Dealcoholized Red Wine Decreases Systolic and Diastolic Blood Pressure and Increases Plasma Nitric Oxide

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ABSTRACT

Rationale: Experimental studies have shown a potential blood pressure (BP) lowering effect of red wine polyphenols, while the effects of ethanol and polyphenols on BP in humans are not yet clear.

Objective: The aim of the present work was to evaluate the effects of red wine fractions (alcoholic and non-alcoholic) on BP and plasma nitric oxide (NO) in subjects at high cardiovascular risk.

Methods and Results: Sixty-seven men at high cardiovascular risk were studied. After a 2-week run-in period, subjects were randomized into three treatment periods in a cross-over clinical trial, with a common background diet plus red wine (30g alcohol/d), the equivalent amount of dealcoholized red wine, or gin (30g alcohol/d), lasting 4 weeks each intervention. At baseline and after each intervention, anthropometrical parameters, BP and plasma NO were measured. Systolic and diastolic BP decreased significantly after the dealcoholized red wine intervention and these changes correlated with increases in plasma NO.

Conclusions: Dealcoholized red wine decreases systolic and diastolic BP. Our results point out through a NO-mediated mechanism. The daily consumption of dealcoholized red wine could be useful for the prevention of low to moderate hypertension. Trial registered at controlled-trials.com: ISRCTN88720134.

Keywords:
Red wine, polyphenols, alcohol, blood pressure, nitric oxide

Non-standard Abbreviations:

ANCOVA	analysis of covariance
ANOVA	analysis of variance
BP	blood pressure
CHD	coronary heart disease
DBP	diastolic blood pressure
DRW	dealcoholized red wine
G	gin
HDL	high density cholesterol
LDL	low density cholesterol
NO	nitric oxide
RW	red wine
SBP	systolic blood pressure

Introduction

Epidemiological evidence has associated moderate alcohol consumption with decreased cardiovascular risk[1]. However, red wine (RW) seems to confer greater protective effects because of its high polyphenolic content. *In vitro* and experimental studies have shown a potential blood pressure (BP)-lowering effect and/or enhancement of endothelial nitric oxide (NO) production by RW[2]. It is unclear whether these effects can be extrapolated to humans, since the amount of RW polyphenols used in these studies is usually higher than that achieved through moderate RW consumption. Recently, small amounts of RW, but not other alcoholic beverages, were shown to increase plasma NO concentrations[3]. While the negative effects of heavy or binge alcohol drinking on BP are well known, the effects of moderate alcohol consumption are controversial, since some studies have observed a linear trend and others a non-linear or J-shaped association, independently of the beverage consumed[4-6]. Therefore, the aim of the present study was to evaluate the effects of RW fractions (alcoholic and non-alcoholic) on BP and plasma NO concentration in high cardiovascular risk subjects.

Methods

The study was an open, randomized, cross-over, controlled clinical trial comprising three 4-week periods. Detailed Methods have been published[7] and are provided in the Online Supplement.

Seventy-three men at high cardiovascular risk, aged between 55 and 75 years were included in the study. All subjects had diabetes mellitus or $\geq$3 cardiovascular disease risk factors[7]. After a 2-week run-in period wherein subjects were asked not to consume any alcoholic beverage, they were randomized using a computer-generated table into three treatments in a cross-over design, with a common background diet plus gin (100mL – 30g ethanol/day), RW (272mL – 30g ethanol/day; total polyphenols: 798 Eq Gallic Acid/day - EGA/day-), and the same amount of polyphenols as RW in the form of dealcoholized red wine (DRW) (272mL - total phenols: 733 EGA/day), resulting in six possible beverage sequences lasting 4 weeks each intervention. No washout periods were included between the interventions.

After the run-in period (baseline) and the day after each intervention period (RW, DRW and gin), BP and heart rate were measured 3 times at 5-min intervals on the nondominant arm with an oscillometer (Omron 705 CP; Omron Matsusaka Co Ltd, Matsusaka City, Japan) after 15 minutes resting in a seated position. The mean of the second and the third measures was considered for statistical analysis.

Fasting blood samples for the NO analyses were collected at baseline and after each intervention, and stored at –80°C until assayed. For measurement of NO, the release of NO_2^- and NO_3^-, the stable breakdown products of NO in plasma, were determined by a chemiluminescence detector in a NO analyzer (Sievers Instruments, Inc., Boulder, CO).

Statistical analyses were performed using the Statistical Analysis Systems (version 9.2, SAS Institute Inc, Cary, NC). To analyze the changes within each treatment a Student's t test for paired samples was performed between the data obtained before and after each intervention. One-factor analysis of variance (ANOVA) for repeated measures and the Bonferroni *post-hoc* test were used to compare the differences of the changes in outcome variables between the interventions. See Online Supplement for further details of statistical analyses.

RESULTS

The baseline characteristics of the 67 subjects who completed the study are detailed in Table 1. Reasons for exclusion of 6 participants are described in the Online Supplement. No

significant differences in body mass index, waist-to-hip ratio and heart rate were observed (Table 2). Systolic BP (SBP) and diastolic BP (DBP) decreased significantly after the DRW intervention (P=0.0001 and 0.017, respectively) (Figure 1). These changes were significantly different from those observed after the gin intervention (P=0.026 and 0.045 for SBP and DPB, respectively) (Table 2). Plasma NO concentration increased after the DRW intervention (P=0.041) and the change was also significantly different from that observed after the gin intervention (P=0.026). The changes in BP and NO after the DRW period were correlated (r=0.598; P<0.001 and r=0.362; P=0.002 for SBP and DBP, respectively; On-line Supplemental Figure I). The intervention with RW did not differ from the DRW and gin interventions, although SBP and DBP tended to decrease and NO tended to increase after the RW intervention compared to the gin period (P=0.069, 0.075 and 0.079 for SBP, DBP and NO, respectively). In addition, changes in SBP correlated with changes in NO after the RW intervention (r=0.251, P=0.035). Exclusion of participants with hypertension or antihypertensive treatment did not materially change the results (Online Supplemental Table I). Intervention compliance and dietary data during the three interventions are also shown in the Online Supplement. No carryover effect was observed for any outcome.

Discussion

After the 4-week interventions with RW, DRW and gin in a crossover study in high cardiovascular risk subjects, we observed that DRW decreased SBP and DBP while increasing plasma NO concentration. RW tended to have similar effects to those of DRW but BP changes were non-significant and gin had no effect. Therefore, the BP-lowering and NO-raising effects should be attributed to the RW polyphenols and not to alcohol, which seems to counteract the effects of the non-alcoholic fraction of RW.

Botden *et al*. observed that RW polyphenol consumption for 4 weeks did not affect the BP in subjects with high-normal BP or grade 1 hypertension[8] or in healthy young women[9] and postulated that RW polyphenols could only favorably affect BP in subjects with endothelial dysfunction[8]. Our study included subjects with high-normal BP or grade 1 hypertension, but we did not measure endothelial function. However, considering the load of cardiovascular risk factors of the study subjects, their probability of having endothelial dysfunction was very high. On the other hand, Huang *et al*.[3] reported increased plasma NO in healthy volunteers consuming 100mL/day of RW during 3 weeks, but not when they consumed equivalent amounts of alcohol as beer or vodka, although no BP changes were reported after any intervention.

The results of our study point out that moderate alcohol consumption does not affect BP. Okubo *et al*.[6] observed a J-shaped association between alcohol consumption and BP changes in a normotensive population, with a threshold effect at 18mL of daily ethanol consumption. Besides, the meta-analysis of Xin *et al.*[10] described a dose-response relationship between the reduction of alcohol consumption in heavy alcohol drinkers (≥3 drinks/d) and the reduction of BP. We studied moderate alcohol consumers who followed a run-in period with abstention from alcohol, and 4 weeks of moderate consumption of RW or gin had little effect on BP, suggesting that moderate alcohol consumption does not affect BP, at least in high cardiovascular risk subjects. These results concur with those of Frisoli *et al*.[4] and Stranges *et al.*[11], who observed no consistent association of beer, wine or liquor consumption with the risk of hypertension. Stranges *et al.*[11] also observed that drinking outside meals increased the risk of hypertension independently of the amount of alcohol consumed. Our study subjects were advised to consume the beverages during meals and this may explain, in part, why moderate alcohol consumption did not affect BP.

Finally, although the BP reduction after DRW consumption was modest (5.8 and 2.3 mmHg of SBP and DBP, respectively), decreases of 4 or 2 mm Hg in SBP or DBP respectively,

have been associated with a 14% and 20% reduction in CHD and stroke risk, respectively[12], conferring clinical significance to our results, especially in the case of DRW.

Our study has limitations. A 4-week intervention may not represent the potential effects of long-term consumption. In addition, the specific substances responsible for the observed effects could not be identified and endothelial function was not measured. In conclusion, DRW decreases SBP and DBP, possibly through a NO-mediated mechanism. Therefore, the daily consumption of DRW may be useful for the prevention of low to moderate hypertension.

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DISCLOSURES

None.

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FIGURE LEGEND

Figure 1. Blood pressure and plasma nitric oxide in the 67 subjects studied. *Comparisons between before and after the interventions (Student's *t* test for paired samples). Before each intervention is the value of the previous intervention or the baseline in the first intervention.

TABLES

Table 1. Baseline characteristics of the study subjects.

	Mean ± SD*
Age (years)	60 ± 8
Current smokers [*n (%)*]	16 (23.9)
Sedentarism [*n (%)*]	40 (59.7)
Family history of premature CHD [*n (%)*]	52 (77.6)
Type-2 diabetes [*n (%)*]	15 (22.4)
Hypertension [*n (%)*]	38 (56.7)
Dyslipemia [*n (%)*]	16 (23.9)
Medications [*n (%)*]	
ACE Inhibitors	28 (41.8)
Diuretics	5 (7.5)
Statins	22 (32.8)
Oral hypoglycemic drugs	14 (20.9)
Aspirin or antiplatelet drugs	15 (22.4)
Triglycerides (mg/dL)	128 ± 60
Total cholesterol (mg/dL)	204 ± 33
LDL-cholesterol (mg/dL)	133 ± 32
HDL-cholesterol (mg/dL)	43 ± 7
LDL/HDL ratio	3.08 ± 0.10

*Mean ± SD or *n* (%), when indicated (n=67). CHD, coronary heart disease; ACE, angiotensin-converting enzyme.

Table 2. Changes in anthropometric parameters, blood pressure and plasma concentrations of nitric oxide in the 67 subjects studied after the 3 interventions.

	Red wine intervention	Dealcoholized red wine intervention	Gin intervention	P^*
Body mass index (kg/m^2)	0.6 (-0.7, 0.2)	-0.1 (-0.4, 0.1)	-0.1 (-0.3, 0.1)	0.200
Waist-to-hip ratio	-0.006 (-0.013, 0.001)	-0.001 (-0.006, 0.005)	0.007 (-0.002, 0.015)	0.118
Systolic blood pressure (mm Hg)	-2.3 (-5.1, 0.5)[a,b]	-5.8 (-8.9, -2.7)[a,]	-0.8 (-4.1, 2.5)[b]	0.028
Diastolic blood pressure (mm Hg)	-1.0 (-2.5, 0.5)[a,b]	-2.3 (-4.1, -0.4)[a,]	0.1 (-1.8, 1.9)[b]	0.027
Heart rate (beats/min)	-0.2 (-1.9, 1.5)	-1.7 (-3.4, 0.1)	1.1 (-0.8, 3.0)	0.187
Nitric oxide (μmol/L)	0.6 (-3.3, 4.3)[a,b]	4.1 (0.5, 7.6)[a,]	-1.4 (-4.1, 1.3)[b]	0.022

Results expressed as mean differences (95% CI) between after and before each intervention. Before each intervention is the value of the previous intervention or the baseline (run-in period) in the first intervention. $^*P$ value of the repeated-measures ANOVA from the differences between interventions. Values in a row with different superscript letters are significantly different ($P<0.05$, Bonferroni *post-hoc* test).

Novelty and Significance

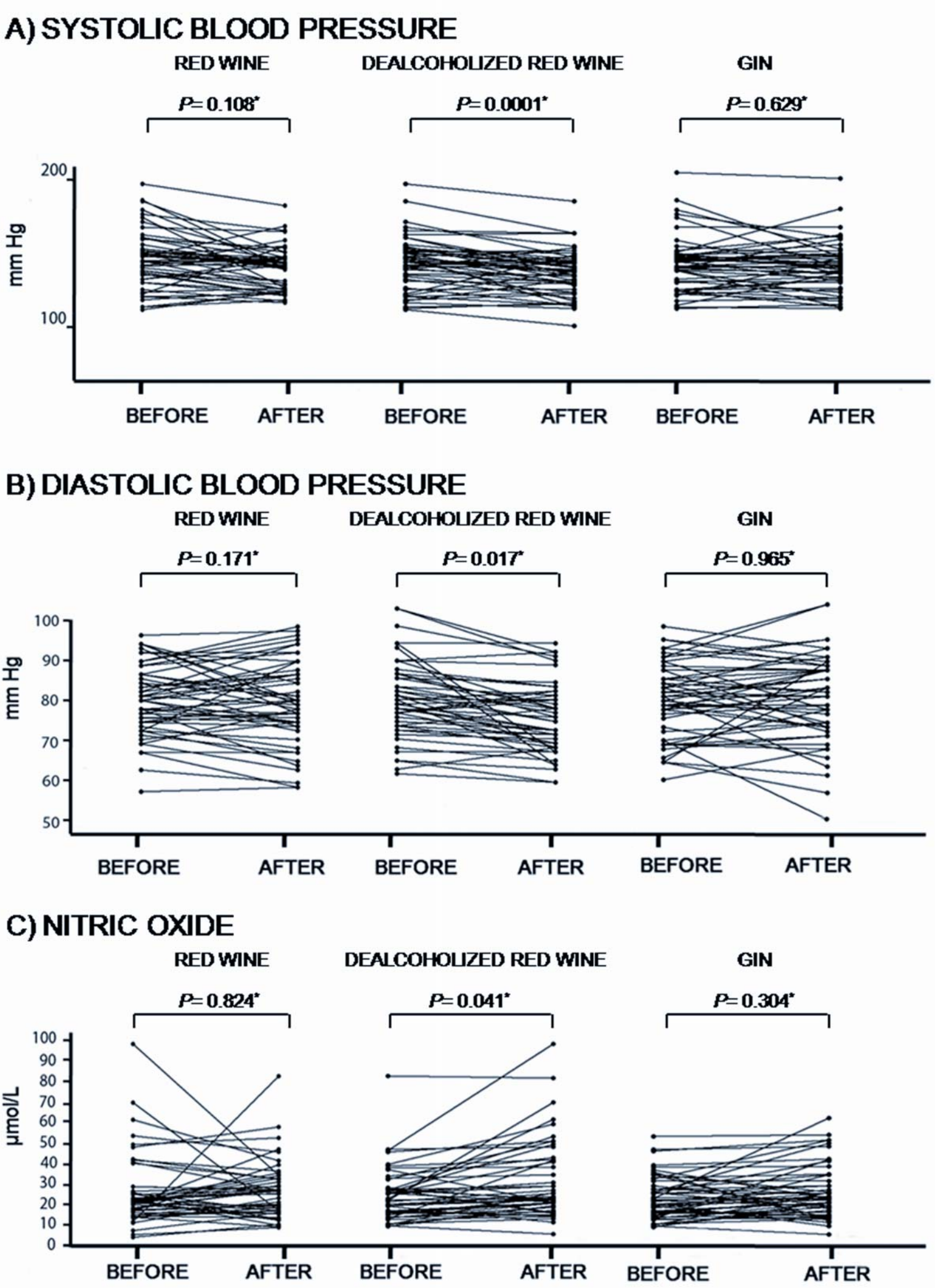
What Is Known?

- Hypertension is a major cardiovascular risk factor, and is associated with decreased life expectancy.
- Endothelial secretion of nitric oxide (NO), a potent vasodilator, contributes to lower blood pressure.
- In experimental studies, dietary compounds such as polyphenols (contained in fruits, vegetables and fermented alcoholic beverages such as red wine) have been shown to stimulate the secretion of endothelial NO, potentially decreasing blood pressure.
- The relationship between moderate alcohol consumption and blood pressure has not been clearly established.

What New Information Does This Article Contribute?

- Moderate red wine consumption (alcohol plus polyphenols) does not significantly affect blood pressure or NO production.
- Moderate gin consumption (alcohol without polyphenols) does not significantly affect blood pressure or NO production.
- Dealcoholized red wine consumption (red wine polyphenols without alcohol) significantly decreases systolic and diastolic blood pressure and increases plasma NO concentration.

Although an inverse relationship between moderate alcohol consumption and the incidence of hypertension has been described, the effects of the different alcoholic beverage fractions (alcoholic and non-alcoholic) on blood pressure are unclear. We observed that moderate alcohol consumption, independently of beverage type (red wine or gin) did not significantly affect blood pressure, but dealcoholized red wine, decreased blood pressure and these changes were correlated with plasma NO increases. The findings provide new insights into the role of t dietary components such as red wine polyphenols in cardiovascular health, particularly in blood pressure regulation. Consumption of dealcoholized red wine might be useful in preventing low to moderate-degree hypertension.


A) SYSTOLIC BLOOD PRESSURE
RED WINE
DEALCOHOLIZED RED WINE
GIN
P= 0.108*
P= 0.0001*
P= 0.629*
200
100
mm Hg
BEFORE
AFTER
BEFORE
AFTER
BEFORE
AFTER
B) DIASTOLIC BLOOD PRESSURE
RED WINE
DEALCOHOLIZED RED WINE
GIN
P= 0.171*
P= 0.017*
P= 0.965*
100
90
80
70
60
50
mm Hg
BEFORE
AFTER
BEFORE
AFTER
BEFORE
AFTER
C) NITRIC OXIDE
RED WINE
DEALCOHOLIZED RED WINE
GIN
P= 0.824*
P= 0.041*
P= 0.304*
100
90
80
70
60
50
40
30
20
10
0
µmol/L
BEFORE
AFTER
BEFORE
AFTER
BEFORE
AFTER

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Supplemental Material

Dealcoholized red wine decreases systolic and diastolic blood pressure and increases plasma nitric oxide

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Supplemental Detailed Methods

Subjects

A total of 73 high-risk subjects aged between 55 and 75 years were recruited for the study in the outpatient clinic of the Internal Medicine Department at our Institution. The subjects included in the trial were moderate alcohol consumers (1-3 drinks/day) and had diabetes mellitus or ≥3 of the following cardiovascular disease risk factors: active smoking, hypertension, plasma LDL cholesterol >160 mg/dL, plasma HDL cholesterol <35 mg/dL, overweight or obesity (body mass index ≥25 kg/m^2), and/or family history of premature coronary heart disease (CHD). Exclusion criteria included documented CHD, stroke or peripheral vascular disease, human immunodeficiency virus infection, alcoholic liver disease, malnutrition and neoplastic or acute infectious diseases. None of the study subjects were consumers of multivitamin or vitamin E supplements or anti-inflammatory drugs (steroids, non-steroidal anti-inflammatory agents or aspirin at doses >100 mg/day).

After a 2-week run-in period wherein subjects were asked not to consume any alcoholic beverage, they were randomized using a computer-generated table into three treatments in a cross-over design, with a common background diet plus gin (100mL – 30g ethanol/day), RW (272mL – 30g ethanol/day; total polyphenols: 798 Eq Gallic Acid/day -EGA/day-), and the same amount of polyphenols as RW in the form of dealcoholized red wine (DRW) (272mL - total phenols: 733 EGA/day), resulting in six possible beverage sequences lasting 4 weeks each intervention. No washout periods were included between the interventions.

The Institutional Review Board of the hospital approved the study protocol, and all participants gave written consent before participation in the study. This trial was registered at controlled-trials.com as ISRCTN88720134.

Diet and exercise monitoring

Subjects were asked to exclude alcoholic beverages 2 weeks before the first intervention (run-in period) and throughout the study. They were also asked not to change their dietary habits or level of physical activity during the study. Natural foods rich in antioxidants, especially fruit and vegetables, were especially monitored so that individual diets had similar antioxidant content throughout the study. Given the characteristics of the tested beverages, participants were not blinded to the type of drink they ingested. At the beginning of the study and after each intervention period, a medical record and Minnesota Leisure Time Physical Activity Questionnaire validated in Spain[4] were administered, and a 7-d food record questionnaire (5 weekdays and 2 weekend days), also validated in our population[5] was used to assess nutrient intake and to monitor adherence to the study protocol. The dietary information was converted into nutrient data using the Food Processor Nutrition and Fitness Software (*esha* Research, Salem, OR). Subjects were asked to maintain their lifestyle habits and to report any illness or abnormality presented during the study period. At the end of each study sequence, a clinician assessed any adverse effects from the interventions by administering a checklist of symptoms, including bloating, fullness, or indigestion, altered bowel habit, dizziness and other symptoms possibly associated with consumption of the test beverages.

Composition of wines

The RW and DRW were from the Penedès appellation and elaborated with the Merlot grape variety. The total phenolic content of the three beverages was determined with the Folin-Ciocalteu method[1], the phenolic profile of RW and DRW was determined by HPLC-DAD as described previously[2] and resveratrol and piceid content was determined by HPLC-DAD as described by Romero-Perez *et al*[3]. There were no significant differences between the phenolic composition of RW and DRW (ref. 7 of the main manuscript).

Laboratory Analyses

After the run-in period (baseline) and the day after the end of each intervention period (RW, DRW and gin), BP and heart rate were measured 3 times at 5-min intervals on the nondominant arm with an oscillometer (Omron 705 CP; Omron Matsusaka Co Ltd, Matsusaka City, Japan) after 15 minutes resting in a seated position. The mean of the second and the third measures was considered for statistical analysis.

Fasting blood samples for the NO analyses and safety biochemistry determinations were collected at baseline and after each intervention, and stored at –80℃ until assayed. For measurement of NO, the rele ase of NO_2^- and NO_3^-, the stable breakdown products of NO in plasma, were determined by a chemiluminescence detector in a NO analyzer (Sievers Instruments, Inc., Boulder, CO). Plasma aminotransferases (ASAT and ALAT), gamma glutamyl transpeptidase (GGT) and albumin were measured by molecular absorption spectrometry and vitamin B12 and serum and intraerythrocytary folic acid concentrations by immunoanalyses.

Compliance assessment

Resveratrol conjugates derived from phase II metabolism were measured in 24-h urine samples from the last day of the run-in period and the last day of each intervention, using the validated methodology described by Urpi-Sarda *et al*.[6] quantitatively adapted to the commercial and available standards. trans- and cis-Resveratrol-3-O-glucuronide (98% purity each), *cis*-resveratrol-4'-O-glucuronide (96% purity) and *trans*-resveratrol-3-O-sulfate (98% purity) were purchased from Toronto Research Chemicals Inc. (North York, ON, Canada). *trans*- and *cis*-Resveratrol-4'-O-sulfate and *cis*-resveratrol-3-O-sulfate were quantified using the *trans*-resveratrol-3-O-sulfate calibration curve. Ethylglucuronide was measured in 24-h urine samples as a biomarker of alcohol intake by liquid chromatography (LC) (Agilent series 1200) coupled with a hybrid quadrupole time-of-flight (TOF) QSTAR Elite (Applied Biosystems/MDS Sciex).

Statistical analyses

Statistical analyses were performed using the SAS Statistical Analysis Systems (version 9.2, SAS Institute Inc, Cary, NC). Descriptive statistics [mean ± standard deviation (SD)] were used to describe the baseline characteristics of the participants. To exclude the presence of a carryover effect for the three periods, the interaction between treatment (RW, DRW and gin) and period (1^{rst}, 2^{nd} and 3^{rd}) was analyzed by the repeated measures Analysis of Covariance (ANCOVA) with the baseline values (the values of the previous intervention or the run-in period if the first intervention) as the covariates. To analyze the changes within each treatment a Student's t test for paired samples was performed between the data obtained before and after each intervention. One-factor analysis of variance (ANOVA) for repeated measures and the Bonferroni *post-hoc* test were used to compare the differences of the changes in outcome variables between the interventions. Pearsons' correlation analysis was used to quantify relationships between changes in blood pressure and nitric oxide plasma concentrations. Within- and between-group differences are expressed as means and 95% confidence intervals (CI). *P* was considered significant when <0.05.

Supplemental Results

Baseline characteristics, intervention compliance, diet, exercise monitoring, and side effects

Of the 73 subjects included, six withdrew before completing the three phases of the study because of physical illness (n = 2), journeys (n = 2) or taste intolerance to DRW (n = 2). Therefore, 67 subjects completed the study. Most were overweight or obese (~91%), more than half the population had hypertension (~57%), more than three

quarters had a family history of cardiovascular disease (~78%), and more than one fifth had dyslipemia (~24%), type-2 diabetes (~22%) or were active smokers (~24%). Biochemical safety analytes (serum and intraerythrocytary folic acid, vitamin B12, albumin, ASAT, ALAT and GGT) remained within the normal range throughout the study. None of the subjects reported adverse effects related to the interventions.

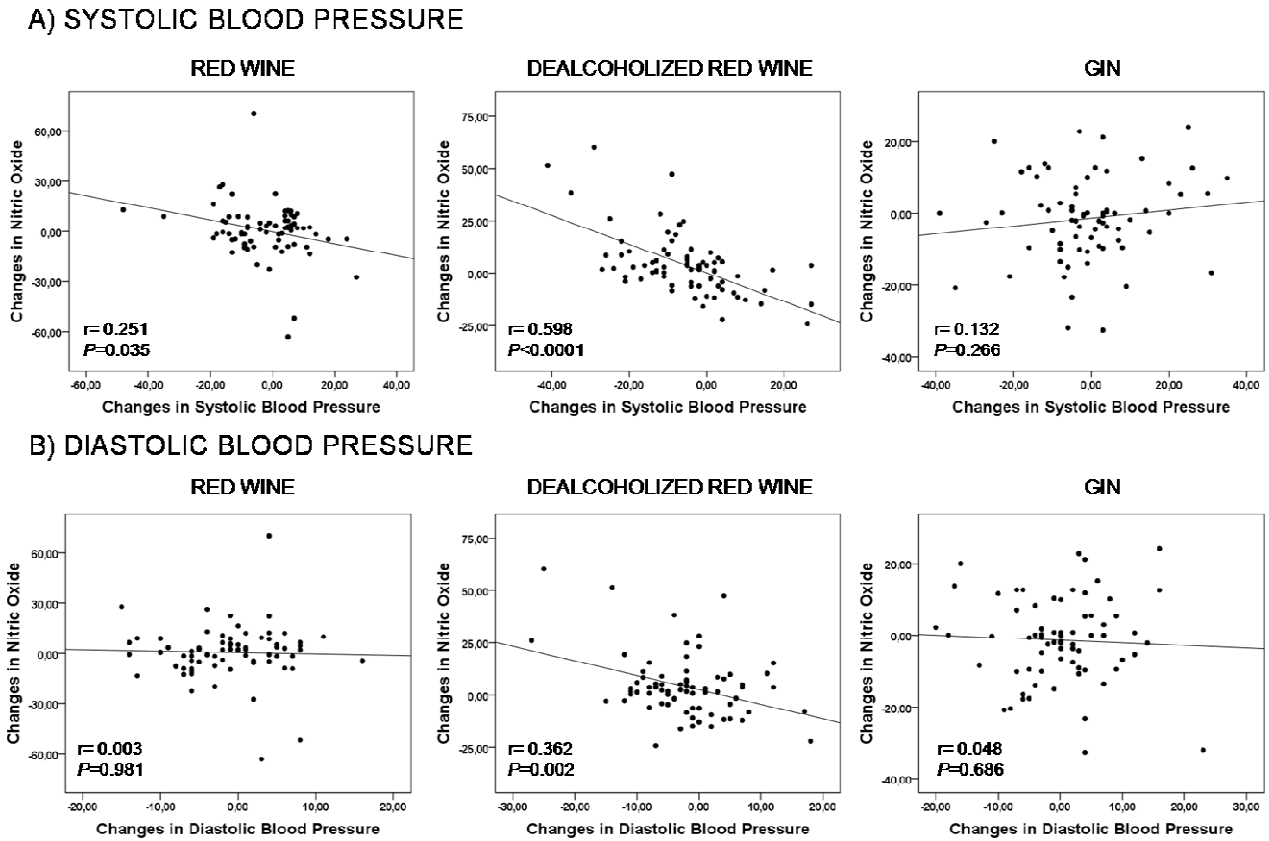
Protocol adherence was optimum in all subjects, and complete agreement was observed between the participants' reports and the number of empty bottles returned. As a measure of intervention compliance, a sum of total resveratrol metabolites -a marker of RW and DRW consumption[7]- was determined in 24-h urine samples collected the last day of the run-in period and the last day of each intervention. After consumption of RW and DRW, 24-h urinary excretion of total resveratrol metabolites increased above baseline from 0.94 µmol (95% CI: 0.43, 1.46 µmol) to 6.04 µmol (95% CI: 4.76, 7.31 µmol) and 6.28 µmol (95% CI: 5.10, 7.46 µmol), respectively ($P < 0.001$, both). Resveratrol metabolites concentrations were not statistically different after DRW and RW interventions ($P = 1.00$) and were significantly higher after RW and DRW interventions compared to gin period [0.51 µmol (95% CI: 0.08, 0.94 µmol); $P \leq 0.001$]. After the gin intervention, urinary resveratrol metabolites were similar to baseline values ($P = 1.00$). Urinary ethylglucuronide concentrations, a biomarker of alcohol consumption, increased significantly after the RW and gin periods compared to baseline values, with increases of 342% (95% CI: 245, 773%) and 256% (95% CI: 179, 599%), respectively ($P < 0.001$, both). Moreover, concentrations after the RW and gin interventions were also higher than those obtained after DRW: 634% (95% CI: 468, 1424%) and 491% (95% CI: 359, 1121), respectively ($P < 0.001$, both). No significant differences were observed between the DRW and baseline periods [66% (95% CI: 64, 75%); $P = 1.000$] and between the RW and gin interventions [24% (95% CI: 24, 25%); $P = 1.000$]. According to these results, compliance with the three interventions was excellent.

Exclusion of the participants with hypertension or under antihypertensive treatment did not materially change the results (**Online Table I**). Nevertheless, in the hypertensive subgroup, the diastolic blood pressure and the plasma nitric oxide concentrations remained practically unchanged during the study. Interestingly, we observed that baseline BMI and waist-to-hip ratios were significantly different between the hypertensive and the non-hypertensive subgroup (P=0.009 and 0.047, respectively, Student's t test for independent samples), without changes throughout the study.

Dietary intake data for the three intervention periods are shown in **Online Table II**. No significant changes from baseline in energy, nutrient, mineral, and antioxidant intake or in the daily average energy expended in physical activity were observed. Likewise, none of the study subjects reported changes in medication use throughout the study. No carryover effect was observed for any outcome, and the values before each intervention were not significantly different between them for any of the outcomes.

Supplemental Data

On-line Supplemental Figure I: Correlation between the changes in blood pressure and nitric oxide between before and after each intervention in the 67 subjects studied.



Online Supplemental Table I: Comparison of anthropometric parameters, blood pressure and plasma concentrations of nitric oxide between 38 hypertensive and 29 non-hypertensive subjects at baseline and after the 3 interventions (n=67).

		Red wine intervention			Dealcoholized red wine intervention			Gin intervention			
		Mean±SD*		Mean differences (95% CI)†	Mean± SD *		Mean differences (95% CI)†	Mean± SD *		Mean differences (95% CI)†	
		Before	After		Before	After		Before	After		*P*‡
Body mass index (kg/m^2)	Total	29.4 ± 3.8	29.5 ± 3.9	0.6 (-0.7, 0.2)	29.3 ± 3.9	29.4 ± 4.1	-0.1 (-0.4, 0.1)	29.4 ± 3.8	29.5 ± 4.0	-0.1 (-0.3, 0.1)	0.200
	Hypertensive	30.9 ± 4.2	30.9 ± 4.3	0.2 (-0.1, 0.4)	30.9 ± 4.5	30.9 ± 4.3	-0.1 (-0.2, 0.2)	30.8 ± 4.4	30.9 ± 4.3	-0.2 (-0.5, 0.1)	0.084
	Non-hypertensive	28.4 ± 3.1	28.3 ± 3.2	-0.1 (-0.2, 0.1)	28.4 ± 3.0	28.3 ± 3.2	0.1 (-0.3, 0.4)	28.4 ± 3.2	28.2 ± 4.0	-0.1 (-0.5, 0.2)	0.876
Waist-to-hip ratio	Total	0.97 ± 0.04	0.97 ± 0.05	-0.006 (-0.013, 0.001)	0.97 ± 0.04	0.97 ± 0.05	-0.001 (-0.006, 0.005)	0.98 ±0.05	0.98 ± 0.05	0.007 (-0.002, 0.015)	0.118
	Hypertensive	0.99 ± 0.06	0.98 ± 0.05	-0.009 (-0.020, 0.003)	0.98 ± 0.05	0.98 ± 0.05	-0.001 (-0.010, 0.008)	0.98 ± 0.05	0.99 ± 0.06	0.003 (-0.010, 0.017)	0.400
	Non-hypertensive	0.97 ± 0.04	0.96 ± 0.04	-0.003 (-0.012, 0.006)	0.96 ± 0.04	0.96 ± 0.04	-0.001 (-0.008, 0.006)	0.97 ± 0.04	0.97 ± 0.05	0.011 (-0.001, 0.022)	0.202
Systolic blood pressure (mm Hg)	Total	137 ± 16	135 ± 15	-2.3 (-5.1, 0.5)[a,b]	138 ± 18	132 ± 16	-5.8 (-8.9, -2.7)[a,§]	138 ± 17	136 ± 19	-0.8 (-4.1, 2.5)[b]	0.028
	Hypertensive	139 ± 19	138 ± 15	-2.4 (-6.2, 1.5)[a,b]	143 ± 20	136 ±18	-7.7 (-12.2, -3.0)[a,§]	138 ± 19	139 ± 18	0.8 (-3.5, 5.1)[b]	0.024
	Non-hypertensive	134 ± 13	131 ± 14a	-2.1 (-6.5, 2.3)[a,b]	133 ± 16	129 ±15	-3.5 (-7.8, -0.8)[a,§]	135 ± 15	134 ± 19	-1.0 (-8.0, 2.0)[b]	0.042
Diastolic blood pressure (mm Hg)	Total	80 ± 8	79 ± 9	-1.0 (-2.5, 0.5)[a,b]	79 ± 10	77 ± 8	-2.3 (-4.1, -0.4)[a,§]	79 ± 8	79 ± 10	0.1 (-1.8, 1.9)[b]	0.027
	Hypertensive	80 ± 10	78 ± 10	-2.1 (-4.6, 0.3)	80 ± 11	77 ± 9	-2.2 (-4.8, 0.5)	78 ± 10	78 ± 9	-0.4 (-2.4, 1.7)	0.401
	Non-hypertensive	79 ± 7	79 ± 9	-0.8 (-4.2, 0.5)[a,b]	78 ± 9	77 ± 8	-2.7 (-5.6, -0.6)[a,§]	79 ± 7	80 ± 11	2.3 (-0.1, 4.9)[b]	0.015
Heart rate (beats/min)	Total	68 ± 10	68 ± 10	-0.2 (-1.9, 1.5)	67 ± 9	68 ± 9	-1.7 (-3.4, 0.1)	68 ± 10	69 ± 11	1.1 (-0.8, 3.0)	0.187
	Hypertensive	69 ± 12	68 ± 12	-1.2 (-3.7, 1.2)	67 ± 9	67 ± 10	-0.7 (-4.2, 1.0)	68 ± 11	69 ± 12	1.2 (-2.0, 4.4)	0.428
	Non-hypertensive	68 ± 8	69 ± 9	0.3 (-2.0, 2.6)	68 ± 10	69 ± 8	-1.4 (-3.9, 1.1)	68 ± 8	69 ± 11	1.1 (-1.3, 3.6)	0.499
Nitric oxide (µmol/L)	Total	27.5 ± 15.8	27.8 ± 13.0	0.6 (-3.3, 4.3)[a,b]	26.1 ± 12.1	29.6 ± 17.8	4.1 (0.5, 7.6)[a,§]	27.1 ± 11.4	25.8 ± 12.0	-1.4 (-4.1, 1.3)[b]	0.022
	Hypertensive	28.1 ± 19.1	27.4 ± 13.7	-0.8 (-8.2, 6.5)	24.8 ± 9.7	27.1 ±14.1	2.2 (-2.6, 7.1)	27.1 ± 10.7	25.8 ± 11.2	-2.0 (-6.4, 2.3)	0.464
	Non-hypertensive	26.8 ± 10.9	28.2 ± 12.5	2.1 (-0.5, 4.8)[a,b]	27.8 ± 14.1	32.0 ± 19.3	5.7 (0.2, 11.2)[a,§]	27.0 ± 12.4	25.8 ± 12.8	-1.0 (-4.6, 2.5)[b]	0.015

Results are expressed as *mean ± SD (n=38 and n=29 for the hypertensive and the non-hypertensive population) and †mean differences (95% CI) between after and before each intervention. Before each intervention is the value of the previous intervention or the baseline in the first intervention. ‡P value of the repeated-measures ANOVA from the differences between interventions. §Significant differences (*P*<0.05) between

after and before the intervention, measured by a Student's *t* test for paired samples. No significant differences were found between the values before each intervention for any of the outcomes (repeated-measures ANOVA).

Online Supplemental Table II: Daily energy and nutrient intakes in the 67 subjects studied at baseline and after the three interventions[*].

	Red wine intervention			Dealcoholized red wine intervention			Gin intervention			
	Mean± SD [†]		Mean differences	Mean± SD [†]		Mean differences	Mean± SD [†]		Mean differences	
	Before	After	(95% CI)[‡]	Before	After	(95% CI)[‡]	Before	After	(95% CI)[‡]	$P^{§}$
Energy (kcal/d)	1863 ± 256	1782 ± 325	-85 (-238, 67)	1804 ± 396	1862 ± 320	90 (-63, 244)	1896 ± 403	1887 ± 336	-15 (-183, 153)	0.359
Total protein (g/d)	92.63 ± 16.92	89.06 ± 18.35	-2.3 (-9.9, 9.5)	93.15 ± 15.78	94.29 ± 17.56	2.3 (-8.3, 8.8)	92.1 ± 2.0	95.04 ± 18.73	1.8 (-8.2, 11.8)	0.967
Carbohydrates (g/d)	205 ± 37	201 ± 44	-6.3 (-32, 30)	184 ± 40	193 ± 38	14.4 (-12.3, 41.3)	194 ± 39	206 ± 40	13.2 (-7.8, 34.2)	0.763
Dietary fiber (g/d)	22.31 ± 8.2	20.63 ± 8.08	-2.1 (-5.7, 1.5)	17.85 ± 5.14	19.88 ± 6.82	1.6 (-1.2, 4.4)	19.53 ± 9.04	22.05 ± 10.01	1.6 (-1.1, 4.4)	0.277
Sugars (g/d)	71.77 ± 20.08	66.74 ± 25.59	-9.7 (-19.6, 0.2)	61.36 ± 23.34	67.33 ± 20.08	6.5 (-4.7, 17.8)	68.88 ± 23.84	70.11 ± 20.84	2.1 (-10.3, 14.4)	0.153
Total lipids (g/d)	75.51 ± 13.99	72.72 ± 16.27	-5.7 (-13.9, 2.5)	73.48 ± 19.31	78.55 ± 20.41	9.1 (-1.3, 19.6)	73.70 ± 23.16	79.86 ± 21.89	-0.6 (-11.5, 10.3)	0.118
SFA (g/d)	19.86 ± 4.93	18.40 ± 7.06	-0.7 (-3.4, 2.0)	17.36 ± 8.33	18.90 ± 6.15	3.9 (-0.3, 7.4)	19.20 ± 6.51	19.01 ± 5.48	-1.0 (-4.9, 2.8)	0.128
MUFA (g/d)	36.99 ± 7.30	35.84 ± 8.23	-3.1 (-7.1, 0.9)	36.05 ± 8.38	37.97 ± 10.18	3.8 (-0.5, 8.2)	37.04 ± 11.27	38.31 ± 9.60	0.2 (-4.9, 5.4)	0.171
PUFA (g/d)	11.18 ± 2.93	10.65 ± 4.21	-0.8 (-2.4, 0.7)	10.87 ± 3.81	11.94 ± 4.42	0.7 (0.7, 2.0)	10.53 ± 4.83	10.71 ± 3.29	0.2 (-1.1, 1.5)	0.464
Cholesterol (mg/d)	365 ± 108	355 ± 125	-14 (-65, 36)	336 ± 136	342 ± 103	33 (-21, 86)	345 ± 124	360 ± 162	25 (-55, 61)	0.462
Vitamin C (mg/d)	118 ± 75	125 ± 83	11 (-11, 32)	124 ± 59	121 ± 68	-2.6 (-21, 16)	129 ± 87	133 ± 91	-12 (-37,11)	0.507
Vitamin A (µgRE[‖]/d)	605 ± 255	688 ± 340	89 (-195, 374)	696 ± 532	729 ± 343	83 (-125, 231)	725 ± 452	709 ± 481	-65 (-158,77)	0.234
Vitamin E (mg/d)	9.28 ± 2.82	9.65 ± 3.22	-0.5 (-1.5, 0.5)	9.55 ± 2.86	9.47 ± 3.34	-0.1 (-1, 0.8)	9.37 ± 2.98	10.29 ± 4.65	0.5 (-0.4, 1.3)	0.399
Folic acid (µg/d)	493 ± 166	454 ± 155	-43 (-121,35)	394 ± 138	443 ± 143	64 (-20, 148)	484 ± 186	498 ± 226	11 (-45, 67)	0.335
Calcium (mg/d)	714 ± 73	718 ± 36	61 (-24, 146)	663 ± 21	678 ± 35	39 (-47, 125)	731 ± 31	712 ± 30	-70 (-200, 605)	0.342
Magnesium (mg/d)	398 ± 74	345 ± 92	-87 (-420, 245)	316 ± 94	339 ± 95	97 (-100, 295)	368 ± 96	342 ± 106	-31 (-40.1, 34.0)	0.551
Manganese (mg/d)	2.77 ± 1.17	2.66 ± 1.09	-0.1 (-0.7, 0.5)	2.61 ± 1.06	2.72 ± 1.26	0.1 (-0.3, 0.8)	2.76 ± 1.41	2.82 ± 1.18	0.2 (-1.0, 5.4)	0.617
Potassium (mg/d)	2918 ± 718	3119 ± 673	87 (-149, 323)	2967 ± 751	3089 ± 850	49 (-186, 285)	3254 ± 790	3121 ± 955	-66 (-271, 139)	0.515
Selenium (µg/d)	146 ± 37	139 ± 37	-21 (-186, 144)	134 ± 35	136 ± 38	47 (-121, 215)	131 ± 33	136 ± 33	52 (-85, 189)	0.790
Sodium (mg/d)	2296 ± 161	2298 ± 198	-127 (-434, 180)	2137 ± 202	2205 ± 191	359 (-750, 794)	2293 ± 213	2278 ± 121	-87 (-294, 119)	0.218
Zinc (mg/d)	10.18 ± 1.59	10.48 ± 2.42	1.1 (-4.6, 5.8)	10.32 ± 2.64	9.56 ± 2.40	-2.2 (-5.7, 2.2)	10.07 ± 2.54	10.40 ± 2.21	-2.5 (-5.4, 3.4)	0.549
Total polyphenols (mg/d)	308 ± 134	318 ± 142	31 (-23, 87)	320 ± 168	311 ± 146	-33 (-89,23)	313 ± 169	327 ± 170	-32 (-77, 13)	0.146

[*] Excluding the energy, nutrient and total polyphenol contributions from the tested beverages. Results are expressed as [†]mean ± SD (n=67) and [‡]mean differences (95% CI) between after and before each intervention. Before each intervention is the value of the previous intervention or the baseline in the first intervention. [§]P value of the repeated-measures ANOVA from the differences between interventions. No changes were observed between after and before the intervention, measured by a Student's *t* test for paired samples. [‖]Retinol Equivalents.

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