Virgin Olive Oil Normalizes the Altered Triacylglycerol Molecular Species Composition of Adipose Tissue in Spontaneously Hypertensive Rats

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The present study was conducted in order to evaluate the influence of hypertension on the triacylglycerol (TG) molecular species composition and other lipid classes of rat adipose tissue. In addition, the effect of two dietary oils, with a similar content in oleic acid but different TG moieties, was studied. Virgin olive oil (VOO) or high-oleic sunflower oil (HOSO) was added to a baseline diet (BD) and administrated to Wistar–Kyoto and spontaneously hypertensive rats (SHR) for 12 weeks. Both VOO and HOSO normalized the altered composition of TG molecular species and phospholipid (PL) fatty acids in SHR compared to animals fed BD, although the effect exhibited by VOO was greater. Rats fed HOSO showed a greater palmitic ($p < 0.05$) and lower linoleic acid ($p < 0.05$) incorporation into PL but a greater accumulation of linoleic acid-containing TG species, particularly dioleoyl-linoleoyl-glycerol, with a concomitant displacement of trilinolein. Both oils were capable of increasing the lipoprotein lipase (LPL) activity in normotensive rats, but only VOO did so in the SHR. Therefore, it was concluded that although oleic acid-rich diets improve some of the altered parameters of SHR adipose tissue, VOO is more effective than HOSO in this regard.

KEYWORDS: Spontaneously hypertensive rat; virgin olive oil; adipose tissue; triacylglycerol; lipoprotein lipase

INTRODUCTION

Triacylglycerols (TG) represent the main form of storage for a wide spectrum of fatty acids. Although fatty acid deposition in the adipose tissue bears some resemblance to the fatty acid composition of the diet (*1*), there are differences between dietary and adipose tissue fatty acid composition, probably due to intermediate processes between fat ingestion and deposition in adipose tissue. Fatty acid incorporation into adipose tissue involves several metabolic steps, including TG hydrolysis from lipoproteins by lipoprotein lipase (LPL) (*2*), which is selective. Therefore, fatty acids are not equally released from circulating lipoproteins by LPL and transferred to adipose tissue (*3*).

In fasting conditions, TG are hydrolyzed and the resulting nonesterified fatty acids released to circulation, where they bind albumin molecules. It has been demonstrated that this mobilization is selective and might affect the storage of individual fatty acids in adipose tissue and their subsequent supply to tissues (*4*, *5*). Raclot and Oudart (*6*) suggested that not only is the process of fatty acid release selective but also a selective reabsorption of the fatty acids occurs. Both processes depend on fatty acid chain length, unsaturation, and positional isomerism of fatty acids in adipose tissue TG. Hence, the TG molecular species composition of adipose tissue might be an important

factor affecting the hydrolysis, release, and re-uptake of fatty acids in the adipose tissue.

Monounsaturated fatty acid (MUFA)-rich oil consumption has been one of the recommended strategies for modulating the plasma lipid profile in humans (*7*). Two sources of MUFA, virgin olive oil (VOO) and high-oleic sunflower oil (HOSO), have been suggested to reduce the risk for cardiovascular heart diseases by providing a similar effect on the atherogenic index of normocholesterolemic and hypercholesterolemic hypertensive patients (*8*). However, there is physiological evidence that the two MUFA-enriched diets have different physiological effects on humans (*9*) and rats (*10*), highlighting the fact that other factors such as TG composition, minor fatty acids, and nonfatty acids constituents, rather than the content of oleic acid itself, might be responsible for the benefits in healthy subjects and patients with cardiovascular risk factors after VOO intake. Dietary VOO administration provides a different fatty acid and TG profile in rat adipose tissue compared to oils with a different fatty acid composition, such as sunflower, rapeseed, and palm oils and beef tallow (*11*, *12*).

Essential hypertension is associated with multiple metabolic alterations (*13*). Abnormal lipid metabolism has been detected in spontaneously hypertensive rats (SHR) (*14*) and humans (*15*). Consumption of VOO or HOSO provides a different TG molecular species composition in the liver of normotensive and hypertensive rats, despite both oils having similar fatty acid compositions (*16*, *17*). However, for the moment there are no

Table 1. Fatty Acid Composition of the Baseline Diet (BD) and of the Virgin Olive Oil (VOO) and High-Oleic Sunflower Oil (HOSO) (Percent)[a]

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

[a] Values are expressed as means ± standard deviations. Mean values within a row sharing the same letter are not significantly different ($p < 0.05$). [b] ND, not detected. [c] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 2. Triacylglycerol Molecular Species Composition of the Baseline Diet (BD) and of the Virgin Olive Oil (VOO) and High-Oleic Sunflower Oil (HOSO) (Percent)[a]

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

[a] Values are expressed as means ± standard deviations. Mean values within a row sharing the same letter are not significantly different ($p < 0.05$). [b] 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); S, stearic acid (18:0). Triacylglycerols: LLL, trilinoleoyl-glycerol; OOP, dioleoyl-palmitoyl-glycerol; AOP, arachidonoyl-oleoyl-palmitoyl-glycerol; PXX, palmitoyl-diacyl-glycerol. [c] ND, not detected.

available data concerning the influence of hypertension on the TG molecular species composition of adipose tissue. Therefore, the present study was conducted to evaluate the effect of two dietary oils, with a similar content in oleic acid but different TG moieties (VOO and HOSO) on the TG pool of SHR rat adipose tissue.

MATERIALS AND METHODS

Animals and Diets. Four-week-old Wistar−Kyoto (WKY) and spontaneously hypertensive (SHR) rats weighing 55.7 ± 2.3 g were purchased from Letica (Barcelona, Spain) and weighed 93.3 ± 2.2 g when killed. They were randomly assigned to three groups of 12 rats and were individually housed in a room at 21 ± 2 °C with a light/dark cycle of 12 h. Animals were fed with a fatfree diet (baseline diet, BD) or BD supplemented with 15% (w/w) VOO (Aceites Toledo, Los Yébenes, Sapin) or 15% (w/w) HOSO (Coreysa, Osuna, Spain). Feeding was ad libitum. BD contained 20.8% milk casein, 19.6% cornstarch, 37% glucose, 5.3% cellulose, 6.3% mineral mix, and 1% vitamin mix [(w/w), 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 gas chromatography (GC) and high-performance liquid chromatography (HPLC), respectively, as described below (**Tables 1** and **2**, respectively). After 12 weeks of feeding, rats were sacrificed by decapitation and the epididimal adipose tissue was removed from the carcass, weighed, and preserved at −80 °C until used. At this time no significant differences were found for 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 liver lipids of the adipose tissue were extracted following the method described by Folch et al. (*18*). 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), as described by Ruiz-Gutiérrez et al. (*19*). The TG and phospholipid (PL) fractions were scrapped off

the silica, eluted with hexane/diethyl ether (9:1, v/v), passed through a nitrogen stream, and stored at temperature below −20 °C until analyzed.

Lipid Class Composition of Rat Adipose Tissue. Lipid composition was determined by TLC coupled with a flame ionization detector (FID) in an Iatroscan apparatus (Iatron Laboratories Inc., Tokyo, Japan), using Chromarods S (Iatron Laboratories Inc.), which were first activated by passing through the flame of the Iatroscan detector for 15 min. Four milligrams of total lipids from adipose tissue (3 μL) was spotted on each rod using a 10 μL Hamilton syringe. The rod was developed in hexane/diethyl ether/formic acid (90:10:1, v/v/v) (Merck España) and subsequently scanned under the following conditions: hydrogen flow, 175 mL/min; air flow, 1850 mL/min. An Iatrocorder TC-11 integrator was used for recording and area integration.

Fatty Acid Composition of Rat Adipose Tissue Triacylglycerols and Phospholipids. TG and PL were transmethylated and the resulting fatty acid methyl esters (FAME) analyzed by GC as described by Ruiz-Gutiérrez et al. (*19*), using a model 5890 series II gas chromatograph (Hewlett-Packard Co, Avondale, PA) equipped with a FID and a capillary silica column Supelcowax 10 (Sulpelco Co., Bellefonte, PA) 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. FAME for which no standard was available were identified by gas chromatography−mass spectrometry on a Konik KNK-2000 chromatograph (Konik Co., Barcelona, Spain) interfaced directly to an AEJ MS30/790 VG mass spectrometer (VG Analytical, Manchester, U.K.) using electron impact ionization mode. The ion source temperature was maintained at 200 °C, the multiplier voltage was 4.0 kV, the emission current was 100 μA, and the electron energy was 70 eV. The data were processed with a VG 11/250 data system.

Triacylglycerol Molecular Species Composition of Rat Adipose Tissue. 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 × 4.6 mm) of 4 μm particle size (Waters Co.). The liquid chromatograph was coupled to a light-scattering detector model DDL31 (Eurosep, Inc., Cergy-Pontoise, France). The system was controlled by computer through the Millenium System (Waters Co., Milford, MA). 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 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-

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Table 3. Lipid Classes in Adipose Tissue of Wistar–Kyoto (WKY) and Spontaneously Hypertensive (SHR) Rats Fed the Baseline Diet (BD) and the Diets Enriched in Virgin Olive Oil (VOO) or High-Oleic Sunflower Oil (HOSO) (Percent)[a]

	BD		VOO		HOSO	
lipid[b]	WKY	SHR	WKY	SHR	WKY	SHR
CE	0.2 ± 0.1a	0.1 ± 0.1a	0.2 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.1a	0.1 ± 0.1a
TG	82.0 ± 2.0a	88.3 ± 1.6b	96.5 ± 2.0c	97.5 ± 2.0c	97.2 ± 1.0c	98.2 ± 1.0c
C	1.0 ± 0.3a	1.5 ± 0.2b	0.3 ± 0.1c	0.2 ± 0.1c	0.2 ± 0.0c	0.2 ± 0.1c
PL	16.8 ± 0.6a	10.1 ± 0.7b	3.0 ± 0.4c	2.2 ± 0.5d	2.5 ± 0.6d	1.6 ± 0.4e
C/PL	0.06 ± 0.02a	0.15 ± 0.03b	0.10 ± 0.02c	0.09 ± 0.01c	0.08 ± 0.02c	0.09 ± 0.02c

[a] Values are expressed as means ± standard deviations. Mean values within a row sharing the same letter are not significantly different ($p < 0.05$). [b] CE, cholesteryl esters; TG, triacylglycerols; C, cholesterol; PL, phospholipids; C/PL, cholesterol-to-phospholipids ratio.

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 standard for a chromatographic 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. (*20*).

Determination of Lipoprotein Lipase Activity in Rat Adipose Tissue. Adipose tissue was 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 drying, 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 unit/mL of sodium heparin was added to 10 mg of the powder. The mixture was incubated in ice for 60 min and subsequently centrifuged at 5000g for 10 min at 4 °C. The supernantant was employed as lipoprotein lipase (LPL) source. One aliquot was used for the activity measurement and another one for protein quantification according to the method of Bradford. Both fractions were frozen in liquid nitrogen and stored at −80 °C until used.

Dibutyrylfluorescein (DBF) (Sigma, St. Louis, MO) was prepared as substrate for the reaction with LPL by dissolving 1 mg in 5 mL of ethylene glycol monomethyl ether. One milliliter of this solution was dissolved in 20 mL of 0.1 M phosphate buffer. Two hundred microliters of the enzyme extract was incubated with 1500 μL of DBF solution, 150 μL of ethylene glycol 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 and 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.

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

RESULTS

Lipid Classes Composition. Table 3 shows the lipid classes of the adipose tissue of WKY and SHR rats fed with the experimental diets. Cholesteryl esters (CE), TG, free cholesterol (C), and PL were separated, identified, and quantified by means of TLC-FID (Iatroscan). A very significant increase in the content of TG was observed in the adipose tissue of rats fed the diets enriched in VOO or HOSO ($p < 0.001$), which was reflected in the relative proportion of PL and C. SHR fed the BD showed a greater accumulation of TG in the adipose tissue than the WKY rats ($p < 0.05$). No difference was found in the

content of this lipid class for the rest of groups. However, there was a decrease in the PL content in the adipose tissue of SHR in regard to WKY for the three diets studies ($p < 0.05$).

Phospholipid Fatty Acid Composition. The intake of diets rich in oleic acid (18:1 n−9) was reflected in the adipose tissue PL because this fatty acid represented ∼50% of total in both WKY and SHR rats ($p < 0.05$) (**Table 4**). The saturated fatty acid (SFA) concentration in adipose tissue PL was higher in SHR rats fed BD than in the corresponding WKY ($p < 0.05$), mainly due to the incorporation of myristic (14:0) and palmitic (16:0) acids. On the contrary, in both WKY and SHR fed the diets enriched in either VOO or HOSO the accumulation of SFA was lower. However, the palmitic acid content in the adipose tissue of SHR fed HOSO was higher ($p < 0.05$) than in WKY, and the linoleic acid content was lower ($p < 0.05$). No significant difference was found in the content of these fatty acids between WKY and SHR fed VOO.

Triacylglycerol Fatty Acid Composition. The content of oleic acid (18:1 n−9) in adipose tissue TG was found to be increased nearly to 65−70% in the rats fed the diets rich in this fatty acid (**Table 5**). No significant differences were found between WKY and SHR when fed VOO, but the content of this fatty acid in WKY fed HOSO was slightly lower than in the SHR. Conversely, palmitoleic acid (16:1 n−7) was found to decrease in TG fatty acids of rats fed the two oleic acid-rich diets, accounting for 10% in WKY and 12% in SHR fed the fatfree diet and values close to 3% in animals fed VOO or HOSO. Consumption of BD also led to the accumulation of SFA, such as myristic (14:0) and palmitic (16:0) acids. Although the greatest content in linoleic acid (18:2 n−6) in TG was also found in rats fed the BD, the intake of HOSO significantly increased the content of this fatty acid compared to rats fed VOO ($p < 0.05$). SHR fed BD showed a lower content in linoleic acid (18:2 n−6) when compared to the WKY.

Triacylglycerol Molecular Species Composition. A greater accumulation of TG species containing linoleic acid was found in the adipose tissue of rats fed the BD ($p < 0.05$) (**Table 6**). Indeed, the concentrations of trilinolein (LLL) and dilinleoyl-acyl-glycerol (LLX) moieties represented nearly 10% in these rats, whereas only 2−3% of the TG found in the animals fed the oleic-acid rich diets corresponded to these species. In addition, relevant amounts of TG containing myristic acid were found in rats fed the fatfree diet (MLL, MML, MOL, MPL/MPPo, MMO, PoOL, and PoPoO/MpoO).

Rats fed oleic acid-rich diets showed a higher accumulation of TG rich in this fatty acid ($p < 0.05$). Despite the contents in MOO and SOO being similar after either of these diets, the content in OOL was higher after HOSO ($p < 0.05$), whereas those of OOO and POO were higher after VOO ($p < 0.05$). The latter oil was also responsible for a greater incorporation of palmitic acid-containing TG into the adipose tissue ($p <$

Table 4. Phospholipid Fatty Acids of Adipose Tissue of Wistar–Kyoto (WKY) and Spontaneously Hypertensive (SHR) Rats Fed the Baseline Diet (BD) and the Diets Enriched in Virgin Olive Oil (VOO) or High-Oleic Sunflower Oil (HOSO) (Percent)[a]

	BD		VOO		HOSO	
fatty acid	WKY	SHR	WKY	SHR	WKY	SHR
14:0	0.6 ± 0.2a	1.6 ± 0.1b	1.5 ± 0.2b	0.9 ± 0.1c	0.3 ± 0.1a	0.7 ± 0.1c
16:0	13.9 ± 0.2a	27.1 ± 1.3b	17.3 ± 0.5c	18.1 ± 2.2c	10.6 ± 0.6d	14.0 ± 1.6a
16:1 *n*–9	0.5 ± 0.1a	0.4 ± 0.1a	0.8 ± 0.1b	0.7 ± 0.1b	0.5 ± 0.0a	0.5 ± 0.0a
16:1 *n*–7	5.3 ± 0.1a	9.5 ± 1.4b	5.6 ± 0.3a	4.0 ± 0.3c	1.8 ± 0.5d	3.2 ± 0.2c
18:0	6.5 ± 0.3a	5.1 ± 0.3b	3.6 ± 0.3c	4.7 ± 0.4d	6.4 ± 0.9a	3.5 ± 1.0c
18:1 *n*–9t	0.2 ± 0.0a	0.2 ± 0.2a	0.3 ± 0.0a	0.3 ± 0.1a	ND[b]	0.3 ± 0.1a
18:1 *n*–9	31.2 ± 0.6a	27.7 ± 1.1b	51.1 ± 0.7c	49.0 ± 1.1c	51.6 ± 1.2c	54.8 ± 3.7d
18:1 *n*–7	4.4 ± 0.3a	5.1 ± 0.3a	0.6 ± 0.3b	0.9 ± 0.1b	ND	ND
18:2 *n*–6	26.3 ± 1.5a	19.7 ± 1.0b	16.3 ± 0.5c	15.5 ± 1.2c	26.4 ± 1.6a	20.5 ± 3.4b
18:3 *n*–3	1.1 ± 0.1a	0.8 ± 0.0a	1.0 ± 0.4a	0.6 ± 0.2a	0.3 ± 0.1b	0.4 ± 0.1b
20:0	0.2 ± 0.0a	0.3 ± 0.0a	ND	ND	ND	ND
20:1 *n*–9	3.4 ± 0.1a	1.1 ± 0.4b	ND	ND	ND	ND
20:4 *n*–6	6.8 ± 0.3a	2.1 ± 0.9b	1.6 ± 0.1b	4.0 ± 0.1c	2.0 ± 0.4b	2.0 ± 0.8b
22:5 *n*–3	ND	ND	0.2 ± 0.0a	ND	0.3 ± 0.0a	ND
SFA[c]	21.1 ± 0.8a	34.1 ± 1.7b	22.4 ± 0.9a	23.8 ± 2.7a	17.2 ± 1.6c	18.3 ± 2.7b
MUFA[c]	44.9 ± 1.3a	43.9 ± 3.5a	58.4 ± 1.9b	54.9 ± 1.7c	53.8 ± 1.7c	58.9 ± 3.8b
PUFA[c]	34.2 ± 1.9a	22.5 ± 1.9b	19.0 ± 1.1b	20.0 ± 1.5b	29.0 ± 2.1c	22.9 ± 4.2b

[a] Values are expressed as means ± standard deviations. Mean values within a row sharing the same letter are not significantly different ($p < 0.05$). [b] ND, not detected. [c] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 5. Triacylglycerol Fatty Acids of Adipose Tissue of Wistar–Kyoto (WKY) and Spontaneously Hypertensive (SHR) Rats Fed the Baseline Diet (BD) and the Diets Enriched in Virgin Olive Oil (VOO) or High-Oleic Sunflower Oil (HOSO) (Percent)[a]

	BD		VOO		HOSO	
fatty acid	WKY	SHR	WKY	SHR	WKY	SHR
14:0	2.2 ± 0.2a	3.4 ± 0.4b	1.6 ± 0.2c	2.0 ± 0.2a	2.5 ± 1.0a	1.8 ± 0.3ac
14:1 *n*–5	2.0 ± 0.2a	2.1 ± 0.2a	0.7 ± 0.0b	0.8 ± 0.1b	0.8 ± 0.1b	0.7 ± 0.1b
16:0	27.7 ± 0.8a	28.3 ± 1.3a	14.5 ± 0.5b	15.7 ± 1.7b	13.0 ± 1.4b	11.4 ± 1.0c
16:1 *n*–9	0.4 ± 0.0a	0.3 ± 0.1a	0.7 ± 0.1b	0.7 ± 0.1b	0.6 ± 0.0b	0.6 ± 0.0b
16:1 *n*–7	10.6 ± 0.5a	12.7 ± 0.4b	3.6 ± 0.2c	3.6 ± 0.6c	3.0 ± 0.5cd	2.3 ± 0.2d
18:0	2.9 ± 0.3a	2.6 ± 0.2a	1.7 ± 0.1b	1.8 ± 0.1b	2.2 ± 0.1bc	2.2 ± 0.1bc
18:1 *n*–9	28.0 ± 0.9a	28.5 ± 4.3a	68.3 ± 1.0b	66.9 ± 2.1b	63.1 ± 0.9c	66.9 ± 0.3b
18:1 *n*–7	4.7 ± 0.2a	4.1 ± 0.6b	3.9 ± 0.4b	4.2 ± 0.1b	4.0 ± 0.6b	2.8 ± 0.1c
18:2 *n*–6	19.3 ± 1.1a	15.9 ± 1.7b	4.1 ± 0.6c	3.5 ± 0.3c	9.4 ± 0.9d	10.4 ± 0.7d
18:3 *n*–3	1.0 ± 0.1a	1.0 ± 0.1a	0.3 ± 0.1b	0.3 ± 0.0b	0.1 ± 0.1c	0.2 ± 0.0b
20:0	0.2 ± 0.1a	0.2 ± 0.1a	ND[b]	ND	0.1 ± 0.0a	0.1 ± 0.0a
20:1 *n*–9	0.3 ± 0.1a	0.3 ± 0.0a	0.3 ± 0.0a	0.3 ± 0.2a	0.3 ± 0.0a	0.1 ± 0.0b
20:2 *n*–6	0.3 ± 0.1a	0.2 ± 0.0a	0.1 ± 0.0a	0.2 ± 0.0a	0.3 ± 0.0a	0.3 ± 0.1a
20:3 *n*–6	ND	ND	0.1 ± 0.0a	0.1 ± 0.1a	0.1 ± 0.0a	ND
20:4 *n*–6	0.2 ± 0.1a	ND	0.1 ± 0.1a	0.1 ± 0.0a	0.2 ± 0.0a	ND
22:1 *n*–9	0.1 ± 0.1a	ND	ND	ND	0.1 ± 0.0a	ND
SFA[c]	33.0 ± 1.4a	34.5 ± 2.0a	17.8 ± 0.8b	19.4 ± 2.0b	17.9 ± 2.6b	15.5 ± 3.4b
MUFA	44.0 ± 1.7a	45.8 ± 5.4a	76.9 ± 1.6b	75.7 ± 3.1b	71.3 ± 2.3b	72.8 ± 5.7b
PUFA	20.2 ± 0.1a	16.4 ± 1.9b	4.4 ± 0.4c	3.8 ± 0.3c	10.0 ± 0.4d	11.1 ± 1.8d

[a] Values are expressed as means ± standard deviations. Mean values within a row sharing the same letter are not significantly different ($p < 0.05$). [b] ND, not detected. [c] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

0.05). No differences were found in the TG molecular species composition of the adipose tissue between hypertensive and normotensive rats fed HOSO. Conversely, SHR rats showed higher concentrations of TG rich in linoleic acid (LLL, OLL, PLL, MOL, OOL, POL, and PPL) after the BD ($p < 0.05$) and higher concentrations of TG rich in oleic acid (OOL, MOO, and POL) after the VOO diet ($p < 0.05$).

Lipoprotien Lipase (LPL) Activity. Normotensive rats fed VOO and HOSO showed a higher LPL activity in adipose tissue ($p < 0.05$) (**Figure 1**). No difference between hypertensive and normotensive rats was found in those animals fed HOSO and BD. In contrast, the activity was higher in SHR rats after the VOO diet than in the WKY fed this diet.

DISCUSSION

The TG composition of rat adipose tissue reflected that of the diets administered, which is agreement with data previously published by us and other authors (*11*, *12*). As expected, the total TG concentration in rat adipose tissue was increased after VOO and HOSO compared with BD (**Table 3**), oleic being the fatty acid incorporated to a greater extent into both TG and PL (**Table 5**). However, the content of linoleic acid was higher in the rats receiving the diet rich in HOSO than in those receiving VOO. Klingenberg et al. (*21*) found that the intake of HOSO increased not only the oleic acid concentration in pig adipose tissue but also that of linoleic acid. Weber et al. (*12*) have demonstrated that up to 13% of total fatty acids stored in adipose tissue of rats fed with a diet rich in HOSO can be accounted for by linoleic acid. Despite the content of palmitic and myristic acids being very low in HOSO (**Table 1**), the incorporation of these fatty acids into adipose tissue TG after HOSO was nearly identical to that observed after VOO (**Table 4**). Such an accumulation of SFA after the diet rich in HOSO suggests a de novo synthesis with the aim of providing the adipose tissue with

Table 6. Triacylglycerol (TG) Molecular Species Composition of Adipose Tissue of Wistar–Kyoto (WKY) and Spontaneously Hypertensive (SHR) Rats Fed the Baseline Diet (BD) and the Diets Enriched in Virgin Olive Oil (VOO) or High-Oleic Sunflower Oil (HOSO) (Percent)[a]

	BD		VOO		HOSO	
TG[b]	WKY	SHR	WKY	SHR	WKY	SHR
LLL	1.5 ± 0.1a	1.1 ± 0.1b	0.1 ± 0.0c	0.3 ± 0.1c	0.2 ± 0.0c	ND[c]
AOL	1.4 ± 0.2a	1.6 ± 0.1a	ND	ND	ND	ND
MPoL	0.7 ± 0.1a	1.0 ± 0.2a	ND	ND	ND	ND
MLL/PoPoL	1.0 ± 0.0a	0.8 ± 0.1a	ND	ND	ND	ND
MML	0.5 ± 0.0a	0.6 ± 0.0a	ND	ND	ND	ND
OLL	2.7 ± 0.5a	3.7 ± 0.3b	1.2 ± 0.2c.	1.4 ± 0.2c	2.6 ± 0.4a	2.2 ± 0.7a
PoOL	4.7 ± 0.3a	5.5 ± 0.6a	1.7 ± 0.1b	1.9 ± 0.2b	1.6 ± 0.2b	2.1 ± 0.4b
PLL	3.8 ± 0.2a	5.8 ± 0.5b	1.0 ± 0.0b	1.1 ± 0.1b	1.5 ± 0.2b	1.4 ± 0.3b
PoPoO/MPoO	3.8 ± 0.5	ND	ND	ND	ND	ND
MOL	6.7 ± 0.9a	7.4 ± 0.9b	1.2 ± 0.2c	1.2 ± 0.2c	1.6 ± 0.1c	1.6 ± 0.1c
MPPo	4.7 ± 0.4a	3.8 ± 0.4b	0.7 ± 0.1c	0.7 ± 0.1c	ND	2.2 ± 0.1d
MMO	1.8 ± 0.1a	1.8 ± 0.1a	0.6 ± 0.1b	0.6 ± 0.1b	0.9 ± 0.1b	1.0 ± 0.0b
MPL	1.8 ± 0.1a	1.7 ± 0.1a	ND	0.5 ± 0.1b	1.0 ± 0.0c	0.6 ± 0.1b
OOL	3.8 ± 0.4a	5.1 ± 0.4b	3.0 ± 0.6a	5.4 ± 0.9b	11.8 ± 0.3c	11.0 ± 2.1c
MOO	3.2 ± 0.0a	2.9 ± 0.1a	6.5 ± 0.0b	8.5 ± 0.5c	6.8 ± 0.6b	6.8 ± 1.3b
POL	8.0 ± 0.2a	9.2 ± 0.5b	3.9 ± 0.0c.	4.6 ± 0.3d	7.3 ± 0.5a	6.9 ± 1.4e
MPoS	ND	ND	ND	ND	3.7 ± 0.4a	4.2 ± 1.4a
MPO	9.0 ± 1.2a	6.3 ± 0.3b	2.6 ± 0.0c	5.0 ± 0.6d	1.1 ± 0.1e	1.2 ± 0.2e
PPL/PPPo	3.7 ± 0.6a	4.8 ± 0.6a	ND	ND	ND	ND
MPP	4.3 ± 0.3a	4.0 ± 0.3a	0.8 ± 0.0b	1.5 ± 0.1c	0.9 ± 0.0b	1.4 ± 0.3c
MMS	1.2 ± 0.1a	1.0 ± 0.1a	0.9 ± 0.2a	0.8 ± 0.0a	0.7 ± 0.1a	0.7 ± 0.1a
OOO	2.4 ± 0.2a	3.5 ± 0.3b	24.2 ± 1.5c	23.5 ± 2.3c.	20.5 ± 1.2d	18.6 ± 0.2e
POO	12.2 ± 0.7a	11.0 ± 0.8b	36.2 ± 1.7c	27.7 ± 1.9d	23.5 ± 1.7d	25.6 ± 2.4d
PSL	ND	1.7 ± 0.2a	ND	ND	0.9 ± 0.0b	ND
PPO	10.4 ± 1.0a	9.8 ± 0.5a	9.0 ± 0.4a	7.3 ± 0.2b	4.8 ± 0.0c	3.9 ± 1.4d
PPP	2.3 ± 0.4a	2.0 ± 0.2a	1.6 ± 0.3ab	1.3 ± 0.0b	0.9 ± 0.1c	1.4 ± 0.1b
MPS	1.7 ± 0.4a	0.3 ± 0.4b	2.2 ± 0.2c	1.5 ± 0.1a	1.3 ± 0.1a	1.5 ± 0.1a
SOO	2.0 ± 0.2a	1.4 ± 0.2b	3.6 ± 0.3c	3.3 ± 0.0c	4.3 ± 0.6d	3.8 ± 0.9c
POS	0.7 ± 0.0a	1.8 ± 0.3b	1.7 ± 0.1b	1.6 ± 0.1b	1.4 ± 0.1b	1.6 ± 0.3b
SSO	ND	0.6 ± 0.2a	ND	ND	0.6 ± 0.0a	ND

[a] Values are expressed as means ± standard deviations. Mean values within a row sharing the same letter are not significantly different ($p < 0.05$). [b] For triacylglycerol abbreviations see **Table 2**. [c] ND, not detected.
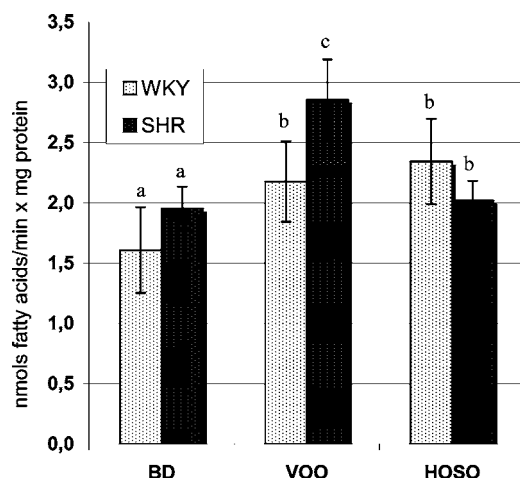

Figure 1. Lipoprotein lipase (LPL) activity in adipose tissue of Wistar–Kyoto (WKY) and spontaneously hypertensive (SHR) rats fed the baseline diet (BD) and the diets enriched in virgin olive oil (VOO) or high-oleic sunflower oil (HOSO). Mean values sharing the same letter are not significantly different ($p < 0.05$).

rapidly oxidizable fatty acids in the case of starvation. The use of SFA avoids employing PUFA for oxidation, allowing their use for other functions (*22*).

Several authors have suggested that the fatty acid release from adipose tissue is selective and based on fatty acid polarity (*22, 23*). In fact, HSL affinity for TG increases with their polarity, for instance, with decreasing chain length of constituting fatty acids and/or increasing number of unsaturations (*23*). Raclot and Groscolas (*24*) suggested that the higher affinity of HSL

for TG is related to their position in the lipid droplet of the adipose cell. The most polar TG would be located closer to the lipid/water interphase and, therefore, more accessible for the enzyme and preferentially hydrolyzed. Furthermore, Raclot and Oudart (*25*) asserted that after their release from adipose tissue, a selective re-uptake of free fatty acids into adipose tissue takes place, the most polar fatty acids being more easily released and more difficultly re-uptaken. This finding supports the idea that the process of fatty acid release (and re-uptake) for adipose tissue is selective in order to avoid the confinement of determined fatty acids in adipose tissue. The picture becomes more complex because the molecular species of TG in adipose tissue are not homogeneous. On the contrary, they are formed of fatty acids of different polarities (*11, 26*).

A higher accumulation of TG containing linoleic acid was observed in rats fed HOSO (**Table 6**). Among these TG, dioleoyl-linoleoyl-glycerol (OOL) was the most abundant. However, the accumulation of this species occurred concomitantly with a reduction of OOO, which is the most abundant TG species found in HOSO. Although the OOO content in HOSO is higher than in VOO, its content in the adipose tissue of rats fed HOSO was significantly lower. In agreement with our results, Weber et al. (*12*) found mainly OOO in the adipose tissue of rats fed HOSO, but also important amounts of OOL and much lower OLL. According to these authors, linoleic acid is usually found in the sn-2 position of the adipose tissue TG. Fatty acids in this stereospecific position are conserved longer during the processes of hydrolysis and reesterification occurring from the TG absorption in the intestine to the deposition in the adipose tissue (*27*). Thus, it is likely that the concentration of the 1,3-oleoyl-2-linoleoyl-glycerol (OLO) isomer might be

higher than that of the 1,2-oleoyl-3-linoleoyl-glycerol (OOL) species. Only a small amount of trilinolein (LLL) was found in the adipose tissue of the normotensive rats, and this species was absent in the SHR rats. HOSO contains a notable amount of LLL, which suggests that the linoleic acid released from this species has not been directed to the synthesis of new LLL and presumably to OLO. Therefore, when rats are fed with HOSO, a redistribution of linoleic acid into different TG species takes place in rat adipose tissue, leading to the reduction of linoleic acid-rich TGs (LLX and LLL).

Hypertension did not modify the fatty acid profile of TG significantly (**Table 5**). However, it was responsible for a dramatic alteration of PL fatty acid composition in the adipose tissue of rats fed BD. Palmitic and myristic acids were increased in this lipid fraction, whereas the contents of oleic, linoleic, and arachidonic acids were decreased (**Table 4**). PUFA biosynthesis and membrane fluidity are impaired in SHR liver (*28*), kidneys (*29*), and adipose tissue (*30*). Fluidity is one of the biophysical characteristics of the cell membrane that is modified in hypertension and usually reflects changes in its lipid composition (*31*). A positive relationship between membrane microviscosity (reciprocal value of membrane fluidity) and the C/PL ratio has been reported (*31*). Therefore, the changes observed here, along with a high C/PL ratio (**Table 3**), suggest lower membrane fluidity in rat adipocytes. VOO administration to rats normalized these parameters, because no significant differences were observed between SHR and WKY with regard to the concentration of these fatty acids and the C/PL ratio.

Both oleic-acid rich oils were successful in increasing LPL activity in the adipose tissue of WKY rats compared to BD. Only VOO significantly increased LPL activity in SHR, though. In the postprandial state LPL mobilizes fatty acids from circulating TG-rich lipoproteins (VLDL and chylomicrons), so they are incorporated into adipose tissue, whereas the HSL activity is suppressed by insulin (*30*). However, in the fasting state very few of the fatty acids released from VLDL in the capillary endothelium are incorporated into the adipose tissue and complement the amount of fatty acids released from adipose tissue by HSL. The proportion of free fatty acids derived from the action of LPL that are incorporated in the adipose tissue is tightly regulated, being virtually zero after an overnight fasting (*31*). Consequently, a high LPL activity in fasting conditions would involve a higher fatty acid release to the bloodstream but not into the adipose tissue. The resulting free fatty acids, bonded to albumin, would be delivered to tissues with greater energy needs. Therefore, in SHR, fatty acids derived from lipoproteins formed after the intake of VOO would be greatly directed to other tissues via the circulation, rather than to adipose tissue, by the action of LPL.

In summary, the data presented here show that administration of two dietary oils with the same oleic acid concentration, but different TG molecular species, affects differently the adipose tissue lipid composition of rats. Both oils were capable of increasing LPL activity in WKY, but only VOO did so in the SHR. Our findings suggest that, in fasting conditions, fatty acid release from lipoproteins (mainly VLDL) into adipose tissue of hypertensive animals would be higher when they are fed VOO, leading to a reduction in plasma TG concentration. In addition, VOO presented a greater capability for normalizing the altered PL fatty acid and TG molecular species composition of adipose tissue in hypertensive rats. Therefore, we conclude that although oleic acid-rich diets improve some of the altered parameters of SHR adipose tissue, VOO is more effective than HOSO.

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