Available online at www.sciencedirect.com

SCIENCE DIRECT

Metabolism Clinical and Experimental 54 (2005) 306–313

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Red wine consumption improves insulin resistance but not endothelial function in type 2 diabetic patients

Raffaele Napoli[a,*], Domenico Cozzolino[b], Vincenzo Guardasole[a], Valentina Angelini[a], Emanuela Zarra[a], Margherita Matarazzo[a], Antonio Cittadini[a], Luigi Saccà[a], Roberto Torella[b]

[a]*Department of Internal Medicine and Cardiovascular Sciences, University Federico II School of Medicine, 80131 Naples, Italy*
[b]*Department of Gerontology, Geriatrics and Metabolic Diseases, Second University of Napoli, 80131 Naples, Italy*

Received 17 May 2004; accepted 17 September 2004

Abstract

Epidemiological studies have shown that red wine consumption is associated with less cardiovascular mortality in the general population and in the diabetic patients. To determine whether red wine improves insulin resistance in diabetic patients and to explore the relation between insulin sensitivity and endothelial function, we studied vascular reactivity and insulin-mediated glucose uptake in 9 type 2 diabetic patients before and after 2 weeks of red wine consumption (360 mL/d, wine-treated diabetics) and 8 type 2 diabetic patients who did not consume wine (control diabetics). Vascular reactivity was evaluated by plethysmography during intraarterial infusion of acetylcholine (Ach), sodium nitroprusside, and L-*N*-monomethylarginine. Forearm nitrite balance was measured during Ach infusion. Insulin sensitivity was measured by euglycemic hyperinsulinemic clamp at 1 mU/kg per minute. The basal forearm blood flow and the response to Ach, to sodium nitroprusside, and to L-*N*-monomethylarginine were unchanged both in the wine-treated and in the control diabetics. In contrast, insulin-mediated whole body glucose disposal improved by 43% after red wine consumption (from 2.79 ± 0.4 to 4.02 ± 0.5 mg/kg of lean body mass per minute, $P = .02$), but did not change in the control group. In conclusion, red wine consumption for 2 weeks markedly attenuates insulin-resistance in type 2 diabetic patients, without affecting vascular reactivity and nitric oxide production.

1. Introduction

In diabetic patients, endothelial dysfunction is involved in the development of both macro- and microvascular complications [1-7]. The altered vascular function in diabetes is attributed to impaired nitric oxide (NO) availability [8-11]. In addition, some studies [12-14] have suggested that endothelial dysfunction may be also involved in the development of insulin resistance in diabetes. Insulin resistance, in turn, is considered as a risk factor for the increased morbidity and mortality for coronary artery disease (CAD) and stroke [15]. This background explains why type 2 diabetic patients have more than twice the risk for developing CAD or stroke than the general population, and more than 70% of total death in diabetic patients is due to CAD or stroke [15].

It is well known that in countries where there is a high procapita consumption of alcoholic beverages, there is also a reduction in the number of deaths for cardiovascular diseases [16]. Recent data suggest that a light to moderate alcohol consumption [17-20], but not an excess [18,21], exerts a protective effect by reducing the number of cardiovascular events. Beneficial effects have also been reported with regard to red wine consumption and explained for improvement of endothelial dysfunction, an effect that does not seem to be entirely dependent on the alcohol content [22-27]. Interestingly, drinking purple grape juice for 2 weeks improves endothelial function in patients with CAD [28], suggesting that the effect of red wine is mediated by its polyphenol content. Epidemiological data have also suggested that alcohol consumption may be associated with improved insulin sensitivity, providing another potential explanation for the reduction in cardiovascular mortality associated with alcohol intake [19].

The issue whether drinking red wine should be the object of open recommendation, particularly for populations at

* Corresponding author. Tel.: +39 081 746 3736x3519; fax: +39 081 746 3199.
E-mail address: raffaele.napoli@unina.it (R. Napoli).

0026-0495/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2004.09.010

high risk of CAD or stroke, has been a matter of debate, essentially because the beneficial effects of wine consumption are claimed mostly on the basis of correlative evidence. Indeed, no data are available regarding the effect of red wine drinking on endothelial-mediated vascular reactivity in diabetic patients. Furthermore, there are no clinical studies on the effect of alcohol intake on insulin resistance associated with diabetes. Therefore, the present study was designed to address simultaneously in the same group of diabetic patients the questions whether red wine drinking affects (1) insulin resistance and (2) endothelium-mediated and non–endothelium-mediated vascular reactivity and NO production.

2. Research design and methods

2.1. Subjects

We studied a group of 17 type 2 diabetic patients (15 men and 2 women, 48-72 years of age) treated with diet alone or with low doses of oral hypoglycemic agents (sulphanylureas and/or metformin). All patients were in good glycemic control before the start of the study. Six of the patients were not used to drink alcoholic beverages before being selected for the study. However, all patients were asked not to drink any wine or alcoholic beverage in the 4 weeks preceding the basal evaluation of vascular reactivity and insulin resistance. In addition, all patients were asked to maintain their life style (physical activity, dietary, and smoking habits) as much as possible unmodified for the 4 weeks preceding the study and during the 2-week experimental period. The diabetic patients were studied according to 2 different protocols: one group of 9 diabetic patients, the day after the baseline study, started the daily ingestion of 180 mL of red wine (Chianti Riserva, Marchese Antinori, Italy) at lunch and dinner (wine-treated diabetics); a second group of 8 diabetic patients after the baseline study continued their usual diet without any alcoholic beverage (control diabetics). The 2 groups of patients were matched for the use of oral hypoglycemic agents. The clinical and metabolic characteristics of the patients studied are shown in Table 1. After 2 weeks of treatment, the experiment to measure vascular reactivity and insulin sensitivity was repeated. Informed consent to participate in the study was obtained by all participants and the study protocol was approved by the Ethics Committee of the University Federico II School of Medicine, Naple, Italy.

2.2. Experimental procedure

The studies were performed, as previously described [29,30], in the morning in a quiet room kept at 22°C to 24°C. The subjects were studied in supine position after 12 to 15 hours overnight fast. A plastic cannula (20 G) was inserted into the brachial artery of the nondominant arm under local anesthesia (Xylocaine, 2%) and used for the infusion of the test substances, the monitoring of arterial blood pressure and heart rate, and the arterial blood sampling. In the same arm, a second plastic cannula was introduced into a large antecubital vein to obtain venous blood samples. In the contralateral arm, a third catheter was inserted into an antecubital vein and used for the infusion of insulin and glucose during the euglycemic hyperinsulinemic clamp study. Systolic and diastolic blood pressure and heart rate were recorded by a transducer. Forearm blood flow (FBF) was measured in both the forearms by strain gauge plethysmography with a calibrated mercury-in-silastic strain gauge applied around the forearm connected to a Hokanson plethysmography (Hokanson 045 EC4; PMS Instruments, Berks, UK) associated with a McLab computer. Both arms were supported slightly above the heart level. During the measurement of FBF and blood sampling, a pediatric cuff was inflated around the wrist 100 mm Hg above systolic blood pressure to exclude hand circulation from the measurements. Each subject underwent the following stepwise infusions into the brachial artery: (1) Ach, infused at the rate of 15, 30, 45, and 60 μg/L of forearm per minute, to assess endothelial-mediated vasodilation; (2) sodium nitroprusside (NP), infused at the rate of 1, 3, and 9 μg/L per minute, to get information on the non–endothelial-mediated vasodilation; and (3) to assess the role of endothelial NO release in the maintenance of the basal vascular tone, L-*N*-monomethylarginine (L-NMMA), a competitive, inactive analog of L-arginine, was infused at the rate of 1 mg/L per minute. To determine the peak flow, FBF was measured after 5 minutes of ischemia induced by inflating a sphygmomanometer cuff around the upper arm. Each dose of the test substances was infused for 5.5 minutes and FBF was measured during the last 1.5 minutes of infusion. At least 30 minutes washout was allowed between each substance. The infusion rates were adjusted according to the forearm volume of each subject. Forearm blood flow was measured simultaneously in both arms to ensure that no systemic effects occurred during the experiment. Each FBF value represents the mean of 6 consecutive measurements performed at 10-second intervals. At the end of the evaluation of vascular reactivity, a euglycemic hyperinsulinemic clamp study was performed as previously described [31]. An infusion of human regular insulin (Humulin R; Eli Lilly, Indianapolis, Ind) started intravenously at the rate of 1 mU/kg of body weight to achieve a constant hyperinsulinemia in the high physiological range. The glucose infusion was started when plasma glucose concentration reached euglycemic values and then kept constant all throughout the study. The glucose infusion rate was adjusted on the basis of frequent control of blood glucose concentration measured on samples taken from the brachial artery by a glucose analyzer (Yellow Spring, Palo Alto, Calif). Because hepatic glucose production can be considered suppressed at insulin plasma concentration achieved [32], the glucose infusion rate, adjusted by the lean body mass, necessary to maintain a constant normo-

Table 1
Clinical and metabolic characteristics of 17 patients studied

		Sex (M/F)	Age (y)	Duration of diabetes (y)	BMI (kg/m^2)	Fructosamine (mg/dL)
Red wine-treated	Baseline	8/1	58 ± 2.0[a]	8 ± 3	28.2 ± 1.7	233 ± 12
diabetic patients	After 2 weeks	–	–	–	28.3 ± 1.6	234 ± 16
Control	Baseline	7/1	53 ± 3.7	7 ± 3	26.0 ± 1.3	279 ± 35
diabetic patients	After 2 weeks	–	–	–	26.0 ± 1.0	247 ± 32

BMI indicates body mass index; FPG, fasting plasma glucose. HbA1c, glycated hemoglobin.
[a] Mean ± SE.
* $P = .02$ vs baseline.

glycemia in the last 40 minutes of the 120 minutes glucose clamp study was considered the insulin-mediated whole body glucose uptake (*M* value).

2.3. Analytical methods

Body composition was obtained by measuring the impedance of the body (Bodyfat Analyzer TBF-105; Tanita Corporation, Tokyo, Japan). Serum insulin concentration was measured by radioimmunoassay. Nitrite concentration was measured in plasma samples using EDTA as an anticoagulant. After collection, blood samples were immediately centrifuged at 2000 rpm at 4°C and plasma stored at −20°C. Before assay, plasma was ultrafiltered through a 10-kDa molecular weight cutoff filter (Centricon 10; Millipore, Bedford, Mass). Total plasma nitrite and nitrate were measured using a colorimetric kit (Cayman Chemical Co, Ann Arbor, Mich). Nitrate was converted to nitrite by nitrate reductase and then nitrite was assayed by the standard Griess diazo reaction. All determinations were done in triplicate. The data are referred to as nitrite concentration but they reflect the sum of nitrate and nitrite. Plasma free fatty acid (FFA) was determined by an enzymatic method [33].

2.4. Calculations

The net forearm balance of nitrite was calculated by multiplying the plasma arterial-venous concentration difference of each substrate by the plasma flow. Therefore, a negative balance indicates substrate release, whereas a positive balance indicates uptake. The differences in clinical and metabolic characteristics before and after the 2 weeks of treatment were analyzed using the paired Student *t* test. The data of vascular reactivity were analyzed by a 2-way repeated-measure analysis of variance (SPSS, version 11.0, Chicago, Ill). Vascular reactivity data are expressed as absolute values of FBF. Results are expressed as mean ± SEM.

3. Results

The clinical and metabolic characteristics of the diabetic patients, at baseline and after 2 weeks of observation, are

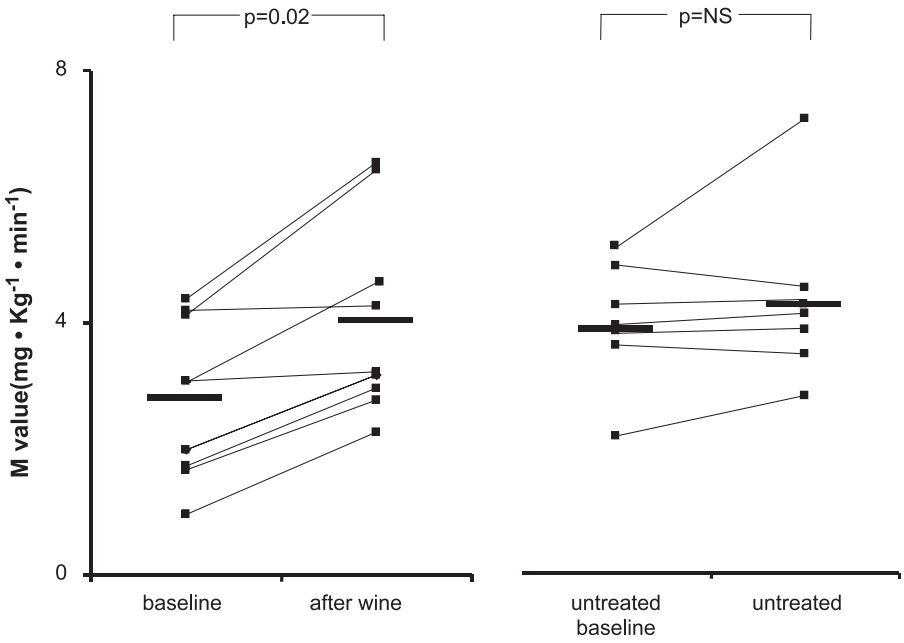

Fig. 1. Insulin-mediated whole body glucose disposal in the 9 wine-treated diabetic patients (left panel) and in the 8 control diabetic patients (right panel). Insulin was infused at the rate of 1 mU/kg of lean body mass per minute. The horizontal bars indicate the mean values.

HbA1c (%)	Plasma insulin (μU/mL)	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	FPG (mg/dL)	Glucose infusion rate (mg/kg per minute)
7.0 ± 0.27	5.2 ± 1.0	211 ± 15	163 ± 31	124 ± 10	2.79 ± 0.4
6.6 ± 0.37	5.4 ± 0.9	205 ± 17	128 ± 17	135 ± 13	4.02 ± 0.5*
7.5 ± 0.5	5.0 ± 0.7	211 ± 8	162 ± 35	135 ± 12	3.97 ± 0.4
7.6 ± 0.5	4.7 ± 0.7	200 ± 15	158 ± 49	117 ± 8	4.31 ± 0.5

reported in Table 1. Body weight remained unchanged. Similarly, fasting blood glucose, fructosamine, and plasma insulin concentration did not change from their basal values.

3.1. Insulin clamp

The mean plasma insulin concentration during insulin infusion increased to steady state levels of 112 ± 11 and 122 ± 16 in the experiments performed at baseline and to 113 ± 10 and 113 ± 10 μU/mL in the wine-treated and control diabetics, respectively. Blood glucose concentration was kept constant in both the experimental settings (97 ± 3 and 98 ± 2 mg/dL, before and after wine, and 97 ± 3 and 98 ± 2 mg/dL in the control diabetics), by virtue of a variable exogenous glucose infusion. In the basal state, FFA concentration was 0.76 ± 0.06 and 0.68 ± 0.07 mmol/L before and after wine in the wine-treated group, respectively, and fell to similar levels during insulin infusion (0.11 ± 0.01 and 0.12 ± 0.02 mmol/L). In the control group, FFA concentration was 0.77 ± 0.1 and 0.75 ± 0.12 mmol/L in the basal state of the baseline and 2-week study, respectively, and fell to similar levels during insulin infusion (0.13 ± 0.01 and 0.10 ± 0.02 mmol/L, respectively).

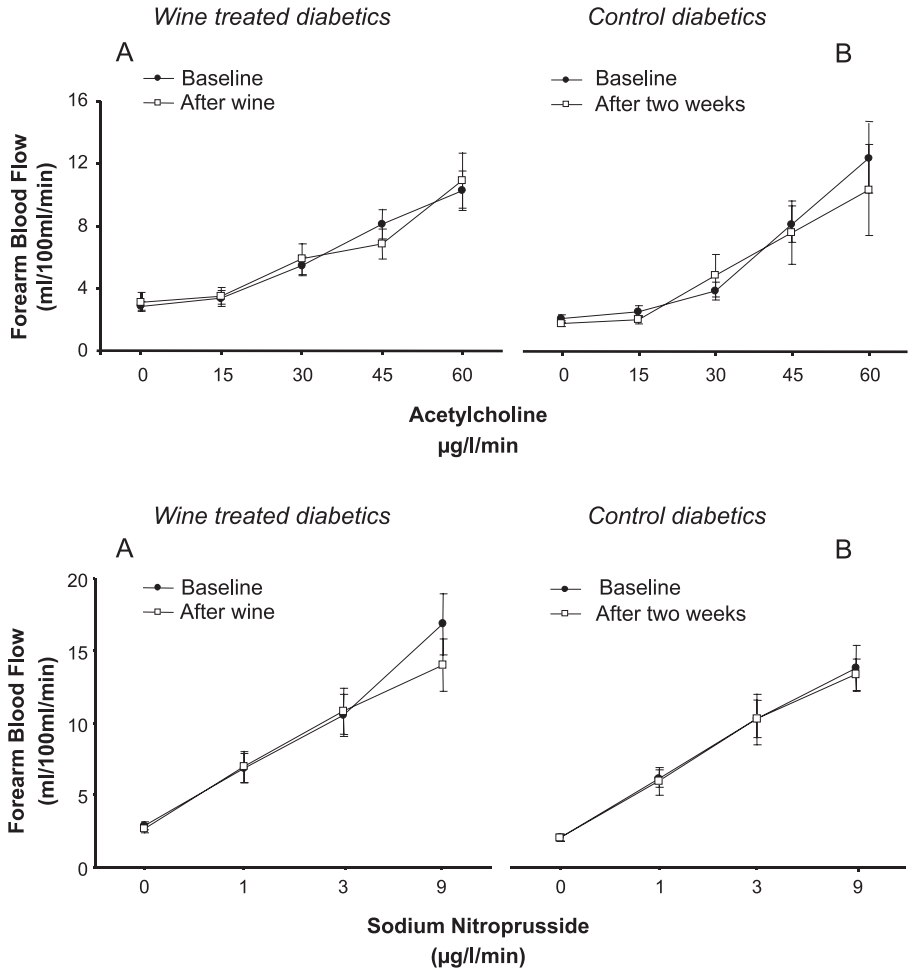

Fig. 2. Upper panel, Forearm blood flow response to Ach infusion in the wine-treated diabetic patients (A panel) and in the control diabetic patients (B panel) at baseline and after 2 weeks. Lower panel, Forearm blood flow response to NP infusion in the wine-treated diabetic patients (A panel) and in the control diabetic patients (B panel) at baseline and after 2 weeks.

The amount of glucose metabolized by the whole body during the euglycemic hyperinsulinemic clamp study in the wine-treated diabetic and in the control diabetic patients is shown in Fig. 1. Two weeks of wine consumption increased by 43% insulin-mediated whole body glucose disposal (from 2.79 ± 0.4 to 4.02 ± 0.5 mg/kg of lean body mass per minute, P = .02). The improvement in insulin sensitivity was remarkable in 7 out of the 9 patients studied, whereas it was minimal in the remaining 2. In contrast, the whole body glucose uptake did not change significantly in the 8 diabetic patients who did not consume wine (from 3.97 ± 0.4 to 4.31 ± 0.5 mg/kg of lean body mass per minute, baseline and 2-week study, respectively, P = not significant [NS]).

3.2. Vascular reactivity

The response to the endothelium-dependent vasodilator Ach is shown in Fig. 2. A dose-dependent increase in FBF was observed in all patients. The values reached with the highest Ach dose were 10.2 ± 1.2 and 10.9 ± 1.8 mL/dL per minute before and after the 2 weeks of wine ingestion, respectively (P = NS). Similarly, the Ach infusion induced a comparable increase in FBF in the baseline and in the 2-week study in the control diabetics (12.6 ± 2.3 and 10.5 ± 2.9 mL/dL per minute, respectively). There were no significant differences in the FBF response to Ach between the 2 groups of diabetics.

In the group of diabetic patients consuming the red wine, we measured the forearm nitrite balance to confirm the lack of effect of chronic alcohol administration on the NO release. In agreement with the FBF data in response to Ach, forearm NO production after Ach infusion remained unchanged in the diabetic patients (0.76 ± 2.2 and 1.39 ± 5.6 nmol/dL per minute, before and after wine treatment, respectively, P = NS) (Table 2).

The response of FBF to NP is depicted in Fig 2. Similar to endothelium-dependent vasodilation, the response of FBF to NP, an endothelium-independent vasodilator, did not differ before and after wine consumption (16.8 ± 2.1 and 14.0 ± 1.8 mL/dL per minute, at the highest NP infusion rate, respectively, P = NS) and in the baseline and 2-week study in the control diabetic patients (14.1 ± 1.6 and 13.6 ± 1.1 mL/dL per minute, at the highest NP infusion rate, respectively, P = NS). The response to NP infusion was similar in the 2 groups of diabetic patients.

Table 2
Forearm nitrite balance before and during intrabrachial Ach infusion at baseline and after 2 weeks of wine consumption in 9 type 2 diabetic patients

		Forearm nitrite balance (nmol/dL per minute)
Baseline	Basal	−0.59 ± 1.2[a]
	After Ach	0.76 ± 2.2
After wine	Basal	0.38 ± 1.3
	After Ach	1.39 ± 5.6

[a] Mean ± SE.

Inhibition of basal NO bioactivity by intraarterial infusion of L-NMMA reduced FBF to similar levels in both studies (2.55 ± 0.4 and 2.18 ± 0.3 mL/dL per minute before and after wine, and 1.62 ± 0.1 and 1.41 ± 0.2 mL/dL per minute in the baseline and 2-week study in the control diabetic patients, respectively, P = NS). No significant differences were present in the response to L-NMMA between the 2 groups of diabetic patients.

4. Discussion

This study demonstrates that red wine consumption for 2 weeks improves insulin-resistance in type 2 diabetic patients without affecting vascular reactivity. In particular, we show that in a population at high risk for cardiovascular diseases, such as the diabetic patients, red wine consumption does not affect either the endothelium-dependent or the non–endothelium-dependent components of vascular reactivity or NO release from the forearm vessels. In contrast, insulin-mediated whole body glucose metabolism improved after 2 weeks of red wine consumption.

Because diabetic patients are at very high risk for CAD or stroke [15], identifying and correcting risk factors for cardiovascular diseases in this population represent an important task of the scientific community. Type 2 diabetic patients are characterized by the coexistence of both endothelial dysfunction and insulin resistance, and both abnormalities are considered as key factors in the development of cardiovascular complications [1-7,15]. It is also relevant that endothelial dysfunction and insulin-resistance are more than simply associated defects in the diabetic patients. There is reason to believe that these defects may interact with each other, thus generating a deleterious vicious cycle [12,13]. Therefore, endothelial dysfunction and insulin-resistance must be considered major targets of any intervention aimed at reducing cardiovascular mortality in diabetic patients.

Alcohol consumption is associated with reduction in cardiovascular mortality both in the general population and in type 2 diabetic patients as well [17-28]. This observation is so consistent that the American Diabetes Association has included the suggestion of allowing moderate alcohol drinking into its recommendations for the treatment and prevention of diabetes [34]. However, the exact mechanisms behind the reduction in cardiovascular complications due to the use of alcoholic beverages are still a matter of speculation. First, alcohol consumption may be associated with reduction of serum concentration of total cholesterol and low-density lipoprotein cholesterol, and increase in high-density lipoprotein cholesterol [35-37]. However, several epidemiological data have failed to demonstrate that alcohol consumption is consistently effective in triggering such mechanism both in the general population and in type 2 diabetic patients, even in the presence of positive effects on cardiovascular complications [38-43]. In our study, we did not observe any change in total cholesterol concentration, suggesting that 2

weeks of red wine consumption in type 2 diabetic patients might be too short a treatment to modify this parameter.

Some studies [27,38] have shown that red wine consumption might have more beneficial effects than other alcoholic beverages. Substances with antioxidant properties, such as flavonoids, present in red wine, fruits, vegetables, and nuts improve endothelial dysfunction in patients with CAD [28]. In the present study, we decided to use red wine to combine the potential properties of ethanol and other substances contained in red wine, supposed to improve endothelial dysfunction. Compared with a group of 11 matched healthy subjects studied in our laboratory (data unpublished), the diabetic patients show severe endothelial dysfunction (at the highest Ach dose, FBFs were 23.8 ± 3.3 and 10.2 ± 1.2 mL/dL per minute in healthy subjects and diabetic patients, respectively, $P = .001$). However, we demonstrate that 2 weeks consumption of red wine was ineffective in improving endothelial dysfunction in our diabetic patients. On the other hand, the fact that 2 weeks of purple grape juice drinking has been able to improve endothelial dysfunction in patients with CAD [28] suggests either that endothelial dysfunction in diabetes is linked to different mechanisms or that the correction of this defect requires a longer period of wine use.

A consistent observation in studies on the effect of alcoholic beverages in diabetic patients is the reduction of circulating insulin concentration [20,36,44-46]. Both circulating insulin concentration and insulin-resistance have been implicated in the development of cardiovascular complications [46-48]. Some epidemiological data suggest that alcoholic beverages might regulate insulin sensitivity [19]. However, these data have been obtained with a very indirect approach, that is, the assessment of insulin sensitivity by HOMA index. In addition, there are no data on the basis of intervention studies [49]. The present study, on the basis of an interventional approach, provide the first evidence for a direct effect of red wine on insulin resistance in type 2 diabetic patients. Indeed, the hyperinsulinemic euglycemic clamp is considered the gold standard for the measurement of insulin sensitivity, and the observed 43% improvement in insulin resistance is hard to achieve by lifestyle measures or any pharmacological intervention.

An interesting implication of our study is that the mechanism by which red wine consumption improves insulin resistance is not mediated by or linked to endothelial dysfunction. Previous studies have suggested that endothelial dysfunction and insulin resistance may be linked by a cause-and-effect relationship [12-14,48]. We show that alcohol ingestion acts on the insulin resistance of diabetic patients by a mechanism that does not involve amelioration of endothelial dysfunction or changes in NO production.

The mechanisms and the signals involved in the effect of red wine on insulin sensitivity remain to be determined. The inhibitory effect of alcohol on gluconeogenesis is unlikely to have played a role, because hepatic glucose output is completely suppressed by the insulin concentration achieved during the clamp studies [32]. Moreover, the plasma concentration of FFAs, which are well recognized competitors of glucose for muscle and adipose tissue uptake, was equally reduced in the studies performed before and after the 2 weeks of wine consumption. Interestingly, moderate alcohol consumption activates Akt, an intermediate of intracellular insulin signaling, as demonstrated in mice myocardial cells [50]. One is tempted to speculate that chronic red wine consumption might have affected insulin resistance in the present study by a direct action on intracellular insulin signaling.

In conclusion, the present study demonstrates that a 2-week consumption of red wine attenuates substantially insulin resistance in type 2 diabetic patients, independent of changes in endothelial function and NO availability. The data are relevant in view of the pivotal role played by insulin resistance in the pathogenesis of diabetes and metabolic syndrome, and the associated cardiovascular complications. They also provide further support to the recommendation to adopt this simple, effective, and, not least, enjoyable lifestyle measure.

4.1. Study limitations

This study provided a negative finding with regard to one of its end points, that is, endothelial dysfunction. It might be argued that the current approach did not have sufficient sensitivity to reveal an effect of red wine on vascular reactivity. We believe this was not the case. Using exactly the same experimental setting, we have been previously able to show relevant differences in vascular reactivity in various clinical conditions, including chronic heart failure and endocrine diseases [29,30]. For obvious reasons, this study could not be placebo-controlled. Therefore, the meaning of including a control group is restricted to the need to provide some reassurance that the main parameters, measured at 2-week interval, were not affected by either biological or technical variability. Finally, the treatment with red wine lasted 2 weeks, which leaves open the possibility that a much longer treatment may also affect vascular dysfunction. However, the novel aspect of the study is that an improvement in insulin sensitivity may be induced by a short-term wine consumption through mechanism(s) independent of endothelial function.

References

[1] Stehouwer CDA, Nauta JJP, Zeldenrust GC, Hackeng WHL, Donker AJM, Den Ottolander GJH. Urinary albumin excretion, cardiovascular diseases, and endothelial dysfunction in non–insulin-dependent diabetes mellitus. Lancet 1992;340:319-23.

[2] Williamson JR, Chang K, Frangos M, Hasan KS, Ido Y, Kawamura T, et al. Hyperglycemic pseudohypoxia and diabetic complications. Diabetes 1993;42:801-13.

[3] Harrison DG. Cellular and molecular mechanisms of endothelial cell dysfunction. J Clin Invest 1997;100:2153-7.
[4] Michel T, Feron O. Nitric oxide synthases: which, where, how and why? J Clin Invest 1997;100:2146-52.
[5] Bucala R, Tracey KJ, Cerami A. Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. J Clin Invest 1991;87:432-8.
[6] Feener EP, King GL. Vascular dysfunction in diabetes mellitus. Lancet 1997;350(Suppl I):SI9-SI13.
[7] Pieper GM, Jordan M, Adams MB, Roza AM. Syngeneic pancreatic islet transplantation reverses endothelial dysfunction in experimental diabetes. Diabetes 1995;44:1106-13.
[8] Williams SB, Cusco JA, Roddy MA, Johnstone MT, Creager MA. Impaired nitric oxide-mediated vasodilation in patients with non–insulin-dependent diabetes mellitus. J Am Coll Cardiol 1996;27:567-74.
[9] Johnston GD, Nugent AM, Hayes RJ, McVeigh GE. Endothelium dependent arterial dilatation in non–insulin-dependent diabetes. BMJ 1996;312(7033):744-5.
[10] McVeigh GE, Brennan GM, Jonhston GD, McDermotton BJ, McGrath LT, Henry WR, et al. Impaired endothelium dependent and independent vasodilation in patients with type 2 (non–insulin-dependent) diabetes mellitus. Diabetologia 1992;35:771-6.
[11] Giugliano D, Marfella R, Coppola L, Verrazzo G, Acampora R, Giunta R, et al. Vascular effects of acute hyperglycemia in human are reversed by L-arginine. Evidence for reduced availability of nitric oxide during hyperglycemia. Circulation 1997;95:1783-90.
[12] Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD. Insulin mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. J Clin Invest 1994;94:1172-9.
[13] Scherrer U, Randin D, Vollenweider P, Vollenweider L, Nicod P. Nitric oxide release accounts for insulin's vascular effects in human. J Clin Invest 1994;94:2511-5.
[14] Petrie JR, Ueda S, Webb DJ, Elliott HL, Connell JM. Endothelial nitric oxide production and insulin sensitivity. A physiological link with implications for pathogenesis of cardiovascular disease. Circulation 1996;93:1331-3.
[15] Nathan DM, Meigs J, Singer DE. The epidemiology of cardiovascular disease in type 2 diabetes mellitus: how sweet it is . . . or is it? Lancet 1997;350:SI4-SI9.
[16] Rimm EB, Klatsky A, Grobbee D, Stampfer MJ. Review of moderate alcohol consumption and reduced risk of coronary heart disease: is the effect due to beer, wine or spirits. BMJ 1996;312:731-6.
[17] Camargo CA, Hennekens CH, Gaziano M, Glynn R, Manson JA, Stampfer MJ. Prospective study of moderate alcohol consumption and mortality in US male physicians. Arch Intern Med 1997;157:79-85.
[18] Fuchs CS, Stampfer MJ, Colditz GA, Giovannucci EL, Manson JE, Kawachi I, et al. Alcohol consumption and mortality among women. N Engl J Med 1995;332:1245-50.
[19] Kiechl S, Willeit J, Poewe W, Egger G, Oberhollenzer F, Muggeo M, et al. Insulin sensitivity and regular alcohol consumption: large, prospective, cross sectional population study (Bruneck Study). BMJ 1996;313:1040-4.
[20] Kiechl S, Willeit J, Rungger G, Egger G, Obehollenzer F, Bonora E. Alcohol consumption and atherosclerosis: what is the relation? Prospective results from The Bruneck Study. Stroke 1998;29:900-7.
[21] Boffetta P, Garfinkel L. Alcohol drinking and mortality among men enrolled in an American cancer society prospective study. Epidemiology 1990;1:342-8.
[22] Klatsky AL, Armstrong MA. Alcoholic beverage choice and risk of coronary artery disease mortality: do red wine drinkers fare best? Am J Cardiol 1993;71:467-9.
[23] Gronbaeck M, Deis A, Soresen TIA, Becker U, Schnohr P, Jensen G. Mortality associated with moderate intake of wine, beer or spirits. BMJ 1995;310:1165-9.
[24] Maxwell S, Cruickshank A, Thorpe G. Red wine and antioxidant activity in serum. Lancet 1994;344:193-4.
[25] Whitehead TP, Robinson D, Allaway S, Syms J, Hale A. Effect of red wine ingestion on the antioxidant capacity of serum. Clin Chem 1995;41:32-5.
[26] Agewall S, Wright S, Doughty RN, Whalley GA, Duxbury M, Sharpe N. Does a glass of red wine improve endothelial function? Eur Heart J 2000;21:74-8.
[27] Flesch M, Schwarz A, Bohm M. Effects of red and white wine on endothelium-dependent vasorelaxation of rat aorta and human coronary arteries. Am J Physiol 1998;275:H1183-90.
[28] Stein JH, Keevil JG, Wiebe DA, Aeschlimann S, Folts JD. Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. Circulation 1999;100:1050-5.
[29] Napoli R, Biondi B, Guardasole V, Matarazzo M, Pardo F, Angelini V, et al. Impact of hyperthyroidism and its correction on vascular reactivity in humans. Circulation 2001;104:3076-80.
[30] Napoli R, Guardasole V, Matarazzo M, Palmieri EA, Oliviero U, Fazio S, et al. Growth hormone corrects vascular dysfunction in patients with chronic heart failure. J Am Coll Cardiol 2002;39:90-5.
[31] Lembo G, Napoli R, Capaldo B, Rendina V, Iaccarino G, Volpe M, et al. Abnormal sympathetic overactivity evoked by insulin in the skeletal muscle of patients with essential hypertension. J Clin Invest 1992;90:24-9.
[32] DeFronzo RA, Gunnarson R, Bjorkman O, Olsson M, Wahren J. Effects of insulin on peripheral and splanchnic glucose metabolism in non–insulin-dependent (type II) diabetes mellitus. J Clin Invest 1985;76:149-55.
[33] Noma A, Okabe H, Kita M. A new colorimetric microdetermination of the free fatty acids in serum. Clin Chim Acta 1973;43:317-20.
[34] American Diabetes Association. Evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications. Diabetes Care 2003;26:S51-S61.
[35] Suh I, Shaten B, Cutler J, Kuller LH. Alcohol use and mortality from coronary heart disease: the role of high-density lipoprotein cholesterol. The multiple risk factor intervention trial research group. Ann Intern Med 1992;116:881-7.
[36] Razay G, Heaton K, Bolton C, Hughes AO. Alcohol consumption and its relation to cardiovascular risk factors in British women. BMJ 1992;304:80-3.
[37] Gaziano JM, Buring JE, Breslow JL, Goldhaber SZ, Rosner B, VanDenburgh M, et al. Moderate alcohol intake, increased levels of high-density lipoprotein and its subfractions, and decreased risk of myocardial infarction. N Engl J Med 1993;329:1829-34.
[38] Truelsen T, Gronbaek M, Schnohr P, Boysen G. Intake of beer, wine, and spirits and risk of stroke: the Copenhagen city heart study. Stroke 1998;29:2467-72.
[39] Sacco RL, Elkind M, Boden-Albala B, Lin IF, Kargman DE, Hauser WA, et al. The protective effect of moderate alcohol consumption on ischemic stroke. JAMA 1999;281:53-60.
[40] Criqui MH, Cowan LD, Tyroler HA, Bangdiwala S, Heiss G, Wallace RB, et al. Lipoproteins as mediators for the effects of alcohol consumption and cigarette smoking on cardiovascular mortality: results form the Lipid Research Clinics Follow-up Study. Am J Epidemiol 1987;126:629-37.
[41] Valmadrid CT, Klein R, Moss SE, Klein BE, Cruickshanks KJ. Alcohol intake and the risk of coronary heart disease mortality in persons with older-onset diabetes mellitus. JAMA 1999;282:239-46.
[42] Djousse L, Levy D, Murabito JM, Cupples LA, Ellison RC. Alcohol consumption and risk of intermittent claudication in the Framingham Heart Study. Circulation 2000;102:3092-7.
[43] Ajani UA, Gaziano JM, Lotufo PA, Liu S, Hennekens CH, Buring JE, et al. Alcohol consumption and risk of coronary heart disease by diabetes status. Circulation 2000;102:500-5.

[44] Facchini F, Chen YD, Reaven GM. Light-to-moderate alcohol intake is associated with enhanced insulin sensitivity. Diabetes Care 1994;17:115-9.

[45] Mayer EJ, Newman B, Quesenberry Jr CP, Friedman GD, Selby JV. Alcohol consumption and insulin concentrations. Role of insulin in associations of alcohol intake with high-density lipoprotein cholesterol and triglycerides. Circulation 1993;88:2190-7.

[46] Lazarus R, Sparrow D, Weiss ST. Alcohol intake and insulin levels. The Normative Aging Study. Am J Epidemiol 1997;145:909-16.

[47] Pyorala M, Miettinen H, Laakso M, Pyorala K. Hyperinsulinemia predicts coronary heart disease risk in healthy middle-aged men: the 22-year follow-up results of the Helsinki Policemen Study. Circulation 1998;98:398-404.

[48] Arcaro G, Cretti A, Balzano S, Lechi A, Muggeo M, Bonora E, et al. Insulin causes endothelial dysfunction in humans: sites and mechanisms. Circulation 2002;105:576-82.

[49] Howard AA, Arnsten JH, Gourevitch MN. Effect of alcohol consumption on diabetes mellitus. A systematic review. Ann Intern Med 2004;140:211-9.

[50] Zhou HZ, Karliner JS, Gray MO. Moderate alcohol consumption induces sustained cardiac protection by activating PKC-epsilon and Akt. Am J Physiol Heart Circ Physiol 2002;283:H165-74.