Food and Red Wine Do Not Exert Acute Effects on Vascular Reactivity

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Experimental hyperglycemia and hyperinsulinemia have been shown to affect vascular reactivity. Chronic red wine consumption is associated with less cardiovascular mortality. Whether ingestion of a natural meal and red wine causes acute changes in vascular homeostasis is poorly understood. The aim of the current study was to clarify whether meal ingestion, with and without red wine, exert acute effects on vascular reactivity in healthy humans. We studied vascular reactivity and forearm nitrite balance in 10 healthy subjects under 3 different circumstances: (1) fasting; (2) after ingestion of a standard natural meal (1,050 kcal); and (3) after the same meal enriched with a glass of red wine. We measured forearm blood flow (FBF) by strain-gauge plethismography during intrabrachial, graded infusion of acetylcholine (ACh), sodium nitroprusside (NP), and norepinephrine (NE). We also measured the forearm balance of nitrite before and during ACh infusion. Despite significant increases in plasma glucose and insulin concentrations, the vasodilatory response to Ach and NP after meal ingestion was not different from the fasting response. Similarly, the vasoconstrictory response to NE was similar postprandially and during fasting. Addition of red wine did not modify the response to any of the vasoactive agents. Finally, the forearm nitrite production during Ach infusion was not different in the 3 experimental settings. Food intake, whether associated or not with red wine, does not affect vascular reactivity in normal human subjects.

ENDOTHELIAL DYSFUNCTION has been implicated in the development, progression, and worsening of cardiovascular complications.[1,2] For this reason, any action capable of affecting endothelial nitric oxide (NO) production or to prevent endothelial dysfunction is regarded as potentially relevant to cardiovascular morbility and mortality. Of particular importance are those factors that affect endothelial-mediated vasodilation in a prolonged or repeated fashion. In this regard, hyperglycemia has a strong negative impact on NO production and endothelial function.[3-9] In particular, postprandial hyperglycemia has been implicated in the development of endothelial activation and endothelial dysfunction in diabetic patients.[8,9]

The effects of insulin on vascular physiology have been less straightforward, mostly dependent on the hormonal level and time of exposure.[10-13] Noteworthy, recent evidence suggests that prolonged, moderate hyperinsulinemia may deteriorate endothelial-mediated vascular relaxation.[14] Since an increase in plasma glucose and insulin concentration occurs at each meal, a disconcerting corollary to these findings would be that during each postprandial period the vascular bed is exposed to a major insult by the combined glucose and insulin action. It should be stressed, however, that previous studies are based on experimental hyperglycemia or hyperinsulinemia. It is possible that in a natural setting of food intake a variety of factors are called into play and that their net effect on vascular homeostasis is not necessarily deleterious.

Unfortunately, there are few and contradictory studies looking at the effects of a regular, standard meal on endothelial-mediated and non-endothelial-mediated vascular reactivity.[15-23] Furthermore, no studies have investigated the effects of a regular meal on endothelial- and non-endothelial-mediated vascular reactivity and NO production. Therefore, the first aim of the present study was to clarify whether vascular reactivity and forearm NO balance are altered by the ingestion of a natural meal.

Recent data have suggested that light to moderate alcohol consumption, but not an excess, is associated with reduced number of cardiovascular events.[24-29] Based on these findings, moderate alcohol consumption has been recommended for patients affected by diabetes and cardiovascular diseases.[30-34] However, it is still unknown whether there is a cause-and-effect relation between alcohol use and vascular risk. A preliminary step to get more insight into this issue would be to clarify whether alcohol intake exerts any acute effect on vascular reactivity. We decided to explore this question by adding a glass of red wine to the standard meal. This design was based on the consideration that wine consumption occurs mostly during meals and this habit is becoming increasingly more common in western countries. In addition, some studies suggested that red wine might have beneficial vascular effects, not entirely dependent on the alcohol content per se.[35-41]

MATERIALS AND METHODS

Subjects

We studied 10 healthy, nonsmoking, volunteers (6 men and 4 women, 23 ± 1 years of age, body mass index 23.6 ± 0.6 kg/m^2). Vascular reactivity was measured in each subject under 3 different circumstances: (1) ingestion of a standard meal of 1,050 kcal (meal study); (2) ingestion of the same meal enriched with 200 mL of red wine (meal and wine study); and (3) fasting condition (control study). The experiments were performed on different days and in a random order and, in each subject, they were separated by at least a 3-week interval. Informed written consent was obtained by all participants and the study protocol was approved by the Ethics Committee of the University Federico II School of Medicine.

Experimental Procedure

All the studies were performed, as previously described,[42,43] in the morning in a quiet room kept at 22 to 24°C. The subjects were studied in the supine position after a 12- to 15-hour overnight fast. The day of

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Table 1. Clinical Characteristics of the 10 Subjects Studied

Sex (M/F)	Age (yr)	BMI (kg/m^2)	Body Weight (kg)	SBP (mm Hg)	DBP (mm Hg)	HR (bpm)
6/4	23 ± 1	23.6 ± 0.6	70 ± 3	124 ± 2	60 ± 1	62 ± 3

NOTE. Data are mean ± SEM.
Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, hearts rate.

the study, a plastic cannula (20G) was inserted into the brachial artery of the nondominant arm under local anesthesia (xilocaine 2%) and used for the infusion of the test substances, the monitoring of arterial blood pressure and heart rate, and arterial blood sampling. Systolic and diastolic blood pressure and heart rate were recorded by a transducer. In the same arm, a second plastic cannula (20G) was inserted into an antecubital vein. 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 plethysmograph associated with a McLab computer (Hokanson 045 EC4, P.M.S. Instruments, Berks, UK). 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 to 100 mm Hg above systolic blood pressure in order to exclude hand circulation from the measurements. After the procedures of cannulating the vessels and connecting the forearm to the plethysmograph were completed, a 30-minute interval was allowed before starting the experiment. The subjects were then invited to seat in the bed for the following 30 minutes. During this period of time, either the standard meal (meal study), or the standard meal with 200 mL of red wine (meal and wine study), or nothing (control study) was served. At the end of the 30 minutes, the subjects were invited to lay again in the supine position. Fifteen and 30 minutes later, FBF was measured. Then, each subject received the following stepwise infusions into the brachial artery: (1) acetylcholine (ACh), infused at rates of 15, 30, 45, and 60 μg/L of forearm/min, to assess endothelial-mediated vasodilation; (2) sodium nitroprusside (NP), infused at rates of 1, 3, and 9 μg/L/min, to obtain information on non-endothelial-mediated vasodilation; and (3) norepinephrine (NE), infused at rates of 140, 280, and 560 ng/L/min, to assess the vascular sensitivity to noradrenergic stimulation. The infusions were performed in this order to assure that the same vasoactive agent was infused at the same time after the meal in the different experimental conditions. Therefore, the ACh infusion started 30 minutes after the meal, the NP infusion 85 minutes after the meal, and NE infusion 130 minutes after the meal. 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. A 30-minute wash-out was allowed between each substance. The infusion rates were adjusted according to the forearm volume of each subject. FBF was measured simultaneously in both arms to ensure that no systemic effects of the infused substances occurred during the experiment. Each FBF value represents the mean of 6 consecutive measurements performed at 10-second intervals. Blood samples were simultaneously taken from the arterial and venous cannulas before and at the end of the maximal dose of ACh to measure nitrate/nitrite concentrations. Arterial blood samples for glucose and insulin determinations were taken in the basal state, before and at the end of the maximal dose of ACh and at the end of NP infusion (before, and 30, 60, and 120 minutes after the meal).

Meal and Wine Composition

The standard meal administered to the subjects was cooked right before the experiment and consisted of 120 g of spaghetti with tomato sauce and 25 g olive oil, 50 g of bresaola (partially dehydrated, very low fat content meat), 60 g of bread, and 1 apple, for a total of 1,050 kcal. Therefore, 60% of the calories were provided by carbohydrates, 25% by fat, and 15% by proteins. During the standard meal, water was provided at libitum. As to the meal and wine study, a glass of red wine (200 mL) was added to the standard meal. Since Barolo stored in oak barrel was previously shown to improve vascular reactivity in vitro,[38] we decided to use Barolo Cannubi, vintage 1995, stored in an oak barrel for 24 months (13.93% vol/vol ethanol, 1.8 g/L carbohydrates; Marchesi di Barolo, Barolo, Italy).

Analytical Methods

Plasma glucose concentration was measured using a Yellow Springs glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). 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 2,000 rpm at 4°C and plasma stored at −20°C. Before assay, plasma was ultrafiltered through a 10-kd molecular weight cut-off filter (Centricon 10, Millipore, Bedford, MA). Total plasma nitrite and nitrate were measured using a colorimetric kit (Cayman Chemical, Ann Arbor, MI). 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.

Calculations

The net forearm balance of nitrite was calculated by multiplying the plasma arterial-venous concentration difference of the substrate by the plasma flow. Therefore, a negative balance indicates nitrite release, whereas a positive balance indicates uptake. Vascular reactivity data are expressed as absolute values of FBF. The data were analyzed by a 2-way analysis of variance (ANOVA) for repeated measures (SPSS, version 11.0; SPSS Inc, Chicago, IL). Post hoc analysis was performed by Bonferroni's test. Results are expressed as the mean ± SEM.

RESULTS

The clinical characteristics of the subjects studied are shown in Table 1.

As depicted in Fig 1, glucose and insulin concentrations were similar in the basal state in the 3 different studies. While plasma glucose remained constant in the control study, it rose significantly after meal or meal and wine ingestion and remained above baseline up to 120 minutes ($P < 0.001$ in control study *v* meal and meal and wine studies). Similarly, serum insulin concentration was unchanged in the control study, whereas it increased to comparable levels after meal and meal and wine ingestion ($P < .001$ in control study *v* meal and meal and wine studies).

As shown in Fig 2, basal FBF was similar in the 3 experimental situations. The infusion of ACh, an endothelium-dependent vasodilator, elicited a progressive vasodilatory response of FBF that reached similar values in the 3 groups (22.3 ± 4.1, 19.2 ± 2.0, and 22.0 ± 3.7 $mL \cdot dL^{-1} \cdot min^{-1}$ in meal, meal and wine, and control studies, respectively; $P < .001$ for the

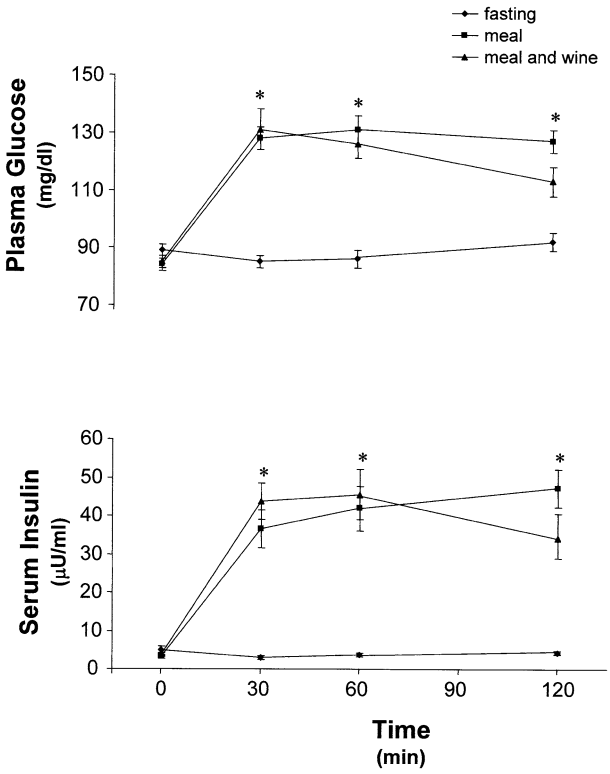

Fig 1. Plasma glucose (top) and serum insulin (bottom) concentration in meal, meal and wine, and control studies. Data were analyzed by ANOVA for repeated measures and post hoc differences evaluated by Bonferroni's test. *$P < .001$ *v* fasting.

ACh effect; P = not significant [NS] for the effect of treatment).

Figure 3 shows the forearm nitrite balance. During the highest Ach infusion rate, there was a net nitrite production in the 3 groups ($P < .001$ for all groups). However, this production was comparable in the 3 experimental settings (-19.1 ± 8.0, -15.4 ± 7.0, and -13.2 ± 7.3 nmol · dL^{-1} · min^{-1} in meal, meal and wine, and control studies, respectively; P = NS for the effect of treatment). Arterial plasma nitrite concentration was similar in the 3 studies basally (24.5 ± 6.2, 20.2 ± 2.7, and 17.4 ± 1.4 nmol/mL in meal, meal and wine, and control studies, respectively; P = NS) and remained unchanged during intra-arterial Ach infusion.

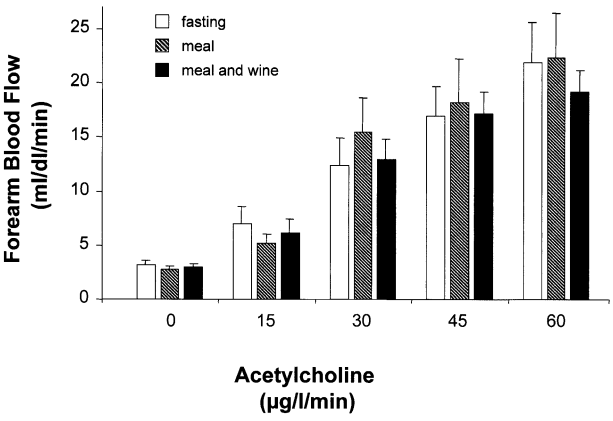

Fig 2. Forearm blood flow response to ACh infusion in meal, meal and wine, and control studies. The ACh infusion rate 0 μg/L/min represents the measurement of FBF performed 30 minutes after the end of the meal.

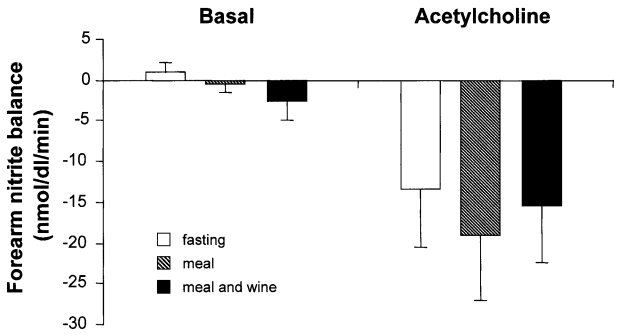

Fig 3. Forearm nitrite balance before and during intrabrachial ACh infusion in meal, meal and wine, and control studies. The signs (+) and (−) indicate uptake and release, respectively.

The dose-response curve for NP, an endothelium-independent vasodilator, is shown in Fig 4. All subjects showed a significant increase of FBF at all NP infusion rates ($P < .001$). At the maximal dose of NP, FBF was 21 ± 2, 24 ± 2, and 21 ± 2 mL · dL^{-1} · min^{-1} in the meal, meal and wine, and control studies, respectively; P = NS for the effect of treatment.

As shown in Fig 5, NE infusion caused a progressive and comparable reduction of FBF in the 3 studies ($P < .001$ *v* basal). At the maximal NE infusion rate, FBF was 2.1 ± 0.3, 1.9 ± 0.2, and 2.6 ± 0.4 mL · dL^{-1} · min^{-1} in the meal, meal and wine, and control studies, respectively; P = NS for the effect of treatment.

As shown in Fig 6, the peak FBF response to ischemia was similar in the meal, meal and wine, and control studies (32 ± 2, 32 ± 4, and 32 ± 3 mL · dL^{-1} · min^{-1}, respectively; P = NS).

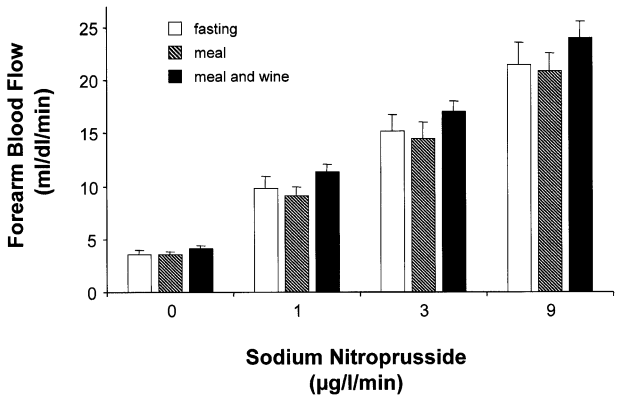

Fig 4. Forearm blood flow response to NP infusion in meal, meal and wine, and control studies.

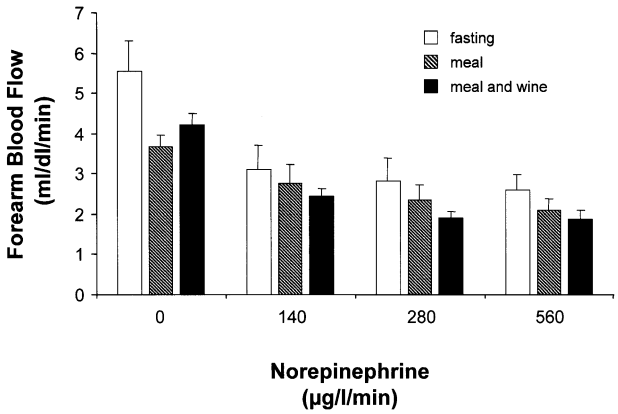

Fig 5. Forearm blood flow response to NE infusion in meal, meal and wine, and control studies.

DISCUSSION

The current study shows that ingestion of a mixed, natural meal does not cause any impairment of vascular reactivity. Both the endothelial and the non-endothelial-mediated vascular relaxation was as effective in the postprandial state as in the fasting condition. In addition, both basal and Ach stimulated forearm nitrite production were unchanged by the ingestion of the meal. Similarly, the vasoconstrictory response to norepinephrine was unchanged by meal ingestion. An additional observation of the present study was that the responses to the vasoactive agents were not altered when red wine was added to the meal.

A large body of data have recently accumulated, suggesting that postprandial hyperglycemia may be the explanation for the high vascular risk associated with diabetes.[5] Because of the pivotal role of the endothelium in the development of cardiovascular complications,[1,2] several studies have attempted to define the relathionship between hyperglycemia and endothelial dysfunction.[3,4,6-7] The results have almost invariably shown a deleterious effect of glucose on vascular reactivity.[3,4,6-9] The mechanism of action seems to be mediated by an increased oxidative stress.[7] These studies, however, are based on experimentally induced hyperglycemia, by either oral glucose load or intravenous glucose infusion.[3,4,6,7,9] Although useful to dissect the contribution of glucose per se, this approach has little relevance to postprandial physiology.

The same arguments used for hyperglycemia also apply to the role of insulin, which has been mostly explored in experimental settings of isolated hormone elevations, never occurring in nature.[13,14] When hyperinsulinemia, associated with hyperglycemia, was evoked by an oral glucose load, vascular reactivity was impaired.[7] Recently, it has been reported that 6 hours of moderate hyperinsulinemia impairs endothelial-mediated vascular reactivity.[14]

If the above findings are transposed to the postprandial state, one might expect that the combined effect of glucose and insulin leads to marked deterioration of vascular reactivity. It should also be remembered that the postprandial state is associated with such factors potentially deleterious to the endothelium as oxidative stress, low-density lipoprotein (LDL) oxidation, and activation of coagulation components. For this reason, the postprandial state has been viewed as a key event with regard to the development and progression of atherosclerosis. However, in the current physiologic model, postprandial hyperglycemia was not associated with any change in vascular reactivity. Similarly, systemic nitrite levels and forearm nitrite production were unaffected, confirming that endothelial function is unaltered by meal ingestion. As simplistic it may be, the only explanation is that, in the myriads of events triggered by food ingestion, those with potentially negative impact on the endothelium are offset by other, vasoprotective factors. Ultimately, it should not be surprising that the most fundamental act of everyday's life does not bear an atherogenic impact.

The present data are in agreement with previous reports of unchanged vascular reactivity following ingestion of a non-fat meal.[15-18,20-23] However, the methods used in previous studies did not allow the combined measurement of NO production and endothelium- and non-endothelium-mediated vascular reactivity during stepwise stimulation by vasoactive substances. Furthermore, the effects of meal ingestion on the response to NE have not previously been addressed.

A number of epidemiological data have suggested that the intake of alcoholic beverages reduces cardiovascular mortality.[24-29] Some data also suggest that wine, in particular red wine, provides an additional benefit in terms of cardiovascular risk prevention.[35] In vitro data indicate that red wine stored in oak barrel is more potent in improving endothelial dysfunction.[38] Based on this evidence, several groups have investigated whether wine intake affects endothelial function.[37-41,44] However, very few studies have explored the acute effects of red wine on vascular reactivity.[38-41] Some of these studies reported a beneficial effect of red wine in vitro or in animal models.[38,39] Others found a positive effect of red wine only when it was deprived of ethanol.[41] However, the previous studies were performed using the flow-mediated dilation (FMD) approach to measure vascular reactivity,[40,41] which does not allow the combined evaluation of FBF response to stepwise infusion of vasoactive substances and the measurement of nitrite balance. In addition, the effect of red wine was not evaluated in the natural context of a mixed meal.[41]

In the current study, we did not find any effect of red wine intake on vascular reactivity. This is confirmed by the lack of changes in nitrite release by the forearm. Particularly interesting was the current observation that red wine is unable to

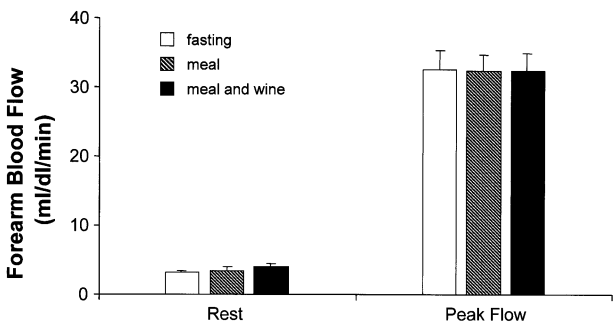

Fig 6. Peak forearm blood flow response to ischemia in the meal, meal and wine, and control studies.

modify the vasoconstrictory response to NE. This finding was unexpected, given the well-documented effect of alcohol per se to induce vasodilation acutely.[41]

The present data are not in contrast with the possibility that chronic red wine consumption might have beneficial effects on vascular reactivity and cardiovascular mortality. However, it is clear that any beneficial effect of wine on the vascular wall is unrelated to the moment-to-moment regulation of vascular reactivity and requires the activation of long-term mechanisms. Caution should be also exercised in recommending red wine consumption under the assumption that wine improves the vascular risk profile. Clearly, more studies based on adequate approach to assess endothelial function are needed before theorizing about the benefits of alcoholic beverages.

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