Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 491 (2004) 43–51
www.elsevier.com/locate/ejphar

Selective vasodilator and chronotropic actions of 3′,4′-dihydroxyflavonol in conscious sheep

Sheng Wang[a,b], Gregory J. Dusting[a,b], Owen L. Woodman[b], Clive N. May[a,b,*]

[a] Howard Florey Institute, The University of Melbourne, Melbourne, Victoria 3010, Australia
[b] Department of Pharmacology, The University of Melbourne, Melbourne, Victoria 3010, Australia

Received 20 November 2003; received in revised form 26 February 2004; accepted 3 March 2004

Abstract

Flavonoids are known to relax isolated large arteries, but the cardiovascular actions of flavonoids are not well studied in vivo. Hence, we determined the systemic and regional haemodynamic responses to a novel synthetic flavonol, 3′,4′-dihydroxyflavonol (DiOHF), in conscious sheep previously instrumented with flow probes on the aorta and coronary, mesenteric, renal, and iliac arteries. Intravenous injection of DiOHF (1.0 mg/kg) caused a delayed but prolonged vasodilatation in the coronary and renal vascular beds, with no changes in the mesenteric or iliac vascular beds. DiOHF induced prolonged increases in heart rate and cardiac output without altering arterial pressure. Pretreatment with N^{ω}-nitro-L-arginine, a nonspecific inhibitor of nitric oxide synthase, abolished all the cardiovascular effects of DiOHF. The results indicate that DiOHF caused a nitric oxide-dependent vasodilatation of the coronary and renal vasculature and an increase in cardiac output due to its chronotropic action.

Keywords: Cardiac output; Chronotropic; Flavonoid; Haemodynamics; N^{ω}-nitro-L-arginine; Nitric oxide; Regional blood flow

1. Introduction

There is growing evidence that an increased dietary intake of flavonoids reduces the risk of cardiovascular disease. The Zutphen Elderly Study demonstrated that men aged 65–84 years with a dietary history of high flavonol and flavone intake had a significantly lower risk of death from coronary heart disease (Hertog et al., 1993). An inverse correlation between flavonoid intake and coronary heart disease has also been demonstrated in population-based surveys (Knekt et al., 1996; Yochum et al., 1999). Furthermore, the Rotterdam Study indicated that increased intake of dietary flavonoids was associated with a lower incidence of myocardial infarction (Geleijnse et al., 2002). In view of these positive epidemiological outcomes, the antioxidant, antiatherosclerotic, anti-inflammatory, and vasodilator actions of flavonoids have been examined to determine which of these actions may contribute to cardiovascular protection (Middleton et al., 2000; Nijveldt et al., 2001).

Flavonoids have direct concentration-dependent relaxant actions on precontracted arterial rings from large arteries (Andriambeloson et al., 1997; Fitzpatrick et al., 1993; Flesch et al., 1998; Herrera et al., 1996). Most of these studies, however, were performed in isolated conductance arteries, and the extent to which vasorelaxation to flavonoids is dependent on the integrity of vascular endothelium remains controversial (Middleton et al., 2000). Recently, it has been reported that endothelium-independent vasorelaxation by quercetin, a major dietary flavonoid, was significantly greater in isolated resistance vessels than in conductance vessels (Perez-Vizcaino et al., 2002; Rendig et al., 2001), but the actions of flavonoids on resistance arteries in vivo have not been assessed thoroughly. Although the actions of flavonoids on blood pressure, either after dietary supplement (Diebolt et al., 2001; Duarte et al., 2001) or after intravenous infusion (Cortes et al., 2001), have been investigated, it is unknown whether flavonoids have any effects on cardiac output or the distribution of blood flow to different vascular beds after systemic administration.

* Corresponding author. Howard Florey Institute, The University of Melbourne, Parkville, Melbourne, Victoria 3010, Australia. Tel.: +61-3-8344-7302; fax: +61-3-9348-1707.
E-mail address: c.may@hfi.unimelb.edu.au (C.N. May).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.03.012

We have demonstrated that a synthetic flavonol, 3′,4′-dihydroxyflavonol (DiOHF), caused relaxation of rat isolated thoracic aorta, which was partially endothelium-dependent (Chan et al., 2000). Importantly, DiOHF was significantly more potent than a number of natural flavonols and flavones (Chan et al., 2000). In further studies, DiOHF enhanced nitric oxide bioavailability and improved vascular function after ischaemia and reperfusion injury in rat hindquarters (Chan et al., 2003). Moreover, DiOHF administered during ischaemia, but shortly before reperfusion, significantly reduced the infarct size and reperfusion injury in anaesthesized sheep (Wang et al., 2002). The level of protection by DiOHF was similar to that afforded by ischaemic preconditioning—currently the most effective method to prevent this injury (Wang et al., 2002). These findings indicate that compounds such as DiOHF have potential as treatments

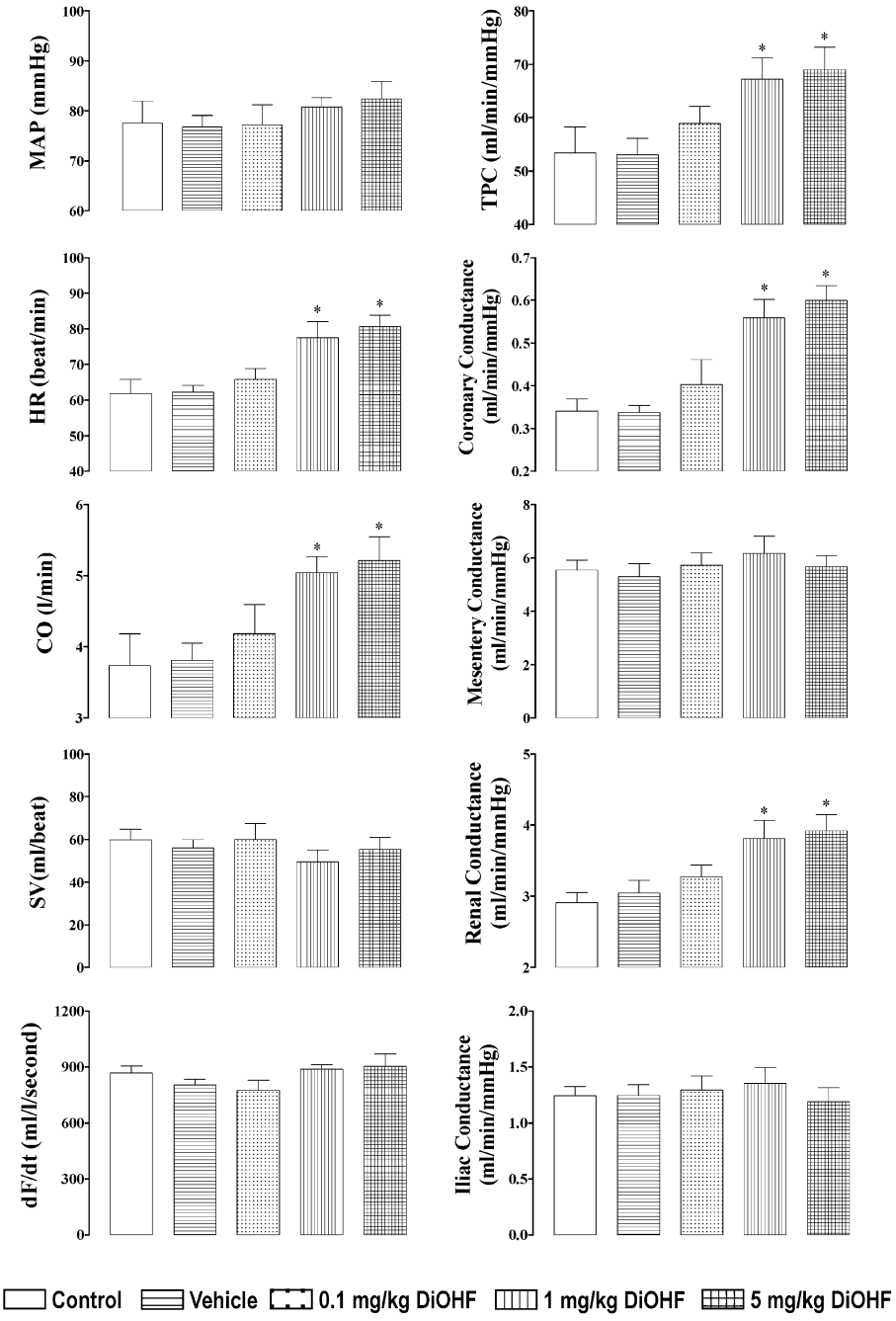

Fig. 1. The cardiovascular responses to DiOHF (0.1, 1.0, and 5.0 mg/kg) in comparison to vehicle in conscious sheep. Maximum responses, at 10 h after administration, are shown for mean arterial pressure (MAP), heart rate (HR), cardiac output (CO), total peripheral conductance (TPC), the first differential of the upstroke of systole (d*F*/d*t*), stroke volume (SV), and regional conductances in the coronary, mesenteric, renal, and iliac vascular beds. Results are mean±S.E.M. **P*<0.05 vs. vehicle (*n*=5 per group).

for cardiac injury following ischaemia and reperfusion, so it is important to understand in detail their cardiovascular actions in response to systemic administration.

The aim of the present study was to determine the cardiovascular actions of DiOHF in vivo. To avoid the complicating effects of anaesthesia, we examined the systemic and regional haemodynamic responses to intravenous administration of DiOHF in conscious sheep. To investigate whether any of the observed actions of DiOHF were dependent on nitric oxide, we examined the effect of pretreatment with a nonspecific inhibitor of nitric oxide synthase (NOS), N^{ω}-nitro-L-arginine (L-NNA).

2. Materials and methods

The study was approved by the Animal Experimentation Ethics Committee of the Howard Florey Institute and performed in accordance with the guidelines of the National Health and Medical Research Council of Australia.

2.1. Animal instrumentation

Merino ewes (35–45 kg body weight), oophorectomized and with carotid arteries enclosed in skin loops, were used in this study. Flow probes were implanted in anaesthesized sheep in two separate procedures to measure aortic and regional blood flows as previously described (Bednarik and May, 1994). Briefly, anaesthesia was induced by intravenous thiopental sodium (15 mg/kg) and maintained by 1.5–2.0% isoflurane in air–oxygen (1:1) after tracheal intubation. Transit time flow probes were implanted on the ascending aorta (20 mm diameter; Transonic Systems, Ithaca, NY, USA) and the left circumflex coronary artery (3 mm probe with coronary flange). Following 2 weeks of recovery, transit time flow probes were implanted on the cranial mesenteric (6 mm), left renal (4 mm), and left external iliac (6 mm) arteries. All leads attached to flow probes were tunneled subcutaneously and exteriorized on the sheep's back. An antibiotic (900 mg of procaine penicillin, i.m.; Troy Laboratories, NSW, Australia) was admin-

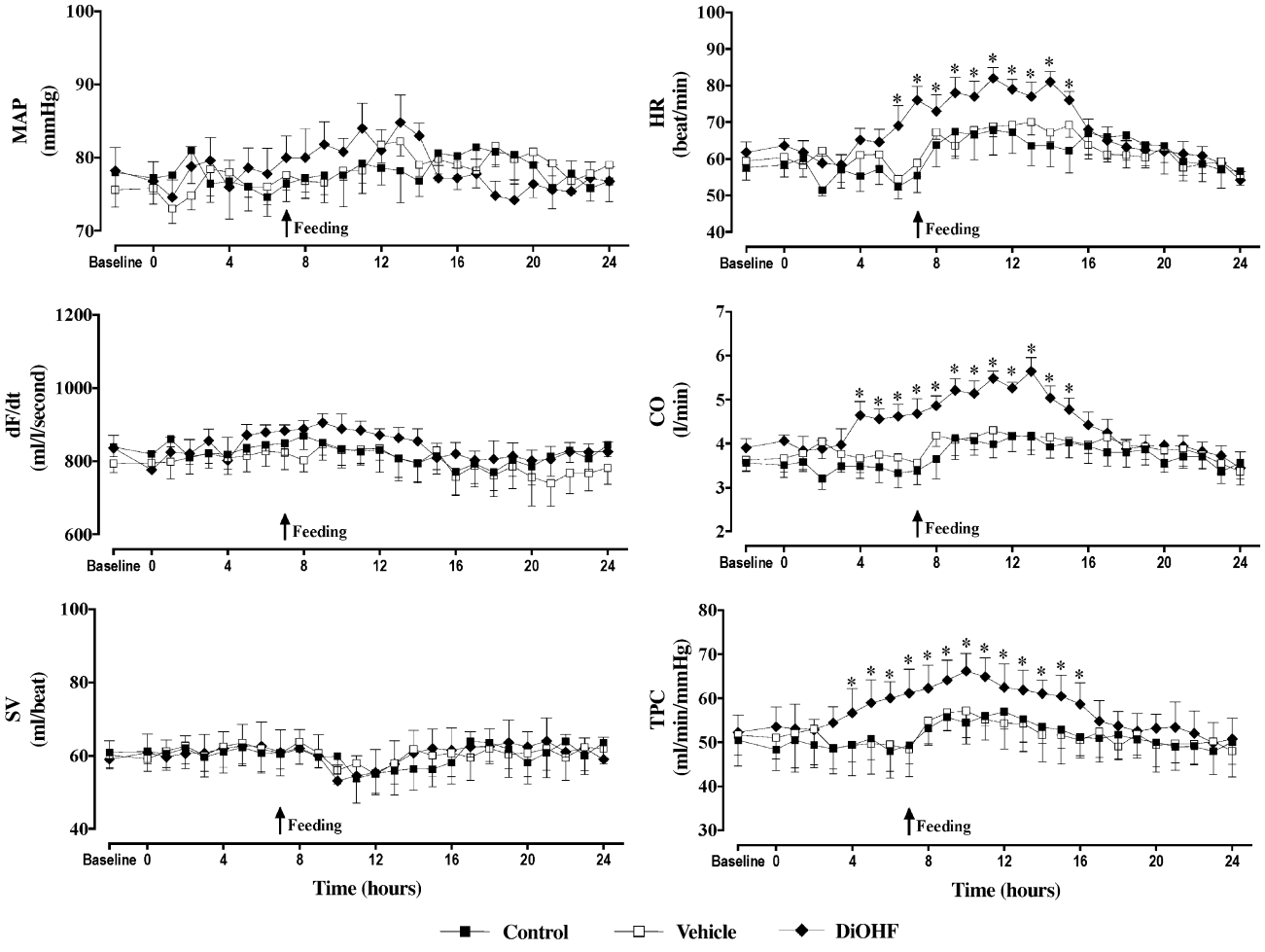

Fig. 2. The cardiovascular responses to of DiOHF (1 mg/kg) or vehicle given at time 0 in conscious sheep. The baseline value is the mean of a 30-min control period. Mean arterial pressure (MAP), heart rate (HR), the first differential of the upstroke of systole (dF/dt), cardiac output (CO), stroke volume (SV), and total peripheral conductance (TPC) were measured for 24 h. Control represents the daily rhythm of cardiac parameters in conscious sheep. "Feeding" indicates the time at which the animals were fed. Results are mean±S.E.M. *P<0.05 vs. vehicle (n=5 per group).

istered prophylactically for 3 days postsurgery. Postsurgical analgesia was maintained with intramuscular injection of flunixin meglumine (1 mg/kg; Mavlab, QLD, Australia) at the end of surgery, then 4 and 16 h postsurgery.

2.2. Data collection

On the day prior to the start of the experiment, a sterile Tygon cannula (ID 1.0 mm, OD 1.5 mm) was inserted 15–20 cm into a carotid artery under aseptic conditions for arterial pressure measurement. Under local anaesthesia, two sterile polyethylene cannulae (ID 1.18 mm, OD 1.7 mm) were inserted 20 cm into a jugular vein for measurement of central venous pressure and intravenous infusion. The patency of the cannulae was maintained by infusing heparinized saline (25 U/ml) at 3.0 ml/h from flush devices (TDF-3WC; Biosensors International, Singapore). The cannulae for measurement of arterial pressure and central venous pressure were connected to pressure transducers (CDXIII; Cobe, Colorado, USA) tied to the wool on the sheep's back and calibrated against a mercury and water manometer, respectively. A correction factor in the data collection program compensated for the height of the transducers above the level of the heart. Flow probes for measurement of aortic flow were connected directly to a transit time flow meter (T206;

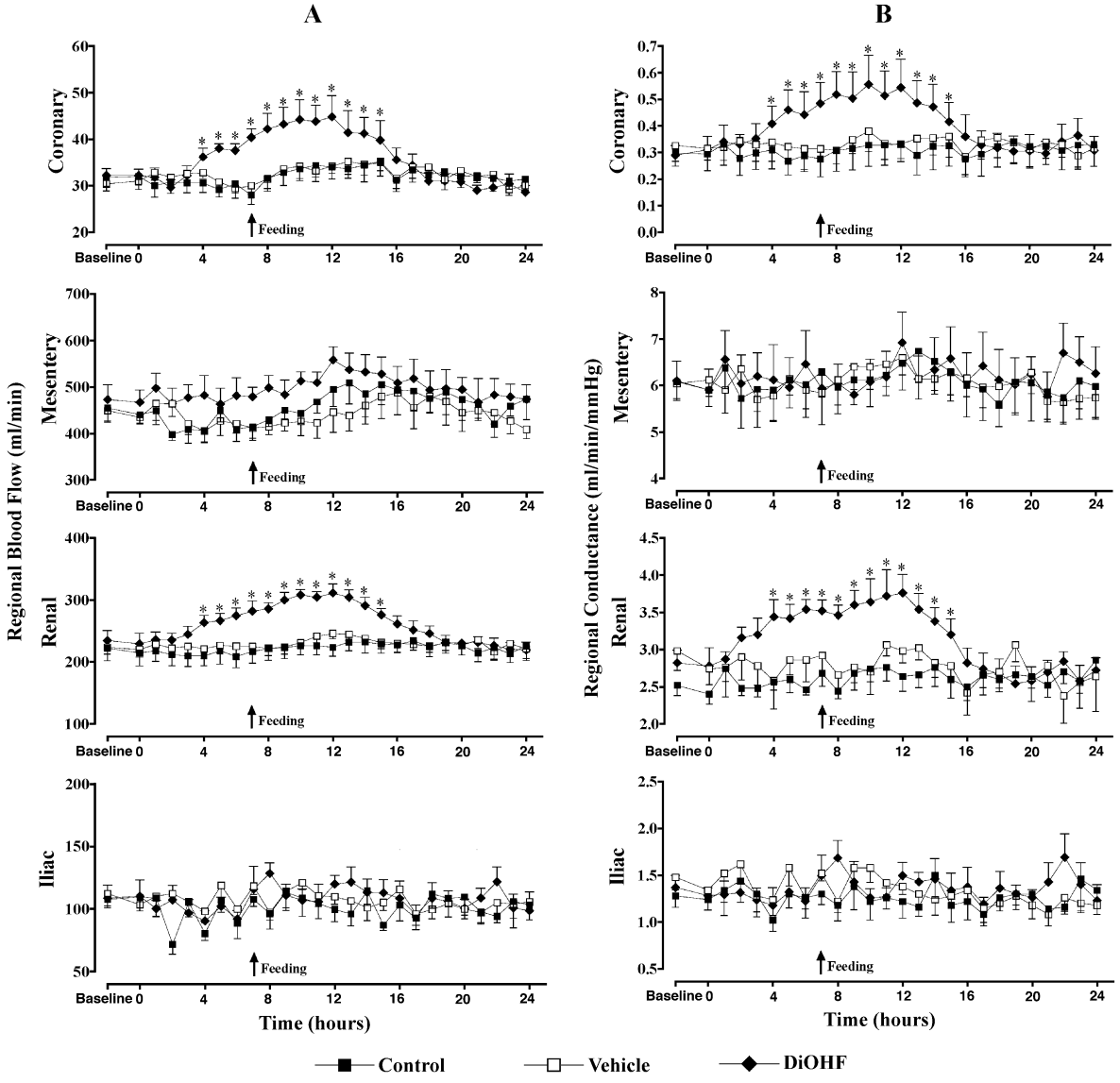

Fig. 3. The effects of intravenous administration of DiOHF (1 mg/kg) or vehicle given at time 0 on regional blood flows (A) and conductances (B). Control represents the daily rhythm of regional haemodynamic variation in conscious sheep. "Feeding" indicates the time at which the animals were fed. Results are mean±S.E.M. *P<0.05 vs. vehicle (n=5 per group).

Transonic Systems) and, for regional flows, the probes were connected via a four-probe sequential scanner (TM04; Transonic Systems) to a transit time flowmeter (T201; Transonic Systems). The accuracy of chronically implanted transit time flow probes for the measurement of regional blood flow in conscious sheep has been validated previously (Bednarik and May, 1995).

Analogue signals of arterial blood pressure, central venous pressure, aortic flow, and regional blood flows were collected on a computer using custom-written software. Following analogue-to-digital conversion (DT 2811 board; Data Translation), data were collected at 100 Hz for 10 s at 1-min intervals. Mean arterial pressure was calculated from the arterial blood pressure signal. Heart rate, cardiac output, the first differential of the upstroke of systole (dF/dt), stroke volume (cardiac output/heart rate), and total peripheral conductance (cardiac output/mean arterial pressure) were calculated from the aortic flow signal. Mean regional blood flows and mean regional conductances (mean flow/mean arterial pressure) were calculated from the regional flow signals.

2.3. Experimental protocols

Sheep were housed in individual metabolism cages in an open laboratory in association with other sheep. They were fed a diet of oaten chaff and water was offered ad libitum. Sheep were not used for an experiment until they had fully recovered from surgery and were accustomed to laboratory conditions and human contact. Experiments were started between 0930 and 1000 h and animals were fed between 1630 and 1700 h. An interval of at least 1 week was allowed between experiments due to the long half-life of L-NNA and the prolonged responses to DiOHF.

2.3.1. Haemodynamic effects of DiOHF

Five treatments, normal control, vehicle, and DiOHF (DiOHF, 0.1, 1.0, and 5.0 mg/kg) were given to conscious sheep (n=5) in random order. Following 30 min of baseline measurement, either DiOHF (Indofine Chemical, Belle Mead, NJ, USA) dissolved in vehicle (0.5 ml of dimethyl sulfoxide and 5 ml of polyethylene glycol) or vehicle was injected intravenously over 3 min. Control animals received

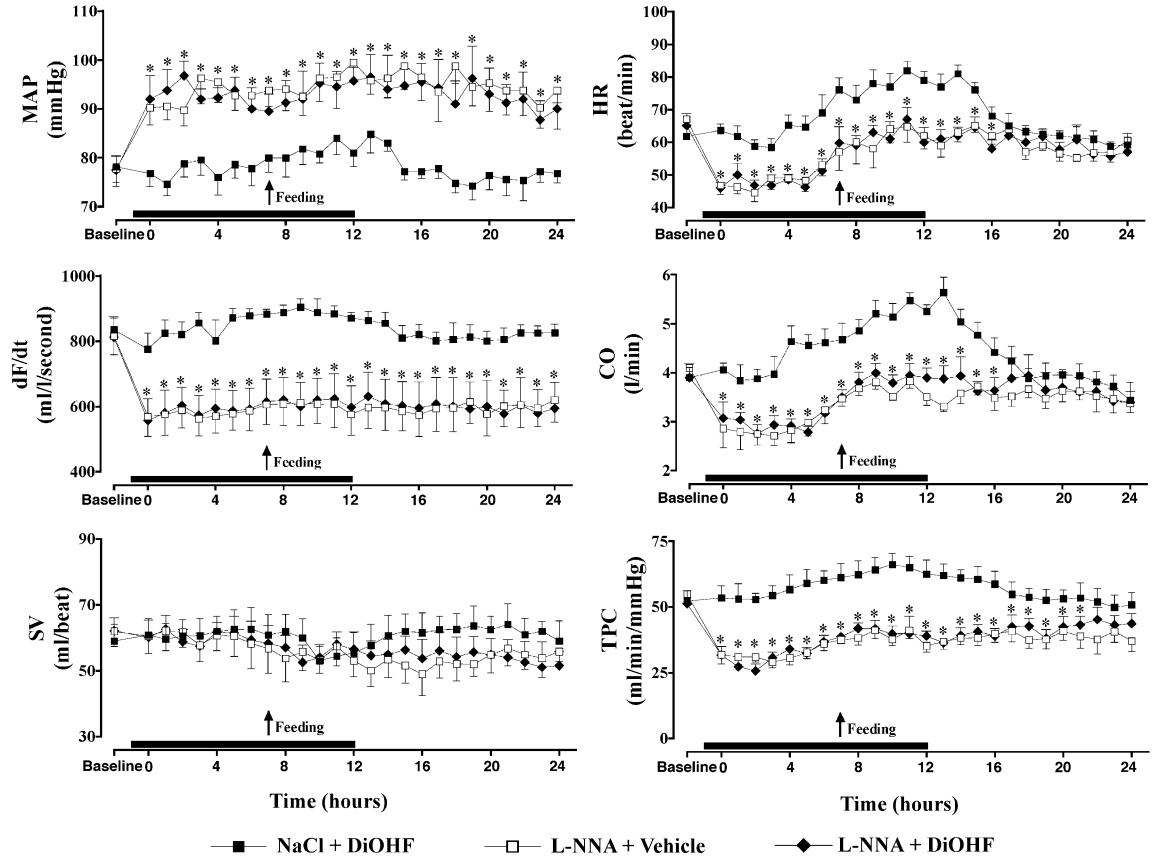

Fig. 4. The effect of intravenous administration of L-NNA on the cardiovascular responses to DiOHF in conscious sheep. L-NNA (10 mg/kg, followed by 5 mg/kg/h for 13 h) was administered 1 hour before DiOHF (1 mg/kg) (L-NNA + DiOHF) or vehicle (L-NNA + VEH). Saline (50 ml followed by 30 ml/h for 13 h) was administered 1 hour before DiOHF (1 mg/kg) (NaCl + DiOHF). The solid bar indicates the period of L-NNA infusion, which started after a 30-min control period, one hour before DiOHF or vehicle administration at time 0. 'Feeding' indicates the time at which the animals were fed. Abbreviations as Fig. 3. Results are means ± S.E.M. *P<0.05 vs. NaCl + DiOHF (n=5 per group).

no treatment. Haemodynamic variables were recorded every 5 min for 24 h.

2.3.2. Effect of L-NNA on the cardiovascular responses to DiOHF

To determine whether the cardiovascular actions of DiOHF depended on the release of endogenous nitric oxide, the effect of pretreatment with the nonspecific NOS inhibitor L-NNA (Sigma, USA) on the responses to DiOHF was examined. Sheep (n=5) were given three treatments in random order: L-NNA plus DiOHF (L-NNA+DiOHF), L-NNA plus vehicle (L-NNA+vehicle), and saline plus DiOHF (NaCl+DiOHF). A dose of L-NNA was used that we have shown causes a significant and prolonged increase in arterial pressure and decrease in total peripheral conductance in conscious sheep (Tresham et al., 1994). After 30 min of baseline recording, L-NNA (bolus dose of 10 mg/kg, followed by infusion at 5 mg/kg/h for 13 h) or saline vehicle (bolus of 50 ml, followed by infusion at 30 ml/h for 13 h) was given intravenously. At 1 h after administration of L-NNA or saline, DiOHF (1 mg/kg) or vehicle (0.5 ml of dimethyl sulfoxide in 5 ml of polyethylene glycol) was injected intravenously. Data were collected at baseline and every 5 min immediately after L-NNA infusion for 25 h.

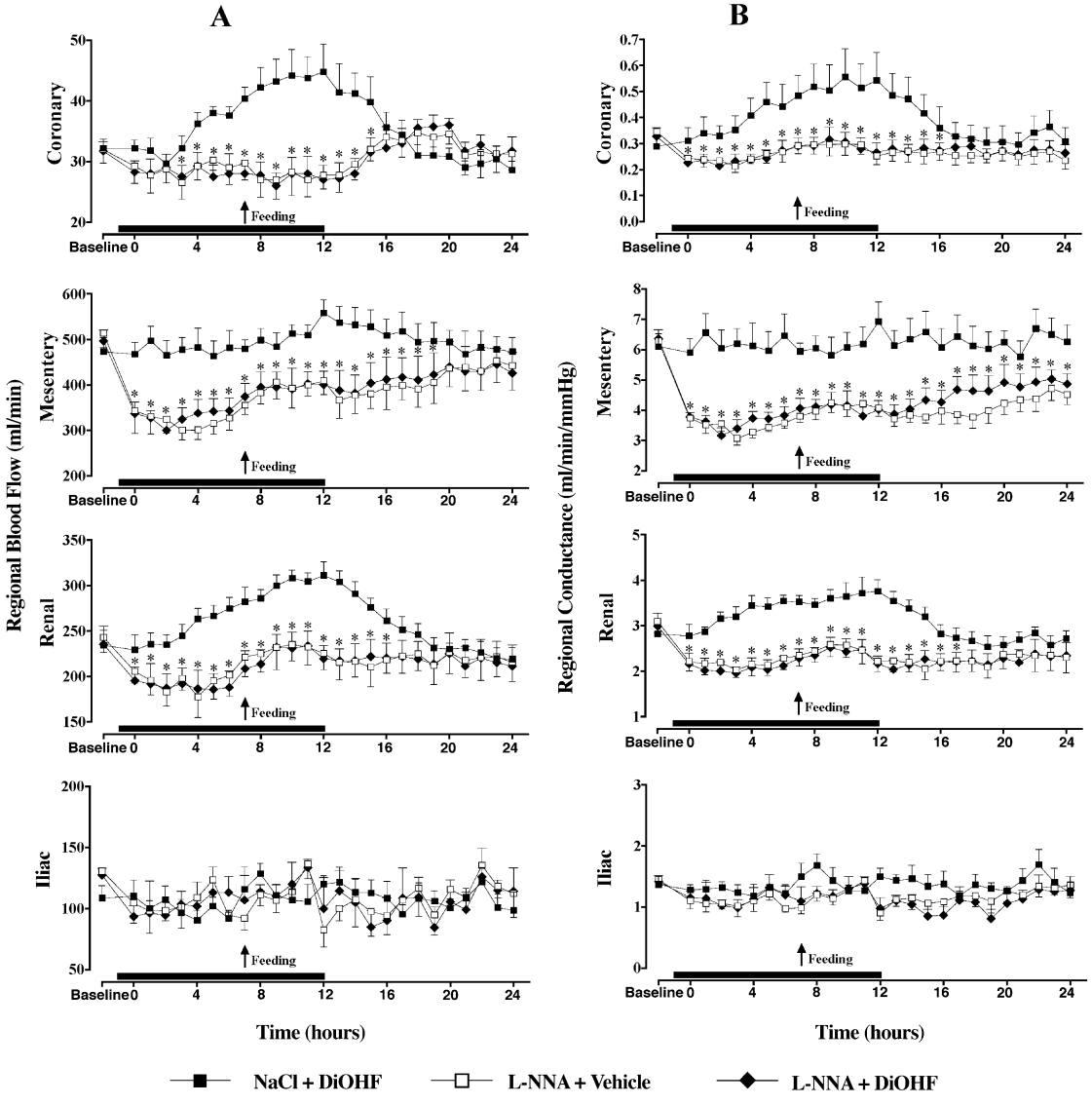

Fig. 5. The effect of intravenous administration of L-NNA on the regional haemodynamic responses to DiOHF in conscious sheep. L-NNA (10 mg/kg, followed by 5 mg/kg/h for 13 h) was administered 1 hour before DiOHF (1 mg/kg) (L-NNA + DiOHF) or vehicle (L-NNA + VEH). Saline (50 ml followed by 30 ml/h for 13 h) was administered 1 hour before DiOHF (1 mg/kg) (NaCl + DiOHF). The solid bar indicates the period of L-NNA infusion, which started after a 30-min control period, one hour before DiOHF or vehicle administration at time 0. 'Feeding' indicates the time at which the animals were fed. Results are means ± S.E.M. *P<0.05 vs. NaCl + DiOHF (n=5 per group). Abbreviations as Fig. 3. Results are means ± S.E.M. *P < 0.05 vs. NaCl + DiOHF (n=5 per group).

2.4. Statistical analysis

Data were analyzed by two-way repeated-measures analysis of variance, followed by Bonferroni's test for multiple comparisons (SigmaStat). Results are presented as mean± S.E.M. $P<0.05$ was considered statistically significant.

3. Results

3.1. Haemodynamic effects of DiOHF

DiOHF (5 mg/kg) had significant prolonged actions on the heart and regional vasculature—the main effects being increases in heart rate and cardiac output, and vasodilatation in the coronary and renal vascular beds (Fig. 1). The actions of this dose were not significantly different from the responses to the intermediate dose (1 mg/kg), which are described in detail below. The lowest dose of DiOHF (0.1 mg/kg) caused qualitatively similar changes, but these did not reach significance. In comparison to vehicle, none of the doses of DiOHF changed mean arterial pressure, stroke volume, or dF/dt.

Treatment with DiOHF (1 mg/kg) caused no significant changes in mean arterial pressure, central venous pressure, dF/dt, or stroke volume, but from 6 h after administration, it increased heart rate (from a basal level of 63±3 to 71±3 beats/min; $P<0.05$) and this effect was maintained for a further 10 h (Fig. 2). Similarly, cardiac output was significantly increased at 4 h after DiOHF treatment (from 4.0±0.2 to 4.8±0.3 l/min; $P<0.05$) and remained increased for a further 12 h (Fig. 2). DiOHF also significantly increased total peripheral conductance (from 51.2±3.9 to more than 58.9±3.7 ml/min/mm Hg; $P<0.05$) for more than 10 h, which resulted from differential actions on individual vascular beds. Coronary and renal conductances were significantly increased from 4 to 12 h after DiOHF injection, resulting in increases in coronary blood flow, from 31±1.5 to more than 38±1.0 ml/min ($P<0.05$), and increases in renal blood flow, from 227±12 to more than 273±11 ml/min ($P<0.05$; Fig. 3). In contrast, DiOHF had no effect on the flows or conductances in the mesenteric and iliac vascular beds. Compared with the control group, vehicle infusion had no effect on systemic or regional haemodynamics.

3.2. Effect of L-NNA on the cardiovascular responses to DiOHF

After inhibition of NOS by L-NNA, there was a large and sustained increase in mean arterial pressure for the 24 h that measurements were made (Fig. 4). This was caused by the significant and sustained peripheral vasoconstriction as indicated by the reduction in total peripheral conductance (Fig. 4), which resulted from substantial reductions in renal and mesenteric conductances, but only minor changes in coronary and iliac conductances (Fig. 5). These responses to L-NNA were accompanied by decreases in heart rate, dF/dt, and cardiac output (Fig. 4).

In the presence of L-NNA, all the systemic and regional haemodynamic actions of DiOHF were abolished (Figs. 4 and 5). The responses to L-NNA were identical in the L-NNA+vehicle and the L-NNA+DiOHF groups, indicating that the responses to L-NNA were not altered by treatment with DiOHF (Figs. 4 and 5).

4. Discussion

The major findings of this study were that intravenous administration of a novel synthetic flavonol, DiOHF, caused a delayed but prolonged increase in total peripheral conductance in conscious sheep. Interestingly, this effect resulted from selective vasodilatation in the renal and coronary vascular beds, with no vasodilatation in the mesenteric or iliac circulations. There was no reduction in arterial pressure because the vasodilatation was accompanied by increases in heart rate and cardiac output. The vasodilator and chronotropic actions of DiOHF were prevented by inhibition of NOS, indicating that they were mediated by endogenous nitric oxide.

We have reported that vasorelaxation to DiOHF in rat isolated thoracic aorta was partially endothelium-dependent (Chan et al., 2000). In the present study, the selective vasodilator action of DiOHF in the coronary and renal vascular beds was completely prevented by the inhibition of NOS, suggesting that the vasodilator action of DiOHF in these resistance vessels was nitric oxide-dependent. This is in accord with several in vitro studies that have shown that vasodilatation in response to a number of flavonoids is mediated by nitric oxide (Andriambeloson et al., 1997; Taubert et al., 2002). The relaxation of rat thoracic aorta by an extract from red wine, which contains flavonoids, was mediated by an increase in aortic nitric oxide production (Andriambeloson et al., 1997) and, in porcine coronary artery, the vasorelaxing activity of flavonoids was positively associated with their ability to stimulate nitric oxide release (Taubert et al., 2002).

The vasodilator action of DiOHF was relatively slow in onset, reaching significance after 4 h, and the action was sustained for more than 10 h. To our knowledge, this is the first report that acute administration of a flavonoid leads to a long-lasting vasodilator action in selective vascular beds. Flavonoids are known to have long half-lives in vivo. For example, quercetin, a major dietary catechol-containing flavonoid similar in structure to DiOHF, has a plasma half-life of more than 20 h (Hollman et al., 1997). In addition, it has been indicated that the metabolites of quercetin had similar or higher vasorelaxation potency than quercetin itself (Perez-Vizcaino et al., 2002). Hence, the most likely explanation for the slow onset and prolonged action is that DiOHF is slowly metabolized and eliminated, and/or it has active metabolites with long half-lives. An

alternative explanation is that DiOHF may have a genomic action, which develops over hours and results in a prolonged action. This possibility is supported by an in vitro study which showed that red wine polyphenol extract increased endothelial NOS expression and subsequent nitric oxide release from endothelial cells (Leikert et al., 2002).

Administration of DiOHF resulted in strikingly different responses in different vascular beds. The vasodilatation and increase in blood flow in the coronary vasculature were probably due to a direct effect of DiOHF, combined with a response to the increased metabolic demand of the heart due to increased cardiac work as cardiac output increased. Although DiOHF caused no vasodilatation in the mesenteric or iliac vascular beds, it caused a significant, prolonged vasodilatation and an increase in blood flow in the kidney. It has been demonstrated that there is a linear relationship between dietary intake and urinary excretion of flavonoids (Lampe, 2003), indicating that the kidney may be exposed to higher concentrations of DiOHF than other organs. Moreover, recent studies indicate that some flavonoid metabolites have more potent biological actions than their precursors (Chin-Dusting et al., 2001; Liew et al., 2003; Perez-Vizcaino et al., 2002). Thus, DiOHF and its metabolites, which may be more active than their parent compound, may accumulate in the kidney and cause renal vasodilatation. The lack of any vasodilatation in the mesenteric and iliac vascular beds may reflect the lack of accumulation of DiOHF or its metabolites in these organs, leading to an insufficient increase in nitric oxide to cause vasodilatation.

The actions of natural flavonoids on arterial pressure have been examined in a number of studies. In normotensive rats, short-term oral administration of red wine polyphenolic compounds caused a progressive decrease in systolic blood pressure (Diebolt et al., 2001), and intravenous infusion of dioclein decreased blood pressure by reducing peripheral vascular resistance (Cortes et al., 2001). Similarly, quercetin was demonstrated to reduce the elevated blood pressure and to reverse functional vascular changes in spontaneously hypertensive rats but not on normotensive controls (Duarte et al., 2001). In contrast, although we found that DiOHF caused vasodilatation, it did not decrease mean arterial pressure because the increase in cardiac output offset the impact of vasodilatation on blood pressure. Following DiOHF injection, there was no significant increase in dF/dt, an accurate index of myocardial contractility when preload and afterload are stable (de Wildt and Sangster, 1983), suggesting that the increase in cardiac output did not result from an inotropic action of DiOHF, but from its chronotropic action. The mechanisms causing the tachycardia are unclear but may also be attributed to nitric oxide, as the chronotropic action of DiOHF was prevented by NOS inhibition. This suggestion is supported by the demonstration that the chronotropic action of nitric oxide donors is not only a baroreflex response to vasodilatation, but that endogenous nitric oxide has direct actions on the autonomic control of the heart (Chowdhary and Townend, 1999) and exerts a tonic chronotropic influence on the denervated human heart (Chowdhary et al., 2002). It is unlikely that the chronotropic effect of DiOHF was due to baroreflex-induced activation of cardiac sympathetic nerves or withdrawal of cardiac vagal tone because arterial pressure was not reduced.

In summary, this is the first report of the vascular and cardiac actions of the synthetic flavonol, DiOHF, revealing a delayed but long-lasting vasodilator effect in conscious animals. The selective coronary and renal vasodilator actions of DiOHF were completely abolished by L-NNA, a nonspecific NOS inhibitor, indicating that endogenous nitric oxide mediates these effects. Constitutive endogenous nitric oxide production is crucial to maintain vascular homeostasis, and impaired endothelial nitric oxide formation is one of the first signs observed in atherosclerosis (Dusting, 1995; Vanhoutte, 1997). Thus, an action of flavonoids on endogenous nitric oxide may contribute to their protective effect demonstrated in epidemiological studies and experimental ischaemia/reperfusion injury.

Acknowledgements

We are grateful to Craig Thomson and Alan McDonald for excellent technical assistance. This study was supported by an Institute Block Grant from the National Health and Medical Research Council of Australia to the Howard Florey Institute (No. 983001).

References

Andriambeloson, E., Kleschyov, A.L., Muller, B., Beretz, A., Stoclet, J.C., Andriantsitohaina, R., 1997. Nitric oxide production and endothelium-dependent vasorelaxation induced by wine polyphenols in rat aorta. Br. J. Pharmacol. 120, 1053–1058.

Bednarik, J.A., May, C.N., 1994. Differential regional hemodynamic effects of corticotropin in conscious sheep. Hypertension 24, 49–55.

Bednarik, J.A., May, C.N., 1995. Evaluation of a transit-time system for the chronic measurement of blood flow in conscious sheep. J. Appl. Physiol. 78, 524–530.

Chan, E.C., Pannangpetch, P., Woodman, O.L., 2000. Relaxation to flavones and flavonols in rat isolated thoracic aorta: mechanism of action and structure–activity relationships. J. Cardiovasc. Pharmacol. 35, 326–333.

Chan, E.C., Drummond, G.R., Woodman, O.L., 2003. 3′,4′-Dihydroxyflavonol enhances nitric oxide bioavailability and improves vascular function after ischemia and reperfusion injury in the rat. J. Cardiovasc. Pharmacol. 42, 727–735.

Chin-Dusting, J.P., Fisher, L.J., Lewis, T.V., Piekarska, A., Nestel, P.J., Husband, A., 2001. The vascular activity of some isoflavone metabolites: implications for a cardioprotective role. Br. J. Pharmacol. 133, 595–605.

Chowdhary, S., Townend, J.N., 1999. Role of nitric oxide in the regulation of cardiovascular autonomic control. Clin. Sci. (Lond.) 97, 5–17.

Chowdhary, S., Harrington, D., Bonser, R.S., Coote, J.H., Townend, J.N., 2002. Chronotropic effects of nitric oxide in the denervated human heart. J. Physiol. 541, 645–651.

Cortes, S.F., Rezende, B.A., Corriu, C., Medeiros, I.A., Teixeira, M.M., Lopes, M.J., Lemos, V.S., 2001. Pharmacological evidence for the activation of potassium channels as the mechanism involved in the hypotensive and vasorelaxant effect of dioclein in rat small resistance arteries. Br. J. Pharmacol. 133, 849–858.

de Wildt, D.J., Sangster, B., 1983. An evaluation of derived aortic flow parameters as indices of myocardial contractility in rats. J. Pharmacol. Methods 10, 55–64.

Diebolt, M., Bucher, B., Andriantsitohaina, R., 2001. Wine polyphenols decrease blood pressure, improve NO vasodilatation, and induce gene expression. Hypertension 38, 159–165.

Duarte, J., Perez-Palencia, R., Vargas, F., Ocete, M.A., Perez-Vizcaino, F., Zarzuelo, A., Tamargo, J., 2001. Antihypertensive effects of the flavonoid quercetin in spontaneously hypertensive rats. Br. J. Pharmacol. 133, 117–124.

Dusting, G.J., 1995. Nitric oxide in cardiovascular disorders. J. Vasc. Res. 32, 143–161.

Fitzpatrick, D.F., Hirschfield, S.L., Coffey, R.G., 1993. Endothelium-dependent vasorelaxing activity of wine and other grape products. Am. J. Physiol. 265, H774–H778.

Flesch, M., Schwarz, A., Bohm, M., 1998. Effects of red and white wine on endothelium-dependent vasorelaxation of rat aorta and human coronary arteries. Am. J. Physiol. 275, H1183–H1190.

Geleijnse, J.M., Launer, L.J., Van der Kuip, D.A., Hofman, A., Witteman, J.C., 2002. Inverse association of tea and flavonoid intakes with incident myocardial infarction: the Rotterdam Study. Am. J. Clin. Nutr. 75, 880–886.

Herrera, M.D., Zarzuelo, A., Jimenez, J., Marhuenda, E., Duarte, J., 1996. Effects of flavonoids on rat aortic smooth muscle contractility: structure–activity relationships. Gen. Pharmacol. 27, 273–277.

Hertog, M.G., Feskens, E.J., Hollman, P.C., Katan, M.B., Kromhout, D., 1993. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet 342, 1007–1011.

Hollman, P.C., van Trijp, J.M., Buysman, M.N., van der Gaag, M.S., Mengelers, M.J., de Vries, J.H., Katan, M.B., 1997. Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. FEBS Lett. 418, 152–156.

Knekt, P., Jarvinen, R., Reunanen, A., Maatela, J., 1996. Flavonoid intake and coronary mortality in Finland: a cohort study. BMJ 312, 478–481.

Lampe, J.W., 2003. Isoflavonoid and lignan phytoestrogens as dietary biomarkers. J. Nutr. 133 (Suppl. 3), 956S–964S.

Leikert, J.F., Rathel, T.R., Wohlfart, P., Cheynier, V., Vollmar, A.M., Dirsch, V.M., 2002. Red wine polyphenols enhance endothelial nitric oxide synthase expression and subsequent nitric oxide release from endothelial cells. Circulation 106, 1614–1617.

Liew, R., Williams, J.K., Collins, P., MacLeod, K.T., 2003. Soy-derived isoflavones exert opposing actions on guinea pig ventricular myocytes. J. Pharmacol. Exp. Ther. 304, 985–993.

Middleton Jr., E., Kandaswami, C., Theoharides, T.C., 2000. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol. Rev. 52, 673–751.

Nijveldt, R.J., van Nood, E., van Hoorn, D.E., Boelens, P.G., van Norren, K., van Leeuwen, P.A., 2001. Flavonoids: a review of probable mechanisms of action and potential applications. Am. J. Clin. Nutr. 74, 418–425.

Perez-Vizcaino, F., Ibarra, M., Cogolludo, A.L., Duarte, J., Zaragoza-Arnaez, F., Moreno, L., Lopez-Lopez, G., Tamargo, J., 2002. Endothelium-independent vasodilator effects of the flavonoid quercetin and its methylated metabolites in rat conductance and resistance arteries. J. Pharmacol. Exp. Ther. 302, 66–72.

Rendig, S.V., Symons, J.D., Longhurst, J.C., Amsterdam, E.A., 2001. Effects of red wine, alcohol, and quercetin on coronary resistance and conductance arteries. J. Cardiovasc. Pharmacol. 38, 219–227.

Taubert, D., Berkels, R., Klaus, W., Roesen, R., 2002. Nitric oxide formation and corresponding relaxation of porcine coronary arteries induced by plant phenols: essential structural features. J. Cardiovasc. Pharmacol. 40, 701–713.

Tresham, J.J., Cooper, E.J., Bednarik, J.A., Dusting, G.J., May, C.N., 1994. Prolonged regional vasoconstriction produced by N^G-nitro-L-arginine in conscious sheep. J. Cardiovasc. Pharmacol. 24, 144–150.

Vanhoutte, P.M., 1997. Endothelial dysfunction and atherosclerosis. Eur. Heart J. 18 (Suppl. E), E19–E29.

Wang, S., May, C.N., Dusting, G.J., Woodman, O.L., 2002. 3′,4′-Dihydroxyflavonol reduces infarct size and injury associated with myocardial ischemia and reperfusion in sheep. J. Am. Coll. Cardiol. 39 (Suppl. B), 11B–12B.

Yochum, L., Kushi, L.H., Meyer, K., Folsom, A.R., 1999. Dietary flavonoid intake and risk of cardiovascular disease in postmenopausal women. Am. J. Epidemiol. 149, 943–949.