BRIEF OBSERVATION

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Red Wine Prevents the Acute Negative Vascular Effects of Smoking

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ABSTRACT

BACKGROUND: Moderate consumption of red wine is associated with fewer cardiovascular events. We investigated whether red wine consumption counteracts the adverse vascular effects of cigarette smoking.
METHODS: Participants smoked 3 cigarettes alone or after drinking a titrated volume of red wine. Clinical chemistry, blood counts, plasma cytokine enzyme-linked immunosorbent assays, immunomagnetic separation of $CD14^+$ monocytes for gene expression analysis, fluorescence-activated cell sorting for microparticles, and isolation of circulating mononuclear cells to measure telomerase activity were performed, and urine cotinine levels were quantified.
RESULTS: Compared with baseline, leukocytosis ($P = .019$), neutrophilia ($P < .001$), lymphopenia ($P < .001$), and eosinopenia ($P = .008$) were observed after only smoking. Endothelial and platelet-, monocyte-, and leukocyte-derived microparticles ($P < .001$ each) were elevated. In monocytes, messenger RNA expression of interleukin (IL)-6 (2.6- ± 0.57-fold), tumor necrosis factor alpha (2.2- ± 0.62-fold), and IL-1b (2.3- ± 0.44-fold) were upregulated, as was IL-6 (1.2 ± 0.12-fold) protein concentration in plasma. Smoking acutely inhibited mononuclear cell telomerase activity. Markers of endothelial damage, inflammation, and cellular aging were completely attenuated by red wine consumption.
CONCLUSION: Cigarette smoke results in acute endothelial damage, vascular and systemic inflammation, and indicators of the cellular aging processes in otherwise healthy nonsmokers. Pretreatment with red wine was preventive. The findings underscore the magnitude of acute damage exerted by cigarette smoking in "occasional lifestyle smokers" and demonstrate the potential of red wine as a protective strategy to avert markers of vascular injury.
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KEYWORDS: Aging; Endothelial function; Inflammation; Smoking; Wine

A previous version of this article misstated the BAC as 0.75% in the Participants and Study Design section and in the Figure 1 legend. The correct BAC is 0.075%, as reflected in this version of the article.

Regular consumption of red wine often is credited as the explanation for the "French paradox", a term coined to describe the observation that the French enjoy a relatively low risk of cardiovascular disease that correlates to their wine consumption.[1] Benefits of red wine were attributed to phenolic compounds, which mediate its antioxidant effects.[2,3] Red wine stimulates the formation of endothelium-dependent relaxation factors such as nitric oxide, thereby improving endothelial function in human coronary arteries[4] possibly because of the high phenol concentration in red wine.[4] Sparse data exist investigating acute potential vasoprotective effects of red wine in smoking healthy individuals. The aim of this study was to investigate "occasional lifestyle smoking" with or without previous red wine consumption on acute vascular effects in healthy medical professionals.

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MATERIALS AND METHODS

Participants and Study Design

Healthy young nonsmokers (14 male, 6 female) were included in the study after providing informed written consent for the study (mean age, 31.1 ± 0.8 years). All volunteers were fasting for at least 6 hours, not performing exercise, or exposed to passive smoking for at least 24 hours. The participants had to smoke 3 cigarettes (Gauloises red, Imperial Tobacco), 10 of them 1 hour after drinking red wine (Chateau Haut-Pontet, Saint-Emilion Grand Cru, 2005) aiming to reach 0.075% blood alcohol content as calculated by the Widmark formula. Blood (each ~40 mL) and urine collection (each 10 mL) were performed before (baseline, T0) and 45 minutes after drinking the red wine and 100 minutes (T2) and 18 hours after smoking (**Figure 1**). The study was approved by the Ethik-Kommission der Ärztekammer des Saarlandes (Compliance No. 14/11).

CLINICAL SIGNIFICANCE

- Acute cigarette smoke exposure induced release of endothelial microparticles.
- Acute cigarette smoke exposure inhibited mononuclear cell telomerase activity.
- Acute cigarette smoke exposure led to proinflammatory changes.
- Preconsumption of red wine prevented most adverse acute effects of smoking.

Blood and Urine Analyses

Blood counts, high-sensitivity C-reactive protein, sodium, potassium, creatinine, urea, creatinine phosphokinase, transaminases, lactate dehydrogenase, and blood gas analyses were taken. Blood gas analysis was performed to measure carbon monoxide hemoglobin. Blood alcohol content was verified by toxicology. Cotinine (threshold 2.0 µg/L), the predominant metabolite of nicotine, was measured in urine samples.

Microparticles

Analyses of microparticles were performed by flow cytometry (FACS Calibur, BD Biosciences, Heidelberg, Germany) as previously described[5] and analyzed by Cell Quest Pro software (BD Biosciences) to detect fluorescence, forward, and sideward scatter. Nonstained samples were used to discriminate events from background noise and to increase the specificity for detection. Concentrations were assessed by comparison with flowcount calibrator beads. Details are shown in the Online Supplement, available online.

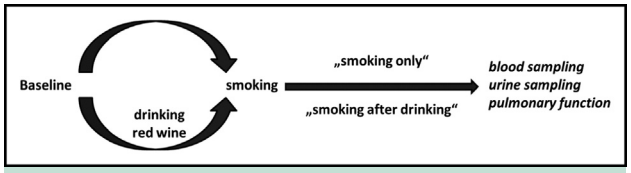

Figure 1 Study design. In the "smoking only" group, baseline data collection was followed by smoking 3 cigarettes. In the "smoking after drinking" group, baseline data collection was followed by drinking a volume of red wine aiming to reach 0.075% blood alcohol content as calculated by the Widmark formula before smoking 3 cigarettes. In both groups, data collection consisting of blood and urine sampling and pulmonary function was performed at baseline and 100 minutes after smoking.

Telomerase Activity

Mononuclear cells were freshly isolated using the Ficoll density gradient centrifugation. Telomerase activity was measured using the Telomerase Repeat Amplification Protocol as described previously.[6,7] Results of the assay were calculated as human embryonic kidney cell equivalents as derived from the standard curve and are shown as percentage versus baseline. Details are shown in the Online Supplement, available online.

Monocytes

Flow cytometric analysis (FACS Calibur, BD Biosciences) of monocyte activation and composition was performed. Interleukin (IL)-6 and soluble intercellular adhesion molecule-1 were determined using enzyme-linked immunosorbent assay kits (R & D Systems, Inc, Minneapolis, Minn). Peripheral blood monocytes were isolated using Ficoll density gradient centrifugation and immunomagnetic negative isolation as previously described.[8] After RNA isolation and reverse transcription, messenger RNA expression of proinflammatory IL-1b, IL-6, tumor necrosis factor-α, and monocyte chemoattractant protein-1 were measured using real-time reverse transcription polymerase chain reaction.[8] Details are shown in the Online Supplement, available online.

Statistical Analyses

Continuous variables were tested for normal distribution by the Kolmogorov–Smirnov test. Changes over time per group were determined by 1-way analysis of variance, and changes over time between protocols (smoking only/smoking after drinking) were determined by 2-way analysis of variance. To compare time points between groups, 2-sided *t* tests were used. Microparticles, telomerase, and inflammation parameters were analyzed in relation to baseline (T0) according to protocol using repeated-measures analysis of variance. *P* values $<.05$ were considered significant. Statistical analyses were performed with SPSS version 18.0 (SPSS Inc, Chicago, Ill) and SigmaStat version 3.5 (Systat Software Inc, San Jose, Calif).

RESULTS

Cotinine, Alcohol, and Blood Counts

Cotinine was not detectable in any volunteer verifying nonsmoking status. After smoke exposure, cotinine increased,

but there were no differences between the "smoking only" and "smoking after drinking" group ($P = .13$). Blood alcohol content was 0.60 ± 0.05 g/dL (equivalent to 0.56% ± 0.05%, $P < .0001$) 45 minutes after wine drinking. Clinical chemistry, blood counts, blood gas analyses, and pulmonary function test results were normal and not different between the groups. Leukocytosis ($P = .019$), neutrophilia ($P < .001$), lymphopenia ($P < .001$), and eosinopenia ($P = .008$) occurred 100 minutes after smoking. When red wine was consumed before smoking, these effects were prevented. Table shows the effects 18 hours after smoking.

Release of Microparticles

Cigarette smoking induced an increase of endothelial-derived microparticles (~3-fold increase, $P < .001$) (Figure 2A). After red wine ingestion, endothelial-derived microparticles did not increase after smoking. Determination of endothelial-derived microparticles' subpopulations showed that activated but not apoptotic (not shown) endothelial-derived microparticles were increased (~8-fold, $P = .015$). Smoking increased platelet-derived microparticles (~2.5-fold increase, $P < .001$) (Figure 2B), whereas after drinking platelet-derived microparticles remained unchanged (Figure 2B). Monocyte-derived (~5-fold) (Figure 2C) and leucocyte-derived microparticles (~2-fold) (Figure 2D) were elevated after smoking ($P < .001$ each). Drinking abolished these increases after smoking (Figure 2C, D).

Telomerase Activity

Smoking reduced mononuclear cells' telomerase activity (32 ± 3 vs 57 ± 9 human embryonic kidney cell

Table Clinical and Laboratory Characteristics of Nonsmokers After "Smoking Only" Versus "Smoking After Drinking" 18 Hours After Smoking and Their *P* Values

Variable	"Smoking Only"	"Smoking After Drinking"	*P* Value
General			
n	20		
Gender	14 male, 6 female		
Drug intake			
Cotinine (µg/g)	22.26 ± 8.26	23.18 ± 13.29	.950
Alcohol level (per mL)	0.00 ± 0.00	No data	
Blood count			
Hemoglobin (g/dL)	14.69 ± 0.34	14.49 ± 0.32	.673
Hematocrit (%)	42.44 ± 0.93	41.60 ± 0.86	.513
Platelets (10^9/L)	248.67 ± 28.19	221.70 ± 17.35	.416
Leucocytes (10^9/L)	5.92 ± 0.34	6.07 ± 0.40	.784
Neutrophils (%)	48.78 ± 1.96	55.40 ± 1.96	.050
Lymphocytes (%)	38.44 ± 1.68	31.80 ± 1.36	.021
Monocytes (%)	8.78 ± 0.40	9.20 ± 0.80	.605
Eosinophils (%)	3.33 ± 0.22	3.40 ± 0.24	.917
Basophils (%)	0.44 ± 0.18	0.40 ± 0.24	.884
Clinical chemistry			
hsCRP n (mg/L)	0.81 ± 0.19	1.92 ± 0.78	.186
Creatinine (mg/dL)	0.85 ± 0.05	0.98 ± 0.04	.048
Creatinine-GFR (mL/min)	100.88 ± 6.6	92.32 ± 6.35	.334
CK (U/L)	132.20 ± 28.43	145.10 ± 18.9	.708
LDH (U/L)	194.20 ± 5.52	169.60 ± 6.83	.012
Blood gas analysis			
pH	7.36 ± 0.01	7.37 ± 0.01	.313
pCO_2 (mm Hg)	46.89 ± 1.60	47.45 ± 1.92	.835
pO_2 (mm Hg)	38.04 ± 3.79	34.54 ± 4.20	.566
BE (mmol/L)	0.70 ± 0.62	1.72 ± 0.65	.295
HCO_3^- (mmol/L)	23.69 ± 0.36	24.39 ± 0.39	.225
Lactate (mmol/L)	1.53 ± 0.21	1.27 ± 0.09	.226
mOsm (mosmol/kg)	284.00 ± 0.89	20.59 ± 0.93	.022
CO-hemoglobin	0.80 ± 0.06	No data	
Pulmonary function			
FVC (L)	4.62 ± 0.28	4.82 ± 0.38	.677
FEV1 (L)	4.28 ± 0.23	4.33 ± 0.39	.898
FEV1/VCM (%)	92.93 ± 1.37	89.44 ± 2.20	.181
PEF (L/sec)	10.60 ± 0.59	11.44 ± 0.88	.434

BE = base excess; CK = creatine kinase; CO = carbon monoxide; FEV1 = forced expiratory volume in 1 second; FVC = forced vital capacity; GFR = glomerular filtration rate; $HCO3^-$ = bicarbonate; hsCRP = high-sensitivity C-reactive protein; LDH = lactate dehydrogenase; mOsm = osmolarity; pCO2 = venous carbon dioxide partial pressure; PEF = peak expiratory flow; pO2 = venous oxygen partial pressure; VCM = vital capacity maneuver.

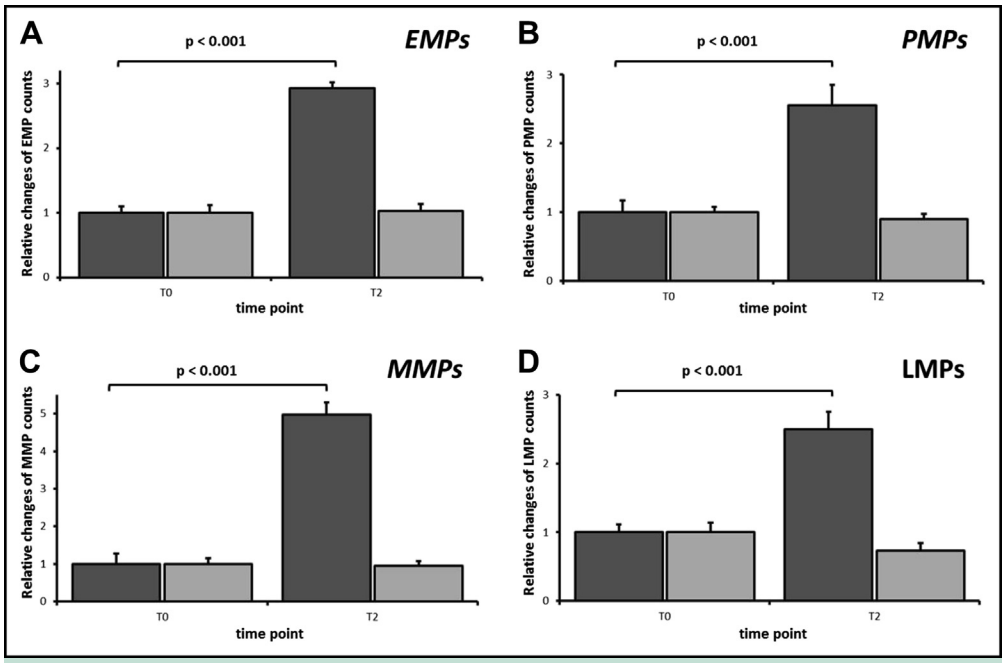

Figure 2 Relative changes of microparticle counts in nonsmokers after "smoking only" (*dark grey*) versus "smoking after drinking" (*light grey*) 100 minutes after smoking (T2) compared with baseline (T0). (**A**) Total change of endothelial-derived microparticles. (**B**) Total change of platelet-derived microparticles. (**C**) Total change of monocyte-derived microparticles. (**D**) Total change of leucocyte-derived microparticles. EMP = endothelial microparticles; LMP = leucocyte microparticles; MMP = monocyte microparticles; PMP = platelet microparticles.

equivalents; decrease to 56% vs baseline; $P < .05$) (Figure 3). Drinking red wine before smoking significantly attenuated telomerase inactivation in mononuclear cells (37 ± 5 vs 46 ± 7 human embryonic kidney cell equivalents; decrease to 80% vs baseline).

Proinflammatory Markers

In $CD14^+$ monocytes, messenger RNA expression of IL-6 (2.6 ± 0.57-fold, $P < .05$) (Figure 4A), tumor necrosis factor-α (2.2 ± 0.62-fold, $P < .05$) (Figure 4B), and IL-1b (2.3 ± 0.44-fold, $P < .05$) (Figure 4C) was upregulated after smoking. Absolute monocyte counts were unchanged with change of $CD16^+CD14^{low}$ monocyte subtypes (not shown). Monocyte chemoattractant protein-1 messenger RNA expression remained unchanged (not shown). Increases of inflammatory cytokine expression were absent when smoking followed ingestion of red wine. Plasma concentrations of IL-6 protein increased 1.2 ± 0.12-fold, from 1576 ± 133 pg/mL to 1836 ± 196 pg/mL ($P < .05$) (Figure 4D). Increase in IL-6 was abolished when red wine was consumed before smoking. Plasma soluble intercellular adhesion molecule-1 remained unchanged on smoking or red wine consumption (not shown).

DISCUSSION

Smoking acutely induced markers of endothelial damage, oxidative stress, vascular inflammation, and cellular aging. Preconsumption of red wine alleviated most of the acute effects of smoking.

Acute cigarette smoke exposure led to endothelial microparticle release. This was demonstrated by fluorescence-activated cell sorting analyses indicating endothelial cell activation and damage, which was prevented by red wine consumption. This is in line with data showing that endothelial dysfunction caused by smoking is lowered with simultaneous consumption of red wine.[3] Microparticles mirrored the pathophysiology of endothelial cell damage (increased endothelial microparticles) followed by platelet activation (increased platelet-derived microparticles) and inflammation (increased leucocyte-derived microparticles and monocyte-derived microparticles). We show for the first time that endothelial microparticle levels acutely increased after smoking and that this is mainly due to endothelial activation after inflammatory microparticles (platelet-, leucocyte-, and monocyte-derived microparticles). When red wine, beer, and vodka were compared, only red wine provided protection against vascular oxidative stress.[9] Bulut et al[10] found that "red wine ingestion prevents microparticle formation induced by a high-fat meal." Our results extend the findings of red wine to acute smoke exposure.

Inflammation leads to enhanced oxidative stress by an imbalance between production of reactive oxygen species and endogenous antioxidant defenses. In our study, we observed acute proinflammatory changes, namely, leukocytosis, neutrophilia, upregulated levels of IL-6 in

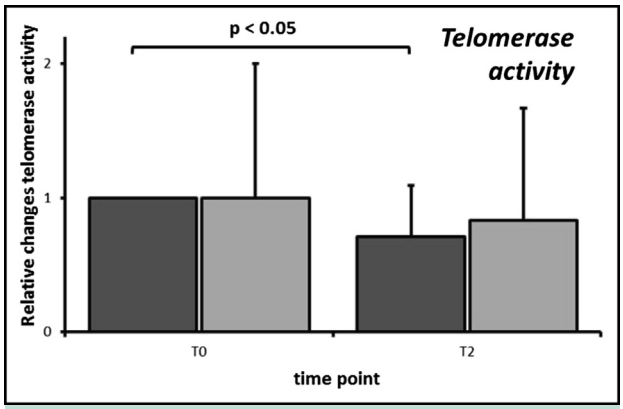

Figure 3 Bar graph of the relative change of telomerase activity (human embryonic kidney cell equivalents) in nonsmokers after "smoking only" (*dark grey*) versus "smoking after drinking" (*light grey*) 100 minutes after smoking (T2) compared with baseline (T0).

serum, and enhanced messenger RNA expression of IL-6 and tumor necrosis factor-α. Our study adds to the present evidence[11] that the proinflammatory effects in nonsmokers with "occasional lifestyle smoking" could be prevented by red wine consumption.

Telomere length and the rate of telomere shortening mark biological aging in replicating somatic cells.[12] Cardiovascular risk factors such as smoking are associated with accelerated leukocyte telomere shortening[13,14] because individuals who smoke have shorter telomeres than those who do not. We extend this concept by showing acute inhibition of telomerase activity by smoking in circulating cells that could be alleviated by red wine consumption. We show that endothelial damage, oxidative stress, vascular inflammation, and cellular aging were completely attenuated when red wine was consumed before smoking. Of note, telomerase activity in the vasculature is upregulated by protective interventions such as statin therapy[15] and physical exercise.[6] Because the interventions elicit anti-inflammatory, antiaging, and endothelial protection effects, these are highly reminiscent to the red wine effects observed in the current study.

Study Limitations

Volunteers were exclusively recruited from medical professions. Furthermore, all participants were healthy, leaving open whether the effect applies to elderly or diseased subjects and chronic smokers. A direct comparison between different alcoholic and nonalcoholic beverages would help to classify molecular mechanisms and molecules to develop therapeutic strategies. Finally, only 1 "dose" of smoking and red wine was compared. Therefore, the results apply only to "occasional lifestyle smokers and drinkers" and cannot be extrapolated to regular users or abusers.

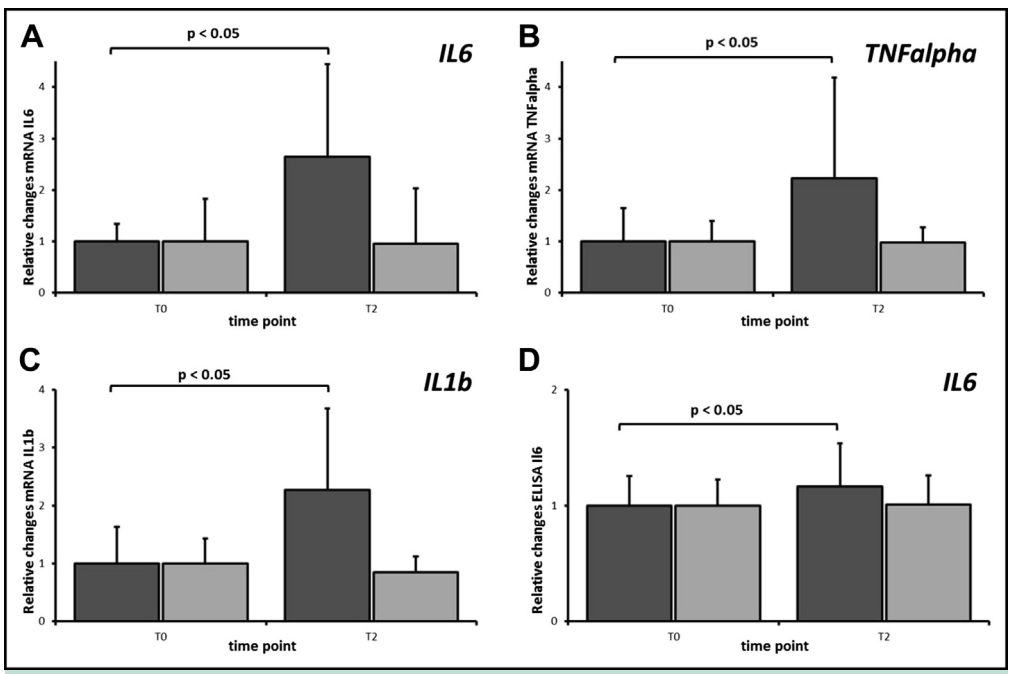

Figure 4 Relative changes of monocyte and serum inflammatory markers in nonsmokers after "smoking only" (*dark grey*) versus "smoking after drinking" (*light grey*) 100 minutes after smoking (T2) compared with baseline (T0). (**A**) Quantitative polymerase chain reaction analysis of monocyte IL-6 (**A**), monocyte tumor necrosis factor-α (**B**), and monocyte IL-1b messenger RNA (**C**) expression. (**D**) Protein quantification of IL-6 in serum (enzyme-linked immunosorbent assay). IL = interleukin; mRNA = messenger RNA; TNF = tumor necrosis factor.

CONCLUSIONS

The presented findings highlight the acute pathophysiologic changes exerted by cigarette smoking in "occasional lifestyle smokers." Evidence for a potential of red wine as a protective strategy to avert some vascular injury was found. The authors would like to point out that they do not intend to motivate occasional smokers to drink or occasional drinkers to smoke. This study identified mechanisms suitable to explore damage and protection on the vasculature in humans, paving the way for future clinical studies.

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SUPPLEMENTARY DATA

Supplementary data accompanying this article can be found in the online version at http://dx.doi.org/10.1016/j.amjmed.2016.08.025.