

Metabolic and Biochemical Effects of Low-to-Moderate Alcohol Consumption

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Background: Alcohol consumption has multiple biochemical consequences. Only a few of these are useful as diagnostic markers, but many reflect potentially harmful or beneficial effects of alcohol. Average consumption of 2 to 4 drinks per day is associated with lower overall or cardiovascular mortality risk than either lower or higher intake. We have analyzed the dose-response relationships between reported alcohol consumption and 17 biomarkers, with emphasis on intake of up to 3 drinks per day.

Methods: Biochemical tests were performed on serum from 8,396 study participants (3,750 men and 4,646 women, aged 51 ± 13 years, range 18 to 93) who had provided information on alcohol consumption in the week preceding blood collection.

Results: Gamma glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, carbohydrate-deficient transferrin, urate, ferritin, and bilirubin showed little or no change with alcohol consumption below 2 to 3 drinks per day, but increased with higher intake. High-density lipoprotein cholesterol and albumin showed increasing results, and insulin showed decreasing results, across the entire range of alcohol use. Biphasic responses, where subjects reporting 1 to 2 drinks per day had lower results than those reporting either more or less alcohol use, occurred for triglycerides, glucose, C-reactive protein, alkaline phosphatase, and butyrylcholinesterase. Increasing alcohol use was associated with decreasing low-density lipoprotein cholesterol (LDL-C) in younger women, but higher LDL-C in older men.

Conclusions: Some markers show threshold relationships with alcohol, others show continuous ones, and a third group show biphasic or U-shaped relationships. Overall, the biochemical sequelae of low-to-moderate alcohol use are consistent with the epidemiological evidence on morbidity and mortality.

Key Words: Alcohol, Biomarkers, Dose-Response Curve, Population Study.

MANY STUDIES HAVE explored the relationships between alcohol intake and biochemical characteristics, either to evaluate the biological effects of alcohol use or to develop biomarkers of hazardous consumption or of relapse in alcoholics. The strongest associations have been found for gamma glutamyl transferase (GGT) and carbohydrate-deficient transferrin (CDT) (Alte et al., 2003; Conigrave et al., 2003; Scouller et al., 2000), but many other commonly measured constituents of serum (e.g., alanine aminotransferase [ALT]; aspartate aminotransferase [AST]; ferritin; high-density lipoprotein cholesterol [HDL-C]; and urate) show highly significant associations with alcohol intake (Alatalo et al., 2009; Nagaya et al., 1999; Whitehead et al., 1996b; Whitfield et al., 2001).

Prospective epidemiological studies have examined the connection between alcohol intake and cardiovascular dis-

ease (see Ronksley et al., 2011), diabetes (Conigrave et al., 2001; Koppes et al., 2005), metabolic syndrome (Alkerwi et al., 2009), or overall mortality (Di Castelnuovo et al., 2006; Doll et al., 1994; Inoue et al., 2012; Thun et al., 1997). These have usually shown biphasic (“U-shaped”) results, in which morbidity or mortality is lower in people who consume some alcohol than in abstainers, but increases once an inflexion point at about 4 drinks per day for men and 2 for women is passed. This inflexion point is often construed as a “safe limit” of alcohol intake. Conclusions about possible health benefits from alcohol have been controversial (Fillmore et al., 2006; Roerecke and Rehm, 2011; but see Fuller, 2011; Ronksley et al., 2011), and criticisms have included potential inclusion of past-drinkers who now abstain because of health problems; reliance on self-report of alcohol use; and the emphasis in most studies on middle-aged subjects at risk of cardiovascular disease when the relationship between alcohol use and mortality is likely to differ in younger people. Nevertheless, alcohol has multiple biological effects and there is no a priori reason why some of them may not be beneficial.

Any favorable effects of alcohol on cardiovascular mortality might be due to effects on known risk factors, such as HDL-C, insulin sensitivity, or coagulation and fibrinolysis. Unfavorable ones could be mediated through increased blood pressure or by changes in the number, size, or atherogenicity of lipoprotein particles. The consensus from meta-analysis of experimental studies, in which volunteers

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take controlled amounts of alcohol, is that daily alcohol administration for a few weeks increases HDL-C and apolipoprotein A1, has no effect on low-density lipoprotein cholesterol (LDL-C) or triglycerides, decreases fibrinogen, and increases adiponectin (Brien et al., 2011).

In view of the evidence that alcohol has cardioprotective effects, it is paradoxical that some markers of alcohol intake, particularly the liver enzymes GGT, ALT, and AST, predict risk of cardiovascular disease, diabetes, and overall mortality (Breitling et al., 2011; Fraser et al., 2007, 2009). Higher enzyme results are associated with increased risk, although the 3-way relationships among alcohol, enzyme results, and risk have not been fully explained. In the population-epidemiology context, these enzymes probably measure mild hepatic steatosis and reflect the liver changes associated with metabolic syndrome. However, the risk of metabolic syndrome as defined by the combination of hypertension, dyslipidemia, impaired glucose tolerance, and obesity (Alberti et al., 2006) is decreased by alcohol; meta-analysis showed a reduction in incidence associated with up to 40 g of alcohol per day in men and 20 g in women (Alkerwi et al., 2009).

Many studies have assessed associations between alcohol intake and markers of systemic inflammation, most commonly by measuring serum C-reactive protein (CRP) in cross-sectional studies. At least 11 such studies have been reported since 2001; most report U-shaped relationships with alcohol, sometimes dependent on sex, body mass index (BMI), or *APOE* genotype. The alcohol intake at which CRP concentration was lowest was usually 1 to 2 drinks per day. In 1 experimental study (Sierksma et al., 2002), 30 to 40 g of alcohol per day for 3 weeks resulted in a 35% decrease in CRP.

Comparison of the response of multiple biochemical markers or cardiovascular risk factors to alcohol intake, particularly to moderate alcohol intake, may allow cross-validation of the independent variable (self-reported alcohol intake) and comparison of the responses of the different dependent variables. This is best achieved by a study in which many biochemical characteristics have been measured, and compared against reliable estimates of alcohol use, rather than through comparisons across studies. We aimed to identify biomarkers or to infer changes in metabolic processes, which are either presumptively favorable across the spectrum of alcohol intake or else favorable at lower levels and unfavorable at higher ones, and to define the average responses to alcohol intake as a preliminary stage before examining genetic variation in such responses.

MATERIALS AND METHODS

Study Participants

This analysis is based on a group of studies on the genetics of alcohol use and dependence, and of smoking or nicotine dependence (Heath et al., 2011; Saccone et al., 2007). These studies were approved by the Queensland Institute of Medical Research and Washington University Ethics Review Committees and participants

gave informed consent. Recruitment was from the general population and was based on twins who had participated in our earlier studies, their first-degree relatives (siblings, parents, or adult offspring) and their spouses or partners. Interview data were available for 16,918 people from 11,700 families; 8,603 people attended for collection of blood and biochemical measurements were obtained from serum for 8,396 of them. Blood was collected into plain tubes to obtain serum samples and into fluoride-oxalate tubes for measurement of plasma glucose. Samples were stored at -80°C until used. At the time of blood collection, participants filled in a retrospective alcohol diary recording the number of standard drinks (containing 10 g of EtOH) in 4 categories (beer, wine, spirits, and other; no distinction was made between red and white wine) consumed on each day in the past week, and a similar retrospective diary for number of cigarettes. Number of drinks was summed across days and types to give the number of drinks per week. Participants who reported any cigarette use during the week were categorized as current smokers, and number of cigarettes was used as a quantitative measure of smoking. BMI was calculated from self-reported weight and height.

Data were also available from telephone interviews, conducted before the blood collection, on usual quantity and frequency of alcohol use over the past 12 months and on symptoms of alcohol dependence. Quantity and frequency data were used to estimate usual number of standard drinks per week (to be distinguished from the reported number in the past week), and symptom data were used to derive a lifetime diagnosis of Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) alcohol dependence. A total of 5,716 people (of the 8,396) had quantity-frequency alcohol estimates, and 6,440 had symptom data, which allowed classification as positive ($N = 1,477$) or negative ($N = 4,963$) for lifetime alcohol dependence.

Biochemical Measurements and Methods

All biochemical measurements except CDT and insulin were made using Roche reagents and methods on 917 or Modular P analyzers (Roche Diagnostics, Castle Hill, NSW, Australia). CDT was measured using the N Latex method on a Dade BN-II nephelometric analyzer (both from Siemens Healthcare Diagnostics, Bayswater, Victoria, Australia) and expressed as a percentage of total transferrin (CDT%). Insulin was measured using Abbott reagents on an Architect analyzer (Abbott Diagnostics Division, North Ryde, New South Wales, Australia). Results were obtained for most of the measurements for 8,390 to 8,396 of the subjects, with lower numbers for CRP (8,308), glucose (6,766), insulin (2,147), and CDT (2,008). LDL-C results were calculated using the Friedewald formula, except for 282 people who had triglyceride concentrations above 4.52 mM. As it was impractical to obtain fasting blood samples from all subjects, results for glucose and insulin were adjusted for reported time between the last meal and blood collection.

Characteristics of Study Participants

Means for alcohol intake and for the biomarker results are shown in Table 1. Alcohol intake in the week before blood collection was used to categorize participants into 7 groups. The groups, with numbers of subjects in each, mean ages, proportions meeting lifetime alcohol dependence criteria and of current smokers, mean past-week and quantity \times frequency alcohol estimates, and mean BMIs, are shown in Table 2. Cumulative frequency distributions for alcohol intake are shown in Fig. S1. Proportions of lifetime alcohol dependent subjects and current smokers were greater in the higher alcohol intake groups. BMI was not associated with alcohol intake group in men, $F(6, 3743) = 0.72$, $p = 0.633$, but there was a highly significant, $F(6, 4639) = 27.14$, $p = 5.54 \times 10^{-32}$, U-shaped relationship in women (Table 2, Fig. S2).

Table 1. Characteristics of the Study Participants

	Men (N = 3,750)	Women (N = 4,646)
Age	51.4 ± 13.4	50.8 ± 13.5
Alcohol intake, reported number drinks in past week (median and 90th centile)	10.0, 40.9	3.0, 16.0
Alcohol intake, estimated usual number of drinks per week (median and 90th centile)	8.5, 19.4	1.5, 10.5
Lifetime DSM-IV alcohol dependence (%)	31.5	15.9
Current smokers (%)	20.6	18.4
BMI (kg/m ²)	27.1 ± 4.4	26.6 ± 5.6
Gamma glutamyl transferase (u/l)	39.2 ± 44.4	24.1 ± 27.2
Alanine aminotransferase (u/l)	30.8 ± 19.7	20.6 ± 12.9
Aspartate aminotransferase (u/l)	27.4 ± 11.4	22.4 ± 8.5
Carbohydrate-deficient transferrin (%)	1.96 ± 0.84	1.60 ± 0.46
Urate (mM)	0.346 ± 0.075	0.263 ± 0.071
Ferritin (μg/l)	261 ± 196	111 ± 113
High-density lipoprotein cholesterol (mM)	1.35 ± 0.37	1.67 ± 0.42
Low-density lipoprotein cholesterol (mM)	3.36 ± 0.91	3.22 ± 0.92
Triglycerides (mM)	2.26 ± 1.39	1.69 ± 0.96
Glucose (mM)	5.34 ± 1.84	4.91 ± 1.42
Insulin (pM)	110 ± 134	93 ± 136
C-reactive protein (mg/l)	3.31 ± 5.91	4.15 ± 7.04
Alkaline phosphatase (u/l)	77.6 ± 21.2	75.3 ± 25.0
Butyrylcholinesterase (u/l)	9,760 ± 1,918	8,810 ± 1,924
Albumin (g/l)	48.8 ± 2.9	47.5 ± 2.8
Bilirubin (μM)	9.9 ± 5.5	8.0 ± 4.3
Urea (mM)	6.76 ± 1.91	6.03 ± 1.74

All statistics are mean ± SD unless otherwise noted.

Data Analysis

In preliminary analysis, biomarker results were subjected to multiple regression to identify effects of sex, age, BMI, alcohol intake, and smoking. Biomarkers results showing skewed distributions were log-transformed. The measure used for smoking was log (N of cigarettes + 1) to allow inclusion of current nonsmokers. Residuals, adjusted for all these effects except alcohol intake, were saved for further analysis.

These adjusted and standardized residuals were tested for associations with alcohol in a 2-step regression procedure, first for the linear effect of log(N of drinks + 1) and then for the quadratic relationship with log(N of drinks + 1) and [log(N of drinks + 1)]². The

improvement in proportion of variance explained (R²) from inclusion of the quadratic term was a measure of deviation from linearity in the log(alcohol)/marker relationship. As subjects were related and therefore not fully independent for variables affected by genetic variation, we used a Huber–White robust variance estimator (Williams, 2000) implemented in STATA (StataCorp, College Station, TX), to adjust estimated standard errors and p-values for the nonindependence of observations on family members.

To illustrate the shape of the alcohol/marker dose–response curves, reported alcohol intake in the week before blood collection was categorized into 7 groups and the means and standard errors for the adjusted residuals were calculated for each group and plotted. Similar plots of adjusted residuals against alcohol intake grouping were made for the quantity-frequency measure, for comparison and validation purposes; and plots based on the past-week alcohol data were constructed after division of the subjects by sex, lifetime alcohol dependence history, and current smoking status.

RESULTS

Multiple Regression

Results of initial analysis to define significant covariates are shown in Table 3. Most of the variables showed significant linear associations with reported alcohol intake. There were positive associations for GGT, ALT, AST, albumin, bilirubin, urea, urate, CDT, ferritin, and HDL-C, and an inverse one for insulin. The strongest associations were found for CDT, GGT, HDL-C, and insulin. All the biomarker results were affected by sex, and all except bilirubin, CDT, and insulin by age. Smoking had independent effects on all results except butyrylcholinesterase and insulin. All results were affected by BMI, although the effect on LDL-C in men was marginal.

Effects of Low-to-Moderate Alcohol Intake

The results from the initial multiple regression represent the linear effects across the observed range of alcohol intake. The 2-stage regression analysis on the standardized residuals of marker results after adjustment for sex, age, BMI, and log (N of cigarettes + 1) showed highly significant quadratic associations with alcohol intake (deviations from linearity) for all markers except insulin and albumin (Table 4). More

Table 2. Grouping of Alcohol Intake, and Associations with Lifetime Alcohol Dependence, Current Smoking Status, and BMI for the 8,396 Study Participants with Biomarker Data

Past-week intake group (Number of reported drinks)	N		Mean age		Percent DSM-IV AD positive		Percent current smokers		Mean past-week number of drinks		Mean Q × F number of drinks		Mean BMI	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
None	733	1558	54.8	54.0	25.3	11.3	16.2	15.3	0	0	1.64	1.04	27.34	27.87
1 or 2	235	684	51.8	50.4	17.9	7.9	16.2	13.7	1.64	1.54	1.90	1.56	27.00	26.62
3 to 5	382	726	50.8	47.7	16.7	11.9	13.8	16.0	4.00	3.91	3.55	2.78	26.87	26.28
6 to 10	544	753	51.2	49.7	21.6	15.4	13.4	15.9	7.83	7.67	5.72	5.27	27.08	25.28
11 to 20	799	645	51.6	49.3	29.0	23.3	15.9	25.7	15.09	14.42	8.70	8.35	27.20	25.43
21 to 40	682	240	49.4	48.2	43.8	45.3	26.8	38.8	28.66	26.80	14.35	13.46	27.01	25.86
Over 40	375	40	48.4	46.1	60.8	64.7	47.5	65.0	59.45	57.15	27.82	26.97	27.21	27.98

M, male; F, Female; AD, alcohol dependence (lifetime); Q × F, quantity-frequency estimate of usual weekly alcohol intake.

Table 3. *p*-Values from Multiple Regression of Marker Results

		Sex	Age	BMI	Total drinks	Smoking
Gamma glutamyl transferase (log)	β	0.241	0.123	0.234	0.241	0.068
	<i>p</i>	9.31×10^{-120}	8.50×10^{-36}	9.04×10^{-127}	2.62×10^{-115}	9.33×10^{-12}
Alanine aminotransferase (log)	β	0.342	-0.074	0.242	0.105	-0.038
	<i>p</i>	1.24×10^{-227}	1.34×10^{-13}	9.83×10^{-133}	2.74×10^{-23}	2.00×10^{-4}
Aspartate aminotransferase (log)	β	0.235	0.040	0.065	0.190	-0.083
	<i>p</i>	2.15×10^{-101}	1.28×10^{-4}	2.06×10^{-10}	1.15×10^{-64}	7.38×10^{-15}
Carbohydrate-deficient transferrin (%)	β	0.138	0.005	-0.122	0.283	0.117
	<i>p</i>	2.30×10^{-9}	NS	3.71×10^{-9}	2.23×10^{-31}	8.28×10^{-8}
Urate	β	0.441	0.163	0.240	0.113	-0.052
	<i>p</i>	$<1.0 \times 10^{-200}$	1.47×10^{-70}	2.45×10^{-154}	9.56×10^{-32}	1.58×10^{-8}
Ferritin (log)	β	0.430	0.170	0.104	0.124	0.035
	<i>p</i>	$<1.0 \times 10^{-200}$	2.03×10^{-69}	1.37×10^{-28}	2.70×10^{-34}	2.92×10^{-4}
Low-density lipoprotein cholesterol (female)	β	-	0.108	0.133	-0.071	0.070
	<i>p</i>	-	6.50×10^{-13}	1.47×10^{-19}	1.60×10^{-6}	3.41×10^{-6}
Low-density lipoprotein cholesterol (male)	β	-	-0.104	0.035	0.018	0.024
	<i>p</i>	-	1.56×10^{-9}	0.034	NS	NS
High-density lipoprotein cholesterol	β	-0.448	0.036	-0.278	0.299	-0.110
	<i>p</i>	$<1.0 \times 10^{-200}$	1.62×10^{-4}	6.23×10^{-189}	2.32×10^{-185}	6.49×10^{-30}
Triglycerides (log)	β	0.236	0.144	0.286	-0.002	0.089
	<i>p</i>	7.25×10^{-107}	2.69×10^{-44}	1.05×10^{-171}	NS	1.24×10^{-17}
Glucose	β	0.112	0.185	0.141	0.004	0.055
	<i>p</i>	3.81×10^{-19}	4.06×10^{-53}	7.32×10^{-33}	NS	6.73×10^{-6}
Insulin (log)	β	0.135	0.031	0.301	-0.221	0.012
	<i>p</i>	4.38×10^{-9}	NS	4.16×10^{-47}	5.47×10^{-20}	NS
Albumin	β	0.195	-0.257	-0.140	0.107	-0.028
	<i>p</i>	2.45×10^{-72}	2.95×10^{-130}	6.65×10^{-43}	2.73×10^{-22}	0.0077
C-reactive protein (log)	β	-0.109	0.133	0.375	0.002	0.072
	<i>p</i>	2.87×10^{-24}	9.09×10^{-38}	6.93×10^{-282}	NS	9.54×10^{-12}
Alkaline phosphatase	β	0.038	0.232	0.200	-0.024	0.090
	<i>p</i>	0.00061	1.40×10^{-101}	8.33×10^{-81}	0.034	1.08×10^{-16}
Butyrylcholinesterase	β	0.229	0.060	0.267	-0.018	-0.019
	<i>p</i>	2.54×10^{-97}	8.13×10^{-9}	4.17×10^{-145}	NS	NS
Bilirubin (log)	β	0.206	-0.012	-0.131	0.046	-0.182
	<i>p</i>	2.10×10^{-75}	NS	9.51×10^{-36}	5.25×10^{-5}	1.87×10^{-61}
Urea	β	0.204	0.342	0.047	-0.056	-0.043
	<i>p</i>	1.45×10^{-81}	1.11×10^{-230}	2.45×10^{-6}	2.45×10^{-7}	3.66×10^{-5}

p-Values > 0.05 are shown as NS, and *p*-values less than the Bonferroni-corrected critical value of 5.9×10^{-4} are shown in scientific notation. Sex is coded as female = 0, male = 1; Total Drinks is the number of drinks reported in the week before blood collection (not log-transformed); and the Smoking variable is $\log(N \text{ of cigarettes} + 1)$.

detailed examination of alcohol–biomarker relationships within the low-to-moderate-intake range was carried out using plots of the means and standard errors for the marker results for the 7 groups defined by reported alcohol intake in the week before blood collection. Results are shown in Figs 1–6. There was good correspondence between the expected marker values, calculated from the regression coefficients and the observed means (Fig. S3).

Many markers showed little or no change with increasing alcohol intake until a threshold level was exceeded. This was found for GGT, ALT, AST, CDT, urate, and ferritin (Figs 1 and 2). In general, the threshold was at around 2 drinks per day. However, some markers were affected by any reported alcohol consumption above zero; this occurred for HDL-C (Fig. 3) and insulin (Fig. 4), with the former increasing and the latter decreasing across the entire range of alcohol intake. Albumin also increased across all alcohol intake groups (Fig. 6). Other variables showed decreases across the lowest intake groups, but increased with larger amounts of alcohol. This “U-shaped” pattern of alcohol effects was seen for triglycerides (Fig. 3), glucose

(Fig. 4), alkaline phosphatase, butyrylcholinesterase, and CRP (Fig. 5).

Other effects were more complex; the response of CDT to alcohol varied with BMI, as reported previously (Whitfield et al., 2008). The relationship between alcohol and LDL-C differed by sex (Fig. 3). Initial results showed significantly significant inverse relationship between alcohol and LDL-C in women, but there was little effect in men. As effects might differ between pre- and postmenopausal women, we repeated the analysis after dividing subjects by age (below or above 50) as well as sex. In men, alcohol had little effect on LDL-C in the younger group, but increased it in the older group; in women, alcohol decreased LDL-C in the younger group, but had little effect in the older group (Fig. 7).

The alcohol–biomarker relationships when the quantity-frequency measure of alcohol intake was used instead of past-week intake are shown in Fig. S4. Comparisons of the effects of alcohol on the biomarker means by sex, lifetime alcohol dependence, and smoking status gave consistent results across categories, shown in Figs S5 to S7.

Table 4. Regression Analysis

	Linear		Quadratic				Proportion of variance		
	β_{Drinks}	P_{Drinks}	β_{Drinks}	P_{Drinks}	$\beta_{\text{Drinks-squared}}$	$P_{\text{Drinks-squared}}$	R^2 linear (%)	R^2 quadratic (%)	R^2 change (%)
Gamma glutamyl transferase (log)	0.2814	1.79×10^{-37}	-0.5156	4.13×10^{-15}	0.5353	5.91×10^{-34}	2.5	4.9	2.4
Alanine aminotransferase (log)	0.1142	8.32×10^{-8}	-0.3087	4.11×10^{-7}	0.2841	1.02×10^{-12}	0.4	1.1	0.7
Aspartate aminotransferase (log)	0.2186	6.87×10^{-24}	-0.3716	3.27×10^{-9}	0.3963	3.00×10^{-20}	1.5	2.8	1.3
Carbohydrate-deficient transferrin (%)	0.3558	1.99×10^{-16}	-0.2375	0.1004	0.3259	5.0×10^{-4}	3.7	4.6	0.9
Urate	0.1878	4.81×10^{-19}	-0.1376	0.0242	0.2185	2.45×10^{-8}	1.1	1.5	0.4
Ferritin (log)	0.1813	3.65×10^{-20}	-0.2159	3.10×10^{-4}	0.2668	4.54×10^{-13}	1.0	1.6	0.6
High-density lipoprotein cholesterol	0.5253	2.17×10^{-133}	0.1122	0.0618	0.2774	7.85×10^{-13}	8.7	9.3	0.6
Low-density lipoprotein cholesterol (LDL-C) (male)	0.0849	0.0046	0.1996	0.034	-0.0700	0.205	0.2	0.3	0.1
LDL-C (female)	-0.1595	7.13×10^{-7}	0.0398	0.670	-0.1595	0.024	0.6	0.7	0.1
Triglycerides (log)	-0.4556	0.0309	-0.2335	1.3×10^{-4}	0.1262	0.0019	0.1	0.2	0.1
Glucose	-0.0455	0.0611	-0.2821	5.3×10^{-5}	0.1615	2.40×10^{-4}	0.1	0.3	0.2
Insulin (log)	-0.3333	1.35×10^{-17}	-0.2658	0.0322	-0.0368	0.582	3.3	3.3	0
Albumin	0.2057	3.66×10^{-23}	0.1135	0.066	0.062	0.118	1.3	1.3	0
C-reactive protein (log)	-0.0628	0.0021	-0.2949	9.48×10^{-7}	0.1557	3.00×10^{-5}	0.1	0.3	0.2
Alkaline phosphatase	-0.1544	2.26×10^{-13}	-0.4992	6.61×10^{-15}	0.2315	3.09×10^{-9}	0.7	1.2	0.5
Butyrylcholinesterase	-0.0584	0.0065	-0.2056	0.0018	0.0989	0.021	0.1	0.2	0.1
Bilirubin (log)	0.1297	9.98×10^{-10}	0.3282	2.44×10^{-7}	-0.1333	0.001	0.5	0.7	0.2
Urea	-0.0392	0.055	0.1662	0.009	-0.1379	3.60×10^{-4}	0.1	0.2	0.1

Coefficients from linear and quadratic regression of biomarker results (adjusted for sex, age, BMI, and log(N of cigarettes + 1)) on log(N of drinks + 1). p-Values are based on use of the robust estimator as described in the text.

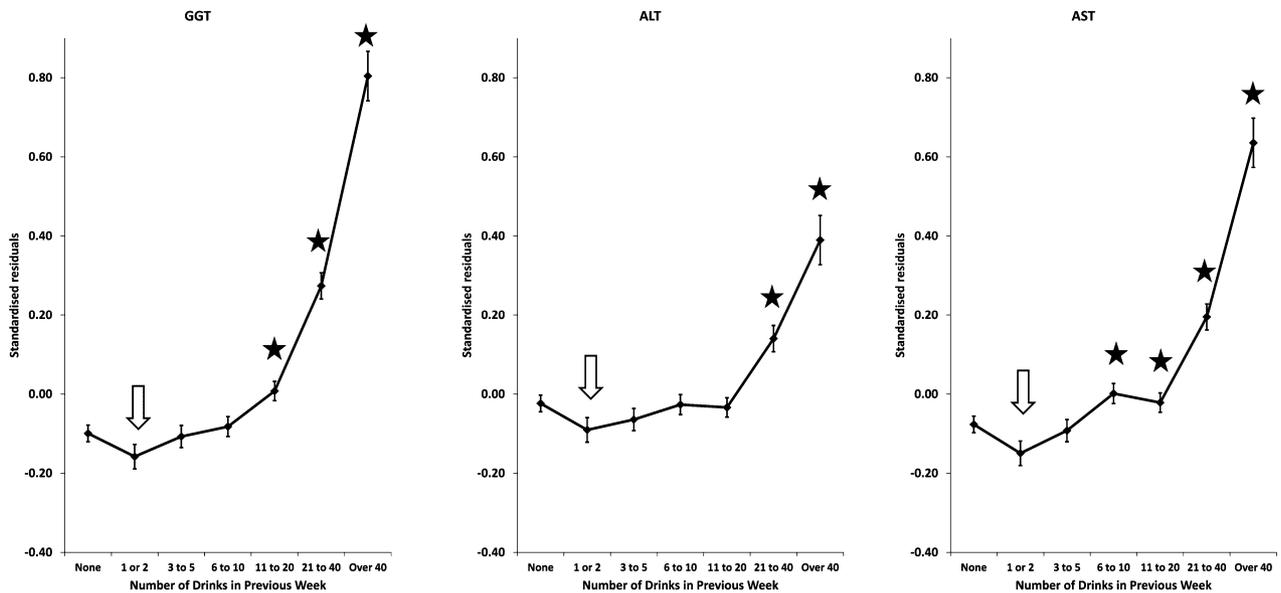


Fig. 1. Effects of alcohol on log₁₀GGT, log₁₀ALT, log₁₀AST; adjusted for sex, age, BMI, and smoking. Points and error bars show mean ± SEM. The arrow shows the reference group and stars indicate the groups which are significantly different from it after allowing for multiple comparisons ($p < (0.05/6) = 0.0083$).

Comparison of 2 Measures of Alcohol Intake

Most study participants had provided information on their usual frequency and quantity of alcohol use. This was used to provide a validation of the past-week intake data; the

sex-adjusted correlation between these 2 measures, using the log(N of drinks + 1) transformation, was 0.76. However, the number of drinks per week estimated from the usual quantity and frequency responses was lower than that reported for the week before blood collection. This is illustrated in Fig.

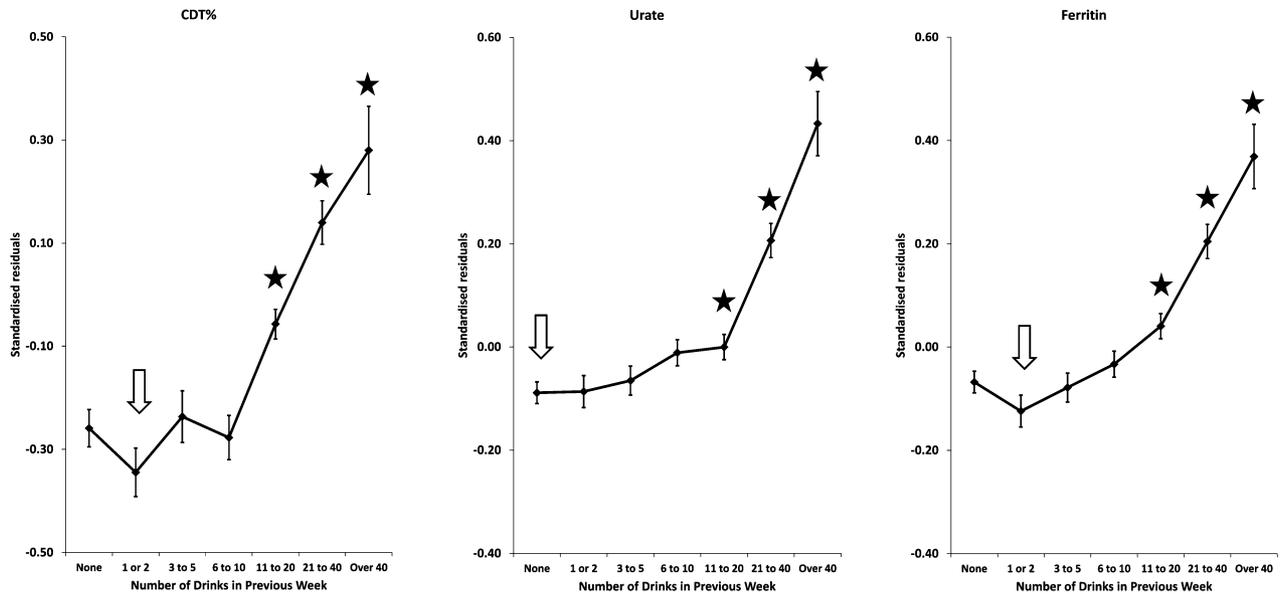


Fig. 2. Effects of alcohol on CDT, urate, \log_{10} Ferritin; adjusted for sex, age, BMI, and smoking. Points and error bars show mean \pm SEM. The arrow shows the reference group and stars indicate the groups which are significantly different from it after allowing for multiple comparisons ($p < (0.05/6) = 0.0083$).

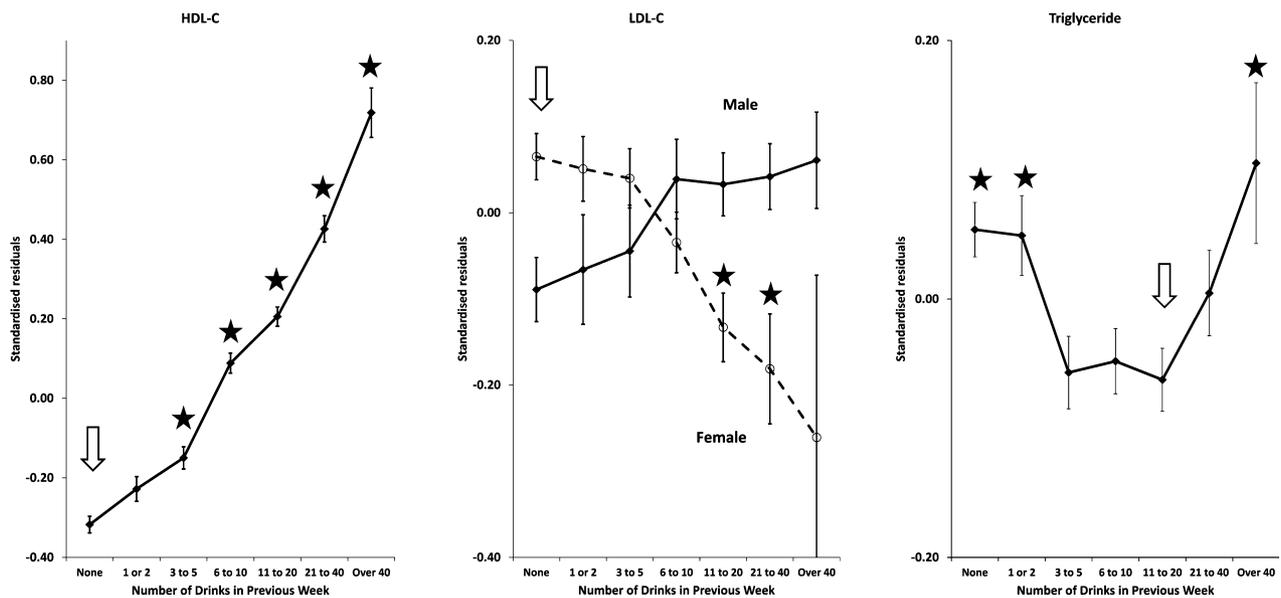


Fig. 3. Effects of alcohol on HDL-C, LDL-C, \log_{10} Triglyceride; adjusted for sex (except for LDL-C), age, BMI, and smoking. Points and error bars show mean \pm SEM. The arrow shows the reference group and stars indicate the groups which are significantly different from it after allowing for multiple comparisons ($p < (0.05/6) = 0.0083$).

S1; the cumulative frequency distributions level out at lower values for the quantity-frequency measure and at higher alcohol intake the past-week number of drinks was approximately double the quantity-frequency estimate. However, the shape of the relationships between the biomarker results and the quantity-frequency estimate of alcohol use were essentially the same as for the past-week estimate (Fig. S4). In particular, glucose, triglycerides, CRP, and alkaline

phosphatase still showed U-shaped relationships with alcohol when this measure was used.

Effects of Past-Alcohol Dependence

Potential effects of lifetime alcohol dependence status on the biomarkers, alone or in combination with current alcohol intake, were examined. This is particularly relevant to the

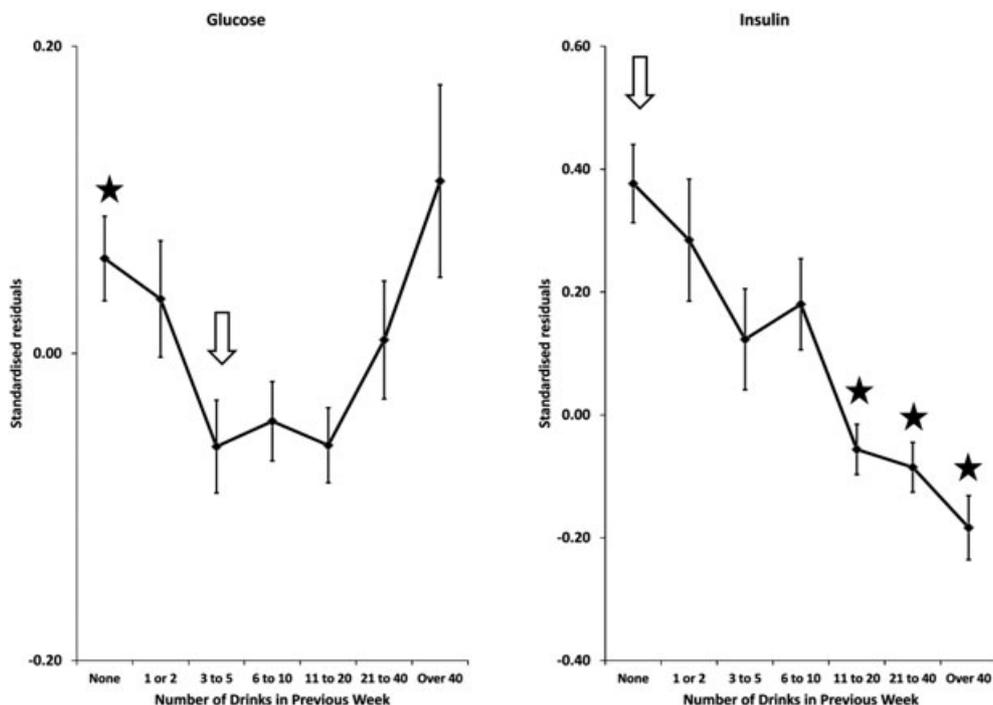


Fig. 4. Effects of alcohol on glucose, \log_{10} Insulin; adjusted for sex, age, BMI, smoking, and time since last meal. Points and error bars show mean \pm SEM. The arrow shows the reference group and stars indicate the groups which are significantly different from it after allowing for multiple comparisons ($p < (0.05/6) = 0.0083$).

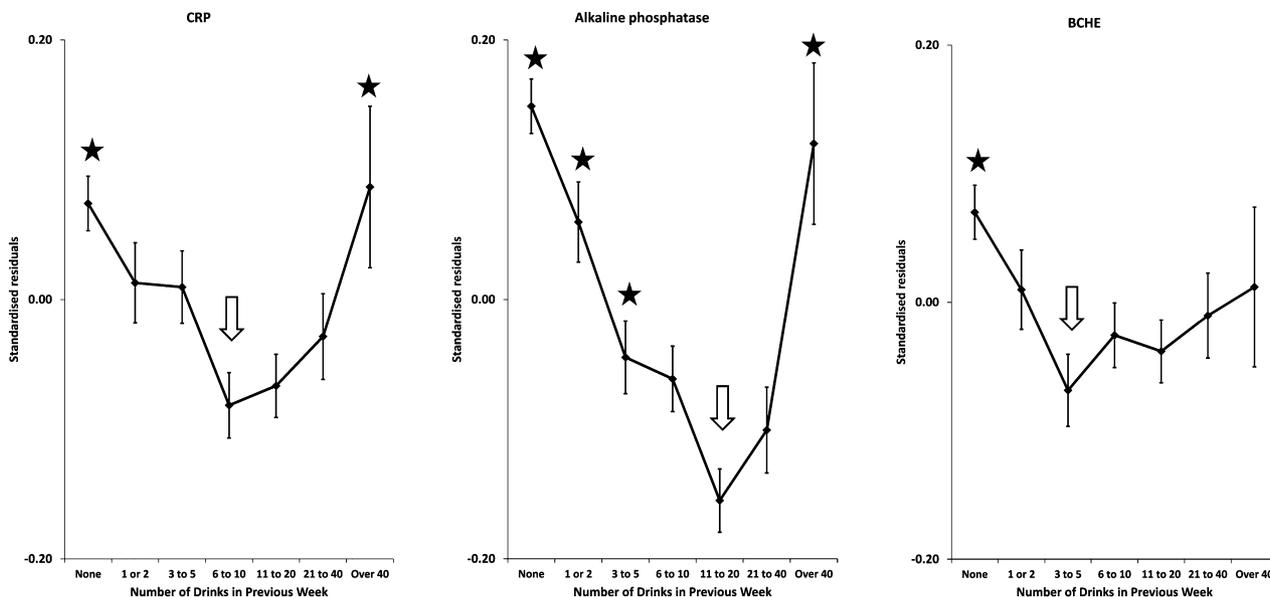


Fig. 5. Effects of alcohol on \log_{10} CRP, alkaline phosphatase, butyrylcholinesterase; adjusted for sex, age, BMI, and smoking. Points and error bars show mean \pm SEM. The arrow shows the reference group and stars indicate the groups which are significantly different from it after allowing for multiple comparisons ($p < (0.05/6) = 0.0083$).

group reporting no alcoholic drinks in the 7 days before blood collection, and it can be seen from Table 2 that the lifetime prevalence of alcohol dependence was higher in the zero-drinks category than in the “1 or 2 drinks” category. This difference was significant (stratified by sex, $G^2 = 9.68$,

2 df, 2-tailed $p = 0.0079$). Arising from this, we considered whether there was a significant difference between biomarker means for alcohol dependence–positive and alcohol dependence–negative people in the zero-drinks category, but none of the differences reached significance at $p < 0.05$ (Fig. S6).

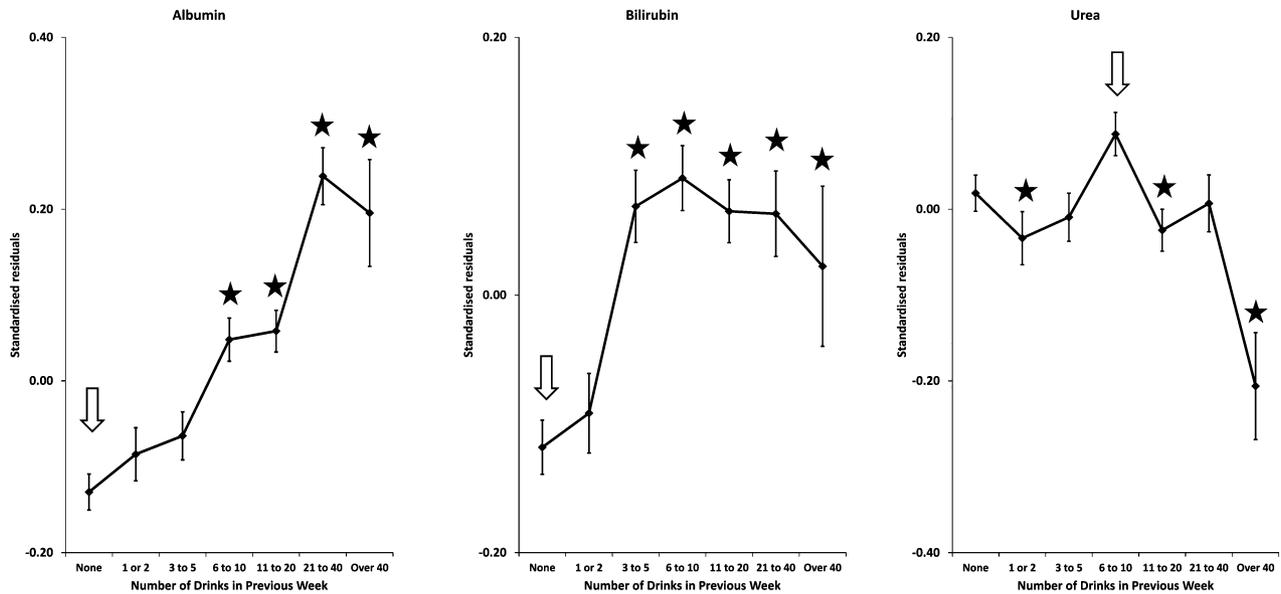


Fig. 6. Effects of alcohol on albumin, log₁₀Bilirubin, urea; adjusted for sex, age, BMI, and smoking. Points and error bars show mean \pm SEM. The arrow shows the reference group and stars indicate the groups which are significantly different from it after allowing for multiple comparisons ($p < (0.05/6) = 0.0083$).

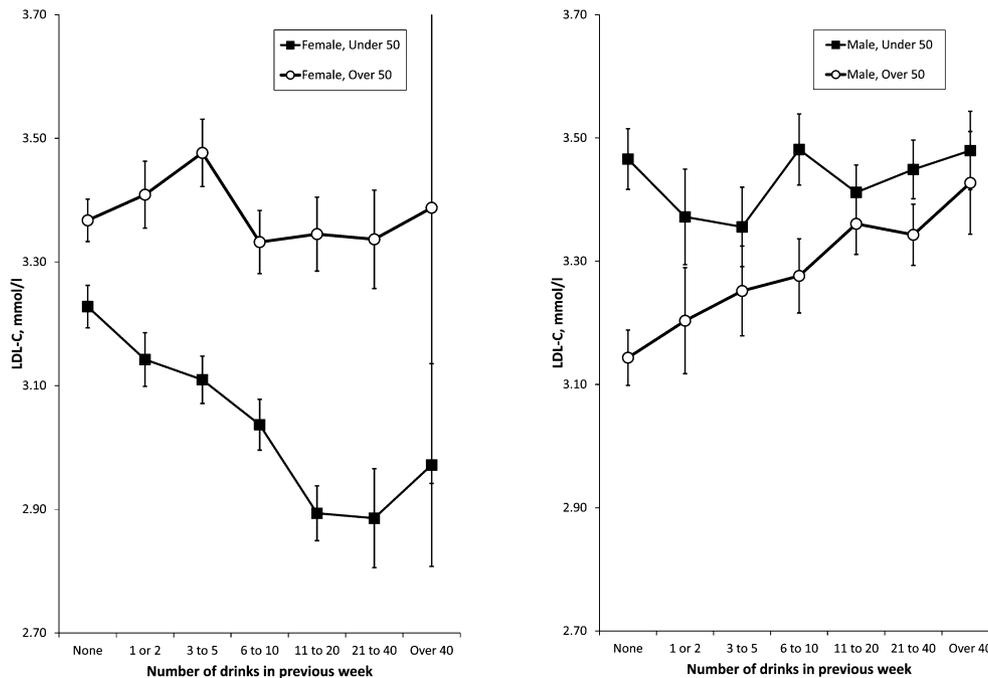


Fig. 7. Associations between alcohol intake and calculated LDL-C in women and men. In each panel, the results are divided by age of the subjects, into those aged less than 50 and those aged 50 or more. Error bars show standard errors. Linear regression showed significant effects of alcohol only in the older men ($p = 0.0045$) and younger women ($p = 7.86 \times 10^{-9}$).

Effects of Current Smoking

Although current smoking status had significant effects on many of the markers (Table 3), the alcohol/marker relationships were essentially parallel in the smokers and nonsmokers (Fig. S7).

Comparison of Beer and Wine

We compared GGT and HDL-C results from participants who reported consumption of only wine, or only beer, in the 7 days before blood collection to test whether the effects of alcohol depend on beverage. There was some confounding

of preferred beverage with sex, because the beer-only drinkers were mostly (83%) men, whereas the wine-only drinkers were mostly (78%) women. Once this was taken into account, the effects of alcohol from wine or from beer on GGT or on HDL-C were essentially the same (data not shown).

DISCUSSION

We concentrate on results which involve small amounts of alcohol, show significantly biphasic (U- or J-shaped) relationships, or have a bearing on the long-term health effects of alcohol.

Effects of Low-to-Moderate Alcohol Consumption

Many of the variables tested show a pattern of little change with increasing alcohol intake at lower levels, below about 2 drinks (20 g of alcohol) per day, and an increase in average values above that. This applies to the liver function tests GGT, ALT and AST, and also to CDT, urate, and ferritin. This suggests that alcohol has little effect below a threshold, but the threshold is marker-specific and there is no evidence of a threshold for HDL-C or insulin.

For the lipids related to cardiovascular risk, the situation is complex. HDL-C increases with increasing alcohol intake even across the low-intake categories, from those who report no alcohol in the previous week to an average of 1 drink (10 g of alcohol) per day. This trend continues across the entire spectrum of alcohol intake. For LDL-C, the associations with alcohol intake differ between men and women, and by age. The apparent effects of alcohol in younger women and older men may not be causal, and we cannot exclude effects from lifestyle or other confounders. For triglycerides, there was a decrease in the mean with increasing alcohol intake up to 1 to 2 drinks a day (10 to 20 g per day), but an increase for the categories equivalent to 4 or more drinks per day.

There is a substantial literature about associations between alcohol intake and plasma lipids in cross-sectional population studies, largely focused on implications for cardiovascular risk. The response of HDL-C is the most consistent feature, and there is some evidence that the higher HDL-C associated with greater alcohol use is accompanied by an increase in reverse cholesterol transport (Kralova Lesna et al., 2010). Overall, high HDL-C is associated with lower cardiovascular risk, but doubts have been raised about the role of HDL-C in mediating alcohol's effects on cardiovascular disease. Adjusting for HDL-C results in a recent prospective study on fatal coronary heart disease made little difference to the hazard ratios associated with alcohol use (Magnus et al., 2011). Against this, results from other studies have shown that adjustment for HDL-C does attenuate alcohol's relationship with cardiovascular mortality (Langer et al., 1992; Mukamal et al., 2005), which suggests that the reduction in HDL-C is important.

Recent meta-analysis of experimental studies in which volunteers took known amounts of alcohol (Brien et al., 2011) showed significant and dose-dependent increases in HDL-C, but no significant effects overall for LDL-C or triglycerides. In our data, triglycerides were lowest in people reporting between 3 and 20 drinks per week, or 4 to 30 g of alcohol per day. There have been previous reports of U-shaped alcohol-triglyceride associations with cross-sectional population studies (Kato et al., 2003; Tolstrup et al., 2009; Whitehead et al., 1996a), with lowest mean triglyceride values at about 20 g of alcohol per day.

Turning to glucose homeostasis, insulin concentration decreased with increasing alcohol consumption across the entire range and the linear association between alcohol intake and insulin concentration ($\beta = -0.33$, Table 4) was one of the strongest we found. Small amounts of alcohol were associated with lower glucose concentration, but reported consumption of over 20 drinks per week was associated with a reversal of this trend. This suggests that low alcohol intake (compared with no alcohol) increases insulin sensitivity and circulating insulin concentration decreases in response, but at higher levels of alcohol use, insulin secretion is insufficient and glucose concentrations increase. There may also be differences in diet or other unmeasured lifestyle factors in the high-alcohol-intake subjects, which could increase glucose concentrations. Our results are consistent with most studies, which have assessed serum insulin or insulin resistance in humans. Observational studies have found that higher alcohol consumption is associated with greater insulin sensitivity or decreased serum insulin (Fueki et al., 2007; Kawamoto et al., 2009; Sung et al., 2007). Experimental studies with humans taking 25 or 30 g of alcohol per day for 6 or 8 weeks gave consistent, but not conclusive results, with 1 showing significant reductions in fasting insulin and insulin resistance (Joosten et al., 2008), whereas the other showed only a trend toward improved insulin sensitivity (Kim et al., 2009). Animal studies support the hypothesis that alcohol improves insulin sensitivity (Hong et al., 2009), and suggest that this is mediated by increased expression of anti-inflammatory factors in adipose tissue (Paulson et al., 2010).

The only marker of inflammation which we measured was CRP. Concentrations were lowest in people reporting around 10 g of alcohol (1 drink) per day, with higher results associated with both lower and higher alcohol use. Although this association between alcohol and CRP was U-shaped and significant, it was not particularly strong, accounting for only 0.3% of the variance in log-transformed CRP concentration. Most cross-sectional studies have shown lower mean CRP concentrations in drinkers taking up to 20 g of alcohol per day compared with abstainers; several have also found higher CRP in excessive drinkers (Raum et al., 2007; Volpato et al., 2004; Wang et al., 2008) and those which did not (Albert et al., 2003; Levitan et al., 2005) were limited by the small number of excessive drinkers and a rather low top category of alcohol intake. Some experimental studies using 20

to 40 g of alcohol daily for 3 to 4 weeks (Sacanella et al., 2007; Sierksma et al., 2002) showed a decrease in CRP, whereas others using similar amounts of alcohol (Rajdl et al., 2007; Retterstol et al., 2005) have found small, but nonsignificant increases.

Alkaline phosphatase also showed a highly significant U-shaped relationship with alcohol use. Few previous studies of alcohol and alkaline phosphatase could be found, although one (Whitfield et al., 1978) found an increase in the prevalence of abnormally high alkaline phosphatase as quantity of alcohol per drinking day increased. The possibility of multiple effects of alcohol with differing thresholds is particularly relevant for alkaline phosphatase, because its activity in serum results from bone, liver, and intestinal isoenzymes. For example, high alcohol intake might increase liver alkaline phosphatase and account for the upward part of the U-shaped relationship, whereas smaller amounts of alcohol could be associated with decreases in bone or intestinal sources. This question cannot be resolved with our data. For butyrylcholinesterase, there was a significant biphasic component as shown in the quadratic regression analysis ($p = 0.009$). This marker was included because of its strong association with obesity and other components of metabolic syndrome. Albumin increased with increasing alcohol intake; there was no indication that excessive intake reduced its hepatic synthesis in this population-based study. Alcohol had no effect on urea except in the extreme group of people taking over 40 drinks per week, where there was a decrease. This may be due to low protein intake, but there is no direct evidence on this point.

Potential Confounders

Biphasic or U-shaped relationships with alcohol intake were found for triglycerides, glucose, CRP, alkaline phosphatase, and butyrylcholinesterase. As with the mortality and morbidity data, these could potentially be due to inclusion of past-drinkers in the zero-alcohol group. We consider this unlikely, because there are no significant biomarker differences between lifetime alcohol dependence–positive and lifetime alcohol dependence–negative people in the zero-alcohol group. Confining the regression analysis performed on all subjects (Table 3) to people who had never met the DSM-IV criteria for alcohol dependence still gave significant quadratic terms for triglycerides, CRP, alkaline phosphatase, and butyrylcholinesterase ($p = 0.0014, 0.0011, 0.0018, \text{ and } 0.021$, respectively), but not for glucose ($p = 0.473$). Similarly, the U-shaped effects do not depend on the association between higher alcohol intake and smoking. The possible effects of inaccurate reporting of alcohol intake are mitigated by the availability of 2 measures of alcohol intake, the number of drinks in the week before blood collection and the usual quantity and frequency measure. These were highly correlated, as shown in previous studies (Whitfield et al., 2004). At high alcohol intake, it seems that people underestimated their usual frequency or quantity of alcohol use and

the past-week estimate may be more accurate, but both measures of alcohol intake showed similar relationships with the biomarkers. In addition to these considerations, any errors in alcohol intake assessment will apply to all the alcohol–marker relationships and could not account for the differences where some are essentially linear, some show thresholds and others show U-shaped curves.

Limitations

Our results are subject to some limitations. We have insufficient information on morbidity and mortality to be able to connect alcohol, the biomarkers, and clinical outcomes into an integrated account, so this article is restricted to the observed alcohol–biomarker relationships. It was not feasible to obtain all blood samples in the fasting state, so additional variation is introduced for at least insulin and glucose and to a lesser extent for triglycerides and LDL-C. However, this is unlikely to generate false associations with alcohol use, nor to distort the shape of the alcohol–marker dose–response curves.

CONCLUSIONS

It is clear that variation in drinking behavior is associated with variation in many biochemical markers and metabolic processes. There may be common pathways from alcohol to several of the observed changes, but the differences in the alcohol–marker dose–response curves indicate that there is not a single pathway for all of them. There are changes which are probably harmful, and others which are probably beneficial. The pattern of potentially beneficial effects of alcohol on HDL-C and insulin increasing progressively as consumption increases, whereas the negative effects show thresholds, is consistent with net U- or J-shaped effects of alcohol on health. Questions for future research include whether genetic variation contributes to differences in responses to alcohol use, and how far the biomarker responses to alcohol predict clinical outcomes, such as liver damage or cardioprotection.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Comparison of cumulative frequency distributions for alcohol intake estimates from past-week recall and usual quantity \times frequency estimate. Data are from study participants for whom both estimates were available. The x -axis shows the reported alcohol intake and the y -axis shows the cumulative frequencies. Open symbols are for intake assessed from reported number of drinks in past week, filled symbols for intake assessed from reported usual quantity \times frequency; red symbols and lines for women and blue for men.

Fig. S2. Relationships between alcohol intake and body mass index in men and women.

Fig. S3. Observed and predicted relationships between log (drinks in past week + 1) and biomarker results (adjusted for sex, age, BMI, and smoking).

Fig. S4. Effects of usual alcohol intake (quantity-frequency measure) on biochemical markers.

Fig. S5. Effects of sex on relationships between reported alcohol intake and biochemical markers.

Fig. S6. Effects of lifetime alcohol dependence on relationships between reported alcohol intake and biochemical markers.

Fig. S7. Effects of current smoking status on relationships between reported alcohol intake and biochemical markers.