Original Article

The effects of alcohol on ambulatory blood pressure and other cardiovascular risk factors in type 2 diabetes: a randomized intervention

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Objective: Although prospective studies suggest light-to-moderate chronic alcohol intake protects against coronary artery disease in type 2 diabetic patients, the balance of effects on individual cardiovascular risk factors needs further assessment. We examined the effects of alcohol consumption on 24-h ambulatory blood pressure (BP) and heart rate (HR), high-density lipoprotein cholesterol, fibrinogen, C-reactive protein, homocysteine, and glycaemic control in well controlled type 2 diabetes.

Methods: Twenty-four participants aged 49–66 year were randomized to a three-period crossover study with women drinking red wine 230 ml/day (~24 g alcohol/day) and men drinking red wine 300 ml/day (~31 g alcohol/day), or equivalent volumes of dealcoholized red wine (DRW) or water, each for 4 weeks. Ambulatory BP and HR were monitored every 30 min for 24 h at the end of each period. Home blood glucose monitoring was carried out twice weekly throughout.

Results: Red wine increased awake SBP and DBP relative to water by $2.5 \pm 1.2/1.9 \pm 0.7$ mmHg ($P = 0.033$, $P = 0.008$, respectively), with a similar nonsignificant trend relative to DRW. Asleep DBP fell with red wine relative to DRW (2.0 ± 0.8 mmHg, $P = 0.016$) with a similar nonsignificant trend relative to water. Red wine increased 24-h, awake and asleep HR relative to water and DRW. Relative to DRW, red wine did not affect glycaemic control or any other cardiovascular risk factor.

Conclusion: In well controlled type 2 diabetic individuals 24–31 g alcohol/day (~2–3 standard drinks) raises awake BP and 24-h HR and lowers asleep BP but does not otherwise favourably or adversely modify cardiovascular risk factors.

Keywords: alcohol consumption, blood pressure, high-density lipoprotein cholesterol, randomized controlled trial, type 2 diabetes

Abbreviations: CAD, coronary artery disease; DRW, dealcoholized red wine; GEE, generalized estimating equations; γ-GT, γ-glutamyl transpeptidase; HOMA-IR, Homeostasis Model Assessment-Insulin Resistance

INTRODUCTION

Cardiovascular disease remains the predominant cause of mortality and morbidity in type 2 diabetes with hypertension as a major risk factor in approximately 40% [1]. Individuals with type 2 diabetes have coexistent dyslipidaemia characterized by increased triglycerides and reduced high-density lipoprotein (HDL) cholesterol, haemostatic and fibrinolytic abnormalities leading to a hypercoagulable state, as well as endothelial dysfunction, inflammation, and heightened oxidative stress [2]. The regular consumption of alcohol has been shown to improve or worsen many of these risk factors, the balance of effects variously linked to the amount of alcohol consumed as well as to type of alcoholic beverage. Light-to-moderate alcohol intake has been associated with lower insulin levels and improved glycaemic control [3], lower levels of fibrinogen [4], and reduced inflammation [5] whereas the antioxidant polyphenolics in red wine have been linked to reduced arterial stiffness [6]. In contrast, heavier alcohol intake, irrespective of beverage type, has been linked to increased blood pressure (BP) [7], triglycerides [8], homocysteine levels [9], arterial stiffness [10], and incidence of the metabolic syndrome [11]. Such contrasting influences may explain, at least in part, why there is a U-shaped relationship between alcohol consumption and incidence of coronary artery disease (CAD) [12], a pattern also seen in type 2 diabetes [13]. More information on the balance of effects of regular mild-to-moderate alcohol consumption on cardiovascular risk factors in type 2 diabetes is, therefore, of considerable public health interest.

Most of the previous reports of the effects of alcohol in men and women with type 2 diabetes have been acute

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studies with a focus on insulin sensitivity. Longer term interventions assessing the overall risks and benefits of alcohol consumption in patients with type 2 diabetes or the metabolic syndrome have been few, of mixed quality and generally of short duration [14–17]. Some have been uncontrolled [18,19] and none has measured the potential impact of alcohol on 24-h ambulatory BP and heart rate (HR). This represents an important oversight given previous reports of a biphasic effect of the acute and chronic ingestion of alcohol on BP, with a fall in BP in the hours immediately following ingestion but an increase 6–14 h after the last drink [20]. We now report the results from an intervention that examined the effects of alcohol consumed as red wine (~24–31 g alcohol/day) over 4 weeks in well controlled type 2 diabetic men and women. The study assessed 24-h ambulatory BP as the primary endpoint, as well as the balance of effects on diabetic control and other cardiovascular risk factors.

METHODS

Study participants

Type 2 diabetic men and postmenopausal women were recruited by media advertisement and community screening. Evidence of diabetes was taken as either being on hypoglycaemic medication, having had a diabetic glucose tolerance test, or at least two fasting plasma glucose measurements more than 7.1 mmol/l. Participants were in the age range 40–70 years and regular drinkers who had maintained the same pattern of alcohol intake for more than 12 months. Women usually consumed 2–3 standard drinks/day (20–30 g alcohol/day) and men 3–4 standard drinks/day (30–40 g alcohol/day). Participants taking antihypertensive or lipid-lowering medication were not excluded. Exclusion criteria included type 1 diabetes, recent (<3 months) symptomatic heart disease, angina pectoris, history of myocardial infarction or stroke, peripheral vascular disease, major surgery 3 months or less, BP higher than 170/100 mmHg, liver or renal disease (plasma creatinine >120 mmol/l), haemoglobin A1c (HbA1c) more than 8.5% (>69 mmol/mol), and current smokers or exsmokers less than 2 years.

Study design

During a 4-week run-in period, participants continued their usual alcohol intake. They then entered a three-period cross-over study of Latin square design, each period of 4 weeks duration. The three study periods were red wine with women drinking 230 ml/day (~24 g alcohol/day) and men drinking 300 ml/day (~31 g alcohol/day), or the equivalent volumes of dealcoholized red wine (DRW) or the consumption of water only. All study participants were advised to consume each beverage with the evening meal. The water only group controlled for any effect of phenolic compounds in red wine or DRW. Participants remained totally abstinent from all alcohol during the DRW and water only periods. There was no washout between each study period. The red wine was a Shiraz Cabernet blend of known composition and 13% v/v alcohol content. The red wine and DRW were from Orlando Wyndham, Rowland Flat, South Australia. Participants were allocated to each study period sequence via block randomization using computer-generated random numbers, devised by the statistician.

The trial was conducted at the clinical trials unit of the School of Medicine and Pharmacology (located at the Medical Research Foundation Building at Royal Perth Hospital) according to the Declaration of Helsinki guidelines and approved by the Royal Perth Hospital Ethics Committee (2003/2004). Written informed consent was obtained from all participants. The participants were recruited between August 2003 and February 2004. The trial is registered with the Australian New Zealand Clinical Trials Registry (ACTRN12615000133538).

Blood pressure monitoring

Ambulatory BP and HR were monitored every 30 min for 24 h at the end of the 4-week run-in period and at the end of each study period. A trained nurse fitted a SpaceLabs Monitor (Model 90217; SpaceLabs Medical Inc., Issaquah, Washington, USA) to the nondominant arm approximately 2.5 cm above the antecubital fossa and explained its use to the participants. They were instructed to continue their usual daily activities and avoid any vigorous exercise. Measurements showing a test code or those with a pulse pressure less than 20 mmHg were excluded from the analysis. BP traces were considered complete if more than 80% of the recordings were valid. BP readings from each study participant were aggregated to calculate average hourly results. Participants kept a diary of when they were asleep or awake from which BP readings were further aggregated into 24-h, awake and asleep means. The pattern of diurnal BP rhythm was defined as nondipping if the fall from awake to asleep SBP was less than 10% and extreme dipping if the fall was more than 20%.

Home blood glucose monitoring

Self-blood glucose monitoring was performed using a blood glucose monitor four times daily (before breakfast, 2 h before lunch, 2 h after lunch and 2 h after the evening meal), on Monday and Thursday of every week. Participants documented their blood glucose level on a sheet provided and returned it to the clinical trials unit every 2 weeks.

Measurement of compliance and concomitant lifestyle change

Compliance with changes in alcohol intake was recorded by 7-day retrospective diaries completed at weekly visits to the clinical trials unit. A fasting blood sample was provided at the end of the 4-week run-in period and the end of each study period for γ-glutamyl transpeptidase (γ-GT) as a biomarker of change in alcohol intake. Twenty four-hour urinary 4-O methylgallic acid was determined as a biomarker of red wine intake [7]. Participants were advised not to change their usual dietary habits. They completed food frequency questionnaires at baseline and at the end of each study period to monitor any dietary changes or nutrient intake. Physical activity logs were completed and weight carefully monitored at the end of run-in and the conclusion of each study period.

Biochemical analyses

Blood was collected at the end of the 4-week run-in period and at the end of each 4-week study period following an overnight fast and plasma samples (including glucose, cholesterol, and triglyceride assay) were analysed by Core Laboratory Services, Royal Perth Hospital, using the Hitachi 917 Biochemical Analyser (Hitachi Limited, Tokyo, Japan). Insulin was measured using a chemiluminescent immunometric assay (Immulite 2000 Biochemical Analyzer; Diagnostic Products, Los Angeles, California, USA). Insulin resistance was estimated by homeostasis model assessment [Homeostasis Model Assessment-Insulin Resistance (HOMA-IR)] score calculated as fasting serum insulin (mU/l) × fasting plasma glucose (mmol/l)/22.5. γ-GT was measured with a Roche kit (Roche Diagnostics GmbH, Mannheim, Germany). HDL cholesterol was measured with the Boehringer Mannheim kit. Low-density lipoprotein cholesterol was calculated using the modified Friedewald formula. Plasma fibrinogen was assayed by the thrombin time titration method using a MLA electra 1600CHD coagulation analyser (MLA, Lexington, Massachusetts, USA). Aliquots from 24-h urine collections stored at −80°C were assayed for 4-O methylgallic acid by gas chromatography–mass spectrometry [7]. Homocysteine was measured using a fluorescence polarization immunoassay with the Abbott Imx Homocysteine kit B3D390 (Axis Biochemicals ASA, Ulvemveoen, Oslo, Norway). C-reactive protein (CRP) was measured using a high-sensitivity monoclonal antibody assay (Dade Behring Marburg GmbH, Marburg, Germany). All samples from each study participant were assayed in the one batch.

Statistics

Twenty-four-hour ambulatory BP records were analysed using repeated measures mixed models allowing for the correlated error structure in the data (Proc Mixed; Statistical Analysis Program; SAS Institute, Cary, North Carolina, USA). Using the data from our previous study of the effects of beer and red wine on ambulatory BP in healthy men [7], there was at least 80% power to demonstrate a 2–3 mmHg change in 24-h ambulatory SBP, more than 90% power to detect similar changes in fibrinogen, γ-GT, homocysteine, and HDL cholesterol, and less than 10% power to detect differences in triglycerides. Weight, compliance biomarkers, and cardiovascular risk factors at the end of each 4-week intervention were analysed with the General Linear Model repeated measures using IBM SPSS Statistics Version 20 software (IBM, Armonk, New York, USA) and were considered significant with $P < 0.05$ after Bonferroni adjustment. Repeated measures analysis of the binary outcome measures for nondipping/extreme dipping SBP pattern were analysed with Generalized Estimating Equations (GEEs) in SPSS. If normally distributed, baseline data are reported as mean ± SD whereas nonbaseline values are reported as mean ± standard error. Log-transformed data are reported as the geometric mean and 95% confidence intervals whereas nonnormal data as the median and interquartile range.

RESULTS

There were 188 respondents by telephone screened against the entry criteria, 33 were invited for more intensive screening at the clinical trials unit. Two participants were excluded on the basis of usual alcohol intake below the specified threshold, another was insulin dependent, one had inadequate diabetic control, and one had symptomatic peripheral vascular disease. Twenty-eight were randomized and 24 (19 men, five women) completed the study whereas four withdrew before completion for personal or family reasons. Participants were aged 46–66 years with a mean of 59.3 ± 5.6 years, a BMI of 29.3 ± 4.8 kg/m^2, and 24-h SBP and DBP of 130.1 ± 11.9/77.7 ± 6.4 mmHg, respectively (Table 1). The mean duration of type 2 diabetes was 4.0 ± 2.3 years. They consumed on average 261 (95% confidence interval, 217, 314) g alcohol/week, 19 predominantly drank wine, three predominantly drank beer, and two drank beer and wine with similar frequency. Most were infrequent consumers of spirits. The majority reported drinking on 5–7 days each week with 11 participants regularly drinking every day of the week. Participants comprised 15 exsmokers and nine who had never smoked. Eleven participants were diet controlled, 13 were on oral hypoglycaemics, eight taking biguanides, eight sulfonylureas, and one a peroxisome proliferator-activated receptor antagonist. Ten participants were regularly taking antihypertensives and eight were on lipid-lowering agents.

Median urinary 4-O-methylgallic acid excretion as a biomarker of red wine intake was similar during the DRW and red wine periods, but was reduced by approximately 80% when water alone was consumed (Table 2). Levels of γ-GT were significantly lower with water compared with red wine. Red wine had no impact on plasma fibrinogen, uric acid, homocysteine, or CRP relative to either DRW or water. Serum total cholesterol and total cholesterol/HDL cholesterol ratio were identical during red wine and DRW but lower with water compared with red wine. There was also no effect of red wine on triglycerides, HDL cholesterol or low-density lipoprotein cholesterol relative to DRW or water. There was no effect of red wine on fasting glucose or insulin levels or the HOMA-IR score, relative to DRW or water. Fasting and postprandial glucose levels on home blood glucose monitoring were not significantly altered during the red wine vs. either the DRW or water periods (data not shown). Weight was unchanged throughout the study and there were no reported changes in diet or level of physical activity. A lower 24-h urinary sodium excretion during the red wine period was no longer significant after correction for creatinine excretion.

Diurnal profiles for 24-h SBP and DBP and HR on the final day of each period are shown in Fig. 1. Table 3 shows the mean 24-h ambulatory, day-time and night-time SBP and DBP and HR during the three study periods. Red wine significantly increased awake SBP and DBP relative to water (2.5 ± 1.2/1.9 ± 0.7 mmHg, $P = 0.033$ and $P = 0.008$, respectively) with a similar but nonsignificant trend relative to DRW. Asleep DBP fell with red wine relative to DRW (2.0 ± 0.8 mmHg, $P = 0.016$) with a similar nonsignificant trend relative to water. The fall in asleep SBP with red wine relative to DRW was not statistically significant (1.8 ± 1.4 mmHg, $P = 0.20$). These divergent diurnal effects resulted in no significant overall effect of red wine on mean 24-h SBP or DBP. A nondipping diurnal SBP pattern was seen in four participants while drinking water, five while

drinking DRW, and three while drinking red wine (GEE repeated measures $P=0.54$). Extreme dipping status was seen in four participants drinking water, two drinking DRW, and eight drinking red wine (GEE repeated measures $P=0.11$). Red wine significantly increased 24-h, awake and asleep HR relative to water and DRW (Table 3 and Fig. 1).

DISCUSSION

This intervention study has identified a biphasic effect of the regular consumption of alcohol as red wine on 24-h ambulatory BP in men and women with type 2 diabetes. Drinking 230–300 ml of red wine with the evening meal reduced BP overnight, but increased awake BP throughout the following day. Red wine significantly increased 24-h, awake and asleep HR, but had no impact on glycaemic control, HDL cholesterol, fibrinogen, CRP, or plasma homocysteine.

Intervention studies using ambulatory BP monitoring in hypertensive participants have also reported a biphasic pattern following the acute or chronic ingestion of alcohol [20]. In a cross-sectional Brazilian study [21] that measured the time since the last drink, BP was lower in men who had consumed alcohol less than 3 h before BP measurement, but higher in those who consumed alcohol 13–23 h before measurement. The prognostic significance of these effects is uncertain. We observed a nonsignificant trend toward an extreme dipping pattern when drinking red wine. Such a pattern with longer term heavy drinking could predispose toward a higher early morning surge in BP, a phenomenon previously linked to both silent cerebral infarction [22] and an increased incidence of early morning stroke [23]. Heavy alcohol intake ($\geq$46 g/day) has been linked to a 2.7-fold increased risk for an early morning surge in BP in a population-based sample [24] and may be relevant to increased risk of stroke in heavy drinkers. A nondipping ambulatory BP pattern can also increase cardiovascular risk but the proportion of participants with a nondipping ambulatory BP pattern in the present study was small and,

TABLE 1. Baseline characteristics ($N=24$)

Characteristic	
Age (year)	59.3 ± 5.6
Sex	19 men/five women
BMI (kg/m^2)	29.3 ± 4.8
Weight (kg)	90.4 ± 19.5
24-h SBP (mmHg)	130.1 ± 11.9
24-h DBP (mmHg)	77.7 ± 6.4
24-h heart rate (bpm)	73.0 ± 9.4
Smoking status	15 exsmokers/nine nonsmokers
Dipping status	Three nondippers/eight extreme dippers
Alcohol intake (g/week)[a]	261 (217, 314)
Glucose (mmol/l)	7.54 ± 2.62
Insulin (mU/l)[a]	10.92 (8.50, 14.03)
HOMA-IR score[a]	3.50 (2.50, 4.89)
HbA1c (%) (mmol/mol equivalent)	6.65 ± 1.30 (48 ± 17)
Cholesterol (mmol/l)	5.12 ± 1.0
Triglycerides (mmol/l)[a]	1.38 (1.09, 1.75)
LDL cholesterol (mmol/l)	3.08 ± 0.97
HDL cholesterol (mmol/l)	1.31 ± 0.47
Total/HDL cholesterol ratio	4.16 ± 1.05
γ-GT (U/l)[a]	32.76 (24.53, 43.77)
Fibrinogen (g/l)	3.21 ± 0.50
CRP (mg/l)[a]	1.58 (1.01, 2.47)
Homocysteine (umol/l)	10.70 ± 2.38
Uric acid (mmol/l)	0.35 ± 0.10
24-h urinary sodium (mmol/d)	164 ± 63
Urinary sodium/creatinine ratio	11.1 ± 0.7
4-O-methylgallic acid (μg/day)[b]	620 (1520)

Values are expressed as mean ± SD, or, when indicated either. CRP, C-reactive protein; γGT, γ-glutamyl-transpeptidase; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment; LDL, low-density lipoprotein.
[a]For log-transformed data as geometric mean (95% confidence intervals).
[b]Median (interquartile range).

TABLE 2. Weight, compliance biomarkers, and cardiovascular biomarkers at end of each 4-week beverage intervention

	Water	Dealcoholized red wine	Red wine	*P* value
Weight (kg)	89.4 ± 4.0	89.6 ± 3.9	89.9 ± 4.0	NS
γ-GT (U/l)[a]	26.55 (20.66, 34.13)[c,†]	28.39 (21.72, 37.10)	30.51 (23.50, 39.62)	0.001
4-O-methylgallic acid (μg/day)[b]	209 (594)[c,d,‡]	1145 (1106)	1169 (954)	<0.001
Cholesterol (mmol/l)	4.82 ± 0.22[c,*]	5.04 ± 0.20	5.04 ± 0.19	0.005
Triglycerides (mmol/l)[a]	1.15 (0.93, 1.42)	1.37 (1.09, 1.71)	1.37 (1.08, 1.73)	NS
HDL cholesterol (mmol/l)	1.29 ± 0.07	1.27 ± 0.08	1.33 ± 0.09	NS
LDL cholesterol (mmol/l)	2.93 ± 0.20	3.07 ± 0.21	3.01 ± 0.21	NS
Total/HDL cholesterol ratio	3.93 ± 0.22	4.25 ± 0.25	4.11 ± 0.27	0.037
Fibrinogen (mmol/l)	3.41 ± 0.18	3.33 ± 0.11	3.27 ± 0.11	NS
Urinary sodium (mmol/24h)	182 ± 17	176 ± 12[c,*]	145 ± 12	0.021
Urinary sodium/creatinine ratio	12.6 ± 0.8	12.4 ± 0.6	11.0 ± 0.8	NS
C-reactive protein (mg/l)[a]	1.27 (0.76, 2.14)	1.10 (0.71, 1.71)	1.33 (0.89, 2.00)	NS
Homocysteine (μmol/l)	10.30 ± 0.46	10.24 ± 0.46	10.71 ± 0.51	NS
Uric acid (mmol/l)	0.35 ± 0.02	0.34 ± 0.02	0.35 ± 0.02	NS
Glucose (mmol/l)	6.65 ± 0.38	6.85 ± 0.39	7.46 ± 0.79	NS
HOMA-IR score[a]	2.78 (1.97, 3.94)	2.99 (2.23, 4.01)	3.02 (2.03, 4.48)	NS
Insulin (mU/l)[a]	9.75 (7.44, 12.77)	10.16 (8.03, 12.86)	9.79 (7.29, 13.13)	NS

Data represent mean ± standard error or, when indicated either. γ-GT, γ-glutamyl-transpeptidase; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment; LDL, low-density lipoprotein.
[a]Geometric mean (95% confidence intervals.
[b]Median (interquartile range); superscript denotes significantly different from.
[c]Red wine.
[d]Dealcoholized red wine. *P*-value is from GLM repeated measures with post hoc analysis by modified *t*-test with Bonferroni correction.
[*]$P<0.05$.
[†]$P<0.01$. Wilcoxon signed ranks test used for 4-O-methylgallic acid.
[‡]$P<0.001$.

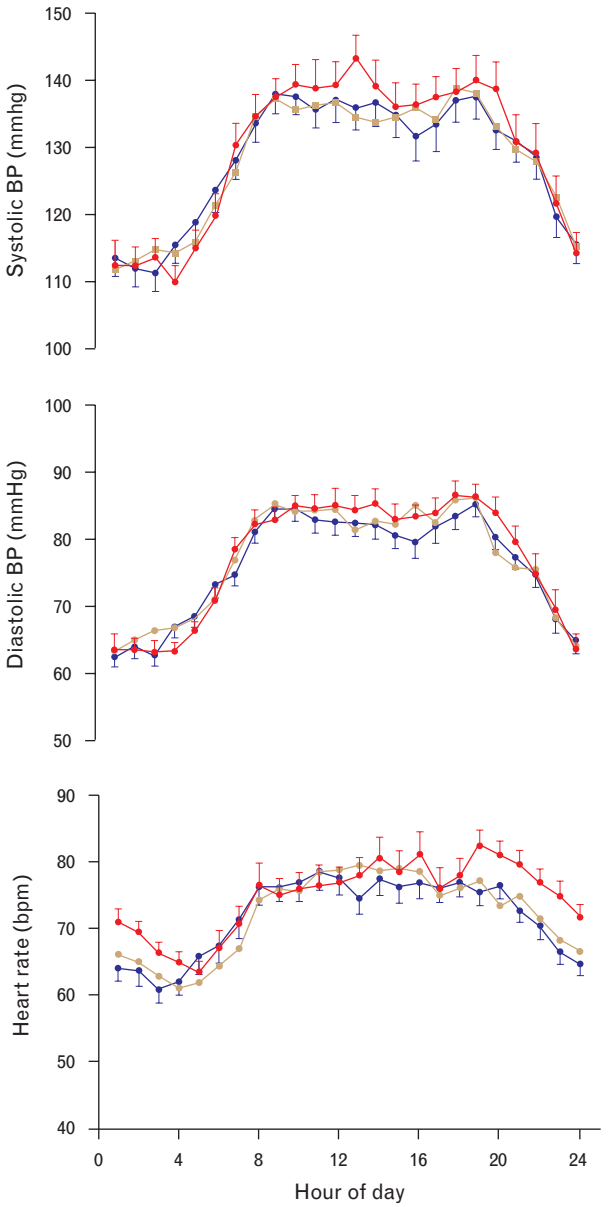

FIGURE 1 Hourly averages of SBP and DBP in 24 men and women on the last day of each 4-week beverage intervention. Red circles = red wine, brown circles = dealcoholized red wine and blue circles = water.

analogous to our previous report in healthy participants [25], was not altered with a change in alcohol intake.

The 2.5/1.9 mmHg increase in awake SBP and DBP with red wine relative to the water control was similar in magnitude to our previous report with red wine relative to an abstinence period in healthy men [7]. It is also similar to the 3.3/2.0 mmHg reduction in SBP and DBP, respectively, calculated from a meta-analysis of 15 randomized controlled trials examining the effects of alcohol reduction on BP [26]. To put such a change in context, a 2 mmHg increase in BP at a population level increases stroke mortality by 10% and CAD mortality by 7% [27].

This study identified higher 24-h ambulatory HR in patients with type 2 diabetes after consuming alcohol, an effect more prominent while asleep but still present throughout the following day. The increase in HR is identical to our previous finding when otherwise healthy participants drank either red wine or beer for 4 weeks [7] and supports data from cross-sectional studies [24]. This increase could ultimately be detrimental, higher HR being predictive of increased microvascular complications [28] and increased cardiovascular mortality in type 2 diabetes [29].

We observed no impact of alcohol on glycaemic control as measured by home blood glucose monitoring for both fasting and postprandial glucose levels or insulin sensitivity as assessed with the HOMA-IR score. This result supports our previous report in healthy men [30]. A recent meta-analysis of 14 intervention studies of at least 2 weeks duration in healthy participants concluded that alcohol consumption did not influence insulin sensitivity or fasting glucose [3] but reduced fasting HbA1c and fasting insulin concentrations. Intervention studies in type 2 diabetic participants have been much fewer and of mixed quality. Bantle *et al.* [14] reported no significant difference between 30 days of wine consumption and abstinence in fasting plasma glucose or HbA1c, but fasting serum insulin was lower after wine than after abstinence causing the authors to question whether alcohol might improve insulin sensitivity. Shai *et al.* [15] showed red or white wine given to previously abstinent type 2 diabetic participants for 12 weeks (or a similar volume of nonalcoholic beer as a control), reduced fasting plasma glucose but there was no change in postprandial glucose levels or HbA1c.

TABLE 3. Results from 24-h ambulatory blood pressure conducted at the end of each 4-week beverage intervention

	Water	Dealcoholized red wine	Red wine		Red wine vs. water	Red wine vs. dealcoholized red wine	Dealcoholized red wine vs. water
24-h SBP	129.1 ± 0.9	129.6 ± 0.9	130.4 ± 0.9	Δ 24-h SBP	1.3 ± 0.9	0.8 ± 0.9	0.5 ± 0.9
Awake SBP	131.2 ± 1.2	132.2 ± 1.2	133.7 ± 1.2	Δ Awake SBP	2.5 ± 1.2*	1.6 ± 1.2	1.0 ± 1.2
Asleep SBP	119.7 ± 1.8	119.9 ± 1.7	118.0 ± 1.8	Δ Asleep SBP	−1.6 ± 1.5	−1.8 ± 1.4	0.2 ± 1.4
24-h DBP (mmHg)	78.6 ± 0.6	79.3 ± 0.6	79.7 ± 0.6	Δ 24-h DBP	1.1 ± 0.6	0.3 ± 0.6	0.7 ± 0.6
Awake DBP	81.3 ± 0.9	82.1 ± 0.9	83.1 ± 0.9	Δ Awake DBP	1.9 ± 0.7†	1.1 ± 0.7	0.8 ± 0.7
Asleep DBP	68.1 ± 1.0	69.2 ± 1.0	67.1 ± 1.1	Δ Asleep DBP	−1.0 ± 0.9	−2.0 ± 0.8*	1.1 ± 0.8
24-h HR (beats/min)	74.8 ± 0.7	75.0 ± 0.7	77.6 ± 0.7	Δ 24-h HR	2.8 ± 0.7‡	2.6 ± 0.7‡	0.2 ± 0.7
Awake HR	76.0 ± 1.1	76.4 ± 1.1	78.7 ± 1.1	Δ Awake HR	2.7 ± 0.9†	2.3 ± 0.9*	0.4 ± 0.9
Asleep HR	70.6 ± 1.2	70.8 ± 1.1	73.9 ± 1.2	Δ Asleep HR	3.2 ± 1.0‡	3.1 ± 0.9†	0.2 ± 0.9

Data is mean ± standard error. Pairwise comparison of estimated marginal means from mixed model analysis. HR, heart rate.
*$P < 0.05$.
†$P < 0.01$.
‡$P < 0.001$.

Differences in study participants, study design, the beverage type and dose of alcohol studied, and approaches to measurement of insulin sensitivity are likely to have contributed to these mixed outcomes.

In contrast to the well documented effects of alcohol in healthy individuals to increase both HDL cholesterol [31] and reduce fibrinogen [4], we observed no change in serum HDL cholesterol or fibrinogen with red wine (24–31 g alcohol/day) relative to DRW or water in our diabetic participants. This was despite the study being more than adequately powered to detect changes similar to those we have seen in healthy men [7], albeit with a somewhat larger dose of alcohol (~40 g/day). The dose range of alcohol that we utilized has been ascertained in a meta-analysis of prospective population studies to result in an ~25% decrease in risk of CAD mortality [32]. An alcohol-induced increase in HDL cholesterol together with the antithrombotic effects of decreased fibrinogen, anti-inflammatory effects and increased adinopectin have been invoked as the major conduits for this decrease in CAD risk [33]. A prospective study in participants with type 2 diabetes similarly observed a 17% decrease in cardiovascular events with the consumption of up to 2–3 standard drinks/day, with red wine the predominant alcoholic beverage associated with the decrease in CAD risk [13]. If alcohol as red wine in the dose range we utilized does not raise HDL cholesterol or reduce fibrinogen levels it brings into question whether such effects are also important in reducing CAD risk in type 2 diabetes. A similar absence of effects of alcohol consumption on HDL cholesterol and/or fibrinogen has been observed in several [14,15,19] but not all [16,18] interventions in type 2 diabetic cases. Apart from differences in study design and the dose of alcohol utilized, a further contributing factor to these diverse outcomes could have been background medication use, with just over half of the participants in our study on oral hypoglycaemic agents, one-third on lipid-lowering agents, and one-third on antihypertensive agents. An absence of an HDL-raising effect has also been reported in obese premenopausal women consuming ~25 g alcohol/day as red wine for 5 days a week for 10 weeks [34]. The authors postulated the metabolic response to alcohol could differ between obese and lean participants, a contention supported by reports of a lack of association between alcohol and HDL cholesterol in obese women [35] and the failure of alcohol to increase HDL cholesterol components in obese compared with normal weight men [36]. Twenty of our 24 participants had a BMI of at least 25 kg/m^2 and 10 had a BMI of at least 30 kg/m^2. A recent prospective cohort study saw no difference at baseline in HDL cholesterol between diabetic participants who drank alcohol and those who abstained suggesting it was unlikely that an effect of alcohol to raise HDL cholesterol could account for the 17% lower risk of cardiovascular events that was seen over a 5-year period with moderate drinking [13]. The finding of lower risks of cardiovascular events in drinkers remains controversial with some observers invoking an overall healthy lifestyle effect in those who regularly drink at mild-to-moderate levels [37], rather than a specific effect of alcohol to improve cardiovascular risk profiles. However, studies in cohorts of participants selected on the basis of healthy lifestyle behaviours have still demonstrated a reduction in risk of myocardial infarction with alcohol consumption vs. abstinence that is equivalent to the risk reduction secondary to the healthy lifestyle behaviours themselves [38]. The degree to which any reduction in risk can be attributed to an increase in HDL cholesterol with alcohol also remains controversial [39].

Cross-sectional population studies have suggested that an association between biomarkers of inflammation and increasing alcohol intake [5] might be responsible, at least in part, for any decrease in CAD risk with alcohol. When confined to men diagnosed with type 2 diabetes alone, there was no effect of alcohol on CRP seen in the Health Professionals Follow-up Study [40]. However, in that study other inflammatory markers such as fibrinogen, soluble tumour necrosis factor receptor-2, and soluble vascular adhesion molecule-1 were all lower with increasing alcohol intake. In the current intervention, we saw no change in plasma CRP after 4 weeks of alcohol (24–31 g/day) as red wine. No change in CRP has been reported in type 2 diabetic patients given alcohol (18 g/day) as red wine for 30 days [14] and, in a study in participants with the metabolic syndrome, 20–30 g/day alcohol reduced CRP with one type of white wine but not with another [19]. The current study suggests an anti-inflammatory effect of alcohol as measured by CRP is unlikely in the dose range we utilized.

Hyperhomocysteinaemia is increasingly recognized as a cardiovascular risk factor in type 2 diabetes and has been linked to both macroangiopathy and nephropathy [41]. Heavier alcohol intake has been associated with an increase in homocysteine levels [9]. We observed no influence of alcohol intake as red wine to elevate plasma homocysteine levels in our type 2 diabetic cases. To our knowledge no previous controlled intervention study has measured effects of alcohol on homocysteine in type 2 diabetes. However, 24 g/day alcohol as red wine given to healthy men for 2 weeks significantly increased homocysteine [9] and 30 g/day alcohol for 6 weeks consumed by healthy social drinkers as red wine, beer or spirits raised homocysteine levels irrespective of the beverage consumed [42]. A cross-sectional study in type 2 diabetic men without overt nephropathy also suggested an increase in plasma homocysteine levels associated with total alcohol consumption [43] but a decrease with increasing beer consumption. Our results would suggest that at least in the mild-to-moderate range of alcohol as red wine, there are no adverse effects on plasma homocysteine levels in otherwise well controlled type 2 diabetes.

A limitation of the study may relate to the degree to which study participants were compliant with the prescribed red wine, dealcoholized red wine and water phases of the study. Obviously, poor compliance with the two abstinence phases could be a contributing factor to our not being able to identify any effect of alcohol on HDL cholesterol, CRP, or homocysteine levels. However, unlike other studies in this area, we were able to closely monitor compliance by measuring 24-h urinary 4-O methylgallic acid excretion as a biomarker of red wine and DRW intake. Levels fell during water ingestion to 18% of those seen with DRW and red wine, a result comparable to our previous

study in healthy men where 4-O methylgallic acid excretion fell to approximately 16% when the abstinence control period was compared with DRW or red wine [7]. Moreover, in the present study, 4-O methylgallic acid excretion during the red wine and DRW periods were nearly identical. These results indicate close compliance with the study protocol. A second limitation may relate to the choice of a crossover rather than parallel design for the intervention. A crossover design has the advantage of an increase in precision with each study participant acting as their own control, and therefore requiring fewer study participants. It has the disadvantage, however, of possible order effects on study outcomes which might confound the results. The Latin square design with randomization of participants into six possible sequences for the three study periods acts to minimize such confounding. The final limitation relates to the impossibility of utilizing a double-blind design, with study participants invariably aware when they are consuming alcohol as red wine vs. abstinence while drinking DRW or water.

In conclusion, the present intervention study has identified a biphasic effect of alcohol consumed daily as 200–300 ml red wine on 24-h ambulatory BP in men and women with type 2 diabetes, with higher awake BP but lower asleep BP. Ambulatory HR was raised during awake and asleep periods. The overall prognostic significance of these observations is uncertain, but alcohol consumed at these levels has generally been associated with an overall reduction of cardiovascular risk in type 2 diabetic participants in longitudinal population based studies. There were no beneficial or adverse effects seen for other cardiovascular risk factors and both glycaemic control and insulin sensitivity were unchanged at a dose of alcohol equivalent to 24–31 g/day.

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Conflicts of interest

There are no conflicts of interest.

Reprints will not be made available.

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Reviewers' Summary Evaluations

Reviewer 1

In this randomized 3-period crossover study conducted among 24 men and women aged 49–66 years with diabetes, participants were assigned to red wine, de-alcoholized red wine or water each for 4 weeks. Ambulatory blood pressure was assessed for 24 h at the end of each intervention phase. Compared with water consumption, red wine consumption increased awake systolic and diastolic blood pressure. Compared to de-alcoholized red wine, red wine consumption decreased asleep diastolic blood pressure. The findings are intriguing but their clinical relevance is uncertain at this time.

Reviewer 2

The idea that the consumption of light-to-moderate amounts of alcoholic beverages is good for health has been liberally accepted by patients and doctors, maybe because this benefit matches with the pleasant effects of wine and other beverages. Nonetheless, the results of experimental studies, such as this controlled trial, have not given mechanistic support for the cardioprotective effect of alcohol. The only consistent cardiovascular effect of alcohol has been the blood pressure-increasing one, something that is undoubtedly harmful for health. The better health of light-to-moderate consumers of alcoholic beverages (health cohort effect) may explain the putative beneficial effects of alcoholic beverages.