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Effect of Thermally Oxidized Oil and Fasting Status on the Short-Term Digestibility of Ketolinoleic Acids and Total Oxidized Fatty Acids in Rats

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ABSTRACT: Western diets contain substantial amounts of lipid oxidation products. The effects of fasting status and oil oxidation on short-term digestibility of oxidized fatty acids (ox-FA) and ketolinoleic acids (keto-LA) of sunflower oils were evaluated. Twelve rats were fasted overnight for 3 days, whereas another 12 rats had free access to diet. From day 4, and for 4 days, two groups of rats, nonfasted (NFT) and fasted (FT), received 1 g/100 g body weight of sunflower oil reused from 40 deep-frying processes, and two control groups of rats, nonfasted (NFC) and fasted (FC), received the same amount of fresh oil. Ox-FA and keto-LA were determined 5 h after the last administration in the various gastrointestinal compartments together with the intraintestinal MDA. Oil digestibility was highest in NFC and lowest in FT rats. NFT and FT rats had higher (at least $P < 0.05$) intraintestinal MDA, ox-FA, and keto-LA than NFC and FC; MDA and keto-LA concentrations correlated with each other ($P < 0.05$). Ox-FA and keto-LA levels found in the gastric lumen suggest that digestion contributes to the formation of these compounds. Total ox-FA and keto-LA were efficiently absorbed during the first 5 h after test oil administration, but poorly absorbed in the case of fresh oils. Oil alteration influenced the digestibility of these compounds more than fasting, although the digestibility of oxidized oil was significantly affected by fasting.

KEYWORDS: absorption, frying oils, ketolinoleic acids, MDA, oxidized fatty acids

INTRODUCTION

Western diets contain substantial amounts of unsaturated oils/fats that have been subjected to various degrees of processing and heat treatment.[1−3] Digestion of triglycerides (TG) depends on the rate of hydrolysis, which affects their absorption. Pancreatic lipase has strict substrate specificity, as TG hydrolysis occurs only at the sn-1 and sn-3 positions, yielding two free fatty acids and two monoglycerides.[2] Pancreatic lipase activity is affected by the degree of oil oxidation,[4−7] the proportion of polymers and nonoxidized TG,[8] and fasting conditions.[9] Monoglycerides are well-absorbed, regardless of their fatty acid composition.[10] However, as study results differ, no consensus has been reached on oil alteration compound absorption.[5,11,12]

It should be noted that fatty acid absorption can be deeply affected by the fasting status;[9] thus, individual eating habits can modify the absorption of lipids and lipid oxidation products. This may mean that a delay in consuming the first meal of the day leads to increased oxidative stress in the intestinal mucosa.[13,14]

Our research group has found that both oil alteration and fasting conditions affect the antioxidant enzyme defense system[14] and concentrations of thiobarbituric acid reactive substances (TBARS) in the small intestine.[9] Several oxidation products have been identified and quantified in frying oils; however, little has been published on the digestibility of certain oxidized fatty acids (ox-FA), including ketolinoleic acids (keto-LA). In addition, the individual roles played by the stomach and intestine in the digestibility of ox-FA have not yet been fully explored.

The hypothesis put forward in this paper is that the digestibility of oil and altered fatty acids is affected by fasting conditions and oil oxidation. A further hypothesis is that the stomach and small intestine play different roles in the digestion of these compounds and that keto-LA and other ox-FA display different digestibility from non-ox-FA.

To test these hypotheses this work studied (1) the ox-FA profile of the oils and gastrointestinal lumen 5 h after administration of oxidized and nonoxidized sunflower oils; (2) differences between the gastrointestinal ox-FA profiles of fasted and nonfasted animals; (3) digestibility of ox-FA and some specific compounds such as keto-LA; (4) differences in the digestibility levels of these compounds between fasted and nonfasted rats; and (5) peroxidation level of the intestinal mucosa in fasted and nonfasted animals given thermally oxidized and nonoxidized sunflower oil. This study also aims to investigate the separate roles of the stomach and small intestine in the digestion of these compounds.

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MATERIALS AND METHODS

Frying Performance. Forty domestic deep-fryings of different foods were performed over a 4 week period, at the rate of 10 procedures per week, using 2.5 L steel vessels (Solac, Vitoria, Spain). Refined sunflower oil (Koipesol, Andújar, Jaén, Spain) was used for frying. Details of foods fried, portion sizes, and frying sequence have been previously reported.[9] The following frying conditions were used: thermostat temperature, 180 °C; heating time, 9−12 min/frying; frying time, 2−6 min/frying; and cooling time, ∼4 h/frying. The frying oil was replenished with unused oil every 10 frying operations to maintain a constant food/oil ratio.

Oil Alteration and Oxidation Assessment. The total polar material of the sunflower oil used in the 40 frying operations, and of the fresh sunflower oil administered to the rats, was determined by silica column chromatography.[15] To obtain further information the polar fractions of used and unused sunflower oils were analyzed using HPSEC, following a slight modification of the AOCS method.[15] Details of the method and reagents used have been previously reported.[14]

Ox-FA and keto-LA were determined in the administered oils using a modified version of the Rovellini and Cortesi HPLC-UV method.[16] Samples, many of which were solid at room temperature, were dissolved in chloroform, passed through filter paper containing anhydrous sodium sulfate, and methylated with diazomethane.[17] Eighty milligrams of each sample was diluted in methanol and filtered through 5 μm filters (Whatman, Barcelona, Spain). To obtain benzyl derivates, samples were first methylated with diazomethane, converting the free fatty acids into methyl derivates. These derivates were then transesterified with sodium benzylate, producing fatty acid benzyl derivates from both glycerol and methyl esters. Sample extracts were redissolved in *n*-hexane and mixed with 1 mL of internal standard (IS) mixture. Accurately weighed amounts of tricaproine (IS 1) [0.010 g (±0.001 g)] and trieptadecanoine (IS 2) [0.020 g (±0.001 g)] were placed in a 50 mL flask and dissolved in hexane of HPLC-grade. Benzylation was performed using 50 μL of benzyl solution (1 M in alcohol) for 15 min at room temperature; 10 μL of glacial acetic acid was subsequently added to stop the reaction.

The hexane phase was recovered after centrifugation at 3000 rpm for 10 min. After evaporation of the solvent under a flow of nitrogen at 40 °C, 1 mL of isopropanol was added, and the final solution was filtered through a 0.45 μm nylon filter. This method ensures control of the transesterification reaction by calculating the RRF ratio between the response factors of benzylcaproine RF (IS 1) and RF benzylheptadecanoine (IS 2). This value must be constant in both blank samples (transesterification between the two standards) and in the analyzed samples. It allows evaluation of the smooth conduct of the transesterification reaction.

A HPLC Shimadzu model LC-10ADVP was used, equipped with a 20 μL sample loop and a UV−vis spectrophotometric detector at 255 nm or photodiodes (SPD-M10AVP, Shimadzu, Milan, Italy), interfaced with an acquisition software Class-VP chromatography data system version 4.6 (Shimadzu), reverse phase column ODS2 250 × 4.6 mm in diameter (Waters Spherisorb, Milford, MA). Compounds were eluted with the following linear gradient (1 mL min^{-1}): T1 = min 0, water/acetonitrile 40:60 (v/v); T2 = min 50, water/acetonitrile 0:100 v/v; T3 = min 70, water/acetonitrile 0:100 v/v; T4 = min 71; water/acetonitrile 40:60 v/v; T5 = min 85, water/acetonitrile 40:60 v/v. Structural identification of benzyl esters of oxidized fatty acids was performed by HPLC-ESI-MS.[18] The compounds quantified were isomers of keto-LA, arising from the alkoxy radical in LA hydroperoxides without homolytic beta-scission. Compound identification was performed by LC-MS and diode array detector (DAD) by comparison of UV spectra.

Animals and Treatments. Animals were obtained from the Animal Research Center, University of Alcalá (Madrid, Spain), certified by the Ministry of Agriculture, Spain, ref. 28005-22A, R.D. 233-88. Rats were housed under standard conditions of temperature (21 ± 2 °C) and humidity (55 ± 10%), with a 12 h light/12 h dark cycle. All experiments were performed in compliance with the Council Directive of November 24, 1986, on the protection of animals for experimental and other scientific purposes (86/609/EEC).

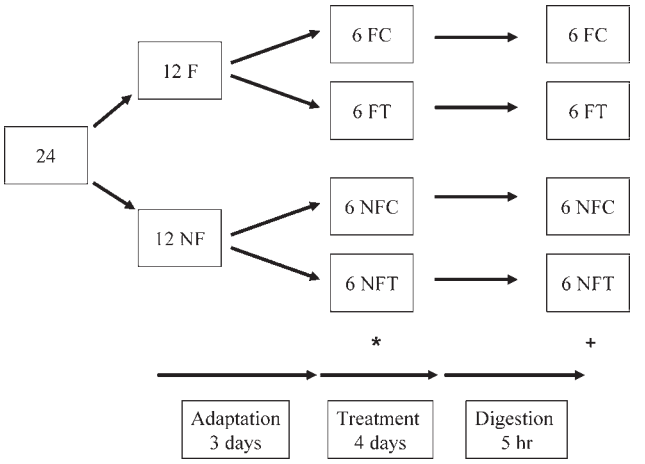

Figure 1. Study design. NF, nonfasted animals; F, fasted animals; NFC, nonfasted animals given control oil; FC, fasted animals given control oil; NFT, nonfasted animals given test oil; F, fasted animals given test oil. *, oil administration; +, anesthesia, gastrointestinal luminal fat and intraintestinal fat obtaining, animal euthanasia.

During a 3 day adaptation period, two groups of 12 rats were subjected to a 15 h/day fast, whereas the two other groups of 12 rats had ad libitum access to food and water. On days 4−6 and starting at 9:00 a.m., one animal at a time was taken at random from each of the four groups. Half of the fasted and nonfasted rats were administered control sunflower oil at a dosage of 1 g/100 g of body weight (nonfasted control and fasted control rats, NFC and FC groups, respectively), whereas the other half received via esophageal probe the same dosage of thermally oxidized sunflower oil (nonfasted test and fasted test rats, NFT and FT groups, respectively) (Figure 1). On day 7 starting at 9:00 a.m.animals were given an additional dose (1 g/100 g of body weight) of thermally oxidized or control sunflower oil, according to group. After a 5 h exposure to the oil, anesthesia was induced by isofluorane (4% in oxygen stream) and maintained with isofluorane (1.5−2.5% in oxygen stream). Rats were euthanized by extracting blood from the descending aorta with a syringe.

As reported in a previous paper,[9] 25 mL of isotonic saline solution was slowly flushed from the distal esophagus to the proximal duodenum to obtain the nonabsorbed gastric fat. To collect the fat present in the intestine, 50 mL of isotonic saline solution was slowly flushed from the proximal duodenum to the distal ileum. Samples were collected in tubes and stored at −80 °C until analysis.

Luminal Gastric and Intestinal Fats. After the saline solution wash-out, luminal gastric and intestinal fats were extracted using a slight modification of the Folch et al. method[19] and gravimetrically determined.

Extraction of Intestinal Tissue Lipids. Briefly, fat from intestinal tissue samples was extracted using choloroform/methanol (1:1, v/v). The extracted fat was homogenized in an Ultraturrax model T 25 B (Kilka-Werke, Germany) for 2 min. The chloroform phase was obtained after centrifugation at 2500 rpm for 10 min. This procedure was performed twice. The fat extract was then purified by adding 0.5% NaCl solution, centrifuged for 30 min at 2500 rpm, and dehydrated by filtration through anhydrous sodium sulfate. The solvent was evaporated under a nitrogen flow at 40 °C. To remove the most polar compounds, the fat extract was

Table 1. Percentage (Grams per 100 g of Oil) of Triglycerides, Oxidized Triglycerides, Polymerization Compounds, and Thermal Oxidation Present in Unused Sunflower Oil (Control Oil) and Sunflower Oil Used in 40 Deep-Frying Procedures (Test Oil)[a]

oil compounds administered (mg/%)	control oil	test oil
polymers of triglycerides (PTG)	nd	7.8 ± 0.3***
dimers triglycerides (DDG)	0.4 ± 0.1	12.4 ± 0.3***
nonoxidized triglycerides (NOTG)	96.4 ± 0.3	68.4 ± 0.2***
oxidized triglycerides (OTG)	1.5 ± 0.1	7.4 ± 0.2***
diglycerides (DG)	1.3 ± 0.1	1.9 ± 0.1
monoglycerides (MG)	nd	nd
free fatty acids (FFA)	0.4 ± 0.1	0.3 ± 0.0
non-ox-FA	92.6 ± 0.3	78.3 ± 0.2***
ox-FA	2.4 ± 0.1	16.7 ± 0.1***
keto-LA	1.5 ± 0.0	11.8 ± 0.1***

[a] Values are the mean ± SD of three determinations. Non-ox-FA, nonoxidized fatty acids; ox-FA, oxidized fatty acids; keto-LA, ketolinoleic acids; ***, $p < 0.001$; nd, not detected.

passed through a silica gel column containing a hexane/diethyl ether mixture.

Oxidized Fatty Acids and Ketolinoleic Acids Assessment in Luminal Fats and Intestinal Tissue. Ox-FA and keto-LA were determined in the luminal gastric and intestinal fats, and intraintestinal fat using a modified version of the HPLC-UV method as detailed under Oil Alteration and Oxidation Assessment.[17−19]

Oil Compound Digestibility. Apparent fat digestibility for the studied oils and their components was calculated as 100 × (administered fat minus luminal fat/administered fat).

Lipid Peroxidation. Lipid peroxidation was determined by measuring malondialdehyde (MDA) in intestinal tissue using HPLC with UV detection. A slightly modified version of the Mateos et al. method[20] was performed. Briefly, intestinal homogenates were prepared by mixing 100 mg of intestinal tissue with 1 mL of 50 mM Tris-HCl (pH 7.4) and homogenizing the mixture using a mechanically driven Teflon glass instrument. Homogenates were sonicated for 30 min and centrifuged at 5000*g* in an automatic, high-speed cold centrifuge for 10 min at 4 °C (Biofuge Primo R; Osterode, Heraeus, Germany). The supernatant fraction was collected and kept for MDA determination. A 125 μL volume of the supernatant fraction was placed in a 1.5 mL Eppendorf tube, and 25 μL of 6 M sodium hydroxide was added. Alkaline hydrolysis of protein-bound MDA was achieved by incubating this mixture in a 60 °C water bath for 30 min. Protein was then precipitated using 62.5 μL of 35% (v/v) perchloric acid, and the mixture was centrifuged at 2800*g* for 10 min. This step was performed before derivatization with 2,4-dinitrophenylhydrazine to minimize sample oxidation during analysis.

A 125 μL volume of supernatant fraction was transferred to an Eppendorf vial and mixed with 12.5 μL of 2,4-dinitrophenylhydrazine prepared as a 5 mM solution in 2 M hydrochloric acid. The mixture, protected from light, was then incubated for 30 min at room temperature. A 50 μL sample of this reaction mixture was injected into a Shimadzu SPD-M10A VP HPLC-DAD with a SphereClone 5 mm ODS 250 × 4.6 mm column (Phenomenex, Castel Maggiore, Italy). Samples were isocratically eluted at a flow rate of 0.8 mL/min at room temperature with a mixture of 0.2% (v/v) acetic acid in deionized water and acetonitrile (62:38, v/v). Chromatograms were obtained at 310 nm. MDA was quantified by means of a calibration curve constructed by injecting increasing amounts of standard MDA obtained by acid hydrolysis of tetraethoxypropane, as described by Mateos et al.[20] In short, to minimize any possible interferences during extraction, experimental samples were treated with 6 M NaOH for 30 min at 60 °C followed by protein precipitation with 35% perchloric acid before derivatization with 2,4-dinitrophenylhydrazine. MDA concentration was expressed as millimoles of MDA per gram of tissue.

Statistical Analysis. All analyses were performed in triplicate. Data were expressed as the mean ± SD. Fasting and oil oxidation global effects were tested by two-way ANOVA. To obtain statistical information on the NFC versus FC, NFT versus FT, NFC versus NFT, and FC versus FT group comparisons, a post hoc study was performed using the unpaired Student *t*test. *P* values of <0.05 were considered to be statistically significant. Statistical analyses were performed using the SPSS statistical software package (version 15.0).

RESULTS

TG polymer and dimer concentrations of sunflower oil used in 40 discontinuous procedures to fry various fresh and frozen foods were notably higher than those of unused sunflower oil. Oxidized TG and diglycerides increased, whereas free fatty acids decreased. Ox-FA and keto-LA increased around 6.9 and 7.8 times, respectively (Table 1). Overall, the data indicated that test rats receive significantly more ox-FA ($P < 0.001$) than control animals.

Luminal Gastric and Intestinal Fats. The HPLC method developed permitted the quantification of ox-FA and keto-LA in oil and biological fluids. Panels A and B of Figure 2 show the fatty acid pattern of sunflower oil both fresh and used for frying, respectively. Panels C and D of Figure 2 show the intestinal luminal fat pattern of control and test rats, respectively.

The fat in the gastric and intestinal lumens was affected by oil alteration (both, $P < 0.001$). NFT and FT animals presented more luminal gastric fat than their NFC and FC counterparts ($P < 0.01$ and $P < 0.05$, respectively). The amount of nonabsorbed fat in the intestinal lumen was also higher in NFT and FT rats than in the corresponding control groups (both, $P < 0.001$). FC animals displayed more luminal gastric fat after 5 h than NFC rats ($P < 0.05$). Total luminal gastrointestinal fat was affected by oil oxidation ($P < 0.001$) and fasting ($P < 0.05$). NFT and FT animals presented more total luminal gastrointestinal fat than their NFC and FC counterparts (both $P < 0.001$). More fat remained in the gastrointestinal lumen of FC rats after 5 h than in the gastrointestinal lumen of NFC animals ($P < 0.05$) (Table 2).

Oxidized Fatty Acids and Ketolinoleic Acids in Gastric and Intestinal Lumens. Ox-FA and keto-LA in the gastric lumen were affected by oil oxidation (both $P < 0.001$). NFT and FT animals had significantly higher levels of ox-FA and keto-LA in the gastric lumen than NFC and FC rats (both $P < 0.05$ and $P < 0.001$, respectively). Concentrations of ox-FA and keto-LA in the gastric lumen were significantly higher in NFC than in FC rats ($P < 0.01$ and $P < 0.001$, respectively) (Table 2). The percentage contribution of ox-FA to total FA in the gastric lumen was higher ($P < 0.05$) in FT than in FC rats and also higher in NFC than in FC rats ($P < 0.01$) (Figure 3A).

The concentration of ox-FA in the intestinal lumen was affected by oil oxidation ($P < 0.001$), whereas the concentration of keto-LA was significantly affected by the fasting × oil interaction ($P < 0.01$), oil oxidation ($P < 0.001$), and fasting status ($P < 0.05$). Concentrations of ox-FA and keto-LA in the intestinal lumen were significantly higher in the NFT and FT groups than in their control counterparts (all, $P < 0.001$). In the intestinal lumen of NFT rats a higher concentration of keto-LA was found compared to their FT counterparts ($P < 0.05$) (Table 2). The percentage of ox-FA with respect to the total FA in the intestinal lumen was higher in NFT and FT rats than in NFC and FC

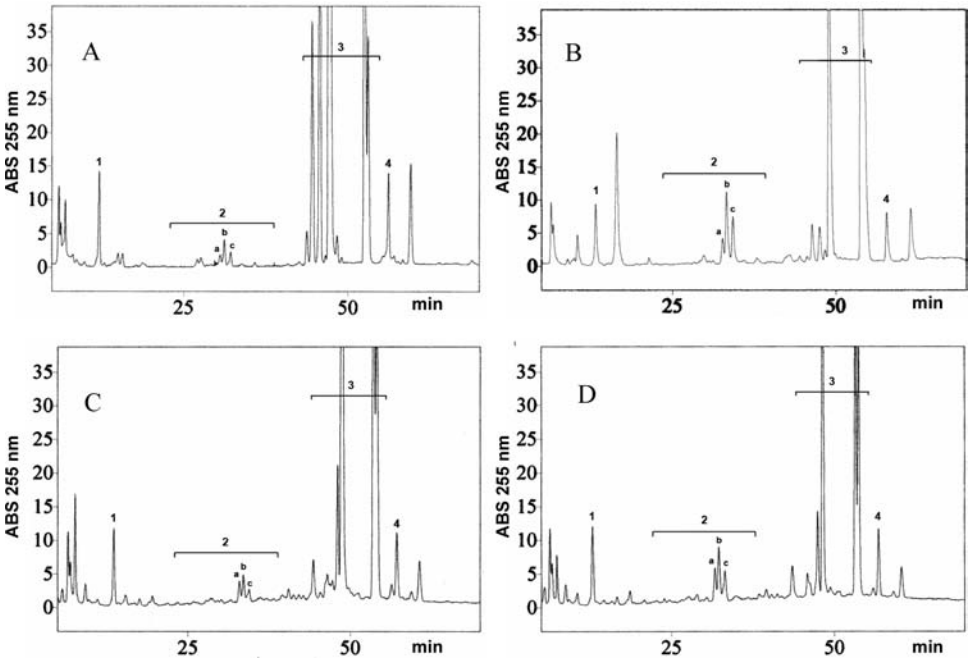

Figure 2. Representative HPLC chromatograms of fatty acid benzyl esters recorded at 255 nm: (A) unused sunflower oil (control oil); (B) sunflower oil used in frying (test oil); (C) luminal intestinal fat of fasting animals given control oil; (D) luminal intestinal fat of fasting animals given test oil. Peaks: 1, internal standard 1; 2, benzylesters of ox-FA; a−c, ketolinoleic acid isomers; 3, benzylesters of non-ox-FA; 4, internal standard 2. For abbreviations NFC, FC, NFT, and FT see the caption of Figure 1. Representative HPLC chromatograms of fatty acid benzyl esters recorded at 255 nm. **A**. Unused sunflower oil (control oil); **B**. Sunflower oil used in frying (test oil); **C**. Luminal intestinal fat of fasting animals given control oil; **D**. Luminal intestinal fat of fasting animals given test oil. 1: internal standard 1; 2: benzylesters of ox-FA; a, b and c: keto-linoleic acid isomers; 3: benzylesters of non-ox-FA; 4: internal standard 2. For abbreviations NFC, FC, NFT, FT see footnote Figure 1. *X*-axis, time in min; *Y*-axis, ABS 255 nm.

Table 2. Gastric and Intestinal Luminal Fats and Oil, Oxidized Fatty Acids (Ox-FA), and Ketolinoleic Acids (Keto-LA) Digestibilities of Fresh and Used in Frying Sunflower Oils at 5 h after in Vivo Administration, in Fasted and Nonfasted Rats[a]

					ANOVA		
	nonfasted controls (NFC)	fasted controls (FC)	nonfasted test (NFT)	fasted test (FT)	oil effect	nutritional status effect	interaction
sunflower oil administered (mg)	2447 ± 58	2132 ± 42 ***	2480 ± 116	2122 ± 52 ***	<0.001	ns	ns
oil in gastric lumen (mg)	124.1 ± 14.7	181.0 ± 57.8 *	258.18 ± 97.4 b	270.6 ± 59.9 c	<0.001	ns	ns
oil in intestinal lumen (mg)	126.8 ± 33.5	163.0 ± 56.9	317.0 ± 75.6 a	362.7 ± 88.1 a	<0.001	ns	ns
total gastrointestinal luminal oil (mg)	250.9 ± 33.2	334.0 ± 78.5 *	575.2 ± 121.8 a	633.3 ± 97.3 a	<0.001	<0.05	ns
oil digestibility (%)	89.8 ± 1.2	83.8 ± 4.2 *	76.7 ± 5.8 b	70.2 ± 4.2 a*	<0.001	<0.01	ns
ox-FA administered (mg)	59.1 ± 1.4	51.5 ± 1.0 ***	415.3 ± 19.4 a	355.3 ± 8.7 a***	<0.001	<0.001	<0.001
ox-FA in gastric lumen (mg)	39.2 ± 5.9	24.1 ± 0.1 **	83.4 ± 34.0 c	113.4 ± 9.9 a	<0.001	ns	ns
ox-FA in intestinal lumen (mg)	15.0 ± 4.6	16.0 ± 4.9	79.7 ± 9.2 a	66.0 ± 13.2 a	<0.001	ns	ns
total ox-FA digestibility (%)	6.9 ± 9.5	19.7 ± 10.2	60.0 ± 7.5 a	49.1 ± 3.7 a*	<0.001	ns	<0.05
keto-LA administered (mg)	36.9 ± 2.1	32.2 ± 1.5 ***	292.4 ± 13.7 a	250.1 ± 6.1 c***	<0.001	<0.001	<0.001
keto-LA in gastric lumen (mg)	32.6 ± 3.6	21.5 ± 1.8 ***	72.1 ± 30.9 c	103.0 ± 9.1 a*	<0.001	ns	<0.05
keto-LA in intestinal lumen (mg)	9.1 ± 2.0	12.2 ± 3.7	56.2 ± 4.3 a	44.0 ± 7.9 a*	<0.001	<0.05	<0.01
total keto-LA digestibility (%)	−16.3 ± 9.9	−5.4 ± 15.5	55.7 ± 7.7 a	41.5 ± 3.7 a**	<0.001	<0.05	<0.05

[a] Data are the mean ± SD of six animals per groups. FA, fatty acids. The digestibility was calculated according to the following expression: 100 × (injected fat minus remainder lumen fat)/(injected fat). NFT and FT data bearing a letter (c, $P < 0.05$; b, $P < 0.01$; a, $P < 0.001$) were significantly different from NFC and FC, respectively. FC and FT data bearing asterisks (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) were significantly different from NFC and NFT, respectively; ns, not significant.

animals ($P < 0.01$ and $P < 0.05$, respectively), and, more interestingly, higher in NFT than in FT rats ($P < 0.05$) (Figure 3B).

Digestibility of Oils and Oxidized FA. The total apparent digestibility of oil was affected by oil oxidation ($P < 0.001$) and fasting conditions ($P < 0.01$). NFC and FC displayed higher oil digestibility values than NFT and FT rats, respectively (at least, $P < 0.01$). Oil digestibility was higher in NFC and NFT than FC and FT rats (both $P < 0.05$). Ox-FA digestibility was affected by

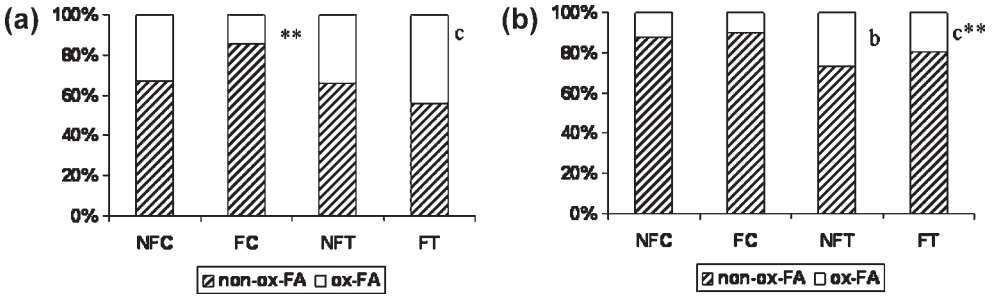

Figure 3. Percentage of nonoxidized fatty acids and oxidized fatty acids present 5 h after administration of unused sunflower oil and sunflower oil used in 40 frying operations. (a) Gastric lumen. Fasting conditions effect, $P < 0.05$; oil effect, $P < 0.05$; fasting conditions × oil alteration interaction, nonsignificant. NFT and FT data bearing a letter (c, $P < 0.05$) were significantly different from NFC and FC values, respectively. FC and FT data bearing asterisks (**, $P < 0.01$) were significantly different from NFC and NFT values, respectively. (b) Intestinal lumen. Fasting conditions effect, nonsignificant; oil effect, $P < 0.01$; fasting conditions × oil alteration interaction, nonsignificant. NFT and FT data bearing a letter (b, $P < 0.01$; c, $P < 0.05$) were significantly different from NFC and FC values, respectively. FC and FT data bearing asterisks (**, $P < 0.01$) were significantly different from NFC and NFT values, respectively. For abbreviations NFC, FC, NFT, FT see the caption of Figure 1. Data are the mean ± SD of six animals per group.

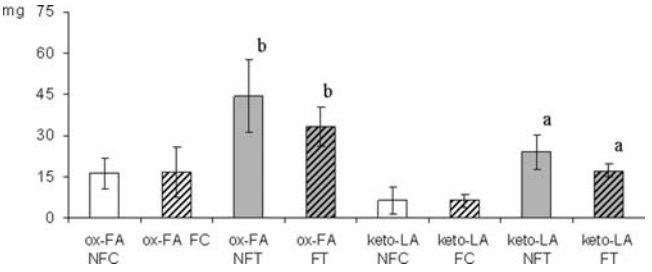

Figure 4. Amount (mg) of total oxidized fatty acids (ox-FA) and ketolinoleic acids (keto-LA) present in the intestinal tissue 5 h after administration of unused sunflower oil and sunflower oil used in 40 frying operations. For ox-FA, fasting conditions effect, nonsignificant; oil effect, $P < 0.001$; fasting conditions × oil alteration interaction, nonsignificant. NFT and FT data bearing a letter (b, $P < 0.01$) were significantly different from NFC and FC values, respectively. For ketolinoleic acids (keto-LA), fasting conditions effect, nonsignificant; oil effect, $P < 0.001$; fasting conditions × oil alteration interaction, nonsignificant. NFT and FT data bearing a letter (a, $P < 0.001$) were significantly different from NFC and FC values, respectively. For abbreviations NFC, FC, NFT, and FT see the caption of Figure 1. Data are the mean ± SD of six animals per group.

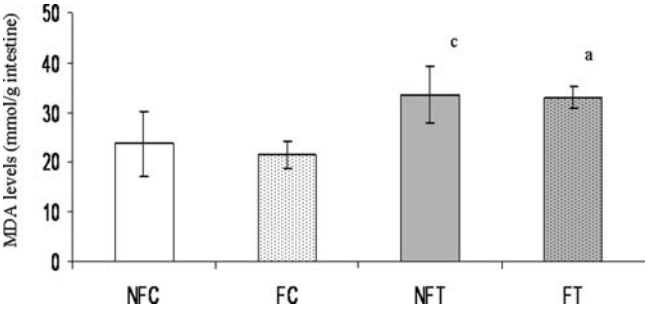

Figure 5. MDA levels (mmol MDA/g intestine) in intestinal tissue of Wistar rats 5 h after administration of unused sunflower oil and sunflower oil used in 40 frying operations. Fasting conditions effect, nonsignificant; oil effect, $P < 0.001$; fasting conditions × oil alteration interaction, nonsignificant. NFT and FT data bearing a letter (a, $P < 0.001$; c, $P < 0.05$;) were significantly different from NFC and FC values, respectively. For abbreviations NFC, FC, NFT, and FT see the caption of Figure 1. Data are the mean ± SD of six animals per group.

oil oxidation ($P < 0.001$) and by the fasting × oil oxidation interaction ($P < 0.05$). Digestibility of keto-LA was affected by the fasting × oil interaction ($P < 0.05$), oil oxidation ($P < 0.001$), and fasting status ($P < 0.05$). NFT and FT rats presented higher digestibility of ox-FA and keto-LA than NFC and FC rats ($P < 0.001$). Digestibilities of ox-FA and keto-LA were higher in NFT than FT rats ($P < 0.05$ and $P < 0.01$, respectively) (Table 2).

Intraintestinal Concentration of Oxidized Fatty Acids, Ketolinoleic Acids, and Malondialdehyde. Ox-FA and keto-LA in the small intestine were influenced by oil oxidation (both $P < 0.001$). NFT and FT animals showed significantly higher levels of ox-FA ($P < 0.01$) and keto-LA ($P < 0.001$) in intestinal tissue than their NFC and FC counterparts (Figure 4). MDA concentrations in the small intestine were affected by oil oxidation ($P < 0.001$). NFT and FT presented higher intestinal levels of MDA than NFC and FC rats ($P < 0.05$ and $P < 0.001$, respectively) (Figure 5). Intraintestinal MDA concentrations were significantly correlated with intraintestinal keto-LA values [$P < 0.05$; $r^2 = 0.180$; slope (mean ± SE) = 0.348 ± 0.162; intercept (mean ± SE) = 23.45 ± 2.45].

DISCUSSION

Nonvolatile ox-FA and keto-LA were detected in the gastric and intestinal lumens and intraintestinal fat 5 h after oil administration. Intestinal MDA content was correlated with that of keto-LA.

During the frying process, culinary oils are subjected to high temperatures in the presence of water and oxygen, conditions favoring their polymerization, oxidation, and hydrolysis,[2,9,21−23] especially under nonadequate frying conditions.[2,23] Keto-LA contributes abundantly to ox-FA in sunflower oils (62.6% in control oil and 70.4% in oxidized oil). The amount of altered FA in the oil increases with the number of frying operations.[24−26] According to Dobarganes et al.[27] and Sánchez-Muniz et al.,[2] 25% polar material corresponds to 8−11% polar FA (ox-FA and polymerized FA). In the present study, 33% polar material in the thermal oxidized oil corresponded with 16.74% ox-FA. After 40 frying operations, ox-FA and keto-LA increased about 7-fold.

The test oil under both fasting and nonfasting conditions showed lower digestibility than the control oil. The more complex the polymers, the more complicated the hydrolysis of the ester bonds by pancreatic lipase.[4,5,12,30] As a result, the formation of

compounds that can be absorbed by the intestinal mucosa (free ox-FA and free non-ox-FA and monoglycerides) slowed.

As shown in a previous study,[9] luminal gastric fat content rose after intake of highly altered oils. Benini et al.[28] observed that total gastric emptying time and the sensation of satiety following the meal was greater when food was prepared with oil previously used in frying than when fresh oil was used. The concentration of luminal gastric fat in the present study was greater than that reported by Olivero-David et al.[9] despite a longer experiment time (5 vs 4 h). However, the difference can be explained by considering the difference between the two studies in the duration of the adaptation period and in the oil administration and fasting conditions.

In line with the results of the previous study,[9] animals given the thermally oxidized oil had a higher amount of luminal intestinal fat than the control animals, suggesting that pancreatic lipase is less effective in hydrolyzing oxidized oils than the control oil. Márquez-Ruiz and Dorbarganes[29] and Arroyo et al.[30] reported that in vitro studies showed that oil polymerization decreased pancreatic lipase activity. The longer absorption period in the present study compared to that in Olivero-David et al.'s study[9] is probably responsible for the lower amount of luminal intestinal fat. Previously, this research group[31] found that the presence of nonabsorbed oil decreased as the length of the absorption period increased.

Fasted rats given the oxidized oil (FT) displayed the greatest amount of luminal gastrointestinal fat 5 h after oil administration. This group previously reported similar results, suggesting that this could be due to the fact that fasting conditions limited the amount/activity of pancreatic lipase available following oil administration.[9] This means that the practice of skipping breakfast ought to be considered an unhealthy eating habit.

As expected, levels of ox-FA and keto-FA in the luminal gastric fat in test animals were higher than in control rats. An interesting finding is that keto-LA in the gastric lumen represents 83.2, 89.2, 86.5, and 90.8% of the total amount of ox-FA of NFC, FC, NFT, and FT, respectively, where this contribution was 62.6 and 70.4% in the control and test oils, respectively. These data suggest that the percentage contribution of keto-LA to ox-FA was higher than that of the oil administered. This observation reinforces the role of lipid hydroperoxide formation in foods and during digestion, especially in the low-pH gastric fluid, which contains dissolved oxygen and iron.[32] However, according to present data, the contribution of the stomach to the luminal keto-LA seems relevant only in the case of oil with low oxidation.

Concentrations of ox-FA and keto-LA in the intestinal lumen of NFC and FC rats 5 h after oil administration were approximately one-fourth of those observed in NFT and FT animals. Keto-LA in the intestinal lumen represents about 70% of the total amount of ox-FA. This suggests that the percentage contribution of keto-LA to the ox-FA was similar to that of the oil administered, but lower than that observed in the gastric lumen.

However, the percentage contribution of ox-FA to the total fatty acids present in the intestinal lumen of NFC and FC rats (12.5 and 10.3%, respectively) was lower than that of NFT (26.5%) and FT (19.2%) animals, suggesting that oxidized fatty acids of test oils are absorbed less than those of control oils. The absorption of non-ox-FA also decreased in altered oil. Previous in vitro [7,30] and in vivo studies[31,33] suggested that the presence of thermally oxidized compounds in oil partially inhibits hydrolysis of nonoxidized TG by pancreatic lipase.

Although the digestibility of test oil was lower than that of control oil, the digestibility of ox-FA and keto-LA was much higher in FT and NFT than in FC and NFC rats, respectively. The digestibility of keto-LA was also negative, suggesting keto-LA production during the digestion process and reinforcing the role of lipid hydroperoxide formation mostly in the gastric fluid.[32] Fasting significantly affected the digestibility of oxidized oils but not of control oil. Simultaneous intake of food containing antioxidants or non-ox-FA together with the test oil may improve the effectiveness of pancreatic lipase on oxidized TG, facilitating the digestibility of ox-FA and keto-LA. This effect was less important in the case of control oil due to its low ox-FA and keto-LA content.

Penumetcha et al.[34] observed that Caco-2 cells displayed a relatively high absorption rate of nonoxidized oleic, nonoxidized linoleic, and oxidized linoleic acids (55, 70, and 40−58%, respectively). These authors reported that the uptake of oxidized linoleate by Caco-2 cells was not saturable even at 150 μM and that this uptake was diluted in the presence of nonoxidized linoleate.

Perkins et al.[35] studied the absorption of nonvolatile oxidized compounds obtained from corn oil heated at 200 °C for 24 h and observed that male rats administered fatty acid methyl esters preferentially absorbed the less polar nonvolatile oxidized compounds during the hours immediately after intake.

The amount of total ox-FA and keto-LA in the intraintestinal tissue of NFT and FT animals was approximately 2.5 times higher than that observed in their NFC and FC counterparts. However, fasting conditions did not significantly affect the amount of intraintestinal ox-FA and keto-LA. Animals given the test oil displayed higher intraintestinal levels of MDA than their control counterparts. This finding was directly related to the presence of keto-LA in these animals, indicating that the prolonged presence of high concentrations of these compounds is damaging to the intestinal cells.

In a previous related study[14] thermally oxidized sunflower oil was administered to rats, during the three fist days of the treatment period, with less amount of oil than in the present paper. A decrease was observed in glutathione levels, superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities, and SOD, glutathione reductase, and GPx gene expression, whereas a TNF-α expression increase occurred 4 h after oil administration. These effects were most evident in the rats fasted for 15 h on the last day of treatment.[14] According to Olivero-David et al.,[3] polymers formed in the intestinal mucosa contribute to the formation of intraintestinal TBARS in rats fed palm olein used to fry potatoes several times.

Ringseis et al.[36] reported that DNA-binding activity of PPARγ increased in intestinal epithelial cells of growing pigs given a diet enriched with very slightly heated sunflower oil, in contrast to those given a diet with fresh fat. These authors also observed that animals given the oxidized fat displayed higher TBARS concentrations than rats fed fresh fat.

In conclusion, this is the first report on short-term absorption of ox-FA in the gastrointestinal tract of rats given several doses of thermally oxidized oil. Results suggest that digestion contributes to ox-FA and keto-LA formation, mostly in fresh oil. This contribution seems less relevant in the case of highly oxidized oils. Total ox-FA and keto-LA were efficiently absorbed during the first 5 h after thermally oxidized oil administration, but less efficiently in the case of fresh oils. Their concentrations in intraintestinal tissue appear to be correlated with the amount administered and intraintestinal tissue MDA levels. Although oil alteration influenced the digestibility of these compounds more than fasting, the latter significantly affected the digestibility of oxidized oil.

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Author Contributions

All authors have significantly contributed to the paper and agree with its present version. F.J.S.-M. is the corresponding author and guarantor of the paper and has contributed to the study design, data discussion, and writing of the paper. A.P., P.V., and R.O.-D. have contributed to the data acquisition and analysis. J.B., M.J.G.-M., R.S., and V.F. have contributed to data discussion and critical review of the paper. R.S. and VF have suggested the use of HPLC methods to monitor Ox-FA, keto-LA and lipid peroxidation.

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Notes

We declare there are no conflicts of interest.

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ABBREVIATIONS USED

DAD, diode array detector; FA, fatty acids; ox-FA, oxidized fatty acids; FC, fasted control; FT, fasted test; keto-LA, ketol-inoleic acids; LA, linoleic acid; MDA, malondialdehyde; NFC, nonfasted control; NFT, nonfasted test; TBARS, thiobarbituric acid reactive substances; TG, triglycerides.

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