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Basic nutritional investigation

Early endothelial nitrosylation and increased abdominal adiposity in Wistar rats after long-term consumption of food fried in canola oil

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ABSTRACT

Objective: The aim of this study was to establish whether the long-term consumption of reused canola oil contributes to the development of dyslipidemia, obesity, and endothelial function.
Methods: Canola oil was used for one frying cycle (1 FC) of corn flour dough or reused 10 times (10 FC). Rats received chow diet (control) or supplemented with 7% raw oil (RO), 1 FC or 10 FC oil (n = 10 per group). Food consumption, blood pressure (BP), and body weight plasma glucose, plasma lipids were monitored. Vascular reactivity was analyzed using aorta rings stimulated with phenylephrine and acetylcholine. Nitrotyrosine presence in aorta rings was analyzed by immunohistochemistry.
Results: After 10 wk of follow-up, visceral adipose tissue was significantly more abundant in 1 FC (7.4 ± 0.6 g) and 10 FC (8.8 ± 0.7 g) than the RO (5.0 ± 0.2 g; $P = 0.05$ versus 10 FC group) or control group (2.6 ± 0.3 g; $P = 0.05$ versus all groups). Despite similar plasma cholesterol, triglycerides, and BP among groups, a significantly reduced acetylcholine-induced vascular relaxation was observed in the three groups receiving the oil-supplemented diet (47.2% ± 3.6%, 27.2% ± 7.7%, and 25.9% ± 7.6% of relaxation, for the RO, 1 FC, and 10 FC, respectively; $P < 0.05$ for all versus 62.4% ± 9.7% of the control group). Endothelial dysfunction was concomitant with the presence of nitrotyrosine residues at a higher extent in the groups that received heated oils compared with the RO group.
Conclusion: High canola oil intake over 10 wk was associated with increased adipose tissue and early endothelial dysfunction probably induced by peroxinitrite formation. Such deleterious effects were significantly potentiated when the consumed oil had been used repeatedly for frying.

Introduction

Reuse of vegetable oils for frying food is a common practice in the culinary tradition in several countries. However, numerous potential toxic compounds are produced when vegetable oils are heated or reheated during long periods and/or high temperatures. The toxic compounds include unsaturated aldehydes [1], cyclic fatty acids [2], and trans-fatty acids [3]. These compounds

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have been suggested to produce liver damage [2,4], altered body weight gain [5], endothelial dysfunction [6,7], increased oxidative stress [8], vascular inflammation [9], lipoprotein abnormalities [10,11], and risk for coronary heart disease [12].

Previous studies have demonstrated that chronic consumption during 6 mo of reused vegetable oils was associated with hypertension in rats [6,7]. In contrast, these results have not been consistently confirmed during shorter periods (10 wk), not even on spontaneously hypertensive rats [8], suggesting that clinical manifestations appear only after long periods of chronic consumption of reused vegetable oils. Nevertheless, it cannot be discarded that reused oils for frying induce early cardiometabolic effects before the detectable clinical symptoms. Thus, in this study we explored the possible early damage to endothelial cells, and occurrence of obesity or dyslipidaemia derived from the ingestion of reused canola oil in Wistar rats.

Materials and methods

Animals and study design

Forty-eight male Wistar rats weighing 300 to 330 g were randomly assigned to four dietary groups comprised of 12 animals each. Animal manipulations followed with the recommended guidelines; the Scientific and Ethics Committees of the Instituto Nacional de Cardiología "Ignacio Chávez" approved all procedures.

The rats were maintained at a temperature of 25°C with a 12-h light/dark cycle. All rats had free access to food and water during the study period. During the 10 wk of the study, each group of rats received during one of the following diets: group 1 was given only commercial rat chow diet (control group); the other groups were fed with the chow diet supplemented with 7% weight/weight of raw canola oil (RO group), canola oil used once (one cycle) for frying (1 FC group), or used 10 times (10 cycles) for frying (10 FC group).

Systolic blood pressure was measured using a non-invasive method previously described [13] at baseline and at 1-wk intervals along the study. Body weight and caloric consumption were monitored periodically along the study. Blood samples were drawn from the tail vein for periodic biochemical analyses. After 10 wk of treatment, under pentobarbital anesthesia, blood samples were drawn from the hepatic artery; then, animals were sacrificed and abdominal aortas were collected for histologic analysis and vascular reactivity studies as described below. The blood samples were immediately processed for total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides (TGs), and glucose plasma concentrations, and aliquots were stored at −70°C for further studies. Abdominal adipose tissue was carefully dissected, washed in isotonic saline solution, gently wiped, and weighed on an analytical balance.

Preparation of oil for dietary supplement

Canola oil used in this study was a local commercial trademark available in most local stores. It was used either fresh, used for frying once, or reused 10 times following the method previously described [7] with some slight modifications. Briefly, 1 L of oil was heated at 190°C ± 5°C in a stainless steel wok and used to deep-fry 100 g of cornmeal dough. The heating process lasted for 10 min. The hot oil was then left to cool at room temperature for 30 min. This corresponds to the canola oil of one frying cycle (1 FC). The cooled oil was used to deep-fry another new batch of 100 g of corn flour dough and the process was repeated up to 10 times. This corresponds to the canola oil with 10 frying cycles (10 FC). The frying process was carried out without replenishing with fresh oil. The experimental diets were prepared twice a week by spreading the oil on the pellets of the laboratory rodent diet 5001 (LabDiet, San Louis, MO, USA) at a final proportion of 7% w/w. Used oils and supplemented pellets were kept under nitrogen atmosphere at 4°C until use.

Biochemical analyses

Glucose, TGs, and cholesterol plasma concentrations were determined by enzymatic colorimetric assays commercially available (Randox, England). Low-density lipoprotein cholesterol (LDL-C) and HDL-C were determined in plasma after ultracentrifugation because rats' apoB containing lipoproteins do not precipitate quantitatively with the polyanion solutions usually used for human samples [14–16].

Table 1
Chemical composition and nutritional facts of the rat chow diet and the supplemented diet with 7% of canola oil

Nutriment	Chow diet*	Supplemented with 7% canola oil†
Protein (%)	23.9	22.2
Fat (%)	5.7	12.3
Cholesterol (ppm)	200	186
Linoleic acid (%)	1.22	2.54
Linolenic acid (%)	0.10	0.83
Total saturated fatty acids (%)	1.56	1.92
Total monounsaturated fatty acids (%)	1.60	2.89
Crude fiber (%)	5.1	4.7
Nitrogen-free extract (%)	48.7	45.3
Starch (%)	31.9	29.6
Glucose (%)	0.22	0.20
Fructose (%)	0.30	0.27
Sucrose (%)	3.70	3.44
Lactose (%)	2.01	1.87
Energy (kcal/g)	4.07	4.40
Calories provided by:		
Protein (%)	28.507	23.694
Fat (ether extract) (%)	13.496	28.100
Carbohydrates (%)	57.996	48.205
Vitamin E (IU/kg)	42	55

* As reported by the manufacturer (LabDiet, St. Louis, MO, USA).
† Calculated on the basis of the 93% chow diet and 7% of canola oil.

Vascular reactivity of aorta rings

Rats were anesthetized with pentobarbital (45 mg/kg of body weight, i.p.), the thoracic aorta was dissected free from surrounding tissues and cut into rings of 3 mm in length. The preparation was then transferred into organ baths with 10 mL of Krebs solution (composition, mM: NaCl 118.1, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.20, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, glucose 11.1 at pH 7.4), continuously bubbled with 95% O_2 to 5% CO_2, at 37°C. Each aortic ring was mounted between two L-shaped stainless steel hooks. One of the hooks was mounted at the bottom of the bath while the other was connected to a force displacement transducer and 1 g basal tension was applied to all experiments. After an equilibrium period of 2 h, endothelium integrity was verified by the presence of the relaxant response (>80% relaxation) to 3 μM acetylcholine (ACh) at the start of each experiment. The contractile capacity of vascular smooth muscle cells was evaluated through the dose–response curve to phenylephrine (PE, 6.25×10^{-9} to 1×10^{-4} M). The dose–response curve to ACh (6.25×10^{-9} to 1×10^{-4} M) was determined in rings precontracted by 3×10^{-6} M of PE. Vasorelaxation is expressed as the percentage of precontraction with PE. Contractions were measured isometrically with an FT-03 Grass Force Displacement Transducer and recorded on a Grass Polygraph (Model 7 D, Grass Medical Instruments, Quincy, MA, USA) [17].

Immunohistochemistry

Immunohistochemistry was performed to determine the relative extent of protein nitration (a stable biomarker of endogenous peroxynitrite formation), using an antibody raised against 3-nitrotyrosine (3-NT) [18]. Aorta samples were cut and prepared as paraffin-embedded sections. The sections were then dewaxed in xylene for 5 min and dehydrated in graded alcohol. The slides were immersed in H_2O_2 (3%) for 15 min at room temperature to block endogenous peroxidase activity. Non-specific protein binding was blocked by incubation with 1.5% goat serum in phosphate buffer saline 10 mM, pH 7.4 for 1 h. The slides were incubated overnight at 4°C with anti3-NT (1:200 dilution, Upstate Biotechnology, Lake Placid, NY, USA). Biotinylated secondary goat antimouse was then applied (1:200) followed by horseradish peroxidase complex reagent (ABC staining system, Santa Cruz, CA, USA). Positive immunoreactivity was visualized through the development of diaminobenzidine (DAB) chromogen. Hematoxylin was used for nuclear counterstaining. Unmodified images were analyzed using research-based image analysis software (Image-Pro Plus, Media Cybernetics Inc., Bethesda, MD, USA). Ten endothelial regions of each aortic ring were systematically captured at 200×, representing 90% of the total vascular endothelium circumference.

Statistical analysis

Data are expressed as the mean ± SEM if not otherwise indicated. Comparisons among groups were made using analysis of variance (ANOVA) test and least significance difference post hoc analysis. Significance of differences was set at $P < 0.05$.