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Effects of dietary oleic-rich oils (virgin olive and high-oleic-acid sunflower) on vascular reactivity in Wistar–Kyoto and spontaneously hypertensive rats

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The effects of two monounsaturated fatty acid (MUFA)-rich diets, containing virgin olive oil (OO) and high-oleic-acid sunflower oil (HOSO), on development of vascular response from isolated thoracic rat aorta and lipid composition and fatty acid composition were studied and compared with samples from rats fed on a control diet. Dietary MUFA oils were fed for 6 weeks to spontaneously hypertensive (SHR) and Wistar–Kyoto (WKY) rats from 4 weeks of age. The maximum contraction of aortic ring preparations in response to phenylephrine (10^{-6} M) was significantly decreased in SHR rats fed with OO (0·81 (SEM 0·05) *v.* 1·18 (SEM 0·09) ***g***, $P < 0·01$) and treatment with HOSO did not alter the phenylephrine-induced contractions. The relaxant responses to acetylcholine (10^{-5} M) were significantly enhanced (30·03 (SEM 0·70) *v.* 18·47 (SEM 0·28) %, $P < 0·001$) in the rings from SHR rats treated with OO, and were more pronounced than in WKY rats ($P < 0·05$). In the same way, OO attenuated the dose–response curves induced by phenylephrine (10^{-8}–10^{-5} M) from SHR rats, accompained with a slower contraction. These results suggest that only the chronic feeding of OO diet was able to attenuate the vascular response of rat aorta. In addition, an increase in phospholipid content (186·7 (SD 3·2) *v.* 159·1 (SD 11·3) g/kg, $P < 0·01$) and changes in the fatty acid composition of aorta (mainly a decrease in arachidonic acid) could contribute to improving endothelial function. Therefore, the effects can not be attributed exclusively to the content of MUFA (mainly oleic acid). Other components of OO, such as polyphenols, not present in HOSO, may help to explain the vascular protective effect of OO consumption.

Olive oil: High-oleic-acid sunflower oil: Vascular reactivity: Rat aorta

Hypertension is characterized by an increased sensitivity of vascular smooth muscle to vasoconstrictor stimuli (Triggle & Laher, 1985; Bohr & Webb, 1988). Hyperactivity to vasoactive agonists has been correlated with an enhanced protein kinase C activity (Turla & Webb, 1987; Silver *et al.* 1992; Bazan *et al.* 1993), an augmented phosphoinositide metabolism (Turla & Webb, 1990), and an increased Ca^{2+} mobilisation (Thorin-Trescases *et al.* 1994). The endothelium plays an important role in controlling the α-agonist-induced contraction and the reduced influence of the endothelium in the stroke-prone spontaneously hypertensive (SHR) rats aorta is most likely to be due to a decreased activity of this endothelium (Osugi *et al.* 1990). The changes in endothelium-dependent relaxation may also affect the contractile response of blood vessels and, as a consequence, may be involved in controlling blood pressure (Shirasaki *et al.* 1988; Tesfamariam & Halpern, 1988). In fact, the vascular endothelial function has been shown to be impaired in blood vessels of hypertensive animal models (Sunano *et al.* 1989), as well as in patients with essential hypertension (Egashira *et al.* 1995; Taddei *et al.* 1995).

The Mediterranean region has a very low prevalence of cardiovascular disease. The life expectation in Mediterranean countries is currently greater than that in countries of northern Europe and in the USA. However, the mechanisms underlying the cardiovascular benefits of Mediterranean-style diets and the effect on endothelial dysfunction are not fully understood (Massaro *et al.* 1999; Tsimikas *et al.* 1999). The diet consumed by these populations have a common characteristic, namely the high proportion of olive oil (OO;

Abbreviations: HOSO, high-oleic-acid sunflower oil; MUFA, monounsaturated fatty acids; OO, olive oil.

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rich in monounsaturated fatty acids (MUFA), mainly oleic acid), whereas the northern European diet includes a large proportion of saturated animal fats (Keys, 1995).

Despite OO being the most common highly monounsaturated oil used for nutritional studies to evaluate the effects of MUFA-enriched diets (mainly oleic acid), other sources of dietary MUFA (such as high-oleic-acid sunflower oil; HOSO) are now becoming available. However, there is virtually no information concerning their effects on endothelial function. It is of interest that the beneficial effects of dietary OO are not found to be the same as for the HOSO-rich diet, despite the fact that both vegetable oils provide a similar concentration of MUFA (Ruiz-Gutiérrez *et al.* 1996, 1999). Therefore, the wealth of minor components (including various polyphenols, such as oleuropein) contained in the unsaponificable fraction of virgin OO (Monteodoro *et al.* 1992) and not present in HOSO, appears to be the key to the beneficial effects of virgin OO on cardiovascular events (Visioli & Galli, 1994, 1995).

The present work has been designed to compare the effects of two diets rich in oleic acid (from virgin OO and from refined HOSO) and a control diet, on the vascular reactivity of Wistar–Kyoto (WKY) rats and SHR rats, in order to provide the first evidence of the effects and mechanisms of action of both oils on endothelial dysfunction present in blood vessels of the hypertensive animal model. In addition, we evaluated the effects of both MUFA diets on lipid and fatty acid composition phospholipids of the vascular wall, in order to document further the beneficial effects on endothelial function.

Methods

Animals and composition of experimental diets

Male SHR and WKY rats were obtained at the age of 4 weeks from Harlan Interfauna Ibérica, S.A. (Barcelona, Spain). They were housed at 24 ± 2°C with 60 ± 20 % relative humidity, with a 12 h light–dark cycle. All experiments were performed on 10–12-week-old rats.

Both SHR and WKY rats were divided into three groups of six animals each. Each group was fed on one of the following diets for 6 consecutive weeks: a semisynthetic diet (basal diet) with 20 g unspecified lipid/kg, purchased from Panlab SRL (Barcelona, Spain) (control group) or basal diet modified by supplemention with 100 g virgin OO (*Olea europea*)/kg (OO group) or 100 g HOSO (*Helianthus annus*)/kg (HOSO group). The composition of the experimental diets is shown in Table 1. To minimise oxidation, all diets were prepared once per week and stored at 4°C under an atmosphere of N_2 until needed. Changes in composition were not detected during storage.

The fatty acid composition of the oils was analysed and is shown in Table 2. The non-fatty acid components of the oils are presented in Table 3.

Pharmacological measurements

The experiments were performed on the thoracic aorta of male SHR and normotensive WKY rats. After 6 weeks of

Table 1. Composition of experimental diets (g/kg)

Ingredients	Control	OO	HOSO
Casein	209	203	203
Sucrose	450	374	374
Cornstarch	202	180	180
Lipid	20	20	20
OO	–	100	–
HOSO	–	–	100
Cellulose powder	52	56	56
Mineral mix*	57	57	57
Vitamin mix†	10	10	10
Total energy (MJ)	15·2	17·1	17·1
Protein (% energy)	23·1	19·7	19·7
Lipid (% energy)	4·9	26·3	26·3
Carbohydrate (% energy)	71·9	53·9	53·9

OO, olive oil; HOSO, high-oleic-acid sunflower oil.

* Mineral mix (g/kg): NaCl 139·3, K_2HPO_4 386·1, $CaCO_3$ 381·4, Mg $SO_4.7H_2O$ 57·3, $FeSO_4.7H_2O$ 27·0, $MnSO_4.H_2O$ 4·0, $ZnSO_4.7H_2O$ 1·25, KI 0·8, $CuSO_4.5H_2O$ 0·5, $CoCl_2.6H_2O$ 0·02.

† Vitamin mix (/kg): retinol 5·9 mg, cholecalciferol 0·15 mg, thiamin 20 mg, riboflavin 15 mg, nicotinic acid 70 mg, pyridoxine 10 mg, inositol 150 mg, cyanocobalamin 50 μg, α-tocopherol 170 mg, phylloquinone 40 mg, choline 1·36 g, folic acid 5 mg, *p*-aminobenzoic acid 50 mg, biotin 0·3 mg.

Table 2. Fatty acid composition (g/kg total fat) of the experimental oils

Fatty acid	Control	OO	HOSO
16:0	132	118	43
16:1 *n*-9	12	9	1
17:0	–	4	1
18:0	31	28	47
18:1 *n*-9	368	792	802
18:2 *n*-6	417	35	94
18:3 *n*-3	41	6	1
20:0	–	3	4
20:1 *n*-9)	–	2	2
24:0	–	4	4

OO, olive oil; HOSO, high-oleic-acid sunflower oil.

Table 3. Composition of non-fatty acid components of the experimental oils

Non-fatty acid	OO	HOSO
Total unsaponifiable fraction (g/kg)	15	10
Total sterols (mg/kg)	1357	1534
Cholesterol (g/kg)	1·7	0·1
Brasicasterol (g/kg)	–	1·2
Campesterol (g/kg)	3·8	102·5
Stigmasterol (g/kg)	8·1	115·9
β-Sitosterol (g/kg)	939·9	593·5
Δ-5-Avenasterol (g/kg)	–	28·8
Δ-7-Stigmasterol (g/kg)	2·2	118·4
Δ-7-Avenasterol (g/kg)	1·5	32·7
Squalene (mg/kg)	3000	90
Tocopherols (mg/kg)	47	484
α-Tocopherol (mg/kg)	34	484
γ-Tocopherol (mg/kg)	13	–
Fraction of polyphenols (mg/kg)	300	–
Total polyphenols (mg/kg)	322	–
Orthodiphenol (mg/kg)	22	–

OO, olive oil; HOSO, high-oleic-acid sunflower oil.

receiving the respective diets (10-week-old animals), the rats were killed by a blow on the head and the descending thoracic aorta was rapidly dissected and placed in a modified Krebs–Henseleit solution, containing (mM): NaCl 118, KCl 4·75, $NaHCO_3$ 25, $MgSO_4$ 1·2, $CaCl_2$ 1·8, KH_2PO_4 1·2, glucose 11. After excess fat and connective tissue were removed, the aorta was cut into 2–3 mm rings. Aortic rings were mounted under the basal tension of 1 *g* in 20 ml organ baths containing modified krebs–Henseleit solution and attached to a force-displacement transducer (Pioden UF-1, Harvard, Kent, UK) to isometric contraction force as previously described (Herrera *et al.* 1996). The signal was recorded by a Powerlab® data acquisition system (AD Instruments, Castle Hill, Australia). The tissue bath was maintained at 37°C and bubbled with a O_2–CO_2 (95:5, v/v). All experiments were performed on aortic rings with endothelium. The presence of endothelium was confirmed by the occurrence of relaxations induced by acetylcholine (10^{-5} M) in rings contracted with noradrenaline (10^{-5} M). Each preparation was allowed to equilibrate for at least 90 min prior to initiation of experimental procedures and during this period the incubation media were changed every 20 min. After equilibrations, the following experiments were performed: (1) aortic rings were contracted by single submaximal concentrations of 10^{-6} M phenylephrine. When the contractile response to the agonist was stable, acetylcholine was added (10^{-5} M) in order to analyse the endothelium-dependent vasodilating response (Alvarez de Sotomayor *et al.* 1999). All results were expressed as a percentage of the maximal contraction of phenylephrine induced responses; (2) dose–response curves for phenylephrine-induced contraction in endothelium-intact preparations. The cumulative concentration–response curve was obtained with phenylephrine (10^{-8}–10^{-5} M) at 3 min intervals. Different aortic rings were used in each experiment.

Extraction and separation of lipids

The intima were carefully scraped with glass slides. Extraction of total lipids from intima scrapings was carried out following the method of Folch *et al.* (1957) in the presence of butylated hydroxytoluene as antioxidant. Tissue dissociation was achieved by homogenization in ice-cold chloroform–methanol (2:1, (v/v) containing 0·001 ml butylated hydroxytoluene/l using an UltraTurrax homogeniser (model Type TP-18-1, Ultra Turrax, Vineland, NJ, USA).

Total lipids were quantified by means of the Iatroscan TLC/FID technique (De Schrijver & Vermeulen 1991). The Iatroscan MK-5, Iatron Laboratories Inc., Tokyo, Japan was used in combination with Chromarods S Iatron Laboratories Inc., Tokyo, Japan having a precoated thin active layer of silica. Chromarods S were routinely stored in 4·5 M-H_2SO_4. Prior to use, the rods were washed with distilled water, dried at 110°C for 15 min and activated by passing through the flame of the Iatroscan detector. Total lipids or phospholipids (3 μl) were spotted on each rod using a 10 μl Hamilton syringe. To separate total lipids, rods were developed in hexane–diethyl ether–formic acid (90:10:2, by vol.). The phospholipids were resolved in two steps, starting with an initial development of rods in chloroform–methanol–acetic acid–water, (67:28:2:3, by vol.), drying at 70°C for 10 min, then a second development in hexane–diethyl ether–formic acid (90:10:2, by vol.). Rods were scanned under the following conditions: H_2 flow 175 ml/min, air flow 1850 ml/min, scanning speed 47 mm/s, chart speed 42 mm/min. An Iatrocorder TC-11 integrator, New Technology System, Rome, Italy, was used for recording and area integration (Ruiz-Gutiérrez *et al.* 1995).

Fatty acid analysis

Lipids from the samples were separated by TLC on plates of silica gel 60 (Kieselgel 60 F254; Merck, Barcelona, Spain) using a solvent system of hexane–diethyl ether–acetic acid (80:20:1, by vol.). After development of the plate, the solvent was allowed to evaporate. This system separates phospholipids, cholesterol, triacylglycerol and cholesteryl esters in increasing order of Rf (distance of spot centre from start point/distance of solvent front from start point). Individual lipid zones were scraped from TLC plates and eluted from the silica gel with chloroform–methanol (2:1, v/v) according to the individual lipids. Fatty acid contents were determined by GC, as previously described (Ruiz-Gutiérrez *et al.* 1992; Muriana *et al.* 1995).

Fatty acid methyl ester analysis

Phospholipids were saponified by heating for 25 min with 5 ml of 0·2 M-sodium methylate at 120°C and heated again at 80°C for 25 min with 60 g H_2SO/l anhydrous methanol. The fatty acid methyl esters thus formed were eluted with hexane and analysed in a Hewlett-Packard 5890 series II GC (Hewlett-Packard Co., Avondale, PA, USA) equipped with a flame ionization detector and using an Omegawax 320 fused-silica capillary column (30 m × 0·32 mm i.d., 0·25 μm film), obtained from Supelco (Bellafonte, PA, USA). The initial column temperature was 200°C, which was held for 10 min, then programmed to change from 200 to 230°C at 2°C/min. Peak areas were calculated by a Hewlett-Packard 3390A recording integrator. Individual fatty acid methyl esters were identified on isothermal runs by comparison of their retention times against those of standards. Fatty acid methyl esters for which no standard was available were quantified using calibration tables of relative response ratios constructed according to C number using GC–mass spectrometry, performed on a Hewlett-Packard 5890 GC interfaced directly to an AEJ MS30/70 VG mass spectrometer (VG Analytical, Manchester, UK), using the electron impact ionization mode. The ion source temperature was maintained at 200°C, the multiplier voltage was 4·0 kV, the emision current was 100 μA and the electron energy was 70 eV. The data were processed with a VG 11/250 data system (VG Analytical).

Non-fatty acid components

For the extraction of the unsaponifiable matter, 20 g oils were saponified for 30 min with 75 ml KOH (100 g/l ethanol). The solution was put in a 5000 ml decanting funnel, 100 ml distilled water was added, and the mixture was extracted with 100 ml aliquots of hexane. The hexane

solution was evaporated to dryness in a rotary evaporator at 30°C under reduced pressure. The sterol fraction was analysed by capillary GLC (Garcia Regueiro *et al.* 1994). Tocopherols were analysed by HPLC (Kramer *et al.* 1997). For the assay of squalene, the hydrocarbon fraction was separated from the oils by column cromatography on silica gel and analysed by capillary GLC (Sulpioce & Ferezou, 1984). The polyphenol fraction was determined by capillary GLC (Arce *et al.* 1998).

Drugs

Lipid and phospholipid standards were obtained from Sigma Chemical Co, (St Louis, MO, USA). Fatty acid methyl ester/standards were obtained from Larodan Fine Chemicals (Malmo, Sweden). The internal standard solution was prepared by dissolving 200 mg tricosanoic acid methyl ester (23:0) in 100 ml hexane. The calibration solutions were prepared by dissolving known amounts of fatty acid methyl ester standards in hexane containing butylated hydroxy-toluene (2,6-di-*tert*-butyl-*p*-cresol) obtained from Sigma (Poole, Dorset, UK).

For pharmacological experiments, the following drugs were used: noradrenaline bitartrate, acetylcholine chloride and phenylephrine chloride, all obtained from Sigma Chemical Co. The drugs were dissolved in distilled water.

Statistical analysis

Results are expressed as mean values with their standard errors (pharmacological experiments) or mean values and standard deviations (lipid and fatty acid composition) of preparations obtained from at least six animals per group. One-way ANOVA followed by Bonferroni tests were used for statistical analysis. P values $< 0{\cdot}05$ were considered as showing a significant difference. The analyses were performed with the GraphPad Prism® statistical package (version 2.01, 1996; Graphpad Software Inc., San Diego, USA).

Results

Effects of virgin olive oil and high-oleic-acid sunflower oil on vascular reactivity in Wistar–Kyoto and spontaneously hypertensive rats

During contractions caused by phenylephrine (10^{-6} M) for 15 min, addition of acetylcholine (10^{-5} M) evoked endothelium-dependent relaxation in aortic ring of WKY and SHR rats (Fig. 1).

The maximum phasic contraction in response to phenylephrine (10^{-6} M) was greater ($P < 0{\cdot}01$) in aortic rings from SHR (1·18 (SEM 0·09) ***g***) than from WKY (0·88 (SEM 0·14) ***g***) rats. This contraction was significantly decreased in aorta from SHR rats fed with OO (0·81 (SEM 0·05), ***g***, $P < 0{\cdot}01$) but not in those from WKY rats (0·89

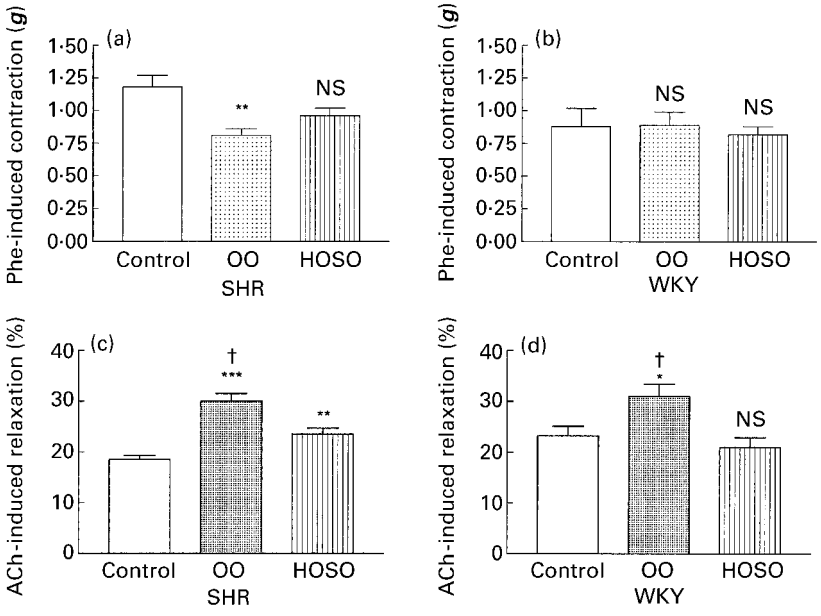

Fig. 1. Evaluation of maximum vascular responses for phenylephrine (Phe)-induced contraction (***g***) (a, b) and maximum vascular responses for acetylcholine (ACh)-induced relaxation (%) (c, d) in rat isolated aorta preparations obtained from control rats, and from those receiving an olive oil (OO)- or a high-oleic-acid sunflower (HOSO)-enriched diet. SHR, Spontaneously hypertensive rats; WKY, Wistar–Kyoto rats. For details of diets and procedures, see Tables 1–3 and p. 350. Values are means for six rats per group with their standard errors represented by vertical bars. Mean values were significantly different from those of the control group: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$. Mean value was significantly different from that of the HOSO diet group; † $P < 0{\cdot}01$.

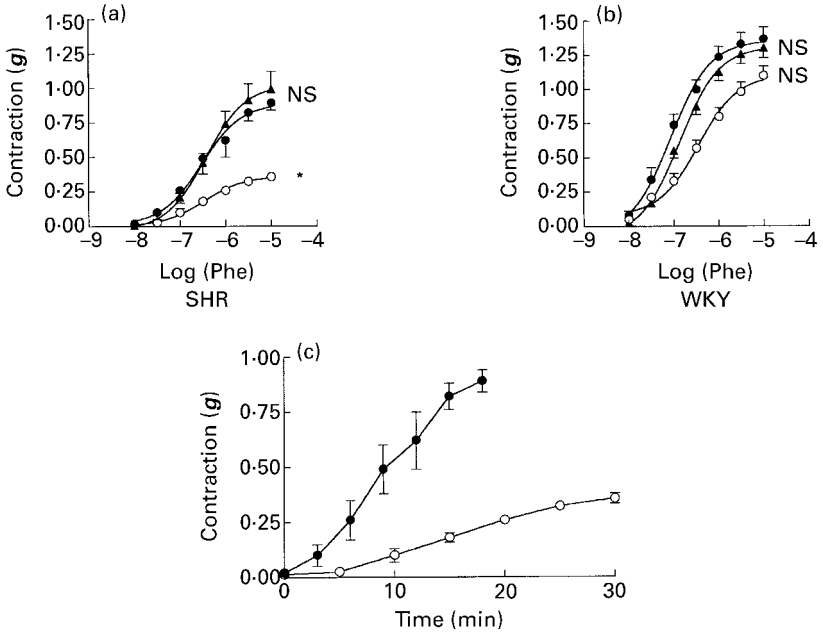

Fig. 2. Dose–response curves for phenylephrine (Phe)-induced contractions (***g***) in rat isolated aorta preparations obtained from control rats (●), and from those receiving an olive oil (OO)- (○) or a high-oliec-acid sunflower (HOSO)-enriched diet (▲) (a, b) and mean time course (min) of Phe-induced contractions (c). Note the difference in the time course in rat isolated aorta preparations from control rats and those receiving the OO diet ((c), ● *v.* ○). SHR, spontaneously hypertensive rats; WKY, Wistar–Kyoto rats. For details of diets and procedures, see Tables 1–3 and p. 350. Values are means for six experiments with their standard errors represented by vertical bars. Mean value was significantly different from that of the control group: * $P < 0{\cdot}01$.

(SEM 0·10) ***g***, NS.). The treatment with HOSO did not alter the phenylephrine-induced contractions (0·96 (SEM 0·06) ***g*** for SHR and 0·82 (SEM 0·06) ***g*** for WKY rats) (Fig. 1).

The relaxant responses to acetylcholine (10^{-5} M) were significantly enhanced by treatment with OO in both SHR (30·03 (SEM 0·70), *v.* 18·47 (SEM 0·28) %, $P < 0{\cdot}001$) and WKY (30·97 (SEM 2·40), *v.* 23·23 (SEM 1·9) %, $P < 0{\cdot}05$) rings and the increase of relaxant responses were significantly weaker in the rings from SHR rats (23·47 (SEM 0·36) %, $P < 0{\cdot}01$) treated with HOSO ($P < 0{\cdot}01$ *v.* OO treatment) (Fig. 1).

Dose-response curves for phenylephrine-induced contractions (10^{-8}–10^{-5} M) in endothelium-intact preparations from SHR and WKY rat isolated aorta were used as an indicator of Ca^{2+} release through the inositol 1,4,5-triphosphate receptor pathway. Phenylephrine evoked similar concentration–contraction curves in the SHR control rings and in the SHR rings from rats treated with HOSO, whereas treatment with OO produced a pronounced decrease of the contraction of aortic ring in response to phenylephrine affecting the attenuation of the maximal response (Fig. 2). Moreover, the time course of the contraction of this last preparation was slower, needing more time to become stable (Fig. 2)

Diets

In order to document the influence of the two experimental oils on distinct behaviour of the contractile and relaxant responses of thoracic aortas, we examined the composition of two oils exhaustively.

The fatty acid composition of OO and HOSO was characterized by a high content of MUFA, mainly oleic acid (about 800 g/kg total fats) (Table 2). The major differences were greater concentrations of palmitic acid (16:0) in OO and linoleic acid (18:2) in HOSO. In relation to composition in non-fatty acid components (minor components), significant differences ($P < 0{\cdot}001$) were noted for tocopherols, squalene and polyphenols between the experimental oils (Table 3). The concentration of tocopherols was greater (484 mg/kg) in HOSO than in OO (47 mg/kg) unlike squalene content, which was 3000 mg/kg OO and 90 mg/kg HOSO. However, polyphenols were only found in OO.

Effects of virgin olive oil and high-oleic-acid sunflower oil on the lipid composition of intima aorta from Wistar–Kyoto and spontaneously hypertensive rats

Table 4 shows the effects of experimental diets on lipid composition of intima aorta from WKY and SHR rats.

The content of membrane phospholipid showed reduced in animal model of hypertension (SHR) (159·1 (SD 11·3) g/kg, $P < 0{\cdot}001$) compared with non-hypertensive animals (WKY) (195·6 (SD 6·0) g/kg). Phospholipid concentration was increased significantly in SHR rats fed on the OO diet (186·7 (SD 3·2) g/kg, $P < 0{\cdot}01$) approaching the values of control WKY rats. However, the HOSO diet led to a significant decrease in both groups of animals ($P < 0{\cdot}001$ *v.* control group). The total cholesterol level remained

Table 4. Lipid composition and distribution (% w/w) of the major phospholipid of intima aorta from normotensive (Wistar–Kyoto; WKY) and spontaneously hypertensive (SHR) rats fed experimental diets‡

(Mean values and standard deviations for six rats per group)

Diet…	Control				OO				HOSO			
	WKY		SHR		WKY		SHR		WKY		SHR	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PL	19·56	0·60	15·91†††	1·13	21·40	1·59	18·67**††	0·32	16·56***	2·13	12·09***†††	0·59
PE	24·84	1·82	14·7†††	1·22	20·41*	3·5	11·2†††	3·5	21·32	0·3	11·2†††	0·3
PS	1·9	0·21	1·5	0·21	2·03	0·7	1·5	0·9	2·23	0·32	1·6	0·3
PC	27·5	6·2	27·5	6·2	27·41	1·3	27·41	1·3	28·02	4·4	27·02	4·4
SM	45·23	6·4	55·23††	5·4	49·56	3·8	59·56††	3·8	48·04	2·7	60†††	0·7
LysoPE	0·53	0·2	0·63	0·2	0·55	0·3	0·32	0·3	0·49	0·3	0·42	0·3
CHOL	1·28	0·32	1·82	0·39	1·80	0·55	1·12*	0·36	1·02	0·28	1·27	0·29
TG	79·00	1·96	81·64	3·23	76·74	0·00	79·72	0·22	82·30*	1·98	86·56**††	0·33
CE	0·16	0·09	0·58††	0·03	0·16	0·06	0·52†	0·12	0·50*	0·41	0·11**	0·01

OO, olive oil; HOSO, high-oleic-acid sunflower oil; PL, phospholipid; PE, phosphatidyl ethanolamine; PS, phosphatidyl serine; PC, phosphatidyl choline; SM, sphingomyelin; LysoPE, lysophosphatidyl ethanolamine; CHOL, free cholesterol; TG, triacylglycerol; CE, cholesteryl ester.
Mean values were significantly different from those of the control group: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
Mean values were significantly different from those of the WKY group † $P < 0·05$, †† $P < 0·01$, ††† $P < 0·001$.
‡ For details of diets and procedures, see Tables 1–3 and p. 350.

practically unaltered in HOSO- and OO-fed groups, except for SHR treated with OO, for which the value was significantly less than the control value (11·2 (SD 3·6) *v.* 18·2 (SD 3·9) g/kg, $P < 0·05$) and triacylglycerol levels increased with HOSO treatment (823·0 (SD 19·8), $P < 0·05$ for WKY and 865·6 (SD 3·3) g/kg, $P < 0·01$ for SHR rats). The HOSO-fed group showed a significant decrease in the concentration of membrane esterified cholesterol in SHR rats (1·1 (SD 0·1) *v.* 5·8 (SD 0·3) g/kg, $P < 0·01$).

To determine the phospholipid composition of intimal aorta, the major phospholipid species were quantified and the results of the analyses are given in Table 4. In this table, we can observe how the major phospholipid species is sphingomyelin (SHR values greater than those from WKY, $P < 0·01$), followed by phosphatidyl choline, phosphatidly ethanolamine (WKY values greater than those from SHR, $P < 0·001$), phosphatidyl serine and lisophosphatidyl ethanolamine (these latter two had very low values).

Effects of virgin olive oil and high-oleic-acid sunflower oil on the phospholipid fatty acid composition of intima aorta from Wistar–Kyoto and spontaneously hypertensive rats

Table 5 shows the proportions of fatty acids (g/100 g total fatty acids) in phospholipids of intima aorta in rats fed on the different diets. In the present study, the most significant

Table 5. Fatty acid composition (g/100 g total fatty acids) of phospholipids of intima aorta from normotensive (Wistar–Kyoto; WKY) and spontaneously hypertensive (SHR) rats fed experimental diets‡

(Mean values and standard deviations for six rats per group)

Diet…	Control				OO				HOSO			
	WKY		SHR		WKY		SHR		WKY		SHR	
Fatty acid	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
16:0	12·99	2·65	18·03†††	0·09	13·92	0·86	15·80	0·86	11·62	1·49	16·15†††	1·49
16:1 *n*-9	2·81	0·77	2·39	0·02	3·28	0·19	1·47**†††	0·19	1·63***	0·17	1·10***	0·17
18:0	22·76	3·76	22·05	1·32	21·54	1·50	17·81	1·50	22·88	2·69	22·69	2·69
18:1 *n*-9	17·95	2·33	17·53	1·66	27·59***	0·42	32·58***†††	0·42	22·87***	0·07	22·34***	0·07
18:1 *n*-7	5·85	1·22	3·42†††	0·01	3·89***	0·01	3·82	0·42	3·93***	0·00	2·72††	0·08
18:2 *n*-6	13·91	1·35	13·45	0·01	8·16***	0·10	9·05***	0·10	13·96	0·03	10·24****†††	0·03
20:0	0·38	0·01	0·1†††	0·01	0·38	0·03	0·14†††	0·03	0·39	0·02	0·85***†††	0·04
20:3 *n*-6	2·07	0·63	1·07†	0·63	1·03*	0·01	2·00*	0·63	0·38***	0·44	0·32	0·04
20:4 *n*-6	18·33	2·86	17·39	1·09	18·49	1·60	13·68***†††	0·01	20·12	0·05	19·22*	0·05
22:5 *n*-3	1·04	0·27	0·69†††	0·02	0·68***	0·01	0·6	0·02	1·02	0·01	1·61***†††	0·01
22:6 *n*-3	1·44	0·33	1·70	0·58	1·10	0·33	0·78	0·33	1·20	0·78	1·45	0·78
SFA	36·13	6·42	40·18	1·42	35·84	2·39	33·75**	2·39	34·89	4·2	39·69	4·22
MUFA	26·61	4·32	23·34	1·69	34·76***	0·62	37·87***	1·03	28·43	0·24	26·16	0·32
PUFA	36·79	5·44	34·30	2·33	29·46***	2·05	26·11**	0·46	36·68	1·31	32·84	0·91

OO, olive oil; HOSO, high-oleic-acid sunflower oil; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
Mean values were significantly different from those of the control group * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
Mean values were significantly different from those of the WKY group: † $P < 0·05$, †† $P < 0·01$, ††† $P < 0·001$.
‡ For details of diets and procedures, see Tables 1–3 and p. 350.

effects in the hypertensive state were on 16:0, 18:1 *n*-9, 20:0 and 22:5 *n*-3 content. A reduction in palmitic acid and an increase in oleic acid, eicosanoic acid and eicosapentanoic acid in hypertensive animals in relation to control animals (WKY). In the hypertensive and normotensive groups, both diets increased the content in the rat aorta of oleic acid (18:1 *n*-9), whereas the rise with the OO diet was more significant. A significant decrease in palmitoleic (16:1) and linoleic acid (18:2) was also evident in SHR rats compared with baseline. On the other hand, 20:4 *n*-6 was reduced by 25 % only in hypertensive animals fed the OO diet. This reduction was significant with respect to both WKY control and WKY fed OO. The differences between the two MUFA diets in the study were a decrease in total saturated fatty acids and polyunsaturated fatty acids in SHR group fed on the OO diet. However, the total MUFA increased in both WKY and SHR rats treated with this diet.

Discussion

Two clear trends emerge from the present study. First, it is apparent that the oil OO diet attenuates phenylephrine-induced contractions, and reduced vascular reactivity, which would contribute to decreasing vascular tone. In addition, an increase in phospholipid content and changes in fatty acid composition of aorta could be the reason for the improvement of endothelial function.

The vascular smooth muscle in genetic hypertension is characterised by modifications in the signal-transduction system (Thorin-Trescases *et al.* 1994) and this is accounted for by medial hypertrophy (Sudhir & Angus, 1990). Phasic contractions of rat aorta induced by phenylephrine were used as an indicator of intracellular Ca^{2+} release through the inositol 1,4,5-triphosphate receptor pathway. NO selectively inhibits intracellular Ca^{2+} release stimulated by inositol 1,4,5-triphosphate in vascular smooth muscle (Ji *et al.* 1998). NO has been extensively shown to be a potent vasodilator produced by vascular endothelial cells and NO release is triggered by pharmacologically vasoactive substances such as acetylcholine (Moncada *et al.* 1991; Moncada & Higgs, 1993). The intracellular NO in smooth muscle cells involves the generation of cGMP (Rapoport & Murad, 1983; Moncada *et al.* 1992), a decrease in Ca mobilization (Twort & van Breemen, 1988; Komori & Bolton, 1989) and inhibition of phosphoinositol breakdown (Hirata *et al.* 1990; Ruth *et al.* 1993). NO plays an active role in the balance between the contraction and dilation of vascular smooth muscle (Pérez-Guerrero *et al.* 2000).

In the present study we describe the important role of endothelium in controlling the phenylephrine-induced contraction, as the greater relaxation response to acetylcholine is in relation to the weaker response to phenylephrine, in both preparations from SHR fed with the OO diet, indicating an improvement of endothelial function of this hypertensive model. It is also clear that the improvement of the endothelium plays an important role in this reduction, inhibiting intracellular Ca^{2+} release by inositol 1,4,5-triphosphate pathway. The time course of the contraction by phenylephrine was also affected, especially in the preparation from SHR treated with OO, the contraction becoming slower than in SHR control, as well as having a smaller response.

It is known that the degree of unsaturation of dietary oil influences the fatty acid composition of cells, fundamentally in the phospholipids of the cell membrane (Ruiz-Gutiérrez *et al.* 1990, 1998), and OO feeding leads to changes in lipid metabolism of the vascular compartment that could be favourable in the prevention of thrombosis and atherosclerosis (Navarro *et al.* 1992). We wanted to verify that the same changes occurred with regard to endothelial function. We can prove, after analysis of lipid composition, that the content of membrane phospholipid, which was found to be reduced significantly ($P < 0{\cdot}001$) by about 20 % in the animal model of hypertension (SHR) compared with non-hypertensive animals (WKY), was increased significantly ($P < 0{\cdot}01$) in SHR rats fed on the OO diet approaching the values to those of WKY rats. However, the HOSO diet led to a significant decrease ($P < 0{\cdot}001$). It is well known that phospholipids, in many cases, are the main components of the cell membranes in a living body. Defects in phospholipid synthesis, besides affecting the absolute amounts of phospholipids per cell, lead to changes in the membrane structure and consequently the function. Except for a few diseases, the information available is very scanty. In relation to the cardiovascular system, phospholipid changes have been observed in blood plasma or serum of patients with heart disease (Cevc, 1993). The plasma levels of total phospholipids are decreased compared with those of normal subjects. Similarly, a decrease in phospholipids content was seen in the present study in hypertensive animals.

Analysis of the fatty acid composition in rat aorta showed a significant increase ($P < 0{\cdot}001$) of 18:1 in aorta lipids obtained from OO- and HOSO-fed rats and lower saturated fatty acid levels in SHR fed with OO. This increase in oleic acid content was probably related to the higher content of oleic acid in the diet of these animals. It has been reported that when endothelial cell cultures are directly supplemented with oleic acid, an increase in its content is found, accompained by a decrease in the saturated acid content (Spector & Yorek, 1985). Conversely, a lower concentration of arachidonic (20:4) was detected in the same group of rats. It is apparent that the OO diet suppressed the formation of arachidonic acid in aortic smooth muscle phospholipids from SHR rats.

In addition, phospholipids can have a role as diagnostic markers. In general, such diagnostic procedures can be used for detecting changes in part of the phospholipids, usually the fatty-acid chain (Badley *et al.* 1993). To be exact, when saturated and unsaturated fatty acids of phospholipids have been examined in relation to heart disease (angina, heart attack), arachidonic acid is significantly raised, implying important links with prostaglandin activity and metabolism (Skulladottir *et al.* 1985).

Therefore, the changes caused by the OO diet, i.e. the content of total membrane aorta phospholipids (elevation) and the content of arachidonic acid (decrease), may help to explain the vascular protective effect of OO consumption. The effects cannot be attributed exclusively to the content of MUFA (mainly oleic acid) in the diet, as high-oleic-sunflower oil was unable to induce favourable changes.

Consequently, other components of OO, like polyphenols, not present in HOSO, may be responsible for the beneficial effects on the cardiovascular system. There are references to the protector effect of polyphenols (from red wine) on endothelial dysfunction in human subjects (Cuevas *et al.* 2000). These polyphenols produce endothelium-dependent relaxation as a result of enhanced NO synthesis rather than enhanced biological activity of NO or protection against breakdown by O_2^- (Andriambeloson *et al.* 1997; Andriantsitohaina 1999).

We conclude that HOSO and OO, providing a similar concentration of MUFA, have different effects on vascular reactivity of isolated rat aorta and lipid composition and fatty acid composition of phospholipids of intima aorta. Only the chronic feeding of the OO diet was able to attenuate vascular response of rat aorta, probably through an improvement of endothelial function.

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