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Fried Sunflower Oil intake affects Bone Quality, in Growing Rats

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Abstract

The effect of consuming a diet containing sunflower oil, which was repeatedly used to fry potatoes, on bone mass and biomechanical competence in growing rats, was investigated. Male Wistar rats (21±1 days old) (n=34) were assigned at weaning to one of three diet groups for 8 weeks: those fed a control diet (C; n=10), a sunflower oil (SFO; n=12) diet or a SFO diet which was repeatedly heated (SFOx; n=12); both mixed with a commercial rat chow at weight ratio of 13% (w/w). Zoometrics, food intake, serum fatty acids composition and lipid profile, total skeleton bone mineral density (BMD), bone mineral content (BMC), and femur biomechanical competence were assessed. Animals fed SFOx diet had altered body growth and showed both increased serum total cholesterol (T-chol) and non-high-density lipoprotein cholesterol (non-HDL-chol). SFOx rats also showed marked differences in BMC, their femur and tibiae had the lowest structural and geometrical properties, but material properties were unchanged. This study showed that growing male rats consuming a SFOx diet adversely affected their body growth, and lipid metabolism. Additionally their BMC and bone biomechanical properties were also altered due to modifications in bone geometric design.

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Introduction

Dietary oxidized lipids, resulting from frying fats and oils, have been shown to induce negative biological effects.[1,2] Frying oil intensifies the oxidation rate and the unsaturated fatty acids content. During heating and frying the polyunsaturated fatty acids (PUFA) yield more free fatty acids and more polar compounds which alter fatty acid composition and oil stability, altering the flavor and quality of food.[3,4]

Thermal oxidation of unsaturated triglycerides (TAGs) engenders oxidized TAGs, with formation of decomposition products and polymerized foodstuffs.[5] Triglycerides are oxidized and degraded, initially forming oxidized triglycerides 3 and upon further oxidation yielding aldehydes and ketones.[6] These products negatively impact the quality food,[7] and some of the frying compounds are potentially harmful.[5]

In Argentina, sunflower oil is the most frequent oil-used for frying and is consumed in large amounts, fried potatoes being the most consumed deep-fried food.[8] Heating this oil produces the highest emissions of total aldehydes[5,9] mainly generated from polyunsaturated triacylglycerols (TAGs) and from other components and reactive intermediates of fried food[10] and from the cis-trans isomerization occurring during the deep-fat frying process.[11] The intermittent heating and cooling of oils foster increases in mutagenic and carcinogenic products, such as aldehydes.[6,12,13] Furthermore, not only the consumers of fried-food are endangered, those exposed to the repeated cooking with frying oils, specially soy oil or sunflower oil, may also be harmed by the regular exposure to the harmful volatiles compounds generated.[14,15] The total polar compounds and distribution of lipids extracted from deep-fried potatoes were like to those obtained from other oils.[16]

It has been demonstrated that the fried foods intake contributes to the development of chronic diseases such as obesity,[17] atherosclerosis,[18] liver damage[19,20] and deterioration of bone histomorphometric parameters.[21] However, the effects of fried sunflower oil on bone mineral content and bone mechanical competence during growth have not been reported.

In the current study, the negative effect of sunflower-fried food consumption on bone mass and strength during growth was hypothesized. Thus, the present study investigated the effect of fried sunflower oil diet on bone mass and bone biomechanical competence of growing rats.

Materials and Methods
Animals

Thirty-four male weanling Wistar rats, weighing 46.4 (SD= 5.0) g were provided by the Animal Resources of the Department of Biochemistry, School of Dentistry, University of Buenos Aires, Argentina. The rats were housed in steel individual cages under Laboratory conditions (light-dark 12:12 hours, 21 ± 1 °C and 50–60% humidity). Animals were kept according to the Principles of Laboratory Animal Care established by the National Institute of Health (NIH), USA. Ethics Committee. University of Buenos Aires approved the protocol (UBACyT 20020170100138BA, N° 004/17).

Diets

The composition of the three diets utilized in the experiment is shown in Table 1. The control diet (C) was a commercial stock diet (Purina chow; Gilardoni SA, Buenos Aires, Argentina). The two experimental diets (SFO and SFOx) differed from the C in fat content and composition as shown in Table 1. Every other day diets were prepared and kept refrigerated. Food intake was measured (Mettler scale PC 4000; accuracy ± 1 mg) and expressed as kcal per 100 g of body weight and per day (kcal/100 g W/day).

C and SFO or SFOx provided 14% and 46% per calories from lipids, respectively. SFO and SFOx were mixed with commercial rat chow at weight ratio of 13% (w/w) of diet. The oils composition of the experimental diets were as follows: (1) SFO composition was: Saturated Fat: 10.1%; Monounsaturated Fat: 27.8%; Polyunsaturated Fat: 61.3%; TFA: 0.7%; (2) SFOx composition: Saturated Fat: 18.09%; Monounsaturated Fat: 33.15%; Polyunsaturated Fat: 46.78%; TFA: 1.66%.

Diets contained calcium carbonate anhydrous at 40.04% (Ca = 143 mg/kg mix), potassium phosphate monobasic at 22.76% (P=44.60 mg/

kg mix) and Vitamin D-3 (400 000 IU/g = 250 mg/kg mix) (AIN-93G) 22; 0.6 mg alpha-tocopherol equivalents/g. PUFA were added to the high fat diets as recommended by Valk & Hornstra.[23]

Frying Procedure
Commercial deep-fat fryers with pots (8L) were used for the repeated frozen potato-frying procedure. To assess the oil oxidation, sunflower-oil was heated for 6 hours/day in order to achieve a total of 20, 40 or 60 hours of frying procedure and then, a Testo Equipment, Model 270 at 50°C was used to analyze the polar compounds (triacylgycerol polymers, dimers, monomers, and oligomers, oxidized triacylglycerols, diacylglycerols and non-esterified fatty acids).

Table 1: Diets composition

Ingredients (g/100g)	C	SFO	SFOx
Starch	48	30.7	30.7
Protein (mix of wheat, corn, fish, soybean and meat flour)	23	14.7	14.7
Fat (acid hydrolysis) (mix of fish oil and corn oil)	7	4.5	4.5
Other source of fats:			
Cholesterol (ppm)	200	128	128
Linoleic acid	1.3	0.83	0.83
Linolenic acid	0.11	0.07	0.07
Arachidonic acid	<0.01		
Omega-3 fatty acids	1.9	1.9	1.9
Total saturated fatty acids	1.89	1.22	1.22
Total monounsaturated fatty acids	1.98	1.27	1.27
SFO		13	
SFOx			13
Fiber	6	3.8	3.8
Minerals	4.09	2.61	2.61
Calcium	1.2	0.77	0.77
Phosphorous	0.8	0.5	0.5
Magnesium	0.17	0.11	0.11
Potassium	1.1	0.7	0.7
Iron (ppm)	95	61	61
Copper (ppm)	9	5.8	5.8
Zinc (ppm)	47	28	28
Sulfur	0.14	0.09	0.09
Selenium (ppm)	0.3	0.19	0.19
Manganese (ppm)	25	16	16
Sodium	0.2	0.13	0.13
Chlorine	0.3	0.19	0.19
Iodine (ppm)	0.6	0.38	0.38
Vitamins mixture	1.5	0.96	0.96
Water	8.5	28.5	28.5
Total kcal	347	339.1	339.1

C: Standard diet; SFO: C diet supplemented with fresh sunflower oil; SFOx: C diet supplemented with thermoxidazed sunflower oil.

At 20, 40 and 60 h, the polar compounds were (n=8; mean ± DS) 20.5±1.02, 29.5±1.77 and 30.5±1.28 g/100g, respectively. Acidity (g oleic acid/100g oil) at 20, 40 and 60 h was 0.110±0.004, 0.160±0.001 and 0.200 ±0.000, respectively. Then, taking into account that frying oils for human consumption must be discarded when polar compounds concentration reaches more than 25%,[24-26] the heating time was set at 40 h. Briefly, the frying lasted for 6 h/day at 180ºC; the oil was allowed to cool after each round and it was repeated successively for seven days. Every twelve hours, 500g of potatoes were added to 5 liters of frying oil.

Determination of FA Composition of the Commercial Stock Diet and Oils

Fatty acid composition of the commercial stock diet, the conventional sunflower oil (SFO) and the thermoxidazed sunflower oil (SFOx) were determined by gas chromatography (GC) technique. Samples preparation were as follows: 100 mg of diet or oil was placed in a tube with a screw cap; 0.35 - 0.40 mL of sodium methoxide solution (0.25 g of metallic sodium in 25 mL of methanol) was added to the tube containing the sample and brought to a bath at 80 °C with shaking for 60 seconds. After 3 minutes, sample was removed from the bath and 0.5 g of the desiccant mixture (calcium chloride - silica gel; 50:50 was added. Then, 3 ml of hexane was added and shacked in vortex for one minute. Sample was centrifuge at 1500 - 1800 rpm and the supernatant containing the methyl ethers was injected into the chromatograph.

Chromatographic Conditions were as follows

Chromatograph Perkin Elmer model Claurus 500, column Supelco SP 2560 (100 m x 0.25 mm x 0.20 µm (a polar biscyanopropyl column specifically designed for detailed separation of geometric positional cis / trans isomers of fatty acid methyl esters – FAMEs-), gas carrier: nitrogen, flame ionization detector (FID, Flame Ionization Detector) at 280 °C on samples derivatized according to Argentine Institute for Standardization and Certification (IRAM) 5650 Part II.[27,28]

The fatty acids were identified against patterns SUPELCO F.A.M.E. Mix NHI-C and F.A.M.E. Mix C14-C22; each fatty acid was expressed as the percent (%) of total fatty acids content.

Experimental Design

Rats were randomly assigned to feedings of one of three different diets. The control rats were fed a C diet and the experimental rats received either SFO or SFOx diets. All rats were fed ad *libitum* throughout the experiment. Food consumption was recorded every other day. At eight-week experimental period, zoometrics and dual-energy x-ray absorptiometry (DXA) analyses were performed under light anesthesia (5% ketamine hydrochloride; 5 mg/100 g i.p. Holliday–Scott SA. Buenos Aires. Argentina and 2% xylazine hydrochloride; 0.5 mg/100 g i.p. Konig Laboratories. Buenos Aires. Argentina). The animals were euthanized under anesthesia (0.1 mL of ketamin hydrochloride; 100 mg/mL, Holliday Lab.)/100 g body weight was mixed with 0.02 mL of xylazine; 100 mg/mL, Konig Lab. /100 g body weight) by intramuscular injection. Then, blood samples were collected by cardiac puncture, placed in tubes and the serum was obtained by centrifugation. Serum was stored at ¬–20ºC for biochemical assays. Biochemical determinations and biomechanical assessment were performed in all rats.

Zoometrics

Throughout the experimental period, body weights (W, measured in a Mettler PC 4000 scale; accuracy ± 0.001g) and body lengths (L) were registered weakly, after 2 to 4 hours fasting.[29]

Biochemical Determinations

Blood samples were collected by cardiac puncture at the end of the experimental period. Total serum cholesterol (T-chol; mg/dL of serum), high-density lipoprotein-cholesterol (HDL-chol; mg/dL of serum) and triglycerides (TG; mg/dL of serum) were determined by standardized methods (Roche Diagnostics GmbH, Mannheim, Germany) in a Hitachi 917 autoanalyzer (Hitachi, Tokyo, Japan).

Serum non-HDL cholesterol (mg/dL), a set of atherogenic lipoproteins rich in apo B,[30] was calculated as the difference between the T-chol and HDL-chol, due to the loss of sensitivity of this parameter, usually significantly lower than serum HDL-chol levels, in rats.

Serum Fatty Acid Composition

Serum fatty acid composition was determined by gas chromatography (GC). Serum fatty acid methyl

esters (FAMEs) were determined according to a modified method of Lepage.[28]

Methanol-n-hexane (2ml; 4:1, vol/vol) was added to 200μl of serum. Then, acetyl chloride (0.2ml) was added and heated for 1h at 100 °C. A solution of K_2CO_3 (5ml at 6%) was added to the tube, vorterex and centrifuged. The n-hexane top layer containing FAMEs was analyzed by a Chromatograph Perkin Elmer model Claurus 500 and a flame ionization detector (FID, Flame Ionization Detector).

Fatty acids were expressed as the percent (%) of total fatty acids content.

Bone Mass Assessment
Total skeleton bone mineral content (BMC) and bone mineral density (BMD) were assessed *in vivo* at the end of the experimental period. Under light anesthesia, rats were placed in a total body scanner with software designed specifically for small animals (DPX Alpha 8034, Small Animal Software, Lunar Radiation Corp, Madison, WI), as previously described.[31]

All rats were scanned using the procedure reported by Zeni *et al.*[31] The coefficients of variation (CV) evaluated by repeated measurements of five subjects of similar age and gender on five consecutive days for total skeleton BMC and BMD were 3.03 % and 0.89%, respectively. Analysis of spine, femur and lumbar BMC and BMD were performed using a region of interest (ROI) for each segment on the image of the animal on the screen. The CV for spine, femur and lumbar BMD and BMC was 1.79% ± 0.18. To avoid inter-assay error the same operator performed all DXA measurements.

Biomechanical Testing on Femur
A three-point bending mechanical test (Instron machine, model 4442; Instron Corp) was used to assess right femur biomechanical competence. The bone was positioned horizontally with the anterior side facing down on two transverse supports. Perpendicularly, at the medial aspect of the long axis of the bone, load was applied until fracture. The Instron test machine detects structural bone properties. The resulting load/deformation (W/d) curves measures the bone stiffness and strength. The structural properties are as follows: (i) load at

the yielding point (Wy) and marks the initiation of the appearance of microcracks on the periosteal surface of the bone subjected to tension; (ii) bone rigidity (Wydy); and (iii) structural strength (Wf). Geometric or architectural properties are: (i) bone length, (ii) outer vertical and horizontal diameters (V and H, respectively) and (iii) inner vertical and horizontal diameters (v and h, respectively) of the section (Isomet low-speed diamond saw; Buehler). Measurements were obtained using a stereomicrosc-ope (Stemi DV4; Carl Zeiss Microimaging); accuracy ± 0·001 mm. The second moment of inertia of cortical bone area (Ix) was estimated by the following equation: $Ix = [\varpi(V^3 \times H - v^3 \times h)/64 \ mm^4]$. The cross sectional area (CSA) was calculated by a formula $[CSA = \varpi/4. (V \times H) \ (mm^2)]$. Femur material properties were estimated from structural and geometrical properties.[32] The Young's modulus of elasticity (E) was calculated using the following formula: $E = WyL^3/48 \ dy. \ Ix$ (Wy=load at the yielding point, L=distance between supports, dy= maximal elastic deflection, Ix=second moment of inertia of the cross section in relation to the horizontal axis).

Statistical Analysis
All data were analyzed using the statistical software (SPSS v. 20.0 IBM Corp., Chicago, IL. USA) and Graphpad Prism (version 6.0) statistical package (Graphpad).

Data were expressed as mean values with their standard deviations (SD) or standard errors (SE). The data comparisons among the groups was assessed by One-way analysis of variance (ANOVA); statistically significant differences were detected by Student–Newman–Keul's test or Dunn (not parametric test). Bartlett's test for homogeneous variances was also done. Normal distribution was verified by Kolmogorov–Smirnov test. $P < 0.05$ was considered statistically significant (Glantz 1997).

Results
Effects on Zoometrics, Dietary intake and Nutrients
The initial and final body weight and body length of the rats, as well as body weight gain and food consumption of the three groups of rats are shown in Table 2. All animals had no significant differences in body weights (P = 0.121) and lengths (P = 0.654) at the beginning of the study. However, body size

was significantly affected by the type of dietary fat ingested throughout the experimental period. In fact, body weights and body weight gain per day were significantly lower in the SFOx rats as compared with C and SFO groups (P = 0.030) at week 8. The body weights of SFO were similar to the C group (P >0.05).

Moreover, the frying oil consumed altered linear growth. Rats fed SFOx attained the lowest body length (P = 0.001), while the SFO and C groups showed similar length growth (P >0.05), over the duration of the experiment.

Rats fed SFO diets containing either fresh oil (SFO) or fried (SFOx) did not show significant differences

in energy consumption as compared to C group (P = 0.162). However, rats consuming SFO or SFOx diet showed higher fat and monounsaturated fatty acids (MUFA) intake than C rats. SFO and SFOx did not show significant differences. Although SFOx rats consumed higher amounts of SFA and lower of PUFA, including linoleic, linolenic and arachidonic acids than SFO.

Effects on Serum Lipid Profile

The serum lipid profile of the three groups of rats is shown in Figure 1.

The rats fed the SFOx diet showed an altered serum lipid profile as compared to C and SFO groups. SFOx

Table 2: Zoometrics, dietary intake and nutrients

	CONTROL n=10	SFO n=12	SFOx n=12	P
Initial Body Weight (g)	46.4±5.0	50.5±5.7	47.8±6.0	0.121
Final Body Weight (g)	304.9±52.0b	323.2±23.3b	278.5±45.2a	0.03
Body Weight gain (g/day)	4.61±0.02b	4.87±0.42b	4.11±0.66a	0.001
Initial Body Length (cm)	13.1±0.6	12.9±0.5	13.1±0.6	0.654
Final Body Length (cm)	24.4±2.5b	23.8±0.7b	22.7±0.8a	0.001
Energy Intake (kcal/100gW/day)	45.7±3.1	46.4 ± 5.4	40.2 ± 10.9	0.162
Fat Intake (g/100gW/day)	0.74±0.1a	2.3±0.3b	1.97±0.3b	0.001
SFA (g/100gW/day)	0.15±0.01a	0.24±0.03b	0.28±0.04c	0.001
MUFA (g/100gW/day)	0.30±0.01a	0.69±0.07b	0.63±0.06b	0.001
PUFA (g/100gW/day)	0.29±0.01a	1.30±0.02c	1.13±0.01b	0.001
Linoleic acid (g/100gW/day)	0.14±0.01a	1.20±0.01c	1.04±0.02b	0.001
Linolenic acid (g/100gW/day)	0.01±0.00a	0.02±0.00c	0.01±0.00b	0.001
Arachidonic acid (g/100gW/day)	-----------	0.004±0.00b	0.003±0.00a	0.001

Data are means ± SD. Values with different letters are significantly different (P < 0.05) among groups; a being the lowest.

rats showed the highest serum T-chol (P =0.019) and non-HDL-chol (P = 0.008) levels; whereas serum T-chol of SFO and C did not show significant differences between them (P > 0.05).

SFO, SFOx and C groups attained similar serum concentrations of triglycerides and HDL-chol (P = 0.119 and P = 0.208, respectively).

Effects on Serum Fatty Acids
Results of major serum fatty acids found in SFO, SFOx and C groups are summarized in Table 3.

SFOx group presented significantly lower in total PUFA (P = 0.013), 18:2n-6 (P = 0.021), 20:4n-6 (P = 0.040), 22:6n-3 (P = 0.001) profiles than SFO. Total MUFA concentration was similar in the SFOx rats and in the SFO group (P = 0.491), and there was a significant higher profile of total saturated fatty acids in SFOx group (P < 0.014).

Effects on Bone Mass
The total skeleton body mineral content (BMC), BMC related to body weight (BMC/W), total skeleton body mineral density (BMD); spine BMC/W; spine BMD; femur BMC/W; femur BMD; tibiae BMC/W and tibiae BMD are shown in Table 4.

Rats fed SFOx diet presented significantly diminished total skeleton BMC, BMC/W, femur BMC/W and tibiae BMC/W, as compared with SFO and C groups

(P < 0.0001, P < 0.001, P = 0.028 and P < 0.001, respectively).

No significant differences among rats fed SFO and C diets were found for BMC, femur BMC/W and tibiae BMC/W (P >0.05). A decrease in BMC/W was found in the SFO group as compared to C, as shown in Table 4.

There were no differences in BMD, spine BMC/W, spine BMD, femur BMD and tibiae BMD of rats fed either SFO or SFOx, as compared with the C rats (P = 0.329, P = 0.668, P = 0.883, P = 0.599 and P = 0.826, respectively).

Effects on Bone Biomechanical Competence
The load-bearing capacity and stiffness of the femoral diaphysis are shown in Figure 2. Load-bearing capacity (Wf) and diaphyseal stiffness (Wy/dy) were negatively affected in rats fed sunflower oil either fresh or fried, as compared to the C group (P < 0.001 and P < 0.001; respectively), being significantly lower in rats fed SFOx diet (Figure 2).

The geometrical properties of the femur are shown in Figure 3. The moment of inertia of the fracture section (Ix) and cross-sectional bone area (CSA) were significantly lower in rats fed SFO or SFOx diets than the values found in the C rats (P < 0.001 and P < 0.001; respectively); however, rats fed SFOx diet showed the lowest values (Figure 3).
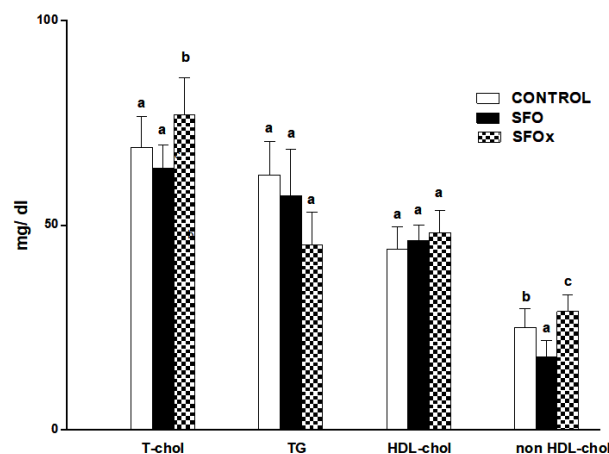
Fig. 1: Serum lípid lipoprotein profile

Young's modulus of elasticity is shown in Figure 4. There were no significant differences among material properties (P > 0.05). The effects of fried SFO did not influence the material bone quality in terms of yield stress.

Discussion

This study demonstrated that rats fed a diet containing thermally oxidized sunflower oil fail to gain adequate weight and length and also develop alterations in the serum lipoprotein profiles, bone mass and bone biomechanical competence.

During the frying process, sequences reactions occur in the oil, such as autoxidation, thermal oxidation, polymerization and cyclisation; unsaturated fatty acids are converted to saturated fatty acids and free radicals.[19] The large amount of thermoxidazed

Table 3: Serum fatty acid (g /100 g of total fatty acids)

Fatty acid	Control	SFO	SFOx	P
Lauric	0.82 ±0.01	0.40 ±0.42	0.59 ±1.00	0.583
Mirístic	1.80 ±0.37	1.32 ±0.59	1.83 ±1.05	0.483
Palmitic	20.49 ±1.33b	18.32±1.39a	21.80 ± 2.25b	0.004
Palmitoleic	1.96±0.06b	1.20±0.25a	1.35 ±0.27a	0.001
Stearic	14.55±0.37a	18.61±2.88b	19.49±2.28b	0.001
Oleic	14.26±0.60	14.70±2.19	14.65±4.60	0.955
Linoleic	17.1±0.31a	20.10±1.89b	16.40±1.90a	0.021
Linolenic	1.54±0.01b	0.53±0.14a	0.48±0.09a	0.001
Arachidonic	12.2±1.77b	12.34±1.02b	10.60±1.05a	0.04
Eicosapentaenoic	0.47±0.06a	0.54±0.10b	0.59±0.08b	0.042
Docosahexaenoic	1.27±0.19c	0.99±0.29b	0.56±0.21a	0.001
SFA	40.0±1.7a	41.06±5.07a	46.75±4.66b	0.014
MUFA	19.37±0.89	17.82±1.96	17.94±4.06	0.491
PUFA	32.75± 2.16a,b	34.92±7.04b	29.02±6.57a	0.013
Trans FA	2.75±0.53a	3.75±1.09b	4.19±0.09b	0.004

Data are means ± SD. Different letters indicate significant differences (P < 0.05) among groups; a being the lowest.

Table 4: Bone mass assessment

	Control	SFO	SFOx	P
BMC (g)	4.846±0.835b	4.357±0.374b	2.988±0.516a	0
BMC/W (g/g)	1.609±0.164c	1.349±0.070b	1.192±0.050a	0
BMD (g/mm2)	0.267±0.007	0.257±0.006	0.247±0.037	0.329
Spine BMC/W (g/g)	0.620±0.050	0.618±0.08	0.653±0.100	0.668
Spine BMD (g/mm2)	0.240±0.07	0.235±0.08	0.217±0.11	0.883
Femur BMC/W (g/g)	0.060±0.007b	0.063±0.008b	0.054±0.011a	0.028
Femur BMD (g/mm2)	0.296±0.110	0.256±0.160	0.238±0.006	0.599
Tibiae BMC/W(g/g)	0.358±0.005b	0.360±0.004b	0.278±0.011a	0
Tibiae BMD (g/mm2)	0.245±0.050	0.214±0.110	0.212±0.130	0.826

Data are means ± SD. Values with different letters denote significant differences (P < 0.05) between groups; a: being the lowest.

compounds in the lumen decrease the digestion and absorption[33] and the pancreatic lipase activity,[34] which negatively affect the hydrolysis of non-oxidized triacylglycerol. The influence of fried-oil consumption on growing rats was also evident by the direct damage by the toxic compounds in the fried-oil associated with histological structural changes. There were also modifications in the microbial profile gut, related to a diminished in total bacterial diversity and density.[35-37] In fact, the potential negative effect of diets rich in fried-oil on the intestine, could be as a consequence of the lowest bacterial microbiota that may set the stage for a more unstable ecosystem.

The current study indicated that SFOx intake led the rats to achieve the lowest body size, suggesting a negative effect of the components from the fried oil on energy metabolism. These findings are in agreement with Zhou *et al.*[35] studies, feeding rats with a stock diet plus fried-canola.

Our results suggests that a decrease in food efficiency could also be associated to the damage of essential food nutrients, such as n-6 and n-3 PUFA. Moreover, a diminished in protein, amino acids and fat[38] digestive utilization may be due to the reaction of amino acids with primary and secondary products
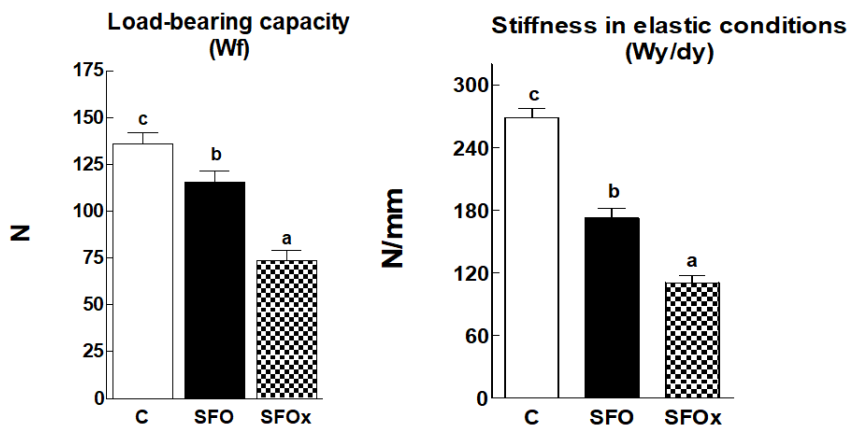

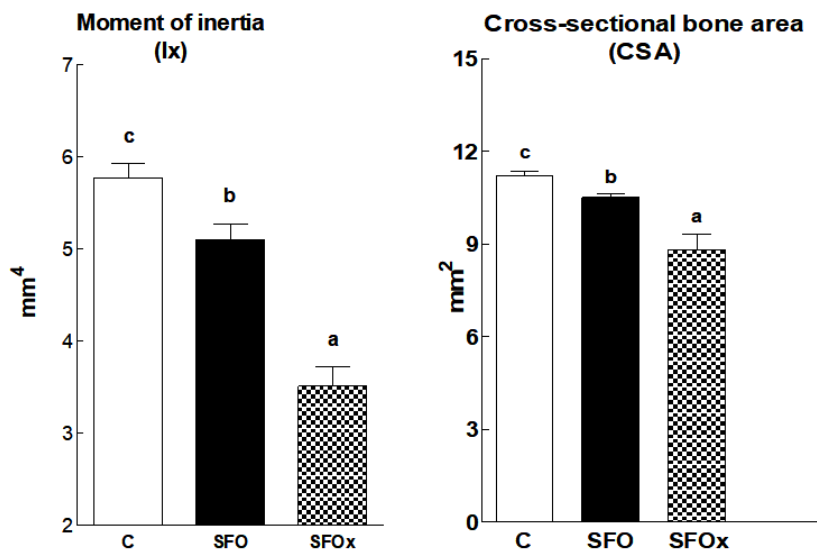**Fig. 2: Bone structural properties**

Fig. 3: Bone geometrical properties

of oxidized lipids. In addition, essential fatty acids deficiency may accompany the ingestion of oxidized fat[19,38] and might affect weight gain.[39]

The rise in total cholesterol and non-HDL-chol serum levels in rats fed SFOx diet may be due to the lower intake of linoleic acid, since PUFA, mainly linoleic acid is recognized to be hypocholesterolemic.[38] The hypercholesterolemia observed in this group could be due to the decrease in about 18 % in the serum linoleic acid.

The higher serum cholesterol levels are factors affecting the risk of bone fractures.[40,41] There is also a negative correlation between T-chol and non-HDL-chol serum levels and bone mass.[42-44] In human and animals, hypercholesterolemia has been associated with deposition and oxidation of lipids in tissues lead to atherosclerosis[45-47] and osteopenia.[48,49]

On the other hand, the diminished PUFA in SFOx diet was accompanied by an increased total saturated fat. Previous studies showed that rats fed dietary saturated fat impaired bone health in growing rats,[50] decreased total skeleton BMD and BMC.[51] This was possible due to saturated fatty acids which promote osteoclast survival by inhibiting apoptosis[52] with the development of alterations in the serum lipid lipoprotein profiles.[51]

It is known that low BMC was considered to increase the risk of developing coronary artery disease.[53,54] The observation of the coexistence of arterial disease and osteoporosis suggests that the same biologically active lipids, lipoproteins and phospholipids involved in atherosclerosis may ultimately affect bone.[55] In the present study, SFOx group attained significantly increased in serum T- chol and non-HDL-chol levels and diminutions in femur and tibiae densitometric bone parameters.

Furthermore, the lower bone mass effects in rats fed SFOx was demonstrated by femur cross-sectional-area measurements. Previous studies in vitro have demonstrated that LDL-chol oxidation was influenced by the type of dietary oil intake. Moreover, this could have an effect on bone mineralization and bone marrow differentiation.[49,56,57] The decrease on bone mass in the group fed SFOx was related to the presence of thermoxidazed sunflower rather than the source of oil or the type and concentration of calcium, phosphorous and vitamin D since both SFO and SFOx diets were otherwise similar.

The importance of dietary n-6 and n-3 PUFA is associated, in part, as being precursors of prostaglandins E (PGE). PGE2 derived from n-6 (arachidonic acid, AA)[58]; PGE3 and Eicosapentaenoic acid (EPA), from n-3 PUFA.[59] Moreover, the dual
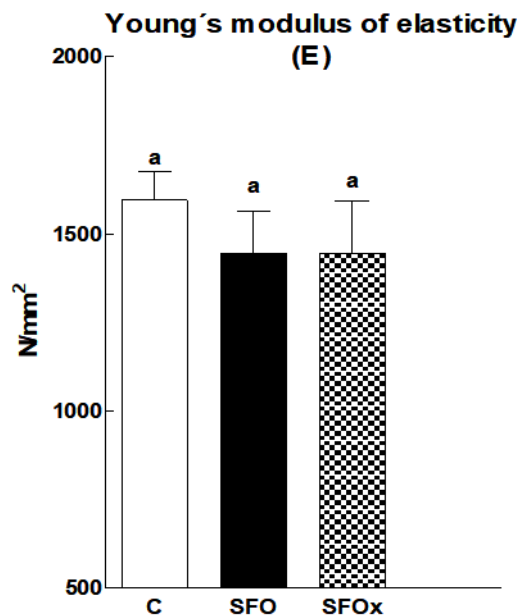

Fig. 4: Bone material property

effect of PGE2 on bone metabolism is related to its amount; inhibiting bone formation at high concentrations. In previous studies by us, a higher serum level of AA in rats consuming a rich-linoleic acid diet was observed.[50] Then, in the SFO group, a great synthesis of PGE2 -formed by AA[60] - rather than PGE3, could be expected.

The lack of decreased bone mass in the SFO group suggests that even though the n-6 content was above the rat requirements, the frying oils and specifically sunflower oil, induce more negative effects on bone than the excess of n-6 PUFA. Moreover, the differences between the SFO and the SFOx dietary intake expose the adverse effects frying oils on the skeleton of a growing animal rather than the oil per se.

Furthermore, the adverse effect of SFOx on bone mass denoted alterations in bone biomechanical competence. The biomechanical concept of bone quality relates bone health to bone strength, rather than bone mass. Even though densitometry is unsuitable to assess bone strength and only provides information concerning bone mass, bone quality can be measured by biomechanical competence assays. The results of this study clearly demonstrated adverse effects of ingestion of SFOx diets on the mechanical strength of femoral diaphysis to external loading. Bone stiffness and mass and architectural distribution were more affected in SFOx rats. Bone mechanical quality depends on the mechanical quality of the mineralized tissue (mainly associated with collagen mineralization) and the architectural quality of the structural bone design[32,61]

However, bone material quality (E)[62] was not modified by fresh sunflower oil or the thermoxidazed oil (SFO or SFOx) diets; this is in congruence with our previous studies in rats fed MUFA-rich diets.[49,63-65] Since the mechanical quality of the mineralized tissue was not affected, the decreased mechanical competence of the femur of SFO and SFOx rats should be regarded as the result of changes in bone mineral content and spatial distribution of mineralized tissue, rather than bone material properties. Bone with a faster turnover rate (modeling), could reveal potential adverse effects of using thermally oxidized oil in the diet on the skeletal and biomechanical bone properties of a growing animal.[66]

Conclusions

The results of this study in growing healthy male rats indicate the prominent role thermoxidazed oil consumption in determining the risk for growth, cardiovascular and bone effects. Consumption of SFOx diet adversely affected body growth, lipid metabolism, bone mass and bone biomechanical competence. The potential adverse effects of thermally oxidized oil in the diet denoted the altered skeletal and biomechanical bone properties of a growing animal in terms of mineralized tissue, rather than bone material properties.

Conflict of Interest
There was no conflict of interest associated with this study by any of the authors.

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