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Effects of Oleic Acid Rich Oils on Aorta Lipids and Lipoprotein Lipase Activity of Spontaneously Hypertensive Rats

JAVIER S. PERONA, ROSALÍA RODRÍGUEZ-RODRÍGUEZ, AND VALENTINA RUIZ-GUTIERREZ*

Instituto de la Grasa (CSIC), Av. Padre García Tejero, 4, 41012, Seville, Spain

Hypertension development in the spontaneously hypertensive rat (SHR) leads to vascular wall widening by smooth muscle cell proliferation. In these cells, triglycerides (TG) and cholesteryl esters (CE) can accumulate until they become foam cells. We administrated two oleic rich oils, virgin olive (VOO) and high oleic sunflower oils (HOSO), to Wistar−Kyoto rats (WKY) and SHR because these oils have been reported to reduce the risk for coronary heart disease in hypertensive patients and SHR. After 12 weeks of feeding, we analyzed the TG and CE composition and the lipolytic (lipoprotein lipase, LPL, and non-LPL) activity in aortas of these animals. HOSO increased the content of linoleic acid in CE and TG of aortas from both WKY and SHR as compared with animals fed VOO by proportionally decreasing the content of oleic acid. Conversely, VOO reduced the LPL and non-LPL lipolytic activities, hence limiting the free fatty acids available for the synthesis of TG and CE in the vascular wall.

KEYWORDS: SHR; aorta; triglyceride; cholesteryl ester; LPL

INTRODUCTION

Hypertension is considered the most important risk factor for coronary heart disease morbidity and mortality (*1*). This pathology is always accompanied by vascular remodeling, which is an adaptive process of blood vessels to the changes of hemodynamics, whose ultimate effect tends to be maintenance of the constancy of tensile and/or shear stresses. Actually, blood flow-induced laminar shear stress has emerged as an atheroprotective feature that inhibits proliferation thrombosis and inflammation of the vessel wall (*2*). Decreased aortic adaptation to the effects of hypertension is an important factor leading to myocardial hypertrophy, which is an independent risk factor for ischemia and heart failure (*3*).

The spontaneously hypertensive rat (SHR) is a model of chronic essential hypertension, in which left ventricular hypertrophy and increased proliferation of smooth muscle cells in aorta are present (*4*, *5*). These animals have been shown to bind monocytes in thoracic aorta with greater avidity than their corresponding control Wistar−Kyoto rats (WKY), which was concomitant to development of hypertriglcyeridemia (*6*). Additionally, it has been reported that lipoprotein lipase (LPL) activity is decreased in skeletal muscle of SHR (*7*).

LPL is a triacylglycerol hydrolase found at the capillary endothelium that hydrolyzes fatty acids from circulating triglyceride (TG)-containing lipoproteins, thereby serving as the major provider of fatty acids to peripheral tissues (*8*). LPL and endothelial lipase (EL) are the only lipolytic enzymes acting in the endothelial surface of blood vessels, producing free fatty acids from circulating lipoproteins (*9*). In contrast to LPL, which is synthesized in muscle cells and transferred to the endothelium (*10*), EL is expressed by endothelial cells (*11*). In addition, whereas LPL has preference for TGs, EL is a phospholipase with little TG activity (*12*−*14*). Free fatty acids released from circulating lipoproteins by these enzymes can be used as energy sources for cells or substrates for lipid biosynthesis (*15*). However, free fatty acids are also a factor for injury to arterial wall cells (*16*, *17*). They can induce foam cell formation in macrophages (*18*) and TG accumulation in smooth muscle cells (*19*). The other lypolitic enzyme acting in blood vessels is the lysosomal acid lipase (LAL), also called cholesterol ester hydrolase, which has its activity in the lysosomes of endothelial and smooth muscle cells (*20*, *21*).

Nutritional supplementation for the treatment of hypertension has acquired an important relevance (*22*, *23*). Monounsaturated fatty acid (MUFA) rich oils consumption has been one of the recommended strategies for modulating the plasma lipid profile in humans (*24*). Two sources of MUFA, virgin olive oil (VOO) and high oleic sunflower oil (HOSO), have been suggested to reduce the risk for coronary heart disease by providing a similar effect on the atherogenic index of normocholesterolemic and hypercholesterolemic hypertensive patients (*25*). However, there is evidence that the two MUFA-enriched diets have selective physiological effects. VOO, but not HOSO, has been shown to reduce blood pressure in healthy and hypertensive patients (*25*, *26*). Additionally, the consumption of VOO or HOSO provides a different TG composition in the liver (*27*) and adipose tissue (*28*) of SHR rats. However, we have recently reported that

* To whom correspondence should be addressed. Tel: 34954611550. Fax: 34954616790. E-mail: valruiz@cica.es.

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Table 1. Fatty Acid Composition of the BD and of the VOO and HOSO (%)[a]

	BD	VOO	HOSO
14:0	0.1 ± 0.0	0.1 ± 0.0	ND
14:1, n-5	0.1 ± 0.0	ND	ND
16:0	10.3 ± 1.5 a	13.5 ± 0.4 b	4.2 ± 0.1 c
16:1, n-9	0.1 ± 0.0	ND	ND
16:1, n-7	0.1 ± 0.0 a	0.5 ± 0.3 b	0.1 ± 0.0 a
17:0	ND	0.3 ± 0.1	0.1 ± 0.0
18:0	2.4 ± 0.4 a	2.3 ± 0.3 a	4.4 ± 0.2 b
18:1, n-9t	0.1 ± 0.0	ND	ND
18:1, n-9	33.0 ± 1.6 a	76.5 ± 0.6 b	79.3 ± 0.6 b
18:1, n-7	1.2 ± 0.2	ND	ND
18:2, n-6	50.9 ± 1.3 a	5.6 ± 0.7 b	10.4 ± 0.7 c
18:3, n-6	0.7 ± 0.2 a	0.7 ± 0.1 b	0.1 ± 0.0 c
18:3, n-3	0.4 ± 0.1	ND	ND
20:0	ND	0.4 ± 0.0	0.4 ± 0.0
20:1, n-9	0.2 ± 0.0 a	0.3 ± 0.1 a	0.2 ± 0.0 a
22:0	0.2 ± 0.0	ND	ND
22:1, n-9	0.1 ± 0.0	ND	ND
22:4, n-6	0.1 ± 0.0	ND	ND
22:5, n-6	0.2 ± 0.1	ND	ND
24:0	ND	0.2 ± 0.1	0.7 ± 0.2
SFA	13.0 a	16.8 b	9.8 c
MUFA	34.8 a	76.8 b	79.5 b
PUFA	52.3 a	6.3 b	10.5 c

[a] ND, not detected. Values expressed as means ± SDs. Mean values within a row sharing the same letter are not significantly different ($p < 0.05$).

neither VOO nor HOSO could ameliorate the lipid changes produced in the myocardium of SHR rats due to cardiac hypertrophy (*29*).

Therefore, the present study was conducted in order to evaluate the effect of two dietary oils, with a similar content in oleic acid but different TG moieties (VOO and HOSO) on the impairments caused by hypertension on the lipid accumulation and on the lipolytic activity of SHR rat aorta.

MATERIALS AND METHODS

Animals and Diets. WKY and SHR weighing 55.7 ± 2.3 g were purchased from Letica (Barcelona, Spain). They were randomly distributed into six groups of 12 rats, according to diet and strain, and were individually housed in a room at 21 ± 2 °C with a light/dark cycle of 12 h. Animals were fed with a low fat diet (baseline diet, BD) or BD supplemented with 15% VOO (Aceites Toledo, Los Yébenes, Sapin) or 15% HOSO (Coreysa, Osuna, Spain). Feeding was ad libitum. BD contained 20.8% milk casein, 19.6% corn starch, 37% glucose, 5.3% cellulose, 6.3% mineral mix, and 1% vitamin mix (PANLAB, Barcelona, Spain). The lipid fraction of the two fatty diets represented 36.6% of total lipid expressed as energy. The fatty acid and TG molecular species composition of the employed oils were analyzed by GC and high-performance liquid chromatography, respectively, as described below (**Tables 1** and **2**, respectively). After 12 weeks of feeding, rats were killed by decapitation and the aorta was dissected from the carcass, free of extraneous fat and connective tissue, and was perfused with saline and preserved at −80 °C until used. At this time, no significant differences were found on food consumption and weight gain among all groups. The protocol for animal handling and experimentation was approved by the Institutional Committee on Investigation in Animals (Universidad de Sevilla, Seville, Spain).

Sample Preparation. Total aortic lipids were extracted following the method described by Folch et al. (*30*). An aliquot of the lipids was separated into fractions by thin-layer chromatography (TLC) on silica gel 60 plates (Kieselgel 60 F254, Merck España, Barcelona, Spain) using an elution system of hexane/diethyl ether/acetic acid (80:20:1, v/v/v) (Merck España). The TG and EC fractions were scraped off the silica, eluted with hexane/diethyl ether (9:1, v/v), passed through a nitrogen stream, and stored at a temperature below −20 °C until analyzed.

Table 2. Triacylglycerol Molecular Species Composition of the BD and of the VOO and HOSO (%)[a]

	BD	VOO	HOSO
LLLn	1.0 ± 0.3	ND	ND
LLL	22.5 ± 3.4 a	0.1 ± 0.0 b	3.0 ± 0.7 c
OLL	23.4 ± 1.5 a	0.1 ± 0.0 b	4.0 ± 1.8 c
PLL	16.1 ± 0.6 a	0.4 ± 0.1 b	0.6 ± 0.1 b
OOL	12.0 ± 1.3 a	6.7 ± 0.2 b	8.3 ± 0.9 b
POL/PoOO	ND	4.3 ± 0.1	2.0 ± 0.2
SLL	14.0 ± 1.7 a	0.1 ± 0.0 b	ND
PPL	1.7 ± 0.5 a	0.1 ± 0.1 b	ND
OOO	4.2 ± 1.0 a	44.7 ± 1.6 b	63.8 ± 4.7 c
POO	ND	30.5 ± 1.5 a	7.8 ± 1.3 b
SOL	1.6 ± 0.4 a	2.5 ± 0.1 b	ND
PPO	ND	3.3 ± 0.2 a	0.2 ± 0.1 b
PPP	0.1 ± 0.0 a	0.1 ± 0.0 a	0.3 ± 0.1 a
SOO	0.6 ± 0.2 a	5.3 ± 0.1 b	ND
PSO	0.4 ± 0.0 a	1.0 ± 0.5 a	ND
SSO	ND	0.2 ± 0.0	ND

[a] ND, not detected. Values expressed as means ± SDs. Mean values within a row sharing the same letter are not significantly different ($p < 0.05$). Fatty acids: A, arachidonic acid (20:4); Ln, linolenic acid (18:3); L, linoleic acid (18:2); O, oleic acid (18:1); P, palmitic acid (16:0); and S, stearic acid (18:0). Triacylglycerols: LLL, trilinoleoyl-glycerol; OOP, dioleoyl-palmitoyl-glycerol; and PSO, palmitoyl-stearoyl-oleoyl-glycerol.

Fatty Acid Composition of Aortic TGs and Cholesteryl Esters (CEs). TG and EC were transmethylated, and the resulting fatty acid methyl esters (FAME) were analyzed by GC, using a model 5890 series II gas chromatograph (Hewlett-Packard Co, Avondale, United States) equipped with a flame ionization detector and a capillary silica column Supelcowax 10 (Sulpelco Co., Bellefonte, United States) of 60 m length and 0.25 mm internal diameter. Individual FAME were identified by means of comparison of their retention times with those of standards.

TG Molecular Species Composition of Rat Aorta. Neutral lipids were passed through a filter with a pore size of 0.2 μm (Waters Co., Milford, MA). The chromatographic system consisted of a model 2690 Alliance liquid chromatograph (Waters Co.), provided with a Novapack (250 mm × 4.6 mm) of 4 μm particle size (Waters Co.). The liquid chromatograph was coupled to a light scattering detector model DDL31 (Eurosep, Ins, Cergy-Pontoise, France). The system was controlled by computer through the Millenium System (Waters Co.). The mobile phase consisted of an initial elution gradient of 20% of acetone in acetonitrile raising the percentage of acetone to 45% in 12 min and then to 80% after 60 min. This percentage was held up to the end of the analysis. The flow rate was 1 mL/min. Standard solutions were of Sigma Grade (99% pure) of tritridecanoyl-glycerol, 1,3-dioleoyl-2-palmitoyl-glycerol, trimyristoyl-glycerol, 1,3-dioleoyl-2-stearoyl-glycerol, 1,3-dioleoyl-2-linoleoyl-glycerol, tripentadecanoyl-glycerol, tripalmitoyl-glycerol, trioleoyl-glycerol, and trilinoleoyl-glycerol. To calibrate the detector and to establish the capacity factor (k') of the system, tripled runs of six concentrations of these standards, between 0.25 and 2.5 mg/mL, were injected in hexane. Various regression models were tested, and finally, fourth regression curves ($r^2 \geq 0.999$) were chosen. Equations resulting from these curves were employed for quantification. When a standard for a chromatograph peak identified was not available, the curve of the standard with a closer retention time was applied. Response factors for each standard relative to closer standards in retention time were calculated, being always between 0.98 and 1.02. TG molecular species composition was calculated as described by Perona et al. (*31*).

Determination of Lipolytic Activity in Rat Aorta. The aortas were homogenized with 20 mL of precooled acetone (−20 °C), filtered, and washed with 100 mL of precooled acetone, 200 mL of acetone at room temperature, and 200 mL of diethyl ether. After it was dried, the remaining powder was frozen in liquid nitrogen and stored at −80 °C until used. Two milliliters of ammonium buffer (25 mM, pH 8.1) containing 1 U/mL of sodium heparine was added to 10 mg of the powder. The mixture was incubated in ice for 60 min and subsequently centrifuged at 5000*g* for 10 min at 4 °C. The supernantant was

Table 3. Cholesteryl-Ester Fatty Acid Composition of the Aortas of WKY and SHR Fed the BD and the Diets Enriched in VOO or HOSO (%)[a]

	WKY			SHR		
fatty acid	BD	HOSO	VOO	BD	HOSO	VOO
14:0	2.4 ± 0.9 a	0.9 ± 0.2 b	0.9 ± 0.2 b	1.9 ± 0.7 c	1.6 ± 0.4 c	1.7 ± 0.5 c
16:0	25.9 ± 1.8 a	14.6 ± 3.1 b	15.1 ± 1.7 b	24.8 ± 1.5 a	19.6 ± 1.2 b	18.4 ± 1.6 b
16:1, n-9	ND	2.6 ± 0.4 a	1.7 ± 0.3 a	5.6 ± 0.9 b	1.3 ± 0.5 a	ND
16:1, n-7	6.8 ± 0.3 a	1.0 ± 0.1 b	1.1 ± 0.6 b	5.0 ± 0.7 a	2.8 ± 0.6 c	3.3 ± 0.5 c
18:0	7.6 ± 1.3 a	7.3 ± 1.7 a	6.2 ± 0.1 b	8.0 ± 0.9 a	6.4 ± 0.4 b	5.1 ± 1.1 c
18:1, n-9	44.4 ± 2.0 a	60.7 ± 2.0 b	67.5± 2.3 b	38.4 ± 1.9 a	57.7 ± 2.3 b	64.1 ± 2.1 b
18:1, n-7	2.6 ± 0.6 a	2.8 ± 1.4 a	2.6 ± 2.1 a	4.7 ± 0.3 b	2.7 ± 0.2 a	2.4 ± 0.6 a
18:2, n-6	8.4 ± 0.4 a	6.9 ± 1.2 a	2.9 ± 0.1 b	8.6 ± 0.7 a	5.9 ± 1.1 c	3.4 ± 0.4 b
20:0	0.2 ± 0.1 a	0.6± 0.2 b	0.5± 0.1 b	0.2± 0.0 a	0.5± 0.1 b	0.3± 0.1 a
20:1, n-9	0.5± 0.1 a	1.5± 0.5 b	0.8± 0.2 b	1.1± 0.4 b	0.6± 0.2 a	0.6± 0.1 a
20:2, n-6	0.2± 0.0	0.4± 0.1	0.3± 0.1	0.2± 0.0	0.2± 0.1	0.3± 0.1
20:4, n-6	0.3± 0.0	0.6± 0.2	0.3± 0.1	0.5± 0.2	0.7± 0.3	0.3± 0.2

[a] ND, not detected. Values expressed as means ± SDs. Mean values within a row sharing the same letter are not significantly different ($p < 0.05$).

Table 4. TG Fatty Acid Composition of the Aortas of WKY and SHR Rats Fed the BD and the Diets Enriched in VOO or HOSO (%)[a]

	WKY			SHR		
fatty acid	BD	HOSO	VOO	BD	HOSO	VOO
14:0	6.3 ± 0.8 a	1.2 ± 0.4 b	1.3 ± 0.2 b	4.8 ± 0.8 c	3.7 ± 0.7 c	4.5 ± 0.4 c
16:0	30.5 ± 1.8 a	15.3 ± 3.1 b	13.9 ± 1.7 b	31.7 ± 1.5 a	17.1 ± 1.2 b	22.9 ± 1.6 c
16:1, n-9	1.8 ± 0.4	1.4 ± 0.3	1.7 ± 0.1	ND	1.4 ± 0.5	1.1 ± 0.2
16:1, n-7	5.3 ± 0.3 a	1.4 ± 0.1 b	1.8 ± 0.6 b	5.4 ± 0.7 a	3.4 ± 0.6 b	5.8 ± 0.5 a
18:0	18.9 ± 1.3 a	8.1 ± 1.7 b	7.6 ± 0.1 b	13.2 ± 0.9 c	6.8 ± 0.4 b	8.8 ± 1.1 b
18:1, n-9	26.3 ± 2.0 a	49.7 ± 2.0 b	54.9 ± 2.3 b	32.6 ± 1.9 a	37.2 ± 2.3 a	48.4 ± 2.1 b
18:1, n-7	4.4 ± 0.6	3.6 ± 1.4	4.5 ± 2.1	5.6 ± 0.3	4.5 ± 0.2	3.8 ± 0.6
18:2, n-6	6.5 ± 0.4 a	15.6 ± 1.2 b	8.7 ± 0.1 c	6.8 ± 0.7 a	19.4 ± 1.1 b	4.1 ± 0.4 c
20:1, n-9	ND	ND	0.4 ± 0.0	ND	ND	0.4 ± 0.1
20:4, n-6	ND	3.8 ± 0.4	4.6 ± 0.2	ND	6.6 ± 1.6	ND

[a] ND, not detected. Values expressed as means ± SDs. Mean values within a row sharing the same letter are not significantly different ($p < 0.05$).

employed as a lipase source. One aliquot was used for the activity measurement and another one for protein quantification by the method of Bradford. Both fractions were frozen in liquid nitrogen and stored at −80 °C until used.

Dibutirylfluorescein (DBF) (Sigma, St. Louis, MO) was prepared as a substrate for the reaction with LPL by dissolving 1 mg in 5 mL of ethilenglycol monomethyl ether. One milliliter of this solution was dissolved in 20 mL of 0.1 M phosphate buffer. A 200 μL amount of the enzyme extract was incubated with 1500 μL of DBF solution, 150 μL of ethilenglycol monomethyl ether, and 1250 μL of phosphate buffer. LPL activity was quantified as the difference between the fluorescence emitted by the released fluorescein when 1 M NaCL was added to the incubation medium to the one emitted in the absence of NaCL. Fluorescence was measured in a fluorescence spectrophotometer (Aminco Bowman Series 2, Urbana, IL) at 490 nm for excitation and 530 nm for emission. The rest of lypolitic activity was quantified as non-LPL activity.

Statistical Analysis. Results are presented as means ± standard deviations (SDs). The significance of the differences between dietary periods was assessed by one-way analysis of variance (repeated measures) with Bonferroni's test for comparison of the means.

RESULTS

CE Fatty Acid Composition. The intake of diets rich in oleic acid (18:1, n-9) was reflected in aorta CE (**Table 4**). The concentration of this fatty acid was higher in rats fed VOO or HOSO than in the groups fed BD ($p < 0.05$), accounting for about 60% of total fatty acids and representing a good marker of consumption of oleic acid rich oils. In contrast, the content of another MUFA, palmitoleic acid (16:1, n-7), was lower in animals receiving the oleic acid rich oils ($p < 0.05$). The concentration of this fatty acid was lower in the hypertensive animals fed the high oleic diets ($p < 0.05$). The BD was responsible for a higher accumulation of saturated fatty acids (SFA) in the aortas of the both normo and hypertensive rats, mostly due to accumulations of palmitic (16:0) and stearic (18:0) acids. The latter was also found in higher concentration in animals fed HOSO. The content of linoleic acid (18:2, n-6) was likewise significantly higher in the aortas of the rats fed BD ($p < 0.05$). The content of this fatty acid in CE from aorta of rats fed VOO was much lower than in those fed the other diets.

Very little differences were found among groups due to hypertension. Stearic acid was lower in SHR rats fed HOSO or VOO as compared to the corresponding WKY ($p < 0.05$). Linoleic acid was in higher concentration in the SHR animals fed HOSO than in the control ones ($p < 0.05$).

TG Fatty Acid Composition. Consumption of oleic acid rich diets was also reflected in aorta TG (**Table 3**). The content of this fatty acid was about 50% in TG form aortas of WKY animals fed VOO or HOSO. However, whereas in SHR rats the concentration was reduced to 37% when HOSO was administrated, no differences were observed as compared to WKY after VOO.

The BD was responsible for a greater amount of stearic and palmitic acids. No differences were observed among WKY rats fed VOO or HOSO for stearic acid, but the content of palmitic acid in SHR fed VOO was higher than in those fed HOSO. The concentrations of linoleic acid were higher in the TG of the rats after the administration of HOSO ($p < 0.05$). VOO consumption caused a reduction in the content of this fatty acid in SHR rats ($p < 0.05$) but not when the animals were fed BD or HOSO.

TG Molecular Species Composition. The diets enriched in oleic acid provided molecular species of TG rich in that fatty acid to the aortas of SHR and WKY rats (**Table 5**). The main

Table 5. TG Molecular Species Composition of the Aortas of WKY and SHR Fed the BD and the Diets Enriched in VOO or HOSO (%)

	WKY			SHR		
TG	BD	HOSO	VOO	BD	HOSO	VOO
OLL	1.3 ± 0.1 a	2.1 ± 0.3 b	0.9 ± 0.0 a	2.2 ± 0.6 b	2.1 ± 0.1 b	0.7 ± 0.0 a
PoOL	2.2 ± 0.8	1.2 ± 0.1	1.1 ± 0.0	1.7 ± 0.2	1.4 ± 0.0	1.1 ± 0.1
PLL	3.0 ± 0.9 a	1.8 ± 0.2 b	1.2 ± 0.0 b	3.3 ± 0.7 a	1.6 ± 0.8 b	ND
PoPoO/MPoO	2.8 ± 0.7 a	1.4 ± 0.2 b	1.1 ± 0.0 b	2.6 ± 0.2 a	2.1 ± 0.2 a	1.1 ± 0.1 b
MMO	1.8 ± 0.5	ND	1.2 ± 0.2	1.6 ± 0.2	1.5 ± 0.1	1.2 ± 0.1
MPL/MPPo	1.5 ± 0.3	1.2 ± 0.0	1.1 ± 0.0	1.4 ± 0.2	1.2 ± 0.2	0.8 ± 0.1
OOL	4.1 ± 0.2 a	9.1 ± 0.4 b	4.8 ± 0.8 a	3.2 ± 0.7 c	8.1 ± 0.1 b	2.7 ± 0.2 c
MOO	3.8 ± 0.7	ND	3.2 ± 0.5	3.3 ± 0.1	ND	3.5 ± 0.2
POL	6.8 ± 0.6 a	7.8 ± 0.4 a	4.8 ± 0.9 b	7.1 ± 0.2 a	8.0 ± 0.4 a	3.3 ± 0.3 b
MPO	7.2 ± 0.3	ND	ND	6.5 ± 2.2	ND	ND
PPL/PPPo	3.4 ± 0.4 a	1.6 ± 0.1 b	4.6 ± 0.8 a	3.1 ± 0.5 a	ND	5.6 ± 0.3 c
MPP	4.0 ± 0.5 a	1.8 ± 0.1 b	1.9 ± 0.0 b	4.2 ± 0.4 a	2.0 ± 0.2 b	2.6 ± 0.1 b
MMS	1.3 ± 0.1 a	0.9 ± 0.1 a	1.0 ± 0.0 a	1.4 ± 0.3 a	2.2 ± 0.2 b	0.7 ± 0.0 c
OOO	6.0 ± 0.6 a	22.3 ± 2.3 b	24.9 ± 4.5 b	5.5 ± 0.3 a	18.8 ± 1.0 c	17.7 ± 1.0 c
POO	21.4 ± 3.4 a	27.5 ± 1.5 b	28.5 ± 3.1 b	21.1 ± 0.7 a	27.4 ± 0.8 b	27.4 ± 2.0 b
PPO	11.5 ± 1.1 a	6.4 ± 0.3 b	7.9 ± 0.6 c	5.2 ± 1.3 b	7.1 ± 0.7 c	10.9 ± 0.3 a
PPP	2.5 ± 0.5 a	ND	ND	2.6 ± 0.5 a	0.6 ± 0.1 b	1.5 ± 0.2 c
MPS	0.7 ± 0.1 a	1.2 ± 0.0 b	1.7 ± 0.1 c	ND	2.6 ± 0.1 d	2.0 ± 0.1 c
SOO	4.7 ± 0.5 a	1.8 ± 0.2 b	7.5 ± 1.1 c	4.3 ± 0.0 a	7.5 ± 0.2 c	8.5 ± 0.7 c
PSO	5.5 ± 1.3 a	8.9 ± 0.6 c	4.1 ± 0.8 a	5.8 ± 0.9 a	4.1 ± 0.2 a	4.6 ± 0.5 a
PPS	1.5 ± 0.3 a	4.1 ± 0.3 b	2.4 ± 0.0 c	1.6 ± 0.3 a	ND	0.9 ± 0.1 a
SSO	1.5 ± 0.2 a	1.1 ± 0.1 a	2.6 ± 0.0 b	1.6 ± 0.3 a	1.3 ± 0.0 a	0.9 ± 0.0 a
PSS	1.9 ± 0.0	1.5 ± 0.1	ND	0.8 ± 0.3	1.4 ± 0.2	1.2 ± 0.1

species were palmitoyl-diloleoyl-glycerol (POO) and triolein (OOO) accounting for more than 45% in both groups of animals. Actually, dioleoyl-acyl-glycerol species accounted for more than 60% of total TG species. In contrast, BD-fed rats had significantly higher amounts of TG rich in SFAs, such as myristic, palmitic, and stearic acids ($p < 0.05$). It is interesting to note that more than 20% of TG contained myristic acid in WKY and SHR rats fed BD whereas in VOO- and HOSO-fed rats it was only about 11%. Rats fed HOSO contained higher amounts of linoleic acid rich species ($p < 0.05$). Oleoyl-dilinoleoyl-glycerol (OLL), dioleoyl-linoleoyl-glycerol (OOL), and palmitoyl-oleoyl-linoleoyl-glycerol (POL) were found in higher concentrations in aortas of rats fed HOSO than in the ones fed VOO ($p < 0.05$). The content OOO was lower in hypertensive rats after the intake of both oleic acid rich oils as compared with the WKY animals ($p < 0.05$). However, this effect was not observed for the other dioleoyl-acyl-glycerol species in which the other fatty acid was saturated [mytistoyl-dioleoyl-glycerol (MOO) and palmitoyl-dioleoyl-glycerol (POO)]. Differences in the content of other oleic acid-containing TG were found between SHR and WKY animals. OOL was lower and dipalmitoyl-oleoyl-glycerol (PPO) was higher in SHR rats fed VOO, and palmitoyl-stearoyl-oleoyl-glycerol (PSO) was lower and stearoyl-dioleoyl-glycerol (SOO) higher in hypertensive animals fed HOSO ($p < 0.05$).

Lipolytic Activity. LPL activity was increased very importantly in SHR rats fed the BD diet as compared to WKY ($p < 0.05$). However, this effect was not observed in animals fed VOO or HOSO. In the latter groups, the LPL activity of the SHR rats was lower than in the control equivalents, although only for HOSO the difference was significant ($p < 0.05$). Hypertension also caused the increment of non-LPL lipolytic activity in animals fed the BD ($p < 0.05$). However, for this activity, the decrease observed after consuming the oleic acid rich oils was not significant. VOO increased non-LPL lipolytic activity in aortas of normotensive animals as compared with the other dietary groups ($p < 0.05$) and in hypertensives as compared with the HOSO group ($p < 0.05$).

DISCUSSION

Adult SHR present cardiac hypertrophy and increased proliferation of smooth muscle cells in aorta, as well as altered LPL activity (*4*, *5*). We administered two MUFA rich oils to WKY and SHR because these oils have been reported to reduce the risk for coronary heart disease by producing similar effects on the atherogenic parameters of hypertensive patients (*26*) and SHR (*27*). The CE composition of the aorta showed a good compliance of the diets as the oleic acid content in the aortas of the animals fed VOO or HOSO was about 50% higher. Murakami et al. (*32*, *33*) reported increased accumulation of CEs in smooth muscle cells and macrophages from SHR than WKY in response to altered LDL, which was attributed to enhanced scavenger receptor activity and intracellular acyl CoA: cholesterol acyltransferase (ACAT) activity. CE comprise the principal lipid class that accumulates within macrophages and smooth muscle cells of the atherosclerotic lesion (*34*).

Increased CE accumulation in vascular cells of SHR is in agreement with our previous results in whole aorta (*35*). In that study, SHR had higher CE accumulation in rat aorta when fed BD. VOO did not exert any effect on the accumulation of this lipid class in either WKY or SHR but administration of HOSO reduced CE accumulation from 0.50 to 0.11%. In the present study, we found very little differences in the fatty acid composition of aorta CE caused by hypertension (**Table 3**). Consequently, the accumulation of CE appears to be nonselective. VOO reduced the content of linoleic acid in CE of aortas from both WKY and SHR as compared with animals fed HOSO by proportionally increasing the content of oleic acid. Additionally, BD increased the content of palmitic acid. Lee and Carr (*36*) have recently shown that dietary palmitic acid increases the activity of ACAT in the liver of Syrian hamsters leading to increased accumulation of CE in apo B-100 lipoproteins. Although these and other authors did not find any difference on ACAT activity between dietary oleic and linoleic acids, others have suggested that linoleic acid may, in fact, inhibit ACAT activity (*37*). Unfortunately, there is very scarce information on the influence of free fatty acids on CE accumulation and/or ACAT activity in smooth muscle cells. Therefore, these

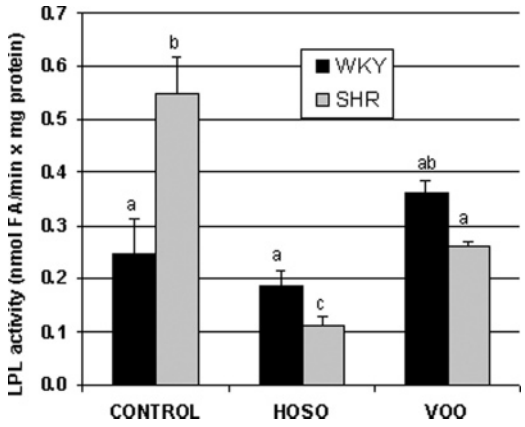

Figure 1. LPL activity in the aortas of WKY and SHR fed the BD and the diets enriched in VOO or HOSO. For the letters a–c, mean values within a row sharing the same letter are not significantly different ($P < 0.05$).

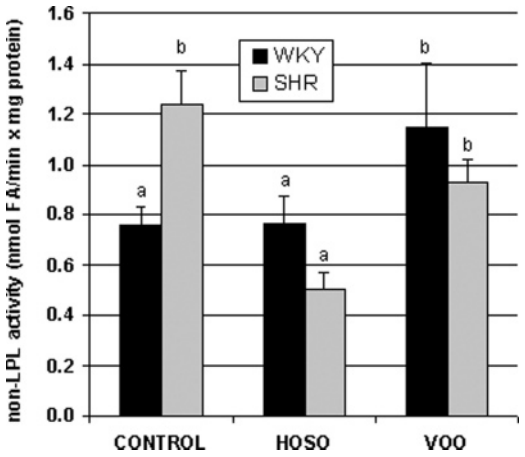

Figure 2. Non-LPL activity in the aortas of WKY and SHR fed the BD and the diets enriched in VOO or HOSO. For the letters a and b, mean values within a row sharing the same letter are not significantly different ($P < 0.05$).

results indicate that BD increases CE accumulation in aortas of SHR by increasing the incorporation of palmitic acid into this lipid class by ACAT. For the same reason, HOSO consumption might reduce CE accumulation in smooth muscle cells by providing more linoleic acid. However, it cannot be discarded that the lower content on linoleic acid in CE of smooth muscle cells is related to its higher oxidizability.

TGs also accumulate in the atherosclerotic plaque, giving to smooth muscle cells the aspect of foam cells, just like macrophages (*34*). In our study, TGs from rat aorta fed HOSO were enriched in linoleic acid, whereas rats fed VOO were enriched in oleic acid (**Table 4**). The consequence was a higher accumulation of linoleic acid rich TG species (OLL, OOL, and POL) in aortas of the HOSO group as compared with the VOO group, although the content in OOO and POO was similar for both dietary groups (**Table 5**). In our earlier study, we found increased TG accumulation in SHR animals after consumption of HOSO as compared with VOO (*35*). With our present results, we can suggest that such TG accumulation occurring in hypertensive animals was due mainly to increased linoleic acid rich species. The presence of one double bond in the molecule makes linoleic acid more susceptible to oxidation than oleic acid, and it is well-known that oxidation products from linoleic acid are responsible for the deterioration of the vascular endothelium (*38*, *39*).

The income of fatty acids into the vascular wall may be due to two main pathways: direct receptor-mediated lipoprotein uptake and lipoprotein hydrolysis by LPL. LPL is a TG hydrolase, acting mainly on TG rich lipoproteins (TRL), whereas EL is a phospholipase acting mainly on HDL, hence releasing high amounts of free fatty acids (*9*). In the present study, hypertension increased LPL activity in SHR fed the BD (**Figure 1**). A negative relationship between the development of hypertension in SHR and the cardiac LPL activity has been found (*7*, *40*). Shepherd et al. (*40*) also found a reduced vascular endothelial-bound LPL activity in SHR. However, in that study, they reported increased LPL activity in SHR diabetic rats. Actually, treatment of these rats with insulin for 2 weeks prevented the increase. It is therefore tempting to speculate that since SHR are known to be insulin resistant (*41*), when on a long term, low fat, high carbohydrate diet, SHR may behave as diabetics. The consequence of enhanced LPL activity implies more free fatty acids available for TG and CE synthesis, which is concordant with the increased accumulation of these lipid classes observed in our previous report (*35*). Although we did not measure EL activity here, this enzyme could contribute to the fatty acid influx to aortas of SHR. Shimokawa et al. (*42*) have very recently shown increased mRNA expression for EL in aortas of SHR, which they related to a decrease in plasma HDL in these animals.

Conversely, when SHR were fed high fat diets, we found the reduced LPL activity due to hypertension reported before (*40*), although it was only statistically significant after HOSO but not after VOO. The consequence of lower LPL activity in the aortic wall would lower lipoprotein hydrolysis and fatty acid incorporation to vascular cells. At the same time, a lower expression of the VLDL receptor has been found in cardiac cells of SHR (*43*). Hence, the higher TG accumulation found in aortas of SHR fed HOSO would be due to higher fatty acid incorporation into cells but to decreased TG hydrolysis. This would be in agreement with the lower non-LPL lipolytic activity shown in the present study, which might be due to a lower preference of the hydrolytic enzymes for linoleic acid-containing lipid classes. A lower LPL activity in SHR fed HOSO as compared to VOO had been observed before in adipose tissue, which was also concomitant to increased TG molecular species containing linoleic acid, mainly OOL (*28*). However, in the heart, we could not find any relevant effect of diets enriched in HOSO or VOO on the LPL activity in these hypertensive animals (*29*).

Free fatty acids released in blood vessels by enzymes attached to the vascular endothelium may injury arterial wall cells (*16*, *17*). They can induce foam cell formation in macrophages (*18*) and TG accumulation in smooth muscle cells (*19*). Lysosome acid lipase (LAL), which can hydrolyze CE and TG, seems to be the only lipolytic enzyme within these cells, since no other TG-hydrolase has been found (*36*). During the progression of the atherosclerotic plaque, a reduction in the content of TG occurs, whereas the amount of CE is increased. Consequently, this process may be affected by the hydrolytic action on TG molecular species. The rate of hydrolysis might depend on the TG fatty acid composition, just as occurs in other tissues (*44*, *45*).

In conclusion, the results shown in the present study indicate that VOO would help to normalize the lipid composition of the aortic wall of SHR by maintaining the LPL and non-LPL lipolytic activity closer to the levels of healthy WKY. However, we also report now that the reduction of the CE content in the aorta of SHR reported before (*35*) was mediated by a lower incorporation of linoleic acid, probably due to reduced ACAT activity. The fate of this fatty acid was, in turn, TG synthesis,

which would explain the higher concentration found in our previous study. Because TGs are more readily hydrolyzed than CE, HOSO would also help to reduce the conversion of smooth muscle cells into foam cells in SHR.

ABBREVIATIONS USED

SHR, spontaneously hypertensive rat; WKY, Wistar−Kyoto rat; TG, triglyceride; CE, cholesteryl ester; VOO, virgin olive oil; HOSO, high-oleic sunflower oil; BD, baseline diet; LPL, lipoprotein lipase; EL, endothelial lipase; LAL, lysosomal acid lipase; ACAT, acyl CoA:cholesterol acyltransferase; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

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