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Alcohol Consumption, Mediating Biomarkers, and Risk of Type 2 Diabetes Among Middle-Aged Women

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OBJECTIVE — The purpose of this study was to investigate whether adiponectin concentrations and biomarkers of inflammation, endothelial dysfunction, and insulin resistance mediate the association between alcohol consumption and diabetes.

RESEARCH DESIGN AND METHODS — In a nested case-control study of 705 women with incident diabetes and 787 matched control subjects, we examined the adjusted relationship between baseline alcohol consumption and risk of diabetes before and after adjustment for markers of inflammation/endothelial dysfunction (C-reactive protein, vascular cell adhesion molecule-1, intercellular adhesion molecule-1, E-selectin, tumor necrosis factor- α receptor 2, and interleukin-6), fasting insulin, and adiponectin concentrations.

RESULTS — Alcohol consumption was associated with a decreased risk of diabetes (odds ratio per 12.5 g/day increment in alcohol use 0.58; 95% CI 0.49–0.69; $P < 0.001$). Adjustment for BMI attenuated the association by 25%. None of the markers of inflammation or fasting insulin appeared to account for $>2\%$ of the observed relationship. Without adjustment for BMI, these biomarkers individually explained slightly more of the association, but $<10\%$ in all cases. Adiponectin accounted for 25% in a fully adjusted model and for 29% without adjustment for BMI.

CONCLUSIONS — In this population of women, alcohol consumption was inversely associated with risk of type 2 diabetes. Adiponectin appeared to be a mediator of this association, but circulating biomarkers of inflammation, endothelial dysfunction, and fasting insulin did not explain this association. These results suggest that further research is needed into the potentially mediating roles of other biomarkers affected by alcohol consumption.

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Moderate alcohol consumption is associated with a decreased risk of both coronary heart disease and type 2 diabetes compared with abstaining and heavier drinking (1,2). Approximately 50% of the association with coronary heart disease (CHD) appears to be attributable to an increase in HDL cholesterol (3), and fibrinogen and glycemia may account for a large part of the remainder (4).

The underlying mechanism for the lower risk of type 2 diabetes among moderate drinkers is not entirely clear, but several factors may be involved. First, high concentrations of markers of both inflammation and endothelial dysfunction are directly related to risk of type 2 diabetes (5,6), and moderate alcohol consumption is associated with lower levels of markers of inflammation and endothelial dysfunction in both cross-sectional

and intervention studies (7,8). Second, a randomized controlled trial (9) and cross-sectional studies (10) have shown improved insulin sensitivity with moderate alcohol consumption, but results of studies are not entirely consistent (11). These changes in insulin sensitivity could be mediated by an increase in adiponectin concentrations that has been consistently shown in several observational and randomized studies (11,12). Finally, BMI is the most important predictor of type 2 diabetes, particularly among women (13). Cross-sectional studies and a recent prospective study suggest that moderate alcohol consumption may be associated with lower BMI and less weight gain over time among women but not men (14,15).

Whether and to what extent markers of inflammation, endothelial dysfunction, fasting insulin, and adiponectin concentrations explain the inverse association between moderate alcohol consumption and type 2 diabetes has not been investigated to date. To address these questions, we investigated these relations in a nested case-control study from the Nurses' Health Study. Previous reports from this study have shown that moderate alcohol consumption, BMI, markers of inflammation and endothelial dysfunction, and fasting insulin are all associated with the risk of type 2 diabetes (5,6) in expected directions.

RESEARCH DESIGN AND METHODS

The Nurses' Health Study began in 1976, when 121,700 female nurses aged 30–55 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 a 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, 714 of these women had a confirmed diagnosis of type 2 diabetes.

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Control subjects providing blood samples were matched to diabetes case patients by year of birth, date of blood draw, race, and fasting status at blood draw. From 1990 until 1996, two control subjects were matched to each case patient on the basis of the above factors. One of the two control subjects was also matched according to BMI within 1 kg/m². After 1996, one control subject was matched to each case patient on the basis of the same characteristics, and another control subject was matched on these characteristics and BMI to each of the case patients in the top decile of the BMI distribution. Women with missing information for alcohol consumption and markers of inflammation and endothelial dysfunction were excluded, leaving 787 control subjects and 705 case patients for analysis.

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

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 one of the following on the questionnaire: treatment with either insulin or an oral hypoglycemic agent, at least one classic symptom of diabetes (for instance, polyuria, polydipsia, or weight loss) plus an elevated plasma glucose level, or an elevated plasma glucose level on at least two 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 h 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). The validity of self-reported diabetes has been confirmed with medical record review in a sample of 62 participants.

Assessment of alcohol consumption

We assessed average alcohol consumption within a semiquantitative food frequency questionnaire including separate items for beer, white wine, red wine, and liquor (16). 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. We used alcohol consumption reported on the food frequency questionnaire in 1990 and replaced information with data from 1986 onward when missing data occurred.

We previously assessed the validity of alcohol consumption estimated with the food frequency questionnaire against intake from two 1-week dietary records collected ~ 6 months apart among 173 women residing in eastern Massachusetts; the Spearman correlation coefficient between these two measures was 0.90. Estimated average alcohol intake was also correlated with HDL cholesterol to an expected degree ($r = 0.40$), and HDL cholesterol levels among drinkers were ~ 15 – 20% higher than those among nondrinkers (17).

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 ($r = 0.96$), and the assessment of physical activity was previously validated. 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 semiquantitative food frequency questionnaire (16).

Laboratory procedures

Women were sent a phlebotomy kit with instructions to return the sample by overnight mail with a frozen water bottle. On arrival, samples were processed and frozen in liquid nitrogen until analysis; 97% arrived within 26 h of phlebotomy. Quality control samples were routinely frozen with study samples; the long-term stability of plasma samples collected and stored under this protocol has been documented. Study samples were analyzed in randomly ordered case-control pairs to further reduce systematic bias and inter-assay variation.

Levels of E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) were measured by a commercial enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN). These biomarkers are released during endothelial cell activation and correlate ($r = 0.04$ – 0.58) with endothelial dysfunction assessed directly by brachial artery flow-mediated vasodilatation or microcirculation iontophoresis methods (18). C-reactive protein (CRP) levels were measured via a high-sensitivity latex-enhanced immunonephelometric assay (Dade Behring, Newark, DE). Interleukin-6 (IL-6) was measured by a quantitative sandwich enzyme immunoassay technique (Quantikine HS Immunoassay kit), and tumor necrosis factor- α receptor 2 (TNF-R2) levels were measured by an ELISA kit using immobilized monoclonal antibody to human TNF-R2 (Genzyme, Cambridge, MA). Insulin levels were measured using a double antibody system with $<0.2\%$ cross-reactivity between insulin and its precursors (Linco Research, St. Louis, MO). C-peptide was measured using antiserum M1230 in an alcohol precipitation nonequilibrium assay. In the C-peptide assay, proinsulin has 10% cross-reactivity, but its contribution to C-peptide immunoreactivity is $<0.5\%$. Proinsulin-like material was measured using antiserum 11E in a nonequilibrium assay with second-antibody precipitation. In the proinsulin assay, human proinsulin cross-reacts 100%, des-31,32 proinsulin cross-reacts 38%, and des-64,65 proinsulin cross-reacts 10%, whereas insulin and C-peptide each cross-react $<0.001\%$. The coefficients of variation were 3.8% for CRP, 5.9% for IL-6, 6.2% for TNF-R2, 6.6% for E-selectin, 3.6% for ICAM-1, 8.5–9.8% for VCAM-1, 3.5–11.7% for fasting insulin, 1.9–3.0% for A1C, 2–7% for C-peptide, and 6–9% for proinsulin. Adiponectin was determined by ELISA (ALPCO Diagnostics, Salem, NH). The sensitivity of this assay is 0.04 ng/ml, and the recovery rate was 99–103%.

Statistical analysis

Analyses were performed in three separate groups that had valid information on a specific set of potential mediating biomarkers: the main dataset for markers of inflammation and endothelial dysfunction (case patients 705; control subjects 787) and three additional sets for fasting insulin (case patients 476; control sub-

Table 1—Descriptive characteristics of control women and women who developed type 2 diabetes

	Control subjects	Case patients	P
n	787	705	
Age (years)	56.7 ± 6.9	56.8 ± 6.8	NS
BMI (kg/m ²)	26.4 ± 6.1	30.4 ± 5.6	<0.001
Physical activity (METs/week)	15.6 ± 27.1	12.1 ± 14.7	0.002
Alcohol consumption (g/day)	1.8 (0–6.9)	0 (0–2.7)	<0.001
Smoking (%)			
Never	46.0	43.7	NS
Past	41.4	42.8	NS
Current	12.6	13.5	NS
Family history of diabetes (%)	20.1	44.8	<0.001
Postmenopausal (%)	78.5	81.1	NS
Nutrients*			
Energy (kcal)	1769 ± 512	1817 ± 561	NS
Saturated fatty acids (g)	20.9 ± 8.4	22.4 ± 9.3	0.002
Trans fatty acids (g)	3.0 ± 1.5	3.2 ± 1.6	0.005
Polyunsaturated fatty acids (g)	11.7 ± 5.1	12.1 ± 5.0	NS
Fiber (g)	19.9 ± 8.0	20.0 ± 8.3	NS
Glycemic load	117.4 ± 41.9	120.8 ± 44.8	NS
Coffee consumption (cups/day)	2.2 ± 1.8	2.0 ± 1.7	0.03
Biomarkers			
Insulin (μU/ml)	7.5 (4.2–11.0)	11.2 (7.1–17.1)	<0.001
CRP (mg/dl)	0.16 (0.07–0.35)	0.37 (0.20–0.63)	<0.001
VCAM-1 (ng/ml)	526.0 (444.0–614.2)	545.1 (458.8–646.8)	0.003
ICAM-1 (ng/ml)	247.3 (218.1–277.4)	264.5 (233.2–316.9)	<0.001
E-selectin (ng/ml)	45.4 (33.8–60.1)	61.5 (45.4–80.6)	<0.001
TNF-R2 (pg/ml)	2396 (2,013–2,861)	2638 (2,209–3,158)	<0.001
IL-6 (ng/ml)	1.8 (1.2–2.7)	2.4 (1.7–3.5)	<0.001
Adiponectin (μg/ml)	17.7 (12.2–22.6)	10.1 (6.8–15.1)	<0.001

Data are means ± SD or median (interquartile range) if not normally distributed. *All nutrients are energy adjusted except energy.

jects 732) and adiponectin concentrations (case patients 694; control subjects 982). In the main dataset individual markers of inflammation and endothelial dysfunction were missing for no more than 4% of the sample. To maintain consistent sample sizes across markers, these missing values were imputed using the median of the sample. An inflammation score was created by summing the quartiles of CRP, IL-6, E-selectin, and TNF-R2 to assess their combined effect.

We examined the relationships of alcohol consumption, biomarkers, and risk of type 2 diabetes with a three-step process. First, we estimated the independent association of alcohol consumption with risk using multivariable unconditional logistic regression models. In these models, we adjusted for matching and confounding factors, including 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 (five 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).

Second, we added biomarkers to the model (as linear terms) to assess the percent change of the β-coefficient (the log of the odds ratio [OR]) for alcohol consumption; these coefficients are scaled linearly. Inclusion of a mediating factor would be expected to attenuate the β-coefficient related to alcohol intake. Third, because BMI could act as both a confounder and a mediator of the effects of alcohol on diabetes (i.e., obesity could affect alcohol consumption and vice versa), we repeated our analyses without BMI in the model and examined the mediating contributions of both BMI and individual biomarkers.

In all analyses, we modeled alcohol consumption as a linear term in increments of 12.5 g (~1 drink) per day. Inclusion of a quadratic term revealed no evidence of a nonlinear relation. We also modeled alcohol consumption on the log scale, which had maximum model fit, with similar results; for ease of interpretation, those results are not shown. Analyses were performed using the SAS statistical package (version 8.2; SAS Institute, Cary, NC).

RESULTS— Baseline characteristics of the case patients and control subjects are shown in Table 1. Women who developed type 2 diabetes had higher BMI, family history of diabetes, and intake of saturated and trans fatty acids and markers of inflammation, endothelial dysfunction, and fasting insulin than control subjects. Alcohol and coffee consumption, physical activity, and adiponectin concentrations were lower among women who developed type 2 diabetes than among control subjects. Of our study population, 640 women did not consume alcohol, 706 consumed 0–12.5 g/day, 88 consumed 12.5–25 g/day, 45 consumed 25–37.5 g/day, 7 consumed 37.5–50 g/day, 1 consumed 50–62.5 g/day, 4 consumed 62.5–75 g/day, and 1 consumed >100 g/day. All mediating biomarkers were significantly correlated with alcohol consumption in expected directions (Table 2).

Mediating biomarkers

Table 3 shows the association between alcohol consumption (modeled per 12.5-g increment) and risk of type 2 diabetes and the extent to which mediating biomarkers explained this association. Alcohol consumption was associated with a decreased risk of type 2 diabetes in this sample of women with an OR of 0.58 (95% CI 0.49–0.69) per 12.5-g increment of alcohol intake ($P < 0.001$), adjusted for matching and confounding factors. Further adjusting this estimate for BMI attenuated the β-coefficient for alcohol intake by 25%.

None of the markers of inflammation and endothelial dysfunction appeared to explain >2% of the association of alcohol consumption with diabetes. Without adjustment for BMI, these percentages increased but were never >10%, although inclusion of CRP attenuated the risk estimate by 9%. Combining CRP, IL-6, E-selectin, and TNF-R2 in an inflammation score, they accounted for 8% of the asso-

Table 2—Spearman correlations between alcohol consumption and mediating biomarkers

Biomarker	n	Spearman correlation (P)
CRP	1,467	−0.14 (<0.001)
VCAM-1	1,491	−0.09 (<0.001)
ICAM-1	1,489	−0.09 (0.001)
E-selectin	1,490	−0.10 (<0.001)
IL-6	1,442	−0.07 (0.005)
TNF-R2	1,477	−0.13 (<0.001)
Insulin	1,055	−0.11 (<0.001)
Adiponectin	1,677	0.18 (<0.001)

ciation in a fully adjusted model and for 17% without adjustment for BMI. Excluding women with clinically relevant elevations of CRP ($n = 128$) did not essentially change our results but only slightly increased the percentage explained for some markers. For example, CRP now explained 12% in a model unadjusted for BMI.

Adiponectin explained the association between alcohol consumption and type 2 diabetes by 25% in a fully adjusted model and by 29% without adjustment for BMI. Fasting insulin did not appear to explain the association between alcohol consumption and type 2 diabetes. Without adjustment for BMI, inclusion of fasting insulin attenuated the observed estimate for alcohol by 7%. In sensitivity analyses, fasting concentrations of proinsulin and C-peptide did not attenuate the association either (data not shown).

Including BMI as a continuous variable in our models did not alter the results of this study (data not shown). Excluding women consuming >50 g alcohol/day did not affect our results.

CONCLUSIONS— In this study, we aimed to quantify the extent to which markers of inflammation, endothelial dysfunction, fasting insulin, and adiponectin could explain the lower risk of type 2 diabetes associated with moderate alcohol consumption. Only adiponectin explained this relation by ~ 25 – 30% . None of the other markers, however, appeared to explain this association to any large extent. This finding suggests that, apart from adiponectin, other pathways may be less important.

It is surprising that markers of inflammation and endothelial dysfunction did not seem to play a role in the relationship of alcohol consumption with risk of diabetes. This contrasts with our previous results for CHD, for which inflammatory markers appeared to explain $\sim 20\%$ of the association (4). Two other studies, however, showed no substantially mediating effect of inflammation on the association of alcohol consumption with CHD (19,20). Our results suggest that inflammation and endothelial dysfunction may have little role in the association between

alcohol consumption and type 2 diabetes. Rather, inflammation and endothelial dysfunction may be indirectly related to type 2 diabetes via associations with BMI (21). Indeed, they were stronger intermediates in this study when BMI was not included as a covariate.

The effect of moderate alcohol consumption on insulin sensitivity is not completely understood. Randomized controlled trials and cross-sectional studies have not always reported consistent results (9–11). Of note, the longest and largest study did find a dose-dependent effect of alcohol consumption on insulin sensitivity and fasting insulin in postmenopausal women similar to our study population. However, our findings suggest that the risk reduction of type 2 diabetes with moderate alcohol consumption is not simply due to improved insulin sensitivity, at least as captured by fasting insulin.

Recent studies have shown that moderate alcohol consumption increases adiponectin concentrations and its oligomers (11), a finding confirmed in observational studies (12). Adiponectin directly improves insulin sensitivity in animal models. Indeed, we show here that adiponectin is an important mediator of the relation between alcohol consumption and type 2 diabetes. However, as adiponectin only explained ~ 25 – 30% of the association, other mechanisms apart from those described here need to be further explored.

It is difficult to assess whether the attenuation of the alcohol-diabetes relationship by BMI reflects confounding by BMI

Table 3—Alcohol consumption (per 12.5 g/day), mediating biomarkers, and risk of type 2 diabetes among 787 control women and 705 women who developed type 2 diabetes

	BMI adjusted			Not BMI adjusted		
	OR (95% CI)	β coefficient	% Attenuated	OR (95% CI)	β coefficient	% Attenuated
MV adjusted*	0.67 (0.56–0.79)	−0.41 \pm 0.09	—	0.58 (0.49–0.69)	−0.55 \pm 0.09	
BMI	NA	NA	NA	0.67 (0.56–0.79)	−0.41 \pm 0.09	25
CRP	0.67 (0.56–0.80)	−0.41 \pm 0.09	0	0.61 (0.51–0.72)	−0.50 \pm 0.09	9
VCAM-1	0.67 (0.56–0.80)	−0.40 \pm 0.09	1	0.59 (0.50–0.69)	−0.54 \pm 0.09	2
ICAM-1	0.67 (0.56–0.80)	−0.40 \pm 0.09	1	0.59 (0.49–0.69)	−0.54 \pm 0.09	2
E-selectin	0.67 (0.56–0.80)	−0.41 \pm 0.09	−1	0.59 (0.50–0.71)	−0.52 \pm 0.09	5
IL-6	0.67 (0.57–0.80)	−0.40 \pm 0.09	2	0.59 (0.50–0.70)	−0.53 \pm 0.09	3
TNF-R2	0.67 (0.56–0.80)	−0.40 \pm 0.09	1	0.60 (0.50–0.70)	−0.52 \pm 0.09	5
MV adjusted*	0.71 (0.59–0.87)	−0.34 \pm 0.10		0.65 (0.53–0.79)	−0.44 \pm 0.10	
Insulin	0.71 (0.58–0.87)	−0.34 \pm 0.10	0	0.67 (0.55–0.82)	−0.41 \pm 0.10	7
MV adjusted*	0.71 (0.61–0.83)	−0.35 \pm 0.08		0.66 (0.56–0.77)	−0.42 \pm 0.08	
Adiponectin	0.77 (0.65–0.91)	−0.27 \pm 0.08	24	0.74 (0.63–0.87)	−0.30 \pm 0.08	29

*Multivariate (MV) models 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. NA, not applicable.

or whether alcohol truly acts by minimizing weight gain in women. A recent study among 49,324 women showed that moderate alcohol consumption was prospectively associated with a decreased 8-year weight gain, whereas heavier drinkers had an increased risk for weight gain (14). Other studies among women have confirmed these results, whereas no or even a positive association with body weight is reported for men (15). To date, few prospective randomized interventions examined the effect of moderate alcohol consumption on body weight (9), but these have not been long enough to exclude such an effect conclusively.

The mechanisms underlying the protective effect of light to moderate alcohol consumption are complex and not completely understood. Our results suggest that other mechanisms apart from adiponectin explain the association between alcohol consumption and type 2 diabetes. Lipotoxicity, excess release of free fatty acids from adipose tissue, is thought to be an important cause of insulin resistance. In the liver, free fatty acids increase glucose production, triglycerides, and secretion of VLDL. Associated lipoprotein abnormalities include reductions in HDL and increased LDL. Alcohol consumption strongly affects lipid metabolism (22) and could thereby possibly also influence insulin resistance and risk of type 2 diabetes. Another possibility is that the effects of moderate drinking are not related to ethanol itself but to acetate, the end product of ethanol oxidation (23). Acetate may affect fat oxidation and decrease lipolysis and free fatty acids and indirectly improve insulin sensitivity (24).

The strengths of this study include its prospective design; detailed assessment of alcohol consumption, diet, and lifestyle; and inclusion of a variety of markers previously related to risk of diabetes. Nonetheless, certain limitations need to be addressed. Because insulin was only assessed for those with fasting blood samples, we used slightly different subgroups for each group of biomarkers, which may have introduced some selection bias. However, within each subgroup, moderate alcohol consumption was associated with a similar decreased risk of diabetes as in the entire case-control study. In addition, we could only include women providing blood samples in this study, which could also be subject to selection. In comparing those women with women not providing blood samples, they had higher prevalences of obesity and family history

of diabetes, which could lead to increased diabetes risk in this sample. However, because we only included incident cases of diabetes, selection of these women is not related to the occurrence of disease and therefore does not lead to selection bias. It could limit generalizability of our results to women with a slightly lower diabetes risk.

We were restricted to fasting insulin as a measure of insulin sensitivity, but it only correlates modestly to the hyperinsulinemic-euglycemic clamp technique. Other studies using more robust markers of insulin sensitivity such as homeostasis model assessment are needed to determine the full degree to which insulin sensitivity mediates the association between alcohol consumption and diabetes. Other biomarkers such as adiponectin, which could potentially mediate a substantial part of the association, should be included in such studies as well.

In summary, adiponectin explains about 25–30% of the relation between alcohol consumption and type 2 diabetes. Markers of inflammation, endothelial dysfunction, and fasting insulin did not appear to play an important role. These results cast some doubt on the physiological importance of the effects of alcohol on inflammation and endothelial function, at least for glucose metabolism, and suggest that further research is needed into the potentially mediating roles of other biomarkers affected by alcohol consumption.

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