Alcohol and Alcoholism Vol. 48, No. 2, pp. 153–159, 2013
Advance Access Publication 2 August 2012
doi: 10.1093/alcalc/ags086

ORIGINAL ARTICLE

Effect of Red Wine Consumption on Biomarkers of Oxidative Stress

Ilse C. Schrieks*, Robin van den Berg, Aafje Sierksma, Joline W.J. Beulens, Wouter H.J. Vaes and Henk F.J. Hendriks

TNO, Utrechtseweg 48, 3704 HE Zeist, The Netherlands
*Corresponding author: TNO, PO Box 360, 3700 AJ Zeist, The Netherlands. Tel.: +31-88-866-42-28; Fax: +31-88-866-87-67; E-mail: ilse.schrieks@tno.nl

(*Received* 18 *January* 2012; *first review notified* 19 *March* 2012; *in revised form* 22 *May* 2012; *accepted* 26 *June* 2012)

Abstract — **Aims**: To evaluate the effect of acute and chronic consumption of red wine or de-alcoholized red wine with a similar antioxidant capacity on plasma total antioxidant capacity (TEAC), nuclear factor-κB (NF-κB) activity and F2-isoprostanes (8-iso-$PGF_{2\alpha}$) in healthy men. **Methods**: Nineteen healthy men with an increased waist circumference (≥94 cm) and a body mass index above 25 kg/m^2 participated in a randomized, controlled crossover design trial. They daily consumed 450 ml of red wine (four drinks; 41.4 g alcohol) or 450 ml of de-alcoholized red wine during dinner for 4 weeks each. On the last day of each treatment period, blood was collected before and 1 h after a standardized dinner with red wine or de-alcoholized red wine and also 24-h urine was collected. **Results**: Absolute TEAC levels were higher 1 h after dinner with red wine compared with dinner with de-alcoholized red wine (1.3 versus 1.1 mmol Trolox equivalents/l; $P = 0.03$). Consumption of dinner together with de-alcoholized red wine acutely stimulated NF-κB activity in peripheral blood mononuclear cells (0.4–0.7 HeLa equivalents/2.5 μg protein; $P = 0.006$), whereas this increase was completely suppressed when the dinner was combined with red wine. A chronic increase in urinary 8-iso-$PGF_{2\alpha}$ after 4 weeks of red wine consumption compared with de-alcoholized red wine consumption (157 pg/mg creatinine and 141 pg/mg creatinine, respectively, $P = 0.006$) was also observed. **Conclusions**: Consumption of a moderate dose of red wine can acutely increase plasma TEAC and suppress NF-κB activation induced by a meal. Controversially, 4 weeks of red wine consumption compared with de-alcoholized red wine consumption increases the oxidative lipid damage marker 8-iso-$PGF_{2\alpha}$.

INTRODUCTION

Epidemiological studies have provided abundant evidence that moderate alcohol consumption is associated with a lower risk for cardiovascular disease (CVD) (Colditz *et al.*, 1985; Stampfer *et al.*, 1988; Grobbee *et al.*, 1999). Atherosclerosis, the underlying cause of CVD, is a process in which lipoproteins, fibrinolytic and inflammatory factors are involved.

The formation of reactive oxygen species (ROS) and an enhanced oxidative stress status is associated with CVD and CVD risk factors, such as obesity, diabetes type II and smoking (Vincent *et al.*, 2007; Lakshmi *et al.*, 2009). It has been shown that red wine consumption can increase plasma antioxidant capacity (Micallef *et al.*, 2007; Boban and Modun, 2010; Covas *et al.*, 2010). Serafini *et al.* (1998) demonstrated that this was probably caused by the polyphenolic content of the wine. However, Arendt *et al.* (2005) found no increase in antioxidant capacity after red wine consumption, while the polyphenolic plasma content was increased. It has been suggested that ethanol in wine is capable of increasing plasma antioxidant capacity in an indirect way, because the absorption of polyphenols is insufficient to explain the total increase of antioxidant capacity (Duthie *et al.*, 1998; Boban and Modun, 2010).

Nuclear factor-κB (NF-κB) is an oxidative stress-related transcription factor involved in the regulation of inflammatory responses. Oxidative stress, for example induced by cigarette smoke (Van den Berg *et al.*, 2001a), activates NF-κB, whereas antioxidants may inhibit this activation (Blackwell *et al.*, 1996; Sen and Packer, 1996; Van den Berg *et al.*, 2001b). *In vitro* studies have shown that exposure to moderate doses of alcohol can inhibit NF-κB activation in human monocytes (Mandrekar *et al.*, 1997; Mandrekar *et al.*, 1999; Mandrekar *et al.*, 2002). Furthermore, Joosten *et al.* (2011) demonstrated that 4 weeks of moderate alcohol consumption resulted in a decreased NF-κB gene expression compared with abstention. This suggests that the down-regulation of NF-κB may be a mechanism involved in the alcohol-induced suppression of inflammatory processes.

Excessive intake of alcohol, however, is associated with increased inflammation (Romeo *et al.*, 2007; Goral *et al.*, 2008; Szabo and Mandrekar, 2009).

To our knowledge, only two human intervention studies have examined the effect of alcohol consumption on NF-κB activation. Dhindsa *et al.* (2004) showed that the consumption of 300 kcal from alcohol did not have acute effects on NF-κB activation, while 300 kcal from glucose increased NF-κB activation. In a study from Blanco-Colio *et al.* (2000) it was demonstrated that the antioxidants in red wine rather than moderate alcohol consumption prevented NF-κB activation induced by a fat-enriched breakfast; however, an alcohol-free control condition was not included. In contrast, previous studies (Caccetta *et al.*, 2001; Hartman *et al.*, 2005; Beulens *et al.*, 2008) showed that alcohol consumption may increase oxidative damage markers (F2-isoprostanes), while red wine consumption may protect against low-density lipoprotein lipid oxidation (Serafini *et al.*, 2000). Therefore, we performed a human intervention study to investigate the effect of moderate alcohol consumption on NF-κB activity and other biomarkers of oxidative stress. In a randomized crossover design, healthy men consumed a moderate dose of red wine or de-alcoholized red wine with a similar antioxidant capacity.

SUBJECTS AND METHODS

Subjects

The study was conducted at TNO (a Dutch acronym for the Netherlands Organization for Applied Scientific Research), in Zeist, The Netherlands. TNO is an independent research organization which collaborates with universities and companies, and has the facilities to carry out clinical studies.

The study was performed according to the ICH Guideline for Good Clinical Practice and approved by the independent Medical Ethics Committee of TNO (authorization no 01/22) complied with the Declaration of Helsinki. Nineteen subjects, all non-smoking, were recruited from the pool of volunteers of TNO. The volunteers received complete information about the study by verbal briefing and in writing and subsequently signed for informed consent.

A questionnaire (self-report) was used for information on alcohol intake, medical history and (family) history of alcoholism. Subjects were considered healthy, based on the values of the pre-study laboratory tests (hematology, clinical chemistry and safety parameters), their medical history and their physical examination. Subjects fulfilled the following inclusion criteria: consumption between 10 and 28 alcohol-containing beverages weekly, waist circumference ≥94 cm and no (family) history of alcoholism, diabetes mellitus type 2 or CVD. Subjects were selected for having an increased waist circumference as this represents the growing group of obese subjects in the western world. Obesity has been associated with an increased state of oxidative stress (Vincent *et al.*, 2007), therefore we hypothesize that moderate alcohol consumption improves the oxidative stress in this population.

Study design

The subjects entered a randomized crossover trial consisting of two periods of 4 weeks in which they consumed red wine or de-alcoholized red wine.

The red wine and the de-alcoholized red wine were specially bottled for this study (Carl Jung GmbH, Rüdesheim am Rhein, Germany). The red wine had an alcohol content of 11.5 vol%. The de-alcoholized wine (alcohol content of 0.13 vol%) was made from exactly the same base wine, however, it was sweetened with sugar (4%). The alcohol was extracted from the wine by vacuum distillation at low temperature (<30°C) to maintain the taste and characteristics of the wine.

Half of the subjects were randomly allocated to the treatment order red wine followed by de-alcoholized red wine. The other half of the subjects consumed de-alcoholized red wine first followed by red wine. In this way, any bias due to the beverage order and a possible drift of variables over time were eliminated.

Subjects were instructed to drink 450 ml (four glasses) of red wine (41.4 g alcohol) or de-alcoholized red wine (control) with dinner, representing the habitual pattern of alcohol intake in The Netherlands. Beverages were provided in bottles at the start and halfway of each treatment period together with a measuring cup marked at 450 ml. Subjects were asked to maintain their normal dietary habits and exercise patterns and not to consume any (additional) alcoholic beverages throughout the study. Each day the subjects completed a questionnaire detailing beverage intake, dietary habits, exercise performed, medications taken and illnesses incurred. The questionnaires were routinely reviewed by the medical investigator and all problems identified were discussed with the subjects during the next visit or by telephone. In addition, compliance was checked by counting the number of bottles returned and measuring those left over. Body weight was determined halfway and at the end of each treatment period, with the subjects wearing indoor clothing, without shoes, wallet and keys.

On the last day of each treatment period subjects collected 24-h urine and had a standardized dinner together with red wine or de-alcoholized red wine at TNO. They were requested not to eat or drink anything starting 3 h before this standard dinner, which consisted of ~19% energy from protein, ~31% energy from fat and ~50% energy from carbohydrate.

Analysis of total antioxidant capacity and flavonoid content of the beverages

The antioxidant capacity was measured using the Trolox equivalent antioxidant capacity (TEAC) assay as described by Van den Berg *et al.* (1999). This method was used to relate total antioxidant capacity to oxidative stress markers (NF-κB, F2-isoprostanes) by all compounds present with an antioxidant potential. Briefly, the beverages were diluted using ethanol and added to an 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonate) (ABTS) radical solution, and the decrease in radical concentration was monitored spectrophotometrically at 734 nm.

Quantitative flavonoid analysis of the major flavonoids myricitin, quercetin and isoramnetin was performed using high-performance liquid chromatography (HPLC) equipped with photodiode array detection according to a method described by Hertog *et al.* (1992). Sample preparation to analyze for free flavonoids consisted of diluting the sample with ethanol (50%, v/v) containing 1% ascorbate, and subsequent filtration, after which the filtrate was analyzed directly. Glycosylated flavonoids were analyzed as their corresponding deglycosylated analogs. For this, samples were treated as described above. The resulting filtrate was diluted 5-fold with water and cleaned using a C18 SPE cartridge. After elution of the glycosylated flavonoids with 50/50 water/methanol, the eluate was hydrolyzed for 2 h at 1.5 M HCl under reflux conditions. The resulting hydrolysate was analyzed using an Alliance 2690 HPLC system, equipped with a Hypersil BDS column (4.6 × 250 mm) and photodiode array detection. Compounds were quantified at their compound-specific absorption maxima.

Biological sample collection and analysis

In vitro studies have shown that ethanol can affect NF-κB as early as after 1 h (Mandrekar *et al.*, 1997, 2002) and therefore blood was collected before and 1 h after the standardized dinner at TNO on the last day of each treatment period. The following morning a fasting blood sample was collected to determine serum high-density lipoprotein (HDL)-cholesterol and plasma adiponectin levels. Staff members who conducted the laboratory analysis were blind to the treatment assignments.

Isolation of peripheral blood mononuclear cells

For determination of NF-κB activity peripheral blood mononuclear cells (PBMCs) were isolated immediately after blood collection. Heparinized blood (20 ml) was transferred into LeucoSep tubes (Greiner-Bio One GmbH, Frickenhausen, Germany) containing Ficoll Paque (Amersham Biosciences, Piscataway, NJ, USA) and diluted with a balanced salt

solution. Samples were centrifuged for 30 min at 800*g* at room temperature and subsequently PBMCs were collected, washed twice and divided over two aliquots containing approximately 1×10^7 cells. These samples were stored at −80°C until the preparation of nuclear extracts.

Preparation of nuclear extracts

Nuclear extracts were prepared using a nuclear extraction kit (Active Motif Europe, Rixensart, Belgium) by using approximately 1×10^7 PBMCs. Briefly, PBMCs were washed with ice-cold phosphate-buffered saline containing phosphatase inhibitors. Cytoplasmic fractions were collected by incubating the cells in a hypotonic buffer for 15 min at 4°C. After addition of detergent, cell suspensions were thoroughly mixed and centrifuged for 30 s at 14,000*g* and 4°C and supernatants were transferred into pre-cooled tubes. Finally, nuclear fractions were collected by the addition of complete lysis buffer, incubated for 30 min on ice and centrifuged for 10 min at 14,000*g* and 4°C. The supernatants (nuclear fractions) were transferred into pre-cooled tubes and stored at −80°C until analysis.

NF-κB activity

For the detection and quantification of NF-κB activation, a TransAM™ NF-κB p65 Kit (Active Motif Europe) containing a 96-well plate to which oligonucleotides with NF-κB consensus binding sites have been immobilized was used. Nuclear extracts were added to the 96-well plate and analyzed using an antibody directed against the NF-κB p65 subunit from the NF-κB complex, which is bound to the oligonucleotides. Addition of a secondary antibody conjugated to horse-radish peroxidase provided a colorimetric readout that was quantified spectrophotometrically at 450 nm.

Plasma total antioxidant capacity

For the determination of plasma total antioxidant capacity, blood was collected in tubes containing ethylenediaminetetraacetic acid. Blood was centrifuged for 10 min at 2000*g* and 4°C. Plasma samples were stored at −80°C until analysis. Plasma total antioxidant capacity as measured by the TEAC assay was determined according to the method described by Van den Berg *et al.* (1999). Briefly, plasma samples were deproteinized by adding an equal volume of ethanol. After centrifugation, the supernatant was added to an ABTS radical solution. The decrease in radical concentration was monitored and related to the decrease obtained with Trolox.

Serum HDL-cholesterol and plasma adiponectin

Serum HDL-cholesterol levels were determined using fasting blood samples which were collected in tubes containing a cloth activator. Between 15 and 30 min after collection blood was centrifuged for 15 min at 2000*g* and 4°C. Serum samples were stored at −80°C until analysis. HDL-cholesterol was determined enzymatically with a commercially available kit (Roche Diagnostics, Mannheim, Germany).

For plasma adiponectin determination, blood was collected in tubes containing citrate-theophylline-adenosine-dipyridamole and centrifuged for 15 min at 2000*g* and 4°C. Plasma samples were stored at −80°C until analysis. Fasting plasma adiponectin concentrations were determined using a validated sandwich enzyme-linked immunosorbent assay employing an adiponectin-specific antibody (ANOC9108), as described by Arita *et al.* (1999).

Urinary F2-isoprostane (8-iso $PGF_{2\alpha}$) analysis

Twenty-four hours urine samples were stored in 10 ml aliquots containing 100 μg butylated hydroxytoluene at −80°C until analysis. Measurement of 8-iso $PGF_{2\alpha}$ was performed using gas chromatography mass spectrometry using negative chemical ionization using a method described by Morrow *et al.* (1999) with some minor modifications.

Statistical analysis

Statistical analyses were performed with SAS statistical software package (SAS/STAT Version 8.02, SAS Institute, Cary, NC, USA). Differences in the characteristics (TEAC and flavonoid concentrations) of the wines were assessed by an independent sample *t*-test. Treatment effects on F2-isoprostanes and fasting clinical parameters at the end of each treatment period were assessed by the mixed model procedure with period as random term and treatment and treatment order as a fixed terms. Treatment effects on NF-κB activity and TEAC before and 1 h after dinner were assessed by the mixed model procedure that included a random term for period and fixed terms for treatment (red wine and de-alcoholized red wine) and moment (before and 1 h after dinner) and an interaction term of treatment and moment. Treatment order was added to the model as fixed factor to correct for possible carry-over effects.

Model terms were considered significant at $P \leq 0.05$. Data are presented as means and SEs.

RESULTS

The characteristics of the 19 subjects are provided in Table 1. The total antioxidant capacity and the major glycosylated flavonoids concentrations were similar for red wine and de-alcoholized red wine (Table 2).

Table 1. Characteristics of the subjects at baseline[a]

	Mean (range)
Age (year)	55 (35–68)
Height (cm)	180.8 (169.2–196.0)
Weight (kg)	95.5 (82.5–154.5)
BMI (kg/m^2)	29.2 (25.0–45.2)
Waist circumference (cm)	109.5 (95.0–151.0)
Hip circumference (cm)	106.6 (99.5–137.0)
Waist-hip ratio	1.03 (0.93–1.12)
Hemoglobin (mmol/l)	9.4 (8.4–10.2)
Triacylglycerol (mmol/l)	1.84 (1.12–4.07)
Total cholesterol (mmol/l)	5.90 (4.54–7.77)
HDL cholesterol (mmol/l)	1.28 (0.97–1.69)
Low-density lipoprotein (LDL) cholesterol (mmol/l)	3.77 (2.23–5.23)
Aspartate aminotransferase (ASAT) (U/l)	25 (12–43)
Alanine aminotransferase (ALAT) (U/l)	34 (9–74)
γ-Glutamyl transpeptidase (GGT) (U/l)	38 (11–79)
Glucose (mmol/l)	5.7 (4.7–6.4)
Insulin (mU/l)	10.9 (4.7–19.3)

[a]$n = 19$.

Table 2. Flavonoids content and antioxidant capacity of red wine and de-alcoholized red wine

	TEAC (mmol TE/l[a])	Free flavonoids			Glycosylated flavonoids		
		Myricitin (mg/l)	Quercetin (mg/l)	Isoramnetin (mg/l)	Myricitin (mg/l)	Quercetin (mg/l)	Isoramnetin (mg/l)
Red wine	24.5	3.0	3.2	0.4	5.1	4.6	1.3
De-alcoholized red wine	24.3	2.8	3.1	0.2	6.2	4.5	1.6

[a]mmol Trolox equivalents/l.

Table 3. Fasting blood parameters (mean ± SE) at the end of each treatment period

	De-alcoholized red wine	Red wine	*P*-value
Body weight (kg)	95.3 ± 4.1	95.8 ± 4.1	0.06
Glucose (mmol/l)	5.44 ± 0.12	5.55 ± 0.12	0.12
Insulin (mU/l)	10.60 ± 1.21	9.95 ± 0.86	0.44
Triacylglycerol (mmol/l)	1.66 ± 0.10	1.63 ± 0.11	0.78
Total cholesterol (mmol/l)	5.59 ± 0.19	5.76 ± 0.19	0.08
HDL cholesterol (mmol/l)	1.06 ± 0.04	1.15 ± 0.04	<0.01
LDL cholesterol (mmol/l)	3.76 ± 0.17	3.86 ± 0.16	0.30
Adiponectin (μg/ml)	5.8 ± 0.4	6.3 ± 0.6	0.02
Leukocytes (10^9/l)	5.6 ± 0.2	5.5 ± 0.2	0.79
Platelets (10^9/l	205 ± 9	200 ± 9	0.24
Creatinine (μmol/l)	77.7 ± 1.9	78.9 ± 2.1	0.30
Alkaline phosphatase (U/l)	64.7 ± 3.8	64.4 ± 4.1	0.72
ASAT (U/l)	22.4 ± 1.2	23.3 ± 1.2	0.36
ALAT (U/l)	25.2 ± 2.6	24.6 ± 2.3	0.54
GGT (U/l)	27.4 ± 2.3	32.2 ± 3.0	<0.01

Compliance with the beverage intake was good, as judged from the daily questionnaire and the return of the empty bottles. Another indication that the subjects were compliant with the beverage intake was their fasting serum HDL-cholesterol which increased ~8.5% after 4 weeks of red wine consumption compared with de-alcoholized red wine consumption (1.15 and 1.06 mmol/l, respectively; $P < 0.01$). Additionally, fasting adiponectin levels was ~8.6% higher after 4 weeks red wine consumption compared with de-alcoholized red wine consumption (6.3 and 5.8 μg/ml, respectively; $P = 0.02$). The effects of 4 weeks of red wine or de-alcoholized red wine consumption on clinical fasting parameters and body weight are shown in Table 3. The average body weight did not differ between red wine and de-alcoholized red wine treatments, suggesting that the dietary habits and exercise patterns did not materially change.

Plasma TEAC before dinner did not differ after 4 weeks of daily consumption of red wine or de-alcoholized red wine ($P = 0.87$; Fig. 1). Post-prandial TEAC was influenced differently by red wine and de-alcoholized red wine consumption. Plasma TEAC was 17% higher 1 h after dinner with red wine compared with the plasma TEAC 1 h after dinner with de-alcoholized red wine ($P = 0.03$; Fig. 1).

Four weeks of daily consumption of red wine did not influence the basal NF-κB activity differently from the daily consumption de-alcoholized red wine ($P = 0.44$), although the NF-κB activity before dinner was on average 19% higher after 4 weeks of red wine consumption. Post-prandial NF-κB activity was influenced differently by dinner with de-alcoholized red wine compared with dinner with red wine. The intake of dinner with de-alcoholized red wine

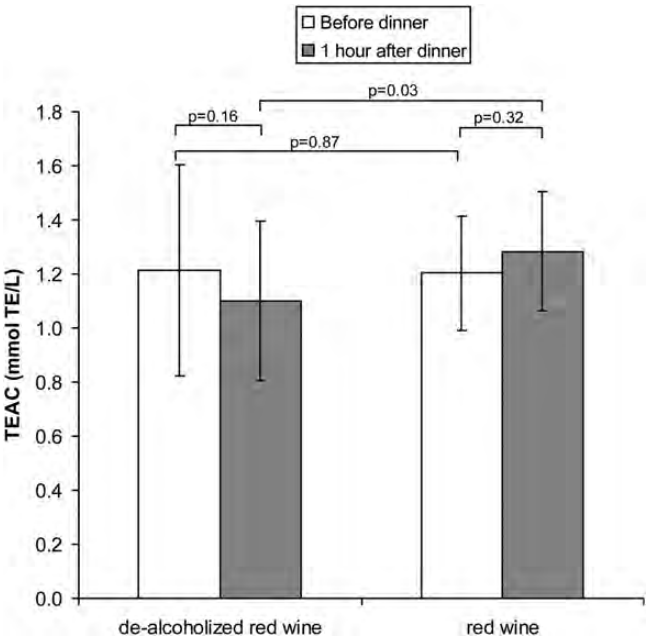

Fig. 1. Plasma TEAC was increased 1 h after dinner with red wine compared with the plasma TEAC 1 h after dinner with de-alcoholized red wine ($P = 0.03$).

increased the NF-κB activity by 65% after 1 h ($P = 0.006$), whereas this effect was absent (+0.3%, $P = 0.99$) when dinner was consumed with red wine. This resulted in an absolute difference in NF-κB activity 1 h after dinner between the red wine and the de-alcoholized red wine condition, with the first being 38% higher ($P = 0.05$) (Fig. 2).

Four weeks of red wine consumption compared with de-alcoholized red wine consumption (Fig. 3) significantly increased the oxidative damage marker isoprostanes (8-iso-$PGF_{2\alpha}$; 157 pg/mg creatinine and 141 pg/mg creatinine, respectively, $P = 0.006$).

DISCUSSION

The results of our study show that red wine or de-alcoholized red wine consumption with dinner differently affected the biomarkers of oxidative stress.

The plasma total antioxidant capacity increased ~17% 1 h after red wine consumption compared with de-alcoholized red wine consumption, despite a similar total antioxidant capacity of both beverages. Since the plasma total antioxidant capacity reflects the absorption of all compounds with an antioxidant capacity, this may suggest that alcohol improves the short-term uptake of these compounds.

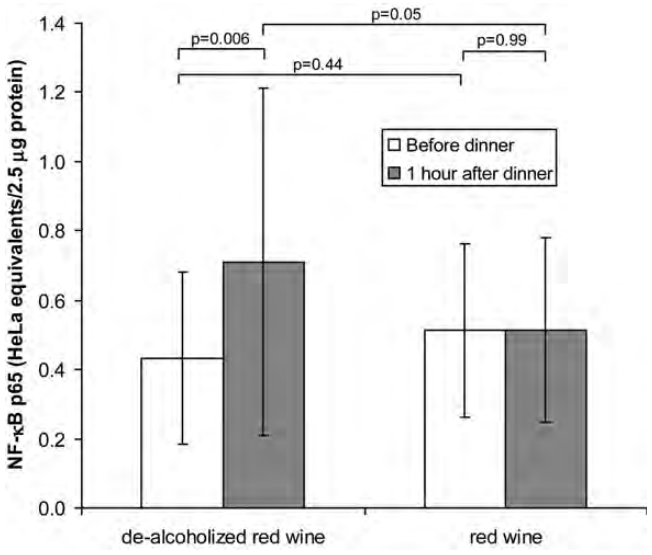

Fig. 2. Intake of dinner with de-alcoholized red wine significantly increased the NF-κB activity ($P = 0.006$), but this effect was absent ($P = 0.99$) when dinner was consumed with red wine. NF-κB activity was higher 1 h after dinner with de-alcoholized red compared with NF-κB activity 1 h after a dinner with red wine ($P = 0.05$).

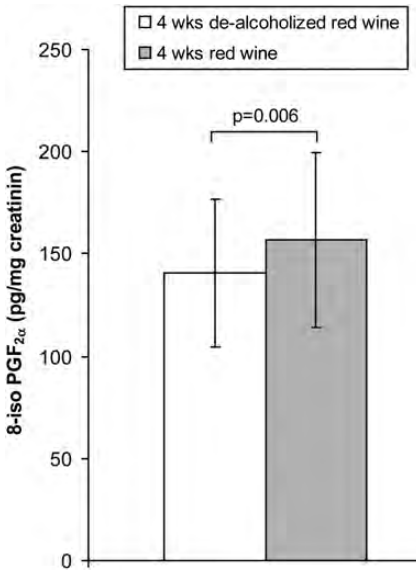

Fig. 3. Four weeks of red wine consumption compared with de-alcoholized red wine consumption significantly increased the oxidative damage marker isoprostane (8-iso-$PGF_{2\alpha}$; $P = 0.006$).

These results suggest that consumption of a dinner decreases the plasma total antioxidant capacity. Post-prandial oxidative stress has been described to occur after the ingestion of high-fat or high-carbohydrate meals (Gregersen *et al.*, 2012). Hyperlipidemia and hyperglycemia are both associated with an increased ROS production. A meal high in oxidized and oxidizable lipids can increase the plasma levels of lipid hydroperoxides, which is associated with a higher susceptibility to low-density lipoprotein oxidation (Sies *et al.*, 2005). High plasma glucose levels may induce labile non-enzymatic glycation and increase the intracellular NADH/NAD^+ ratio, which are both accompanied with ROS production (Ceriello *et al.*, 1999).

We observed no effect on the plasma antioxidant capacity before dinner between the red wine and de-alcoholized red wine period. This indicates that long-term alcohol consumption does not affect the plasma antioxidant capacity.

The results of our study show that the intake of a dinner with de-alcoholized red wine acutely (1 h) stimulated NF-κB activation. Dinner with red wine attenuated this food-induced NF-κB activation. Both red wine and de-alcoholized red wine had a similar total antioxidant capacity, suggesting that the food-induced NF-κB activation is suppressed by alcohol itself or that alcohol possibly facilitates the action of antioxidants present in red wine. The last may be supported by the increased plasma antioxidant capacity measured 1 h after wine consumption compared with de-alcoholized wine consumption.

Since NF-κB plays an important role in the coordinated expression of inflammatory genes, the prevention of diet-induced NF-κB activation suggests a mechanism for cellular regulation of the anti-inflammatory effects of moderate alcohol consumption. This is in agreement with the findings of Joosten *et al.* (2011) indicating a central role for transcription factor NF-κB in altered gene expression profiles in the immune response after 4 weeks of moderate alcohol consumption.

The prevention of diet-induced NF-κB activation after moderate alcohol consumption is in agreement with *in vitro* observations (Mandrekar *et al.*, 1997, 1999, 2002), but does not correspond with findings of Blanco-Colio *et al.* (2000). In their intervention study an increased NF-κB activity was observed after a fat-enriched breakfast with vodka, whereas a fat-enriched breakfast with red wine prevented NF-κB activation. The authors concluded that not alcohol but antioxidants in red wine caused this effect. Unfortunately, they did not include an alcohol-free control condition. A combined effect of alcohol and compounds with antioxidant capacity can therefore not be excluded. The acute reduction of post-prandial oxidation of a meal by antioxidants and wine has been very well described (Natella *et al.*, 2001, 2002; Sies *et al.*, 2005). These observations are in line with our study showing the acute suppression of NF-κB induced by the dinner.

The prevention of food-induced NF-κB activation with moderate red wine consumption was acute and transient, because the NF-κB activity did not differ before dinner between the red wine and the de-alcoholized red wine period. Similarly, Van den Berg *et al.* (2001c) did not observe a chronic effect on NF-κB activation in smokers after 3 weeks of high antioxidant intake. These two studies suggest that the effect of nutritional compounds on NF-κB activation in healthy subjects can only be measured acutely after the activation of NF-κB. The induced NF-κB activation after consumption of a meal or single foods was also shown in previous studies (Bellido *et al.*, 2004; Patel *et al.*, 2007; Dickinson *et al.*, 2008).

In our study sugar was added to the de-alcoholized red wine to improve taste and to compensate for caloric loss due to the de-alcoholization process (4%). The de-alcoholized red wine still had a lower caloric content than the red wine, while the sugar content was somewhat higher (18 g, equal to 72 kcal per 450 ml). The study of Dhindsa *et al.* (2004)

reported an increased NF-κB activity 1 h after intake of 300 kcal from glucose compared with the intake of 300 kcal from alcohol. However, the amount of sugar used by Dhindsa *et al.* (2004) was four times higher than the amount of sugar added to the de-alcoholized red wine in our study. Therefore, we do not expect that the difference in NF-κB activation between the two conditions caused by alcohol may be confounded by the difference in the sugar content.

Coinciding with the acute suppressive effect on NF-κB activity, there was an increase in urinary excretion of 8-iso-$PGF_{2\alpha}$ after 4 weeks of daily consumption of 450 ml red wine consumption compared with the de-alcoholized red wine period. F2-isoprostanes are oxidative products of arachidonic acid and are regarded as a reliable and specific measure of *in vivo* lipid peroxidation. Our results show that (4 weeks) consumption of 450 ml (41.4 g alcohol) of red wine chronically increases the lipid oxidation marker F2-isoprostanes in comparison with de-alcoholized red wine. This effect is independent of the acute dinner effects and the acute suppression of post-prandial oxidative stress by alcohol consumption. Chronic increases in F2-isoprostanes can therefore only be attributed to the difference in the alcohol content of the beverages. This is in line with the observations of Caccetta *et al.* (2001) and Hartman *et al.* (2005). They showed that chronic consumption of alcohol compared with no alcohol resulted in increased F2-isoprostane levels. Beulens *et al.* (2008) also reported increased urinary F2-isoprostane levels after chronic alcohol consumption, although this did not reach significance ($P = 0.09$). Rifici *et al.* (2002) showed that polyphenols in red wine did decrease lipoprotein oxidation *in vitro*, whereas alcohol did not. Wine polyphenols have also been shown to reduce the release of nitric oxide and to scavenge ROS *in vitro* (Cíz *et al.*, 2008). However, the absorption of polyphenols from wine is very low and after entering plasma, they are quickly metabolized and excreted. The effect of plasma polyphenols after wine consumption on oxidative capacity is therefore negligible when compared with endogenous antioxidants (Huisman *et al.*, 2004).

More research is required to establish whether a chronic increase in isoprostanes caused by oxidative stress-induced lipid peroxidation is physiologically relevant. Additional research is also needed to establish whether other nutritional compounds can attenuate NF-κB activation. The relation between NF-κB and inflammation should be further investigated as well as the effect on other transcription- and inflammation-related factors, in order to explain the physiological relevance of both contradictory effects.

In conclusion, red wine consumption can acutely increase the plasma total antioxidant capacity and suppress the NF-κB activation induced by a meal. However, chronic red wine consumption compared with de-alcoholized red wine consumption may increase the oxidative lipid damage marker 8-iso-$PGF_{2\alpha}$.

Acknowledgments — We acknowledge all those involved in the conduct of the study and thank the volunteers for their enthusiastic participation. Carl Jung GmbH (Rudesheim am Rhein, Germany) supplied the red wine and the de-alcoholized red wine, which were specially bottled for this study.

Funding — This work was supported both by the Ministry of Economic Affairs, Agriculture and Innovation and by the Dutch Foundation for Alcohol Research (SAR) representing Dutch producers of and traders in beer, wine and spirits and TNO. Their joint aim is to independently study the health effects of moderate alcohol consumption.

REFERENCES

Arendt BM, Ellinger S, Kekic K *et al.* (2005) Single and repeated moderate consumption of native or dealcoholized red wine show different effects on antioxidant parameters in blood and DNA strand breaks in peripheral leukocytes in healthy volunteers: a randomized controlled trial (ISRCTN68505294). *Nutr J* **4**:33.

Arita Y, Kihara S, Ouchi N *et al.* (1999) Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* **257**:79–83.

Bellido C, López-Miranda J, Blanco-Colio LM *et al.* (2004) Butter and walnuts, but not olive oil, elicit postprandial activation of nuclear transcription factor κB in peripheral blood mononuclear cells from healthy men. *Am J Clin Nutr* **80**:1487–91.

Beulens JWJ, Van den Berg R, Kok FJ *et al.* (2008) Moderate alcohol consumption and lipoprotein-associated phospholipase A2 activity. *Nutr Metab Cardiovasc Dis* **18**:539–44.

Blackwell TS, Blackwell TR, Holden EP *et al.* (1996) In vivo antioxidant treatment suppresses nuclear factor-κB activation and neutrophillic lung inflammation. *J Immunol* **157**:1630–7.

Blanco-Colio LM, Valderrama M, Alvarez-Sala LA *et al.* (2000) Red wine intake prevents nuclear factor-κB activation in peripheral blood mononuclear cells of healthy volunteers during postprandial lipemia. *Circulation* **102**:1020–6.

Boban M, Modun D. (2010) Uric acid and antioxidant effects of wine. *Croat Med J* **51**:16–22.

Caccetta RA, Burke V, Mori TA *et al.* (2001) Red wine polyphenols, in the absence of alcohol, reduce lipid peroxidative stress in smoking subjects. *Free Radic Biol Med* **30**:636–42.

Ceriello A, Bortolotti N, Motz E *et al.* (1999) Meal-induced oxidative stress and low-density lipoprotein oxidation in diabetes: The possible role of hyperglycemia. *Metabolism* **48**:1503–8.

Cíz M, Pavelkova M, Gallova L *et al.* (2008) The influence of wine polyphenols on reactive oxygen and nitrogen species production by murine macrophages RAW 264.7. *Physiol Res* **57**:393–402.

Colditz GA, Branch LG, Lipnick RJ *et al.* (1985) Moderate alcohol and decreased cardiovascular mortality in an elderly cohort. *Am Heart J* **109**:886–9.

Covas MI, Gambert P, Fitó M *et al.* (2010) Wine and oxidative stress: up-to-date evidence of the effects of moderate wine consumption on oxidative damage in humans. *Atherosclerosis* **208**:297–304.

Duthie GG, Pedersen MW, Gardner PT *et al.* (1998) The effect of whisky and wine consumption on total phenol content and antioxidant capacity of plasma from healthy volunteers. *Eur J Clin Nutr* **52**:733–6.

Dhindsa S, Tripathy D, Mohanty P *et al.* (2004) Differential effect of glucose and alcohol on reactive oxygen species generation and intranuclear nuclear factor-κB in mononuclear cells. *Metabolism* **53**:330–4.

Dickinson S, Hancock DP, Petocz P *et al.* (2008) High–glycemic index carbohydrate increases nuclear factor-κB activation in mononuclear cells of young, lean healthy subjects. *Am J Clin Nutr* **87**:1188–93.

Goral J, Karavitis J, Kovacs EJ. (2008) Exposure-dependent effects of ethanol on the innate immune system. *Alcohol* **42**:237–47.

Gregersen S, Samocha-Bonet D, Heilbronn LK *et al.* (2012) Inflammatory and oxidative stress responses to high-carbohydrate and high-fat meals in healthy humans. *J Nutr Metab* **2012**:1–8.

Grobbee DE, Rimm EB, Keil U *et al.* (1999) Alcohol and cardiovascular system. In MacDonald IE (ed). *Health issues related to alcohol consumption*. ILSI Europe, Blackwell Science: Philadelphia, PA, 125–81.

Hartman TJ, Baer DJ, Graham LB *et al.* (2005) Moderate alcohol consumption and levels of antioxidant vitamins and isoprostanes in postmenopausal women. *Eur J Clin Nutr* **59**:161–8.

Hertog MGL, Hollman PCH, Venema DP. (1992) Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *J Agric Food Chem* **40**:1591–8.

Huisman A, van de Wiel A, Rabelink TJ *et al.* (2004) Wine polyphenols and ethanol do not significantly scavenge superoxide nor affect endothelial nitric oxide production. *J Nutr Biochem* **15**:426–32.

Joosten MM, van Erk MJ, Pellis L *et al*. (2011) Moderate alcohol consumption alters both leucocyte gene expression profiles and circulating proteins related to immune response and lipid metabolism in men. *Br J Nutr* First view, 1–8. doi:10.1017/S0007114511005988.

Lakshmi SV, Padmaja G, Kuppusamy P *et al*. (2009) Oxidative stress in cardiovascular disease. *Indian J Biochem Biophys* **46**:421–40.

Mandrekar P, Catalano D, Szabo G. (1997) Alcohol-induced regulation of nuclear regulatory factor-kappa beta in human monocytes. *Alcohol Clin Exp Res* **21**:988–94.

Mandrekar P, Catalano D, Szabo G. (1999) Inhibition of lipopolysaccharide-mediated NFκB activation by ethanol in human monocytes. *Int Immunol* **11**:1781–90.

Mandrekar P, Dolganiuc A, Bellerose G *et al*. (2002) Acute alcohol inhibits the induction of nuclear regulatory factor kappa B activation through CD14/toll-like receptor 4, interleukin-1, and tumor necrosis factor receptors: a common mechanism independent of inhibitory kappa B alpha degradation? *Alcohol Clin Exp Res* **26**:1609–21.

Micallef M, Lexis L, Lewandowski P. (2007) Red wine consumption increases antioxidant status and decreases oxidative stress in the circulation of both young and old humans. *Nutr J* **6**:27.

Morrow JD, Zackert WE, Yang JP *et al*. (1999) Quantification of the major urinary metabolite of 15-F2t-isoprostane (8-iso-PGF2alpha) by a stable isotope dilution mass spectrometric assay. *Anal Biochem* **269**:326–31.

Natella F, Ghiselli A, Guidi A *et al*. (2001) Red wine mitigates the postprandial increase of LDL susceptibility to oxidation. *Free Radic Biol Med* **30**:1036–44.

Natella F, Belelli F, Gentili V *et al*. (2002) Grape seed proanthocyanidins prevent plasma postprandial oxidative stress in humans. *J Agric Food Chem* **50**:7720–5.

Patel C, Ghanim H, Ravishankar S *et al*. (2007) Prolonged reactive oxygen species generation and nuclear factor-κB activation after a high-fat, high-carbohydrate meal in the obese. *J Clin Endocrinol Metab* **92**:4476–9.

Rifici VA, Schneider SH, Khachadurian AK. (2002) Lipoprotein oxidation mediated by J774 murine macrophages is inhibited by individual red wine polyphenols but not by ethanol. *J Nutr* **132**:2532–7.

Romeo J, Warnberg J, Nova E *et al*. (2007) Moderate alcohol consumption and the immune system: a review. *Br J Nutr* **98** (Suppl 1):S111–5.

Sen CK, Packer L. (1996) Antioxidants and redox regulation of gene transcription. *FASEB J* **10**:709–20.

Serafini M, Maiani G, Ferro-Luzzi A. (1998) Alcohol-free red wine enhances plasma antioxidant capacity in humans. *J Nutr* **128**:1003–7.

Serafini M, Laranjinha JAN, Almeida LM *et al*. (2000) Inhibition of human LDL lipid peroxidation by phenol-rich beverages and their impact on plasma total antioxidant capacity in humans. *J Nutr Biochem* **11**:585–90.

Sies H, Stahl W, Sevanian A. (2005) Nutritional, dietary and postprandial oxidative stress. *J Nutr* **135**:969–72.

Stampfer MJ, Colditz GA, Willett WC *et al*. (1988) A prospective study of moderate alcohol consumption and the risk of coronary disease and stroke in women. *N Engl J Med* **319**:267–73.

Szabo G, Mandrekar P. (2009) A recent perspective on alcohol, immunity, and host defense. *Alcohol Clin Exp Res* **33**:220–32.

Van den Berg R, Haenen GR, Van den Berg H *et al*. (1999) Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chem* **66**:511–7.

Van den Berg R, Haenen GR, Van den Berg H *et al*. (2001a) Nuclear factor-kappa B activation is higher in peripheral blood mononuclear cells of male smokers. *Environ Toxicol Pharmacol* **9**:147–51.

Van den Berg R, Haenen GR, Van den Berg H *et al*. (2001b) Transcription factor NF-kappa B as a potential biomarker for oxidative stress. *Br J Nutr* **86**:S121–7.

Van den Berg R, Van Vliet T, Broekmans WM *et al*. (2001c) A vegetable/fruit concentrate with high antioxidant capacity has no effect on biomarkers of antioxidant status in male smokers. *J Nutr* **131**:1714–22.

Vincent HK, Innes KE, Vincent KR. (2007) Oxidative stress and potential interventions to reduce oxidative stress in overweight and obesity. *Diabetes Obes Metab* **9**:813–39.