

Effect of alcohol consumption on biological markers associated with risk of coronary heart disease: systematic review and meta-analysis of interventional studies

Susan E Brien, postdoctoral fellow,¹ Paul E Ronksley, doctoral student,¹ Barbara J Turner, professor of medicine and director,² Kenneth J Mukamal, associate professor of medicine,³ William A Ghali, scientific director and professor^{1,4}

¹Calgary Institute for Population and Public Health, Department of Community Health Sciences, Faculty of Medicine, University of Calgary, Alberta, Canada T2N 4Z6

²REACH Center, University of Texas Health Science Center, San Antonio, TX, USA

³Harvard Medical School, Division of General Medicine and Primary Care, Beth Israel Deaconess Medical Center, Boston, MA, USA

⁴Department of Medicine, University of Calgary, Alberta, Canada

Correspondence to: W A Ghali
wghali@ucalgary.ca

Cite this as: *BMJ* 2011;342:d636
doi:10.1136/bmj.d636

ABSTRACT

Objective To systematically review interventional studies of the effects of alcohol consumption on 21 biological markers associated with risk of coronary heart disease in adults without known cardiovascular disease.

Design Systematic review and meta-analysis.

Data sources Medline (1950 to October 2009) and Embase (1980 to October 2009) without limits.

Study selection Two reviewers independently selected studies that examined adults without known cardiovascular disease and that compared fasting levels of specific biological markers associated with coronary heart disease after alcohol use with those after a period of no alcohol use (controls). 4690 articles were screened for eligibility, the full texts of 124 studies reviewed, and 63 relevant articles selected.

Results Of 63 eligible studies, 44 on 13 biomarkers were meta-analysed in fixed or random effects models. Quality was assessed by sensitivity analysis of studies grouped by design. Analyses were stratified by type of beverage (wine, beer, spirits). Alcohol significantly increased levels of high density lipoprotein cholesterol (pooled mean difference 0.094 mmol/L, 95% confidence interval 0.064 to 0.123), apolipoprotein A1 (0.101 g/L, 0.073 to 0.129), and adiponectin (0.56 mg/L, 0.39 to 0.72). Alcohol showed a dose-response relation with high density lipoprotein cholesterol (test for trend $P=0.013$). Alcohol decreased fibrinogen levels (-0.20 g/L, -0.29 to -0.11) but did not affect triglyceride levels. Results were similar for crossover and before and after studies, and across beverage types.

Conclusions Favourable changes in several cardiovascular biomarkers (higher levels of high density lipoprotein cholesterol and adiponectin and lower levels of fibrinogen) provide indirect pathophysiological support for a protective effect of moderate alcohol use on coronary heart disease.

INTRODUCTION

Moderate alcohol consumption (up to one drink a day for women and up to two for men) has been associated with a decreased risk for certain cardiovascular

diseases, particularly coronary heart disease, in several studies of diverse populations.^{1,2} Most of these studies, however, used an observational design, raising concerns about potential confounding.

Feeding studies (where alcohol is experimentally administered) free of concerns about confounding may help to elucidate the mechanisms by which alcohol affects cardiovascular disease. In 1999, a systematic review of experimental studies of alcohol consumption and changes in lipid levels and haemostatic factors asserted that the protective association of alcohol on certain cardiovascular diseases seemed to be mediated by some of these effects.³ Since that systematic review was published the breadth of research on this topic has expanded substantially. Atherosclerosis, the underlying cause of coronary heart disease and ischaemic stroke, is increasingly understood to be a chronic, low grade inflammatory disease of the arterial wall.⁴ Increased levels of inflammatory markers have been associated with risk of cardiovascular disease.^{5,6} New studies have examined not only the effect of alcohol on lipid levels and haemostatic factors but also on other measures of inflammation and endothelial cell function as well as levels of adipocyte hormones. Furthermore, in addition to haemostatic factors, increased levels of other molecules, such as cellular adhesion molecules and adipocyte hormones, are believed to contribute to the development of the systemic inflammatory response associated with increased risk of cardiovascular disease.^{4,7,8}

A synthesis of the evidence from experimental research in this area may inform clinicians trying to interpret the plausibility of the protective effects of alcohol on certain aspects of cardiovascular disease (coronary heart disease) from observational studies. We therefore systematically reviewed the effect of experimentally manipulated alcohol consumption (alcohol use versus a period of no alcohol use) on the circulating concentrations of selected cellular and molecular biological markers of atherothrombotic conditions associated with increased coronary heart disease risk in adults without pre-existing

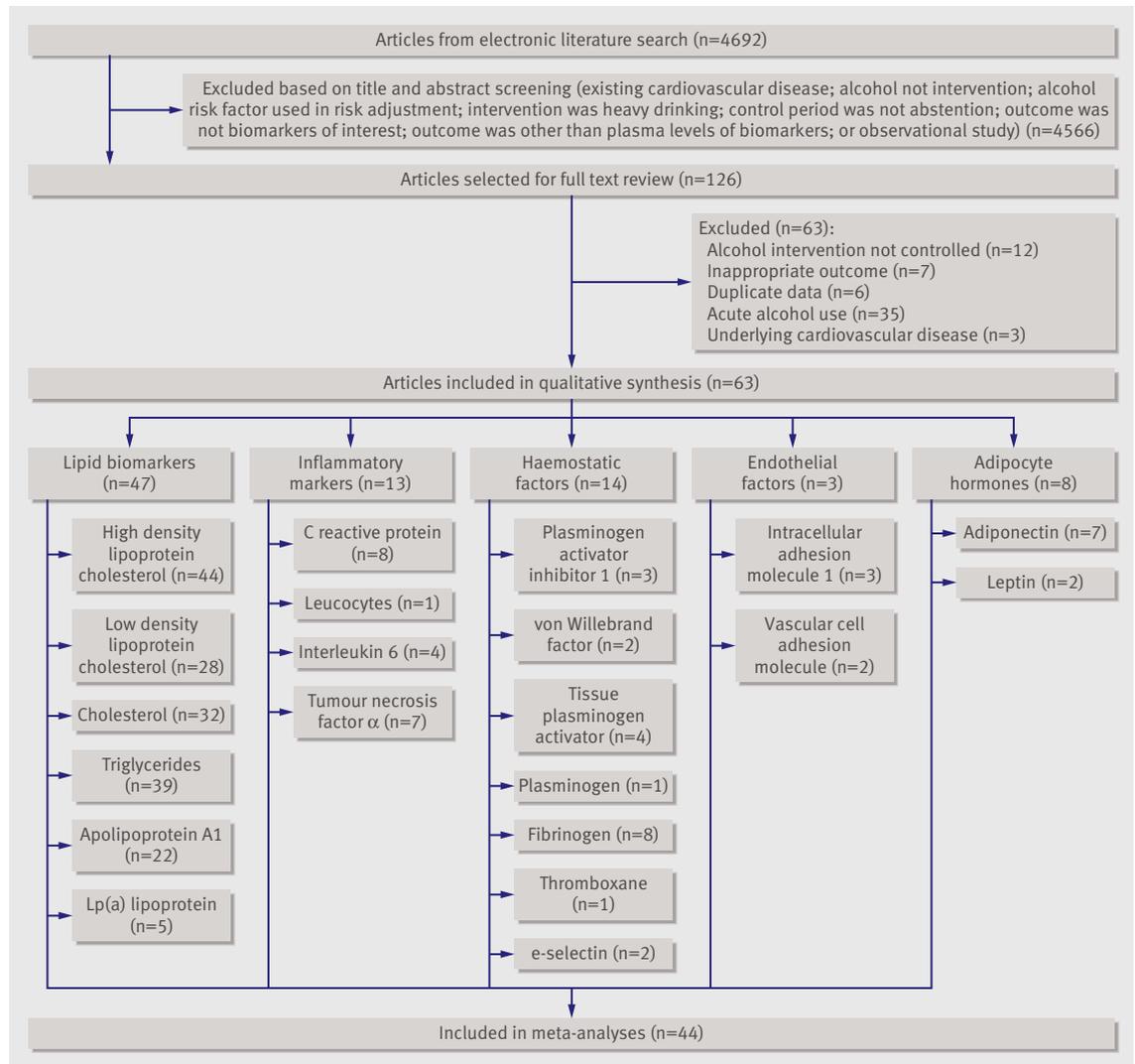


Fig 1 | Flow of studies through review

cardiovascular disease. This review offers complementary, indirect mechanistic evidence to that obtained from the expanding epidemiological research on the apparent protective effect of alcohol on certain aspects of cardiovascular disease.^{9,10}

METHODS

The systematic review was carried out using a predetermined protocol and in accordance with published guidelines for reporting of systematic reviews of randomised controlled trials (PRISMA).

Data sources and searches

We searched for alcohol intervention studies in adults without pre-existing cardiovascular disease in whom circulating levels of specific biomarkers were measured after a specified amount of alcohol had been consumed within a defined timeframe compared with a period of no alcohol use. We searched Medline (1950 to October 2009) and Embase (1980 to October 2009) without language restrictions for potentially relevant articles.

We used a strategy recommended for searching electronic databases for controlled interventional studies.¹¹ Our search focused on the exposure of interest, relevant outcomes, and study designs. The exposure of interest was alcohol consumption. The relevant outcomes were circulating atherothrombotic biological markers associated with coronary heart disease. These included lipids (triglycerides, total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, Lp(a) lipoprotein, and apolipoprotein A1), inflammatory markers (C reactive protein, leucocytes, interleukin 6, tumour necrosis factor α , and haemostatic factors plasminogen activator inhibitor 1, von Willebrand factor, tissue plasminogen activator, fibrinogen, and e-selectin), endothelial cell function markers (intracellular adhesion molecule 1 and vascular cell adhesion molecule), and adipocyte hormones (leptin and adiponectin). Our study designs of interest were experimental studies involving an intentional alcohol intervention to modify levels of biological markers with a no alcohol control. We included randomised controlled trials with two arms,

Table 1 | Characteristics of included studies examining the impact of alcohol interventions (1 week or greater in duration) on fasting plasma concentrations of biomarkers associated with cardiovascular disease

Source	Participants	Study design	Characteristics of participants	Alcohol intervention and diet	Biomarkers sampled	Included in meta-analysis	Reasons for exclusion from meta-analysis
Baer 2002 ²⁰	51; all women; mean age 60	Random crossover	Postmenopausal; no hyperlipidaemia, no diabetes, and no peripheral vascular disease	8 weeks of 15 g/day and 30 g/day 95% ethanol (1 or 2 drinks a day); controlled diet	Triglycerides, total cholesterol, high and low density lipoprotein cholesterol, and apolipoprotein A1	No	Data reported as least mean squares
Bantle 2008 ²¹	17; 7 men; age ≥40	Random crossover	Type 2 diabetes, no hypertension, no heart failure, and not receiving insulin*	1 month (12 g/day women, 24 g/day men) red or white wine (1-2 drinks a day); usual diet	Triglycerides, total cholesterol, low and high density lipoprotein cholesterol, C reactive protein, and plasminogen	Yes	—
Belfrage 1973 ²²	8; all men; age 22-26	Before and after	Healthy (includes some smokers)	5 weeks of 63 g/day beer (5 drinks a day); usual diet	Triglycerides, total cholesterol, and low and high density lipoprotein cholesterol	No	Error measurements not provided
Belfrage 1977 ²³	9; all men; age 22-29	Before and after	Healthy (includes some smokers)	4 weeks of 75 g/day beer or ethanol (6 drinks a day)†	Triglycerides and high density lipoprotein cholesterol	No	Data provided in graph format only
Bertiere 1986 ²⁴	10; all men; age 18-21	Before and after	Healthy (includes some smokers)	4 weeks of 30 g/day red wine (2.5 drinks a day); usual diet	Cholesterol, triglycerides, low and high density lipoprotein cholesterol, and apolipoprotein A1	Yes except for apolipoprotein A1	Data reported as density fraction
Beulens 2008 ²⁷	20; all men; age 18-25	Random crossover	All healthy, lean, or overweight, non-smokers	3 weeks of 40 g/day beer (3 drinks a day); controlled diet	Cholesterol, triglycerides, low and high density lipoprotein cholesterol, and C reactive protein	Yes	—
Beulens 2008 ²⁸	19; all men; age 18-25	Random crossover	Healthy, lean, or overweight	3 weeks of 40 g/day beer (3 drinks a day); usual diet	Adiponectin	Yes	—
Beulens 2007 ²⁶	19; all men; age 18-40	Random crossover	Healthy, lean or overweight	4 weeks of 32 g/day whisky (2.5 drinks a day); partially controlled diet	Adiponectin	Yes	—
Beulens 2006 ²⁵	34; all men; age 35-70	Random crossover	Abdominal obesity, no cardiovascular disease, no diabetes, non-smokers	4 weeks of 40 g/day red wine (3 drinks a day); usual diet	High density lipoprotein cholesterol and adiponectin	Yes, except for high density lipoprotein cholesterol	Error measurements not provided
Burr 1986 ²⁹	100; 48 men (age 20-56). 52 women (age 19-60)	Random crossover	No diabetes and not taking antihypertensive drugs*	4 weeks (19 g/day men, 17.8 g/day women) beer, wine, or spirits (1.5 drinks a day)†	Triglycerides, total cholesterol, low and high density lipoprotein cholesterol, and fibrinogen	Yes	—
Cartron 2003 ³⁰	18; all men; age 20-45	Random crossover	Normal cholesterol and triglyceride levels, no drugs or vitamins, non-smokers	3 weeks of 26 g/day (250 ml/day) white wine, champagne, or red wine (2 drinks a day); controlled diet	Total cholesterol, triglycerides, and apolipoprotein A1	Yes	—
Clevidence 1995 ³¹	34; all women; age 21-40	Random crossover	Premenopausal women, healthy, non-smokers	3 months of 30 g/day grain alcohol (2.5 drinks a day); controlled diet	Triglycerides, total cholesterol, low and high density lipoprotein cholesterol, apolipoprotein A1, and Lp(a) lipoprotein	Yes	—
Contaldo 1989 ³²	8; all men; age 30-47	Crossover	Healthy, non-smoker or light smoker	2 weeks of 75 g/day (750 ml wine) (6 drinks/day); isocaloric diet	Triglycerides, total cholesterol, low and high density lipoprotein cholesterol, and apolipoprotein A1	Yes	—
Couzigou 1984 ³³	7; all men; age 28-31; mean age 29.6	Before and after	Healthy, no drugs, usual smoking habits	1 week 23 g/day, then 4 weeks 31 g/day red wine (1.5, 2.5 drinks a day); usual diet	Low and high density lipoprotein cholesterol and apolipoprotein A1	Yes	—
Crouse 1984 ³⁴	12; all men; age 22-62	Before and after	No liver dysfunction, no metabolic disorders, no diabetes; 3 had hyperglycaemia, 2 atherosclerosis *	4 weeks 90 g/day‡ (7 drinks a day); controlled diet	Triglycerides, total cholesterol, and low and high density lipoprotein cholesterol	Yes	—
Davies 2002 ³⁵	51; all women; mean age 59.5	Random crossover	Post-menopausal, healthy	8 weeks of 15 g/day or 30 g/day‡ (1 or 2.5 drinks a day); controlled diet	Triglycerides	Yes	—

Source	Participants	Study design	Characteristics of participants	Alcohol intervention and diet	Biomarkers sampled	Included in meta-analysis	Reasons for exclusion from meta-analysis
De Oliveira e Silva 2000 ³⁶	14; 9 men; age 21-70; mean age 53.3	Crossover	Without significant disease, non-smokers	2 weeks of 1mL/kg/day vodka (1.5-2 drinks a day); controlled diet	Total cholesterol, triglycerides, and low and high density lipoprotein cholesterol	Yes	—
Djurovic 2007 ³⁷	87; 30 men; age 35-70	Random crossover	Healthy, non-smokers	3 weeks of 16 g/day (150 mL) red wine (1 drink a day)†	C reactive protein, tumour necrosis factor α, interleukin 6, intracellular adhesion molecule 1, vascular cellular adhesion molecule, and leptin	Yes, except for C reactive protein, tumour necrosis factor α, intracellular adhesion molecule 1, vascular cellular adhesion molecule, and leptin	C reactive protein: data reported as correlation with leptin; tumour necrosis factor α: non-detectable change reported; leptin, intracellular adhesion molecule 1, vascular cellular adhesion molecule: only one study reporting usable data
Estruch 2004 ³⁸	40; all men; age 30-50; mean age 37.6	Random crossover	Excludes those with hypertension, diabetes, high low density lipoprotein cholesterol, low high density lipoprotein cholesterol, coronary heart disease, cerebrovascular disease, peripheral vascular disease; non-smokers	28 days of 33 g/day (320 mL) red wine or (100 mL) gin (2.5 drinks a day); isocaloric diet	C reactive protein, fibrinogen, e-selectin, tumour necrosis factor α, intracellular adhesion molecule 1, and vascular cellular adhesion molecule	Yes except for e-selectin intracellular adhesion molecule 1, and vascular cellular adhesion molecule	e-selectin: only study reporting suitable data; intracellular adhesion molecule 1, vascular cellular adhesion molecule: reports on other cellular adhesion molecules
Fraser 1983 ³⁹	10; all men	Crossover	Generally healthy	3 weeks of 10-74 g/day beer or whisky (1-5 drinks a day); controlled diet	High density lipoprotein cholesterol and apolipoprotein A1	Yes	—
Frimpong 1989 ⁴⁰	8; all men; age 21-35	Before and after	Healthy, with normal lipid levels, non-smokers	6 weeks of 40 g/day beer (3 drinks a day); controlled diet	Triglycerides, total cholesterol, and low and high density lipoprotein cholesterol	Yes	—
Glueck 1980 ⁴¹	6; all men; 18-19	Before and after	Healthy, normal lipid profile	1 week of 35 g/day, 1 week 53 g/day vodka (2 then 3.5 drinks a day); controlled diet	Triglycerides, total cholesterol, and high and low density lipoprotein cholesterol	Yes	—
Goldberg 1996 ⁴²	24; all men; age 26-45	Crossover	Healthy	4 weeks of 40 g/day red wine or white wine (3 drinks a day); usual diet	Triglycerides, total cholesterol, high density lipoprotein cholesterol, and apolipoprotein A1	Yes	—
Gottrand 1999 ⁴³	5; all men; mean age 22.8	Random crossover	Healthy, non-smokers, no drugs	4 weeks of 50 g/day red wine (4 drinks a day); controlled diet	Triglycerides, total cholesterol, high density lipoprotein cholesterol, apolipoprotein A1, and Lp(a) lipoprotein	Yes	—
Hagiage 1992 ⁴⁴	14; all men; mean age 28	Before and after	Healthy, light or non-smokers, without history of chronic illness; 7 were normal weight, 7 were obese	2 weeks of 30 g/day red wine (2.5 drinks a day); usual diet	Triglycerides, total cholesterol, high and low density lipoprotein cholesterol, apolipoprotein A1, and Lp(a) lipoprotein	Yes	—
Hansen 2005 ⁴⁵	19; 9 men; age 38-75; mean age 50	Random crossover	No lipid lowering drugs or antihypertensives, includes some smokers	4 weeks (38.3 g/day men, 25.5 g/day women) red wine (1.5 or 2.5 drinks a day); controlled diet	Total cholesterol, high and low density lipoprotein cholesterol, and fibrinogen	Yes	—
Hartung 1983 ⁴⁶	44; all men; age 27-59	Before and after	16 marathoners, 15 joggers and 13 inactive; all healthy*†	3 weeks of 37.5 g/day beer (3 drinks a day); usual diet	Triglycerides, total cholesterol, and high and low density lipoprotein cholesterol	Yes	—
Hartung 1986 ⁴⁷	32; all women; age 30-49	Before and after	Pre-menopausal, half were habitual runners, half inactive; some smokers in inactive group	3 weeks of 35 g/day wine (3 drinks a day); usual diet	Triglycerides, total cholesterol, high and low density lipoprotein cholesterol, and apolipoprotein A1	Yes	—
Hartung 1990 ⁴⁸	49; all men; age 30-54	Before and after	26 habitual runners, 23 inactive; all healthy; some smokers in inactive group	3 weeks of 12.5 g/day or 37.5 g/day beer (1 or 3 drinks a day)†	High density lipoprotein cholesterol and apolipoprotein A1	Yes	—
Imhof 2009 ⁴⁹					Adiponectin	No	

Source	Participants	Study design	Characteristics of participants	Alcohol intervention and diet	Biomarkers sampled	Included in meta-analysis	Reasons for exclusion from meta-analysis
	72; 36 men; age 22-56	Random crossover	Healthy, non-smokers	3 weeks (30 g/day men, 20 g/day women) of beer, wine, or ethanol (2-2.5 drinks a day)			Data presented as percentage change
Jensen 2006 ⁵⁰	80; 28 men; age 35-70	Random crossover	Healthy, non-smokers	3 weeks 15 g/day red wine (1 drink a day)†	Fibrinogen	Yes	—
Joosten 2008 ⁵¹	36; all women; mean age 56.5	Crossover	Postmenopausal, healthy*	6 weeks of 20 g/day white wine (1.5 drinks a day); usual diet	Triglycerides, total cholesterol, high and low density lipoprotein cholesterol, and adiponectin	Yes	—
Karlsen 2007 ⁵²	49; 15 men; age 35-70	Random prospective two arm control	Healthy, non-smokers, no cardiovascular disease, no diabetes, no liver disease, no lipid lowering drugs, no aspirin	3 weeks of 15 g/day red wine (1 drink a day)†	Interleukin 6 and tumour necrosis factor α	No	Data presented as median difference
Malmendier 1985 ⁵³	9; all men; age 23-39	Before and after	Healthy, no drugs, no history of cardiovascular disease, no diabetes, no hyperlipoproteinaemia; 7 normal weight, 2 obese	2 weeks of 60 g/day (normal weight) or 70 g/day (obese) gin or vodka (5-6 drinks a day); isocaloric diet	Triglycerides, total cholesterol, high and low density lipoprotein cholesterol, and apolipoprotein A1	Yes	—
McConnell 1997 ⁵⁴	20; 11 men; age 23-51	Before and after	Healthy, non-smokers, no hyperlipidaemia, no coronary disease, no vascular disease, no hypertension, no diabetes	6 weeks of 16.5 g/day beer (1.5 drinks a day); usual diet	Triglycerides, high and low density lipoprotein cholesterol, apolipoprotein A1, Lp(a) lipoprotein, tissue plasminogen activator, plasminogen activator inhibitor 1, and von Willebrand factor	Yes except for Lp(a) lipoprotein and von Willebrand factor	Lp(a) lipoprotein: incongruent units of analysis; von Willebrand factor: data presented as percentage normal
Mezzano 2003 ⁵⁶	42; all men; mean age 22 years	Before and after	Healthy, non-smokers	30 days of 23.2 g/day red wine (2 drinks a day); specialised diets	von Willebrand factor	No	Too few studies for meta-analysis
Mezzano 2001 ⁵⁵	42; all men; mean age 22	Before and after	Healthy, non-smokers	30 days of 23.2 g/day red wine (2 drinks a day); specialised diets	C reactive protein, fibrinogen, tissue plasminogen activator, and plasminogen activator inhibitor 1	Yes	—
Naissides 2006 ⁵⁷	19; all women; age 50-70 (mean age 58.4)	Random prospective two arm control	Postmenopausal, moderately hypercholesterolaemic, excludes obese, physically active, smokers, poor diet, or those receiving lipid lowering drugs	6 weeks of 40 g/day red wine (3 drinks a day); controlled diet	Triglycerides, total cholesterol, and high and low density lipoprotein cholesterol	No	Data in graph format
Nishiwaki 1994 ⁵⁸	25; all men; mean age 31.4	Before and after	Healthy, no diabetes	4 weeks of 30-49 g/day (0.5 g/kg for 3 hours after dinner) alcohol‡ (2-4 drinks a day); controlled diet	Triglycerides, total cholesterol, high density lipoprotein cholesterol, and apolipoprotein A1	No	Data in graph format
Pace-Asciak 1996 ⁵⁹	24; all men; age 26-45	Crossover	Healthy	4 weeks of 40 g/day red or white wine (2.5 drinks a day); usual diet	Thromboxane	No	Only study reporting on this biomarker
Pikaar 1987 ⁶⁰	12; all men; age 21-29	Random crossover	Healthy, non-smokers	5 weeks of 25 g/day or 50 g/day wine (2-4 drinks a day); usual diet	Triglycerides, total cholesterol, high density lipoprotein cholesterol, plasminogen, and tissue plasminogen activator	No	Error measurements not provided
Retterstol 2005 ⁶¹	87; 30 men; age 35-70	Random crossover	Healthy, non-smokers	3 weeks of 15 g/day red wine (1 drink a day)†	Triglycerides, total cholesterol, high density lipoprotein cholesterol, C reactive protein, and fibrinogen	Yes	—
Romeo 2007 ⁶³	57; 30 men; age 25-50	Before and after	"Medically healthy," no chronic conditions involving immune system	1 month (22 g/day men, 11 g/day women) beer (1-2 drinks a day); usual diet	Leucocytes	No	Only study reporting on this biomarker

Source	Participants	Study design	Characteristics of participants	Alcohol intervention and diet	Biomarkers sampled	Included in meta-analysis	Reasons for exclusion from meta-analysis
Romeo 2007 ⁶²	57; 30 men; age 25-50	Before and after	"Medically healthy," no chronic conditions involving immune system	1 month (22 g/day men, 11 g/day women) of beer (1-2 drinks a day); usual diet	Interleukin 6, tumour necrosis factor α	Yes	—
Roth 2003 ⁶⁴	53; all women; age \geq 49; mean age 59.7	Random crossover	Healthy, non-smokers, postmenopausal	8 weeks of 15 g/day or 30 g/day alcohol \ddagger (1 or 2.5 drinks a day); controlled diet	Leptin	No	Reported as percentage change and geometric mean
Schneider 1985 ⁶⁵	6; 4 men; age 27-33	Before and after	Healthy	4 weeks of 70-80 g/day white wine (5-6 drinks a day); controlled diet	Triglycerides, total cholesterol, and high and low density lipoprotein cholesterol	No	Data in graph format
Senault 2000 ⁶⁶	56; all men; age 18-35	Random crossover	Healthy, no drugs	2 weeks of 30 g/day red wine or hydroalcohol (2.5 drinks a day); usual diet	Triglycerides, total cholesterol, high and low density lipoprotein cholesterol, apolipoprotein A1, and Lp(a) lipoprotein	Yes	—
Sharpe 1995 ⁶⁷	20; 11 men; age 25-60; mean age 37.2	Before and after	Healthy	10 days of 21 g/day red or white wine (1.5 drinks a day); usual diet	Total cholesterol, triglycerides, high and low density lipoprotein cholesterol, apolipoprotein A1, and Lp(a) lipoprotein	Not Lp(a) lipoprotein	Error measurements not provided
Sierksma 2004 ⁷²	23; all men; age 45-65; mean age 52	Random crossover	Apparently healthy non-smokers	17 days of 40 g/day whisky (3.5 drinks a day); controlled diet	Triglycerides, high density lipoprotein cholesterol, tumour necrosis factor α , and adiponectin	No	Error measurements not provided
Sierksma 2004 ⁷¹	18; all women; age 49-65; mean age 57	Random crossover	Postmenopausal, non-smokers, healthy	3 weeks of 24 g/day white wine (2 drinks a day); usual diet	Triglycerides, high density lipoprotein cholesterol, total cholesterol, and apolipoprotein A1	Yes	—
Sierksma 2002 ⁶⁹	19; 10 men (age 45-64), 9 women (age 49-62)	Random crossover	Women postmenopausal, healthy, no prescribed drugs	3 weeks of (men) 40 g/day and (women) 30 g/day (3 and 2.5 drinks a day, respectively) beer; controlled diet	High density lipoprotein cholesterol and apolipoprotein A1	No	Data reported as percentage change
Sierksma 2002 ⁷⁰	19; 10 men (age 45-64), 9 women (age 49-62)	Random crossover	Women postmenopausal, healthy, no prescribed drugs	3 weeks of (men) 40 g/day and (women) 30 g/day (3 and 2.5 drinks a day, respectively) beer; usual diet	Triglycerides, high density lipoprotein cholesterol, C reactive protein, and fibrinogen	Not C reactive protein	Data presented as median change
Sierksma 2001 ⁶⁸	19; 10 men (age 45-64), 9 women (age 49-62)	crossover	Healthy, non-smokers; postmenopausal women	3 weeks of (men) 40 g/day and (women) 30 g/day (3 and 2.5 drinks a day, respectively) beer; controlled diet	Fibrinogen	No	Data in graph format
Suzukawa 1994 ⁷³	12; all men; mean age 31.4	Random prospective two arm control	Healthy, normal anthropometrics	4 weeks of 0.5 g/kg/day brandy (2 drinks a day); usual diet	Triglycerides, total cholesterol, and high and low density lipoprotein cholesterol	Yes	—
Thornton 1983 ⁷⁴	12; 3 men; age 39-57; mean age 47	Before and after	Healthy, normolipidaemic, non-smokers	6 weeks 39 g/day wine (3.5 drinks a day); usual diet	Triglycerides, total cholesterol, and high and low density lipoprotein cholesterol	Yes	—
Tsang 2005 ⁷⁵	12; unclear No of men; age 23-50	Random prospective two arm control	Healthy, non-smokers	2 weeks of 39.7 g/day red wine (3.5 drinks a day); controlled diet	Triglycerides and high and low density lipoprotein cholesterol	Yes	—
Valimaki 1991 ⁷⁷	10; all men; age 27-45; mean age 36	Before and after	Healthy	3 weeks of 60 g/day wine, whisky, or vodka (5 drinks a day); usual diet	Triglycerides, total cholesterol, high density lipoprotein cholesterol, and apolipoprotein A1	Yes	—
Valimaki 1988 ⁷⁶	10; all men; age 30-43	Before and after	Healthy	3 weeks of 30 g/day or 60 g/day wine, whisky, or vodka (2.5 or 5 drinks a day); usual diet	Triglycerides, total cholesterol, high density lipoprotein cholesterol, and apolipoprotein A1	Not apolipoprotein A1	Error measurements not provided
Van der Gaag 2001 ⁷⁹	11; all men; age 45-60	Random crossover	Non-smokers, healthy	3 weeks of 40 g/day red wine, beer, and gin (3.5 drinks a day); controlled diet	Triglycerides, total cholesterol, high and low density lipoprotein cholesterol, and apolipoprotein A1	Yes	—

Source	Participants	Study design	Characteristics of participants	Alcohol intervention and diet	Biomarkers sampled	Included in meta-analysis	Reasons for exclusion from meta-analysis
Van der Gaag 1999 ⁷⁸	11; all men; age 44-59; mean age 51.7	Random crossover	Healthy, non-smokers	3 weeks of 40 g/day red wine, beer, or spirits (3.5 drinks a day); controlled diet	High density lipoprotein cholesterol and apolipoprotein A1	No	Data in graph format
Van Golde 2002 ⁸⁰	6; all men; mean age 34	Before and after	Healthy, non-smokers, normal liver function and lipid profile	2 weeks of 37.5 g/day wine (3 drinks a day)†	Tissue plasminogen activator and plasminogen activator inhibitor 1	Yes	—
Vazquez-Agell 2007 ⁸¹	20; all men; age 25-50	Random crossover	Non-smokers, normal lipids, no hypertension, no diabetes, no cardiovascular disease, no peripheral vascular disease	28 days of 30 g/day gin or white wine (2.5 drinks a day)†	C reactive protein, interleukin 6, e-selectin, adiponectin, tumour necrosis factor α , and intracellular adhesion molecule 1	No	Data presented as percentage change
Watzl 2004 ⁸²	24; all men; mean age 30.6	Random crossover	Healthy, non-smokers	2 weeks of 53 g/day red wine or ethanol (4 drinks a day); usual diet	Tumour necrosis factor α	Yes	—

*Smoking status not specified.

†Diet not specified.

‡Type of alcohol not specified.

before and after studies, and crossover studies. Using the Boolean operator “and” in varying combinations we then combined the three comprehensive search themes. See web extra appendix 1 for the complete Medline search strategy.

In addition to searching the electronic databases we consulted the bibliography of the only pre-existing systematic review on this subject.³ One of the authors (KJM) served as our content expert and provided us with input on captured literature and direction on pertinent studies in the grey literature.

Study selection

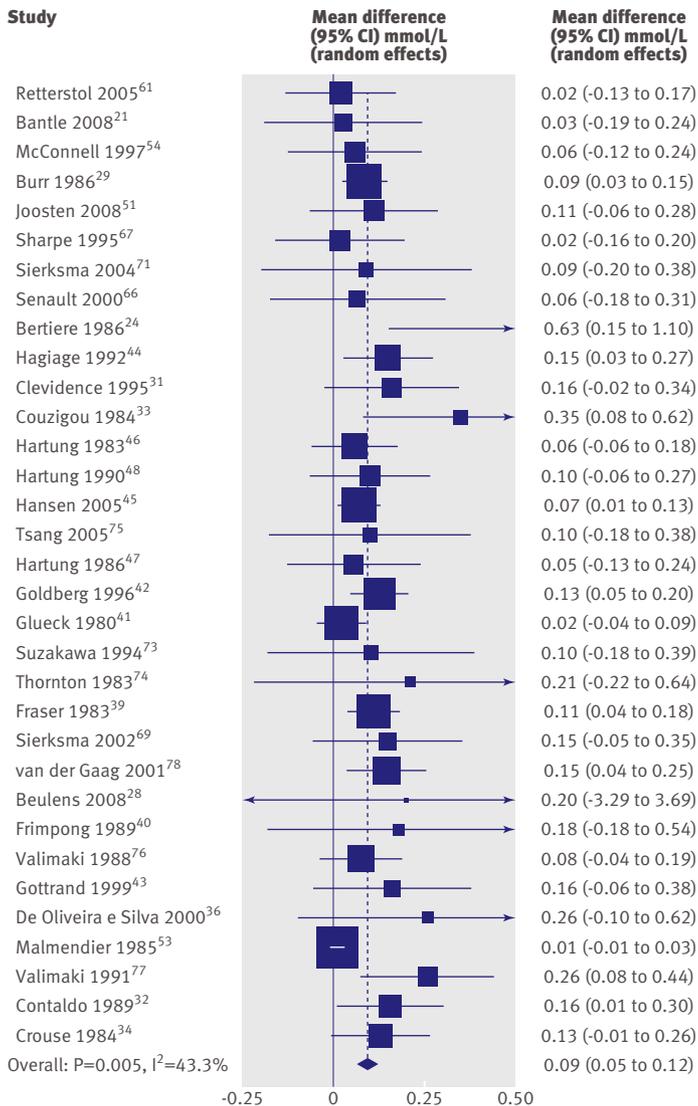
Relevant articles were selected using a two phase process. Two researchers (SEB and PER) independently reviewed all identified abstracts for eligibility. All abstracts reporting on the effect of alcohol consumption and relevant biomarkers in participants without pre-existing cardiovascular disease were selected for full text review. This initial stage was intentionally liberal; we discarded only abstracts that clearly did not meet the aforementioned criteria. The inter-rater agreement for this stage was high ($\kappa=0.80$, 95% confidence interval 0.65 to 0.94). Disagreements were resolved by consensus. Secondly, full text articles assessed by one reviewer (SEB) were verified by a second reviewer (PER) to determine if the study met the specified intervention, study population, and design criteria. Specifically, we included studies if they evaluated the circulating blood levels of the specified biomarkers during a period of intentional, prescribed alcohol feeding versus a period of no alcohol use. We excluded studies if participants had pre-existing cardiovascular disease or continued to drink “usual alcohol” in addition to the amounts of intervention alcohol. Both published and unpublished studies were eligible for inclusion.

Data extraction and quality assessment

From relevant studies we extracted information on sample size, population demographics (age, number of men and women, and mean age or age range, or both), inclusion and exclusion criteria (pre-existing health conditions, smoking status, drugs), study design (crossover, randomised crossover, randomised two arm, and before and after), characteristics of the alcohol intervention (amount, frequency, type, duration), use of a concomitant diet intervention, biomarkers sampled, and the mean concentration and error measurements (standard deviation, standard error, or confidence intervals) of specific biomarkers sampled after the alcohol intervention and after no alcohol use. When available we extracted information on amount of alcohol consumed, using grams of alcohol per day as the common unit of measure. When a study did not specifically report the grams of alcohol per unit, we used 12.5 g alcohol per drink for analysis.^{2 12} For example, if a study indicated that the intervention was 30 g of alcohol a day, we estimated this as 30 g alcohol a day divided by 12.5 g alcohol a drink equals about 2.5 drinks a day. We standardised portions as a 12 oz (355 mL) bottle or can of beer, a 5 oz (148 mL) glass of wine, and 1.5 oz (44 mL) of 80 proof (40% alcohol) distilled spirits.¹ We categorised the volume of alcohol intake as <2.5 g/day (<0.5 drink), 2.5-14.9 g/day (about 0.5-1 drink), 15-29.9 g/day (about 1-2.5 drinks), 30-60 g/day (about 2.5-5 drinks), and >60 g/day (≥ 5 drinks).

We assessed study quality using a previously outlined component approach.¹³ Study design was considered the most important measure of quality, with randomised studies (crossover and prospective two arm controlled studies) judged to have a higher quality than before and after studies. We carried out sensitivity analyses based on study design to evaluate the effect of this aspect of study quality.¹³ We also reported several

High density lipoprotein cholesterol



Low density lipoprotein cholesterol

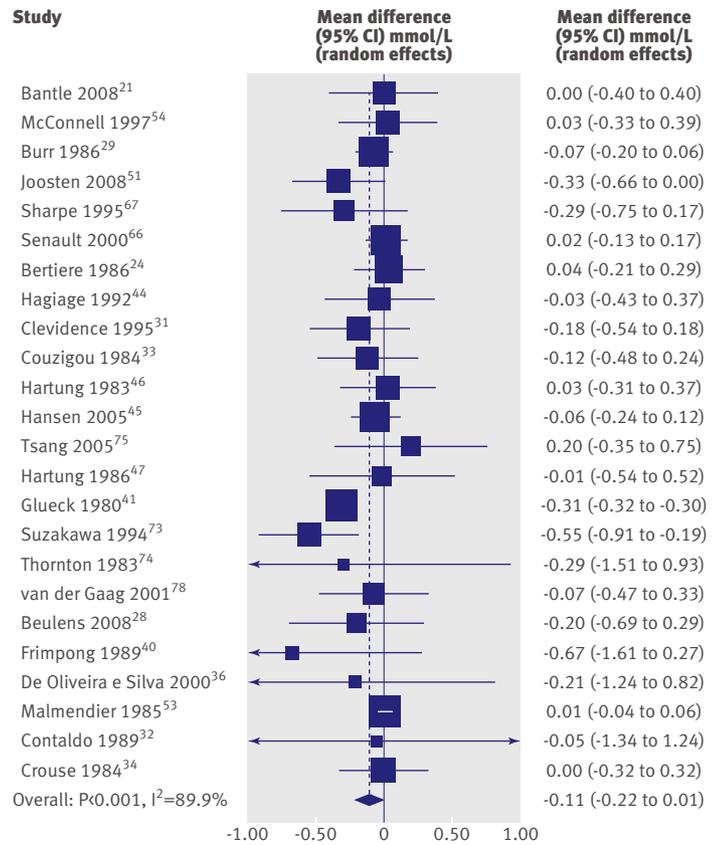


Fig 2 | Forest plot of meta-analysis (random effects) of effect of alcohol consumption on levels of high and low density lipoprotein cholesterol

aspects of the quality of studies in meta-analysis: randomisation, assessment of compliance with alcohol consumption or abstinence, losses owing to attrition, and relevant confounders, such as smoking, diabetes, overweight or obesity or dietary controls. We did not assess blinding of participants to the intervention because it was uncertain if blinding alcohol use was effective. We report these study characteristics based on the quality assessment proposed by Jadad et al.¹⁴

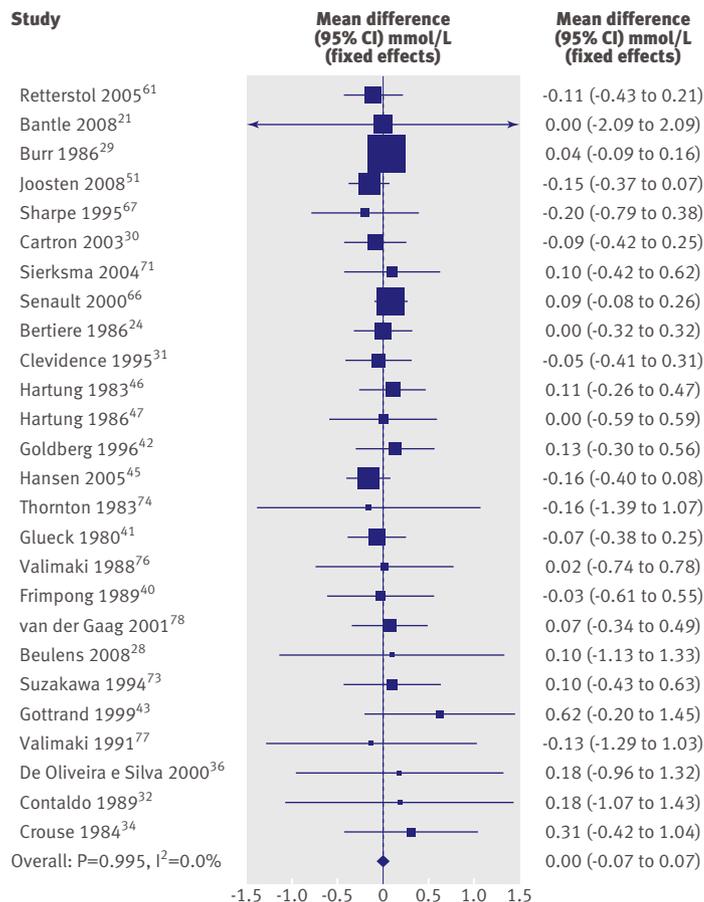
Data synthesis and analysis

The common unit of measurement across all studies was the mean change (standard error) in the level of each biomarker after alcohol consumption compared with the no alcohol control. This was calculated as (mean concentration of biomarker during alcohol consumption)–(mean concentration of biomarker during no alcohol consumption). To determine the standard error of the mean change, we used the calculation: standard error mean change=square root[(standard

deviation_{no alcohol}²/sample size_{no alcohol})+(standard deviation_{alcohol}²/sample size_{alcohol}]. Not all studies included in the systematic review reported results in this manner. We converted error measurements reported as standard errors to standard deviations using the formula standard deviation=(standard error)×square root (sample size).¹⁵ For studies that did not report mean concentrations of biomarkers either before or after the intervention or that reported results in graphical format only, we contacted the study authors to obtain data suitable for inclusion in our study. In all cases the authors failed to supply the necessary data to determine the mean change in biomarker level, and we therefore excluded these studies from meta-analyses.

When at least two studies reported the mean change in level of a specific biomarker we carried out meta-analyses of the effect of alcohol consumption on biomarker concentrations. All analyses were done using Stata 10.0. To assess heterogeneity of the mean change

Total cholesterol



Triglycerides

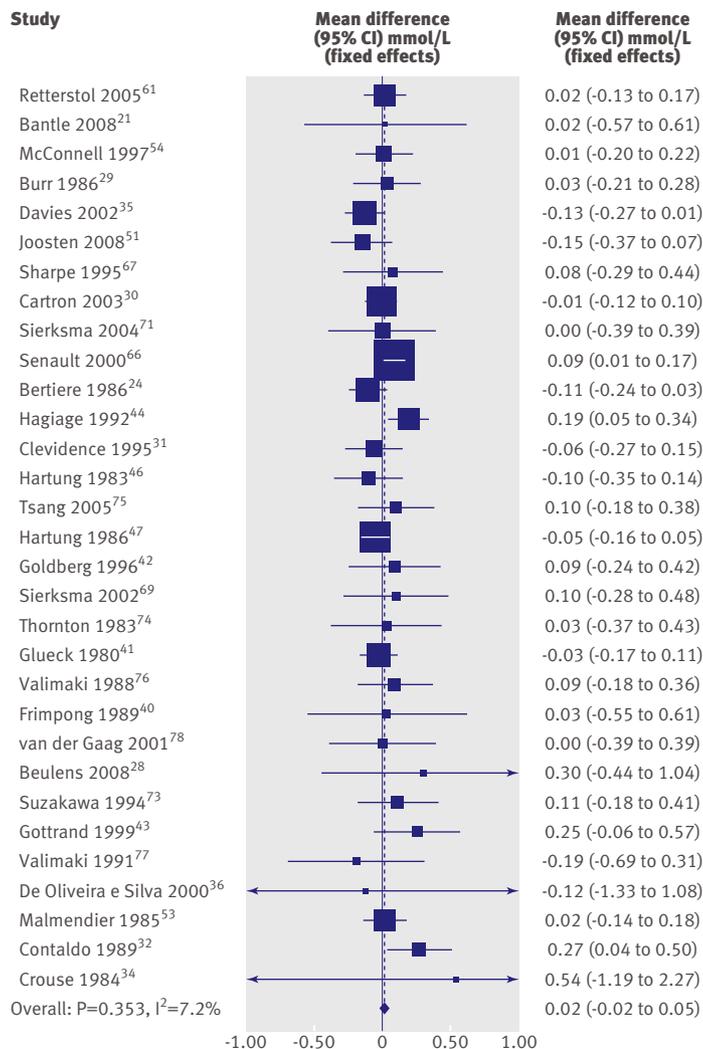


Fig 3 | Forest plot of meta-analysis (fixed effects) of effect of alcohol consumption on levels of total cholesterol and triglycerides

in biomarker concentrations across studies, we calculated the *Q* statistic (significance level $P \leq 0.10$) and the I^2 statistic.^{16,17} This criterion was used to determine whether to use a fixed or random effects model to pool studies. Where appropriate we pooled data according to the dose of alcohol consumed: 12.5–29.9 g/day (about 1–2.5 drinks), 30–60 g/day (about 2.5–5 drinks), and >60 g/day (≥ 5 drinks). To visually assess the mean change estimates and corresponding 95% confidence intervals across studies, we generated forest plots and grouped studies by dose of alcohol.

We carried out sensitivity analyses based on study quality for levels of high density lipoprotein cholesterol, low density lipoprotein cholesterol, total cholesterol, triglycerides, and fibrinogen. For these biomarkers we pooled the results from crossover studies separately from before and after studies. We also pooled results for these biomarkers stratified by beverage type (wine, beer, spirits). Data were pooled by the methods described previously.

Finally, we used the Begg test and visual inspection of funnel plots to assess for evidence of publication

bias.^{18,19} We limited this analysis to biomarkers where a statistically significant effect of alcohol was observed and five or more studies were meta-analysed.

RESULTS

The literature search identified 4690 articles pertaining to the relevant exposure, outcomes, and study designs (fig 1). Two additional articles were added from the bibliographic search. No additional articles were suggested by the content expert (KJM). After the final review, 63 articles were deemed eligible for analysis. Selected studies examined the effect of alcohol consumption on: lipid biomarkers (47 studies), inflammatory markers (13), haemostatic factors (14), endothelial factors (3), and adipocyte hormones (8). Many of the studies examined several of the biomarkers (fig 1).

Table 1 outlines the characteristics of the 63 included studies, totalling 1686 participants (1049 men and 625 women; one study of 12 participants did not indicate sex).^{20–82} Thirty six of the 63 (57%) studies included only men, eight (12.7%) included only women, and 19 (30%) included both. Thirty six

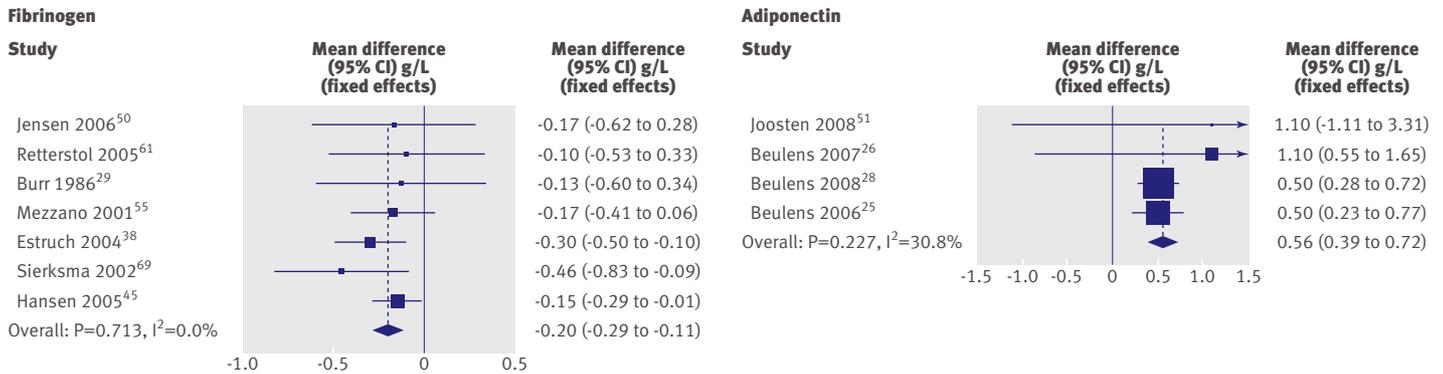


Fig 4 | Forest plot of meta-analysis of effect of alcohol consumption on levels of fibrinogen and adiponectin

studies used a crossover design and 24 used a before and after design, whereas three were parallel arm controlled trials. The control beverage was generally water, fruit juice, or a de-alcoholised drink (wine or beer), and most studies included a washout period of no alcohol use that was of similar duration to the period of alcohol intervention. Several studies included participants whose clinical characteristics may have influenced some of the biomarkers (for example, patients with diabetes, smokers, or those who were overweight or obese). Twenty eight (44%) studies controlled for an increase in caloric intake from alcohol consumption with isocaloric or controlled diets, whereas others maintained their usual diet. Six studies included people who were overweight or obese and three studies specifically examined inactive people compared with regular runners.

Meta-analyses

Of the 63 studies, 44 reported adequate data to permit pooled analyses (see web extra figure). Most commonly, studies could not be included because they did not report data in a format that permitted pooling (for example, graphically or as percentage change), or did not report error calculations. The subsets of identified studies with adequate data dealt with levels of high density lipoprotein cholesterol (33 of 44), total cholesterol (26 of 32), triglycerides (31 of 39), low density lipoprotein cholesterol (24 of 28), apolipoprotein A1 (16 of 22), Lp(a) lipoprotein (3 of 5), C reactive protein (5 of 8), interleukin 6 (2 of 4), tumour necrosis factor α (3 of 7), plasminogen activator inhibitor 1 (3 of 3), tissue plasminogen activator (3 of 4), fibrinogen (7 of 8), and adiponectin (4 of 7; see web extra figure). See web extra appendix 2 for details about exclusion of studies from meta-analysis.

Lipid biomarkers

The pooled analysis of the effect of alcohol consumption on mean high density lipoprotein cholesterol showed a consistent increase in these levels but with significant heterogeneity among studies (fig 2 and table 2; P=0.005). Pooling of studies stratified by dose may partially explain this heterogeneity. A significant dose-response was observed between alcohol

consumption and high density lipoprotein cholesterol levels (fig 2): 12.5-29.9 g/day (1-2 drinks, n=7), mean difference of 0.072 mmol/L (95% confidence interval: 0.024 to 0.119); 30-60 g/day (2-4 drinks, n=24), mean difference of 0.103 mmol/L (0.065 to 0.141); and >60 g/day (≥ 5 drinks, n=2), mean difference of 0.141 mmol/L (0.042 to 0.240; P for trend 0.013). Similar to the effect with high density lipoprotein cholesterol, apolipoprotein A1 also significantly increased in a random effects model pooling 16 studies (table 2).

In contrast, alcohol consumption did not significantly change levels of total cholesterol, low density lipoprotein cholesterol, triglycerides, or Lp(a) lipoprotein (table 2). The 24 studies reporting on low density lipoprotein cholesterol were pooled using a random effects model because heterogeneity was present. Pooled analyses stratified by dose of alcohol also showed no significant effects of alcohol on low density lipoprotein cholesterol. Pooled analysis of the impact of alcohol by dose on triglycerides showed a significant increase at the highest dose of alcohol (>60 g/day) in the two studies reporting alcohol consumption at this dose: mean difference 0.274 mmol/L (0.043 to 0.505), test for heterogeneity P=0.763 (fig 3).

Inflammatory markers

The association of alcohol with levels of C reactive protein, interleukin 6, and tumour necrosis factor α was not significant (table 2). Only one study reported that alcohol (in this case beer) increased leucocyte levels in women (0.51 (SD 0.47) $\times 10^9/L$) but not in men (0.19 (SD 0.31) $\times 10^9/L$).⁶³

Haemostatic factors

Fibrinogen levels significantly decreased after alcohol consumption (fig 4 and table 2). Meta-analyses of the remaining haemostatic biomarkers, however, did not show any significant effect of alcohol, including plasminogen activator inhibitor 1 and tissue plasminogen activator antigens (table 2).

Data were insufficient to permit meta-analysis for plasminogen, thromboxane, von Willebrand factor, and e-selectin levels. One study reported a significant increase in plasminogen levels after red wine consumption in 12 men.⁶⁰ Another study reported a

Table 2 | Summary of pooled mean difference in biomarker level after alcohol use

Biomarker	No of pooled studies	No of pooled participants	Type of model	Pooled mean difference in biomarker level (95% CI)
High density lipoprotein cholesterol (mmol/L)	33	796	Random	0.094 (0.064 to 0.123)*†
Low density lipoprotein cholesterol (mmol/L)	24	513	Random	-0.11 (-0.22 to 0.006)†
Total cholesterol (mmol/L)	26	596	Fixed	0.00 (-0.066 to 0.067)
Triglycerides (mmol/L)	31	752	Fixed	0.016 (-0.018 to 0.051)
Apolipoprotein A1 (g/L)	16	374	Random	0.101 (0.073 to 0.129)*†
Lp(a) lipoprotein (mg/dL)	3	114	Fixed	0.80 (-4.17 to 5.76)
C reactive protein (mg/L)	5	186	Fixed	-0.11 (-0.31 to 0.10)
Interleukin 6 (pg/mL)	2	144	Fixed	0.502 (-3.482 to 4.486)
Tumour necrosis factor α (pg/mL)	3	121	Fixed	-0.469 (-32.02 to 31.08)
Plasminogen activator inhibitor 1 (ng/mL)	3	67	Fixed	3.285 (-0.898 to 7.469)
Tissue plasminogen activator (ng/mL)	3	67	Fixed	0.754 (-0.132 to 1.641)
Fibrinogen (g/L)	7	387	Fixed	-0.20 (-0.29 to -0.11)*
Adiponectin (mg/L)	4	108	Fixed	0.56 (0.39 to 0.72)*

*Indicates significant ($P < 0.01$) change in biomarker level after alcohol use compared with a period of no alcohol use.

†Heterogeneity detected across pooled studies, where Q statistic $P < 0.10$.

significant decrease in thromboxane levels after both white and red wine consumption.⁵⁹ The two studies reporting on the effect of alcohol on von Willebrand factor found no significant change in these biomarker levels.^{54,56} For e-selectin, one study found a significant increase after alcohol consumption,⁸¹ whereas another study found no change.³⁸

Endothelial factors

Three studies reported on the impact of alcohol on intracellular adhesion molecule 1 and two reported on vascular cellular adhesion molecule levels. However, only one study reported data suitable for pooling for each biomarker. Two studies showed no impact of alcohol on intracellular adhesion molecule,^{37,81} whereas one showed a significant decrease in intracellular adhesion molecule 1 after consumption of red wine but not after gin.³⁸ One study reported no change in vascular cellular adhesion molecule after alcohol consumption,³⁷ whereas another found a significant decrease after consumption of red wine but not after gin.³⁸

Adipocyte hormones

Adiponectin levels were consistently significantly increased after alcohol consumption (fig 4). Only two studies reported on the effect of alcohol on levels of leptin; one study found a significant increase after alcohol consumption⁶⁴ and the other no effect.³⁷

Sensitivity analyses

Study quality

Study quality was analysed using the component approach,¹³ focusing primarily on study design as the most important quality factor. Sensitivity analyses were stratified by the two major study designs in selected studies (24 crossover studies, 18 before and after studies), with crossover studies being considered the more robust study design. The findings of these stratified sensitivity analyses are in web extra appendix 3 and show generally similar results for both types of

study design from analyses that include a sufficient number of studies to yield stable pooled estimates—that is, high density lipoprotein cholesterol, low density lipoprotein cholesterol, triglycerides, and fibrinogen. This sensitivity analysis suggests that, regardless of the study design, alcohol had consistent effects on biomarker levels.

See web extra appendix 4 for additional study quality characteristics. Of the 44 studies meta-analysed, 17 randomised the participants into treatment groups but only one study described the randomisation process. Forty three of the 44 studies described the presence of relevant covariates, such as diet, smoking, and physical activity. Twenty of the studies measured compliance with alcohol consumption, whereas 24 described losses to attrition.

Beverage type

Analyses were also stratified by beverage type (wine, beer, spirits). The results were similar to the combined analyses of all beverage types (see web extra appendix 3).

Publication bias

Evidence of publication bias was assessed for high density lipoprotein cholesterol, apolipoprotein A1, and fibrinogen. No asymmetry was found on visual inspection of the funnel plot for each biomarker, suggesting that significant publication bias was unlikely. This was further confirmed by a non-significant Begg test for each outcome of interest (high density lipoprotein cholesterol $P = 0.12$, apolipoprotein A1 $P = 0.064$, and fibrinogen $P = 0.88$).

DISCUSSION

This meta-analysis shows that moderate consumption of alcohol (up to one drink or 15 g alcohol a day for women and up to two drinks or 30 g alcohol a day for men) has beneficial effects on a variety of biomarkers linked to the risk of coronary heart disease. The experimental interventional studies showed that alcohol

consumption significantly increased circulating levels of high density lipoprotein cholesterol, apolipoprotein A1, and adiponectin and significantly decreased fibrinogen levels, all changes reported to be cardio protective.⁴⁻⁸

Comparison with other studies

The findings from our review expand on those reported a decade ago by Rimm et al,³ which focused only on alcohol's effect on lipids and haemostatic factors. Since that review substantial growth has occurred in research on the effect of alcohol on the more traditional biomarkers associated with cardiovascular risk as well as on newer biomarkers such as inflammatory markers, endothelial factors, and adipocyte hormones. To identify eligible studies we followed a similar protocol to that used by the previous reviewers, but we also required that eligible studies had to compare fasting levels of biomarkers after alcohol consumption with those from a period of no alcohol use.

Similar to the review by Rimm et al, our updated meta-analyses found increases in levels of high density lipoprotein cholesterol and apolipoprotein A1. For 30 g alcohol consumed a day (about two drinks) Rimm et al reported an increase in high density lipoprotein cholesterol level of 3.99 mg/dL (0.103 mmol/L) (95% confidence interval 3.25 to 4.73) and an increase in apolipoprotein A1 of 8.82 mg/dL (7.79 to 9.86). For a similar dose of alcohol (30 g a day was the standard dose used by Rimm et al), our results are nearly the same: an increase in high density lipoprotein cholesterol level of 3.66 mg/dL (95% confidence interval: 2.22 to 5.13) and an increase in apolipoprotein A1 of 8.67 mg/dL (6.81 to 10.32). In contrast with the study by Rimm et al, however, we observed that alcohol consumption significantly decreased fibrinogen concentrations. Evidence that alcohol decreases fibrinogen levels from experimental studies supports an important postulated mechanism by which alcohol consumption protects against certain aspects of cardiovascular disease, such as coronary heart disease.^{3,83}

Another finding that varied from the earlier review was that alcohol consumption did not increase triglyceride levels aside from pooled results from two studies of heavy alcohol consumption (>60 g/day, or >4 drinks a day). Although the results of the two studies should be viewed cautiously as they pool two different study designs (a crossover study³² and a before and after study³⁴), they do indicate an adverse effect of heavy alcohol consumption on triglyceride levels. At low levels of alcohol consumption, our findings do not support the previously reported association of alcohol consumption and raised triglyceride levels. Furthermore, we also determined that different types of alcoholic beverage (wine, beer, and spirits) have similar effects on biomarkers. Inferences on beverage type should be viewed with some caution, however, as most of the studies used wine as the alcohol intervention. This preference for using wine, and in most cases red wine, as the type of alcohol for intervention may be related to the other chemical components of

red wine, such as polyphenols, which are believed to have cardioprotective effects.^{30,38,42,45,50} However, it is interesting that in many of these studies, comparisons were made either with a non-red wine alcohol intervention or with de-alcoholised red wine and it was concluded that the effect observed was most likely due to alcohol rather than to the other components in red wine.^{30,42,45}

This review also examined results for several other biomarkers that had not previously been evaluated, most notably adiponectin, an abundant adipocyte hormone that has been associated with lower risk of both diabetes⁸⁴ and coronary heart disease.⁸⁵ In pooled analyses, adiponectin levels were significantly increased by alcohol intake. Taken together, these findings extend previous evidence supporting an apparent causal role for alcohol consumption in preventing coronary heart disease through favourable effects on levels of high density lipoprotein cholesterol, fibrinogen, and adiponectin and limited adverse effects on triglycerides at levels of alcohol consumption that are considered "not risky."

Potential biological mechanisms and clinical context

Our results thus implicate reverse cholesterol transport, haemostasis, and insulin sensitivity in the pathway by which alcohol consumption might prevent cardiovascular disease. The mechanisms by which alcohol influences high density lipoprotein cholesterol, fibrinogen, and adiponectin are not fully understood. In the case of high density lipoprotein cholesterol, various mechanisms have been proposed, including an increased transport rate of lipoproteins³⁶ and increased lipoprotein lipase activity.^{58,86} The effect on fibrinogen is also not well understood, although alcohol seems to influence the conformation and stability of fibrinogen molecules.⁸⁷ For adiponectin, one study showed that alcohol consumption increases expression of the ADIPOQ gene in adipose tissue, but little else is known about this effect.⁵¹

Our findings need to be put into a clinical context. The significant changes in levels of high density lipoprotein cholesterol, fibrinogen, and adiponectin after alcohol consumption were well within a pharmacologically relevant magnitude. In our systematic review, we determined that alcohol consumption increased high density lipoprotein cholesterol levels by about 0.1 mmol/L overall and in a dose-response manner (0.072 mmol/L for 1-2 drinks a day, 0.10 mmol/L for 2-4 drinks a day, and 0.14 mmol/L for ≥ 4 drinks a day). This degree of increase is greater than any currently available single pharmacological therapy, including fibrates (approved by the Food and Drug Administration for people with low levels of high density lipoprotein cholesterol). For example, a systematic review of fibrates on high density lipoprotein cholesterol levels showed an overall increase of 2.6 mg/dL⁸⁸ compared with our findings of alcohol increasing high density lipoprotein cholesterol levels by 3.5-4 mg/dL. Similarly, alcohol consumption decreased fibrinogen levels by 0.20 g/L. Given that an increase of 1 g/L has been

WHAT IS ALREADY KNOWN ON THIS TOPIC

Observational studies suggest that moderate alcohol intake is associated with lower risk of various cardiovascular events, particularly coronary heart disease

Interventional studies showed that alcohol favourably influences various biomarkers associated with risk of coronary heart disease

WHAT THIS STUDY ADDS

Moderate alcohol consumption had favourable effects on levels of high density lipoprotein cholesterol, apolipoprotein A1, adiponectin, and fibrinogen

These results strengthen the case for a causal link between alcohol intake and reduced risk of coronary heart disease

associated with a nearly threefold increase in risk of coronary heart disease in pooled cohort studies,⁸⁹ this magnitude of decrease in fibrinogen could account for a substantial decrease in heart disease among drinkers.⁹⁰ The clinical implications of alcohol's effect on adiponectin is less certain since this biomarker is less commonly examined in the clinical setting. An increase of about 0.6 mg/L represents approximately 1 standard deviation in adiponectin levels in the collected trials, or similar to the effect of thiazolidinediones on this insulin sensitising adipokine.⁹¹

Limitations of the study

Our review has some limitations and caveats. We did not formally search the grey literature, but we are confident that our search of the peer reviewed literature captured all relevant articles. The studies that we pooled did lack uniformity. Duration and dosing of the alcohol interventions were, however, different, as were the characteristics of the participants. Therefore it is possible that potential confounders such as smoking, physical inactivity, body weight, and diet could have affected our findings.⁹² Also, none of the studies blinded participants to alcohol consumption. However, owing to the taste and physiological effects of alcohol, it may not be possible to blind participants to this intervention. We chose to evaluate stable circulating cellular and molecular biomarkers associated with cardiovascular disease, in particular atherothrombotic and coronary heart disease. More variable measures, such as blood pressure, can be influenced by alcohol in complex, biphasic directions after ingestion and hence are less amenable to being summarised. Our selection of biomarkers for study was guided by links to cardiovascular pathophysiology. Other biomarkers may be of relevance to alcohol's effects on other health conditions—for example, cancer.¹² Lastly, although we found that alcohol consumption has favourable effects on some of the biomarkers associated with coronary heart disease, this remains indirect evidence for the mechanisms by which alcohol may cause cardio-protection.

Conclusions and policy implications

This systematic review provides a thorough examination of the literature on the effect of alcohol

consumption on biomarkers associated with cardiovascular disease, and produces compelling, indirect evidence in support of a causal protective effect of alcohol. Our companion systematic review assessing alcohol associations with clinically relevant cardiovascular end points offers parallel evidence of the protective effect of alcohol consumption.⁹³ These combined reviews provide a foundation of knowledge on which clinical and public health messaging can be discussed.

Contributors: All authors conceived the study and developed the protocol. SB and PR carried out the search, abstracted the data for the analysis, and did the statistical analysis. SB, PR, and WG wrote the first draft of the manuscript. All authors critically reviewed the manuscript for important intellectual content and approved the final version of the manuscript. WG will act as guarantor for the paper.

Funding: This work was supported by a contracted operating grant from Program of Research Integrating Substance Use Information into Mainstream Healthcare (PRISM) funded by the Robert Wood Johnson Foundation project No 58529, with cofunding by the Substance Abuse and Mental Health Services and the Administration Center for Substance Abuse Treatment. SB is supported by a postdoctoral fellowship award from the Alberta Heritage Foundation for Medical Research. PR is supported by a Frederick Banting and Charles Best Canada graduate scholarship from the Canadian Institutes of Health Research. WG is supported by a Canada research chair in health services research and by a senior health scholar award from the Alberta Heritage Foundation for Medical Research. Other than funding, research in this manuscript was done independent of funding agencies. None of the funding agencies played an active role in the preparation, review, or editing of this manuscript.

Competing interests: All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: All authors had support from a contracted operating grant from Program of Research Integrating Substance Use Information into Mainstream Healthcare (PRISM) funded by the Robert Wood Johnson Foundation project No 58529, with cofunding by the Substance Abuse and Mental Health Services and the Administration Center for Substance Abuse Treatment for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval: Not required.

Data sharing: Statistical code and datasets available from the corresponding author at wghali@ucalgary.ca.

- 1 Alcoholic Beverages. Dietary guidelines for Americans 2005. US Government Printing Office, 2005.
- 2 Turner C. How much alcohol is in a "standard drink"? An analysis of 125 studies. *Br J Addict* 1990;85:1171.
- 3 Rimm EB, Williams P, Fosher K, Criqui M, Stampfer MJ. Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. *BMJ* 1999;319:1523-8.
- 4 Libby P. Inflammation in atherosclerosis. *Nature* 2002;420:868-74.
- 5 Pai JK, Pischon T, Ma J, Manson JE, Hankinson SE, Joshipura K, et al. Inflammatory markers and the risk of coronary heart disease in men and women. *N Engl J Med* 2004;351:2599-610.
- 6 Kannel WB. Overview of hemostatic factors involved in atherosclerotic cardiovascular disease. *Lipids* 2005;40:1215-20.
- 7 Kannel WB, Wolf PA, Castelli WP, D'Agostino RB. Fibrinogen and risk of cardiovascular disease. *JAMA* 1987;258:1183-6.
- 8 Berg AH, Scherer PE. Adipose tissue, inflammation and cardiovascular disease. *Circ Res* 2005;96:939-49.
- 9 Brien SE, Ronksley PE, Turner BJ, Mukamal KJ, Ghali WA. Effect of alcohol consumption on biological markers associated with risk of cardiovascular disease: a systematic review. *J Gen Intern Med* 2009;24:S65.
- 10 Ronksley PE, Brien SE, Turner BJ, Mukamal KJ, Ghali WA. Association of alcohol consumption with cardiovascular and cerebrovascular disease: a systematic review and meta-analysis. *J Gen Intern Med* 2009;24:S28.
- 11 Egger M, Smith GD. Principles of and procedures for systematic reviews. In: Egger M, Smith GD, Altman DG, eds. *Systematic reviews in health care*. BMJ Publishing Group, 2001:23-42.

- 12 Health risks and benefits of alcohol consumption. *Alcohol Res Health* 2000;24:5-11.
- 13 Juni P, Altman DG, Egger M. Assessing the quality of randomised controlled trials. In: Egger M, Smith GD, Altman DG, eds. *Systematic reviews in health care: meta-analysis in context*. 2nd ed. BMJ Books, 2007.
- 14 Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials* 1996;17:1-12.
- 15 Deeks JJ, Altman DG, Bradburn MJ. Statistical methods for examining heterogeneity and combining results from several studies in meta-analysis. In: Egger M, Smith GD, Altman DG, eds. *Systematic reviews in health care: meta-analysis in context*. 2nd ed. BMJ Books, 2007.
- 16 Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002;21:1539-58.
- 17 Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003;327:557-60.
- 18 Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;315:629-34.
- 19 Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994;50:1088-101.
- 20 Baer DJ, Judd JT, Clevidence BA, Muesing RA, Campbell WS, Brown ED, et al. Moderate alcohol consumption lowers risk factors for cardiovascular disease in postmenopausal women fed a controlled diet. *Am J Clin Nutr* 2002;75:593-9.
- 21 Bantle AE, Thomas W, Bantle JP. Metabolic effects of alcohol in the form of wine in persons with type 2 diabetes mellitus. *Metabolism* 2008;57:241-5.
- 22 Belfrage P, Berg B, Cronholm T, Elmqvist D, Hagerstrand I, Johansson B, et al. Prolonged administration of ethanol to young, healthy volunteers: effects on biochemical, morphological and neurophysiological parameters. *Acta Med Scand* 1973;552:1-44.
- 23 Belfrage P, Berg B, Hagerstrand I, Nilsson-Ehle P, Tornqvist H, Wiebe T. Alterations of lipid metabolism in healthy volunteers during long-term ethanol intake. *Eur J Clin Invest* 1977;7:127-31.
- 24 Bertiere MC, Betoulle D, Apfelbaum M, Girard-Globa A. Time-course, magnitude and nature of the changes induced in HDL by moderate alcohol intake in young non-drinking males. *Atherosclerosis* 1986;61:7-14.
- 25 Beulens JW, van Beers RM, Stolk RP, Schaafsma G, Hendriks HF. The effect of moderate alcohol consumption on fat distribution and adipocytokines. *Obesity* 2006;14:60-6.
- 26 Beulens JW, van Loon LJ, Kok FJ, Pelsers M, Bobbert T, Spranger J, et al. The effect of moderate alcohol consumption on adiponectin oligomers and muscle oxidative capacity: a human intervention study. *Diabetologia* 2007;50:1388-92.
- 27 Beulens JW, van den BR, Kok FJ, Helander A, Vermunt SH, Hendriks HF. Moderate alcohol consumption and lipoprotein-associated phospholipase A2 activity. *Nutr Metab Cardiovasc Dis* 2008;18:539-44.
- 28 Beulens JW, de Zoete EC, Kok FJ, Schaafsma G, Hendriks HF. Effect of moderate alcohol consumption on adipokines and insulin sensitivity in lean and overweight men: a diet intervention study. *Eur J Clin Nutr* 2008;62:1098-105.
- 29 Burr ML, Fehily AM, Butland BK, Bolton CH, Eastham RD. Alcohol and high-density-lipoprotein cholesterol: a randomized controlled trial. *Br J Nutr* 1986;56:81-6.
- 30 Cartron E, Fouret G, Carbonneau MA, Lauret C, Michel F, Monnier L, et al. Red-wine beneficial long-term effect on lipids but not on antioxidant characteristics in plasma in a study comparing three types of wine—description of two O-methylated derivatives of gallic acid in humans. *Free Radic Res* 2003;37:1021-35.
- 31 Clevidence BA, Reichman ME, Judd JT, Muesing RA, Schatzkin A, Schaefer EJ, et al. Effects of alcohol consumption on lipoproteins of premenopausal women. A controlled diet study. *Arterioscler Thromb Vasc Biol* 1995;15:179-84.
- 32 Contaldo F, D'Arrigo E, Carandente V, Cortese C, Coltorti A, Mancini M, et al. Short-term effects of moderate alcohol consumption on lipid metabolism and energy balance in normal men. *Metabolism* 1989;38:166-71.
- 33 Couzigou P, Fleury B, Crockett R, Rautou JJ, Blanchard P, Lemoine F, et al. High density lipoprotein cholesterol and apoprotein A1 in healthy volunteers during long-term moderate alcohol intake. *Ann Nutr Metab* 1984;28:377-84.
- 34 Crouse JR, Grundy SM. Effects of alcohol on plasma lipoproteins and cholesterol and triglyceride metabolism in man. *J Lipid Res* 1984;25:486-96.
- 35 Davies MJ, Baer DJ, Judd JT, Brown ED, Campbell WS, Taylor PR. Effects of moderate alcohol intake on fasting insulin and glucose concentrations and insulin sensitivity in postmenopausal women: a randomized controlled trial. *JAMA* 2002;287:2559-62.
- 36 De Oliveira E, Silva ER, Foster D, McGee HM, Seidman CE, Smith JD, et al. Alcohol consumption raises HDL cholesterol levels by increasing the transport rate of apolipoproteins A-I and A-II. *Circulation* 2000;102:2347-52.
- 37 Djurovic S, Berge KE, Birkenes B, Braaten O, Retterstol L. The effect of red wine on plasma leptin levels and vasoactive factors from adipose tissue: a randomized crossover trial. *Alcohol Alcohol* 2007;42:525-8.
- 38 Estruch R, Sacanella E, Badia E, Antunez E, Nicolas JM, Fernandez-Sola J, et al. Different effects of red wine and gin consumption on inflammatory biomarkers of atherosclerosis: a prospective randomized crossover trial. Effects of wine on inflammatory markers. *Atherosclerosis* 2004;175:117-23.
- 39 Fraser GE, Anderson JT, Foster N, Goldberg R, Jacobs D, Blackburn H. The effect of alcohol on serum high density lipoprotein (HDL). A controlled experiment. *Atherosclerosis* 1983;46:275-86.
- 40 Frimpong NA, Lapp JA. Effects of moderate alcohol intake in fixed or variable amounts on concentration of serum lipids and liver enzymes in healthy young men. *Am J Clin Nutr* 1989;50:987-91.
- 41 Glueck CJ, Hogg E, Allen C, Gartside PS. Effects of alcohol ingestion on lipids and lipoproteins in normal men: isocaloric metabolic studies. *Am J Clin Nutr* 1980;33:2287-93.
- 42 Goldberg DM, Garovic-Kocic V, Diamandis EP, Pace-Asciak CR. Wine: does the colour count? *Clin Chim Acta* 1996;246:183-93.
- 43 Gottrand F, Beghin L, Duhal N, Lacroix B, Bonte JP, Fruchart JC, et al. Moderate red wine consumption in healthy volunteers reduced plasma clearance of apolipoprotein AII. *Eur J Clin Invest* 1999;29:387-94.
- 44 Hagiage M, Marti C, Rigaud D, Senault C, Fumeron F, Apfelbaum M, et al. Effect of a moderate alcohol intake on the lipoproteins of normotriglyceridemic obese subjects compared with normoponderal controls. *Metabolism* 1992;41:856-61.
- 45 Hansen AS, Marckmann P, Dragsted LO, Finne NI, Nielsen SE, Gronbaek M. Effect of red wine and red grape extract on blood lipids, haemostatic factors, and other risk factors for cardiovascular disease. *Eur J Clin Nutr* 2005;59:449-55.
- 46 Hartung GH, Foreyt JP, Mitchell RE, Mitchell JG, Reeves RS, Gotto AM Jr. Effect of alcohol intake on high-density lipoprotein cholesterol levels in runners and inactive men. *JAMA* 1983;249:747-50.
- 47 Hartung GH, Reeves RS, Foreyt JP, Patsch W, Gotto AM Jr. Effect of alcohol intake and exercise on plasma high-density lipoprotein cholesterol subfractions and apolipoprotein A-I in women. *Am J Cardiol* 1986;58:148-51.
- 48 Hartung GH, Foreyt JP, Reeves RS, Krock LP, Patsch W, Patsch JR, et al. Effect of alcohol dose on plasma lipoprotein subfractions and lipolytic enzyme activity in active and inactive men. *Metabolism* 1990;39:81-6.
- 49 Imhof A, Plamper I, Maier S, Trischler G, Koenig W. Effect of drinking on adiponectin in healthy men and women: a randomized intervention study of water, ethanol, red wine, and beer with or without alcohol. *Diabetes Care* 2009;32:1101-3.
- 50 Jensen T, Retterstol LJ, Sandset PM, Godal HC, Skjonsberg OH. A daily glass of red wine induces a prolonged reduction in plasma viscosity: a randomized controlled trial. *Blood Coagul Fibrinolysis* 2006;17:471-6.
- 51 Joosten MM, Beulens JW, Kersten S, Hendriks HF. Moderate alcohol consumption increases insulin sensitivity and ADIPOQ expression in postmenopausal women: a randomised, crossover trial. *Diabetologia* 2008;51:1375-81.
- 52 Karlsen A, Retterstol L, Laake P, Kjolsrud-Bohn S, Sandvik L, Blomhoff R. Effects of a daily intake of one glass of red wine on biomarkers of antioxidant status, oxidative stress and inflammation in healthy adults. *ESPEN* 2007;2:e127-33.
- 53 Malmendier CL, Delcroix C. Effect of alcohol intake on high and low density lipoprotein metabolism in healthy volunteers. *Clin Chim Acta* 1985;152:281-8.
- 54 McConnell MV, Vavouranakis I, Wu LL, Vaughan DE, Ridker PM. Effects of a single, daily alcoholic beverage on lipid and hemostatic markers of cardiovascular risk. *Am J Cardiol* 1997;80:1226-8.
- 55 Mezzano D, Leighton F, Martinez C, Marshall G, Cuevas A, Castillo O, et al. Complementary effects of Mediterranean diet and moderate red wine intake on haemostatic cardiovascular risk factors. *Eur J Clin Nutr* 2001;55:444-51.
- 56 Mezzano D, Leighton F, Strobel P, Martinez C, Marshall G, Cuevas A, et al. Mediterranean diet, but not red wine, is associated with beneficial changes in primary haemostasis. *Eur J Clin Nutr* 2003;57:439-46.
- 57 Naissides M, Mamo JC, James AP, Pal S. The effect of chronic consumption of red wine on cardiovascular disease risk factors in postmenopausal women. *Atherosclerosis* 2006;185:438-45.
- 58 Nishiwaki M, Ishikawa T, Ito T, Shige H, Tomiyasu K, Nakajima K, et al. Effects of alcohol on lipoprotein lipase, hepatic lipase, cholesteryl ester transfer protein, and lecithin:cholesterol acyltransferase in high-density lipoprotein cholesterol elevation. *Atherosclerosis* 1994;111:99-109.
- 59 Pace-Asciak CR, Rounova O, Hahn SE, Diamandis EP, Goldberg DM. Wines and grape juices as modulators of platelet aggregation in healthy human subjects. *Clin Chim Acta* 1996;246:163-82.

- 60 Pikaar NA, Wedel M, van der Beek EJ, van Dokkum W, Kempen HJ, Klufft C, et al. Effects of moderate alcohol consumption on platelet aggregation, fibrinolysis, and blood lipids. *Metabolism* 1987;36:538-43.
- 61 Retterstol L, Berge KE, Braaten O, Eikvar L, Pedersen TR, Sandvik L. A daily glass of red wine: does it affect markers of inflammation? *Alcohol Alcohol* 2005;40:102-5.
- 62 Romeo J, Warnberg J, Nova E, Diaz LE, Gonzalez-Gross M, Marcos A. Changes in the immune system after moderate beer consumption. *Ann Nutr Metab* 2007;51:359-66.
- 63 Romeo J, Warnberg J, Diaz LE, Gonzalez-Gross M, Marcos A. Effects of moderate beer consumption on first-line immunity of healthy adults. *J Physiol Biochem* 2007;63:153-9.
- 64 Roth MJ, Baer DJ, Albert PS, Castonguay TW, Dorgan JF, Dawsey SM, et al. Relationship between serum leptin levels and alcohol consumption in a controlled feeding and alcohol ingestion study. *J Natl Cancer Inst* 2003;95:1722-5.
- 65 Schneider J, Liesenfeld A, Mordasini R, Schubotz R, Zofel P, Kubel F, et al. Lipoprotein fractions, lipoprotein lipase and hepatic triglyceride lipase during short-term and long-term uptake of ethanol in healthy subjects. *Atherosclerosis* 1985;57:281-91.
- 66 Senault C, Betoulle D, Luc G, Hauw P, Rigaud D, Fumeron F. Beneficial effects of a moderate consumption of red wine on cellular cholesterol efflux in young men. *Nutr Metab Cardiovasc Dis* 2000;10:63-9.
- 67 Sharpe PC, McGrath LT, McClean E, Young IS, Archbold GP. Effect of red wine consumption on lipoprotein (a) and other risk factors for atherosclerosis. *QJM* 1995;88:101-8.
- 68 Sierksma A, van der Gaag MS, Klufft C, Hendriks HF. Effect of moderate alcohol consumption on fibrinogen levels in healthy volunteers is discordant with effects on C-reactive protein. *Ann NY Acad Sci* 2001;936:630-3.
- 69 Sierksma A, van der Gaag MS, van Tol A, James RW, Hendriks HF. Kinetics of HDL cholesterol and paraoxonase activity in moderate alcohol consumers. *Alcohol Clin Exp Res* 2002;26:1430-5.
- 70 Sierksma A, van der Gaag MS, Klufft C, Hendriks HF. Moderate alcohol consumption reduces plasma C-reactive protein and fibrinogen levels; a randomized, diet-controlled intervention study. *Eur J Clin Nutr* 2002;56:1130-6.
- 71 Sierksma A, Vermunt SHF, Lankhuizen IM, van der Gaag MS, Scheek LM, Grobbee DE, et al. Effect of moderate alcohol consumption on parameters of reverse cholesterol transport in postmenopausal women. *Alcohol Clin Exp Res* 2004;28:662-6.
- 72 Sierksma A, Patel H, Ouchi N, Kihara S, Funahashi T, Heine RJ, et al. Effect of moderate alcohol consumption on adiponectin, tumor necrosis factor- α , and insulin sensitivity. *Diabetes Care* 2004;27:184-9.
- 73 Suzukawa M, Ishikawa T, Yoshida H, Hosoi K, Nishio E, Yamashita T, et al. Effects of alcohol consumption on antioxidant content and susceptibility of low-density lipoprotein to oxidative modification. *J Am Coll Nutr* 1994;13:237-42.
- 74 Thornton J, Symes C, Heaton K. Moderate alcohol intake reduces bile cholesterol saturation and raises HDL cholesterol. *Lancet* 1983;2:819-22.
- 75 Tsang C, Higgins S, Duthie GG, Duthie SJ, Howie M, Mullen W, et al. The influence of moderate red wine consumption on antioxidant status and indices of oxidative stress associated with CHD in healthy volunteers. *Br J Nutr* 2005;93:233-40.
- 76 Valimaki M, Taskinen MR, Ylikahri R, Roine R, Kuusi T, Nikkila EA. Comparison of the effects of two different doses of alcohol on serum lipoproteins, HDL-subfractions and apolipoproteins A-I and A-II: a controlled study. *Eur J Clin Invest* 1988;18:472-80.
- 77 Valimaki M, Laitinen K, Ylikahri R, Ehnholm C, Jauhiainen M, Bard JM, et al. The effect of moderate alcohol intake on serum apolipoprotein A-I-containing lipoproteins and lipoprotein (a). *Metabolism* 1991;40:1168-72.
- 78 Van der Gaag MS, van Tol A, Scheek LM, James RW, Urgert R, Schaafsma G, et al. Daily moderate alcohol consumption increases serum paraoxonase activity; a diet-controlled, randomised intervention study in middle-aged men. *Atherosclerosis* 1999;147:405-10.
- 79 Van der Gaag MS, van Tol A, Vermunt SH, Scheek LM, Schaafsma G, Hendriks HF. Alcohol consumption stimulates early steps in reverse cholesterol transport. *J Lipid Res* 2001;42:2077-83.
- 80 Van Golde PM, Hart HC, Kraaijenhagen RJ, Bouma BN, van de Wiel A. Regular alcohol intake and fibrinolysis. *Neth J Med* 2002;60:285-8.
- 81 Vazquez-Agell M, Sacanella E, Tobias E, Monagas M, Antunez E, Zamora-Ros R, et al. Inflammatory markers of atherosclerosis are decreased after moderate consumption of cava (sparkling wine) in men with low cardiovascular risk. *J Nutr* 2007;137:2279-84.
- 82 Watzl B, Bub A, Pretzer G, Roser S, Barth SW, Reckemmer G. Daily moderate amounts of red wine or alcohol have no effect on the immune system of healthy men. *Eur J Clin Nutr* 2004;58:40-5.
- 83 Mukamal KJ, Jadhav PP, D'Agostino RB, Massaro JM, Mittleman MA, Lipinska I, et al. Alcohol consumption and hemostatic factors: analysis of the Framingham Offspring Cohort. *Circulation* 2001;104:1367-73.
- 84 Heidemann C, Sun Q, van Dam RM, Meigs JB, Zhang C, Tworogger SS, et al. Total and high molecular weight adiponectin and resistin in relation to the risk for type 2 diabetes in women. *Ann Intern Med* 2008;149:307.
- 85 Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA* 2004;291:1730-7.
- 86 Taskinen MR, Nikkila EA, Valimaki M, Sane T, Kuusi T, Kesaniemi YA, et al. Alcohol-induced changes in serum lipoproteins and in their metabolism. *Am Health J* 1987;113:458-64.
- 87 Gorinstein S, Caspi A, Zemser M, Libman I, Goshev I, Trakhtenberg S. Plasma circulating fibrinogen stability and moderate beer consumption. *J Nutr Biochem* 2003;14:710-6.
- 88 Briel M, Ferreira-Gonzalez I, You JJ, Karanicolas PJ, Akl EA, Wu P, et al. Association between change in high density lipoprotein cholesterol and cardiovascular disease morbidity and mortality: systematic review and meta regression analysis. *BMJ* 2009;338:b92.
- 89 Lewington S, Thompson SG, Lowe GD, Collins R, Kostis JB, Wilson AC, et al. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. *JAMA* 2005;294:2848.
- 90 Kaptoge S, White IR, Thompson SG, Wood AM, Lewington S, Lowe GD, et al. Associations of plasma fibrinogen levels with established cardiovascular disease risk factors, inflammatory markers, and other characteristics: individual participant meta-analysis of 154,211 adults in 31 prospective studies: the fibrinogen studies collaboration. *Am J Epidemiol* 2007;166:867-79.
- 91 Riera-Guardia N, Rothenbacher D. The effect of thiazolidinediones on adiponectin serum level: a meta-analysis. *Diabetes Obes Metab* 2008;10:367-75.
- 92 Naimi TS, Brown DW, Brewer RS, Giles WH, Mensah G, Serdula MK, et al. Cardiovascular risk factors and confounders among nondrinking and moderate-drinking US adults. *Am J Prev Med* 2005;28:369-73.
- 93 Ronksley PE, Brien SE, Turner BJ, Mukamal KJ, Ghali WA. Association of alcohol consumption with selected cardiovascular disease outcomes: a systematic review and meta-analysis. *BMJ* 2011;342:d671.

Accepted: 12 December 2010