beta-coefficient and standard error estimates for men and women using the DerSimonian and Laird random-effects model.

† Adjusted for BMI, physical activity, smoking, family history of diabetes, postmenopausal hormone replacement therapy, energy intake and energy-adjusted intake of saturated fat, trans fatty acids, polyunsaturated fat, dietary fiber, glycemic load and coffee consumption.

Table 4: Alcohol consumption, *ADHIC* polymorphism and risk of type 2 diabetes among 640 cases and 1000 controls from the Nurses Health Study and 383 cases and 382 controls from the Health Professionals Follow-up Study

	Women			P-value for trend	Men			P-value for trend
	<i>*1/*1</i>	<i>ADHIC</i>			<i>*1/*1</i>	<i>ADHIC</i>		
		<i>*1/*2</i>	<i>*2/*2</i>		<i>*1/*1</i>	<i>*1/*2</i>	<i>*2/*2</i>	
	0 g/day				0 g/day			
Cases (n) / Controls (n)	139/ 132	144/ 192	40/ 53		34/ 32	46/ 48	19/ 18	
Multivariate adjusted†	1.0	0.76 (0.53- 1.09)	0.78 (0.46- 1.33)	0.20	1.0	0.64 (0.31- 1.30)	0.96 (0.40- 2.35)	0.72
	0- 4.9 g/day				0- 9.9 g/day			
Cases (n) / Controls (n)	75/ 109	95/ 140	44/ 71		65/ 50	68/ 69	23/ 15	
Multivariate adjusted†	0.60 (0.39- 0.91)	0.74 (0.50- 1.10)	0.62 (0.38- 1.02)	0.76	1.16 (0.58- 2.30)	0.86 (0.44- 1.67)	1.22 (0.49- 3.04)	0.63
	≥ 5 g/day				≥ 10 g/day			
Cases (n) / Controls (n)	24/ 106	49/ 141	30/ 56		39/ 50	67/ 75	22/ 25	
Multivariate adjusted†	0.24 (0.14- 0.43)	0.41 (0.26- 0.66)	0.60 (0.34- 1.07)	0.009	0.73 (0.35- 1.54)	0.96 (0.48- 1.91)	0.93 (0.39- 2.25)	0.29

† Adjusted for BMI, physical activity, smoking, family history of diabetes, postmenopausal hormone replacement therapy, energy intake and energy-adjusted intake of saturated fat, trans fatty acids, polyunsaturated fat, dietary fiber, glycemic load and coffee consumption.

Figure 1: Joint association of alcohol consumption and *ADH1C* genotype with risk of type 2 diabetes among 1023 cases and 1382 controls from the Nurses' Health and Health Professionals Follow-up Study.

Odds ratio are adjusted for BMI, physical activity, smoking, family history of diabetes, postmenopausal hormone replacement therapy, energy intake and energy-adjusted intake of saturated fat, trans fatty acids, polyunsaturated fat, dietary fiber, glycemic load and coffee consumption and pooled for men and women using the DerSimonian and Laird random-effects model.

* Cut-off for alcohol consumption of 5 g/day for women and 10 g/day for men.

