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ORIGINAL CONTRIBUTION

High-fat diets affect energy and bone metabolism in growing rats

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Abstract
Background High-fat diets are usually associated with greater weight (W) gain and body fat (BF). However, it is still unclear whether the type and amount of fat consumed influence BF. Additionally, dietary fat intake may also have consequences on skeletal health.
Objective To evaluate in healthy growing rats the effects of high-fat diets and type of dietary fat intake (saturated or vegetable oils) on energy and bone metabolism.
Methods At weaning, male Wistar rats ($n = 50$) were fed either a control diet (C; fat = 7% w/w) or a high-fat diet (20% w/w) containing either: soybean oil, corn oil (CO), linseed oil (LO), or beef tallow (BT) for 8 weeks. Zoometric parameters, BF, food intake and digestibility, and total and bone alkaline phosphatase (b-AP) were assessed. Total skeleton bone mineral density (BMD) and content (BMC), BMC/W, spine BMD, and bone volume (static-histomorphometry) were measured.
Results Animals fed BT diet achieved lower W versus C. Rats fed high-fat vegetable oil diets showed similar effects on the zoometric parameters but differed in BF. BT showed the lowest lipid digestibility and BMC. In contrast, high vegetable oil diets produced no significant differences in BMC, BMC/W, BMD, spine BMD, and bone volume. Marked differences were observed for LO and BT groups in b-AP and CO and BT groups in bone volume.
Conclusion BT diet rich in saturated fatty acids had decreased digestibility and adversely affected energy and bone metabolisms, in growing healthy male rats. There were no changes in zoometric and bone parameters among rats fed high vegetable oil diets.

Keywords Healthy growing rats · High-fat diets · Saturated and vegetable oil diets · Bone densitometry and histomorphometry

Abbreviations

AA	Arachidonic acid
ALA	Linolenic acid
BMC	Body mineral content
BMD	Body mineral density
BT	Beef tallow
CO	Corn oil
DHA	Docosahexaenoic acid
DXA	Dual-energy X-ray absorptiometry
EPA	Eicosapentaenoic acid
LA	Linoleic acid
LO	Linseed oil

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PGE Prostaglandin E
PUFA Polyunsaturated fatty acids
SO Soybean oil

Introduction

Epidemiologic studies have shown a high incidence of overweight and obesity in humans [1–3]. Public health efforts to reduce dietary fat intake or to limit the proliferation of low-fat foods [3] have not decreased the prevalence of obesity [4, 5]. Intake of high-fat diet is associated with increased body fat content in both humans [6] and rats [7]. However, the role of the source and amount of dietary fat in the development of obesity remains inconclusive, since some fat containing foods may be protective [8].

Dietary fat intake may have consequences on skeletal health [9]. Diets with high-saturated fat content can produce deleterious effects on bone mineralization in growing animals [10]. Conversely, dietary n-3 long-chain polyunsaturated fatty acids (PUFA) play an important role on body growth and bone metabolism [11] and in the prevention and treatment of bone disease [12]. Some reports suggest a correlation between the dietary ratio of n-6/n-3 PUFA and bone formation [13]. Specific dietary fatty acids were found to modulate prostanoid synthesis in bone tissue and improve bone formation rates in animal models [14]. The preponderance of studies regarding the benefits of n-3 PUFA has been primarily focused in fish oil diets [11, 15], while linseed oil as a vegetable source has remained insufficiently investigated. Vegetable oil diets have also been assessed for their potential effects as nutraceuticals [16].

The purpose of the present study was to evaluate the effects of diets high in fat of various types, including an animal source of saturated fat, and high vegetable oil diets with different PUFA on energy and bone metabolism of growing rats.

Materials and methods

Animals

Fifty male weanling Wistar rats (aged 21 days), initial body weight (W) = 38.7 ± 4.4 g (mean ± SD), were obtained from the animal laboratory of the Department of Biochemistry, Faculty of Dentistry, University of Buenos Aires, Argentina. Animals were housed in galvanized cages with meshed floors in order to maintain hygienic conditions and to avoid coprophagy. Rats were kept in individual cages and exposed to a 12-h light/dark cycle throughout the study. Room temperature was maintained at 21 ± 1 °C with a humidity of 50–60%. The rats were maintained in accordance with the USA National Institutes of Health Guide for the Care and Use of Laboratory Animals. The protocols for these experiments were approved by the University of Buenos Aires, Argentina.

Animals were randomized to five groups of 10 animals each: control (C) and four experimental groups: soybean oil (SO), corn oil (CO), linseed oil (LO), and beef tallow (BT) groups. They were fed "ad libitum" one of the five diets for 8 weeks. At the end of the experimental period, nutritional status, biochemical determinations, DXA measurements, percentage of body fat, and static-histomorphometric measurements were performed.

After the rats were fed the diets for 4 weeks, feces were collected for a 7-day period in order to determine lipid absorption diet and lipid digestibility. The total lipids were extracted from the feces with chloroform/methanol (3:1) by volume and quantified. Lipid absorption was calculated by comparing the fat intake minus the fecal lipid content. The apparent digestibility of the diet was calculated as dietary intake (g) minus the fecal weight (g) divided by the amount of dietary intake. The efficiency of energy utilization for daily body weight gain was calculated as daily energy intake (Kcal) minus energy required for maintenance (expressed as $W^{0.75}$) divided by body weight gain (g) over time (8 weeks).

Diets

The composition and fat source of the diets are shown in Table 1. Diets contained 7 g of fat C [14] or 20 g of fat (high-fat, experimental groups) per 100 g of diet. C and experimental diets provided 16 and 40% of total calories from lipids, respectively. Experimental diets varied in the type of fat; three diets had vegetable oils rich in PUFA: SO, CO, or LO, whereas the BT diet was rich in saturated fatty acids. C group was fed the AIN-93G with SO as a source of fat; the SO group received the same source of fat but at a higher level.

All diets contained the same type and concentration of calcium (calcium carbonate anhydrous at 40.04% Ca, equal to 143 mg/kg mix), phosphorus (Potassium phosphate monobasic at 22.76% *P*, equal to 44.60 mg/kg mix), and Vitamin D (Vitamin D-3, cholecalciferol, 400,000 IU/g equal to 250 mg/kg mix) (AIN-93G) [17]; 0.6 mg alpha-tocopherol equivalents/g PUFA was added to the high-fat diets as recommended by Valk et al. [18]. Diets were prepared every 2 days and stored at −4 °C until fed. Fresh diets were offered daily, and food containers were cleaned before refilled. Food cups were refilled once a day, and

Table 1 Composition of the diets given to healthy weaning rats for 8 weeks

Component % (w/w)	Groups				
	C	SO	CO	LO	BT
Energy (kJ)	1,611	1,883	1,883	1,883	1,883
Energy (kcal)	385	450	450	450	450
Protein (g)	17.40	17.40	17.40	17.40	17.40
Carbohydrate (g)	63	50	50	50	50
Total fat (g)	7	20	20	20	20
Saturated (g)	1.30	3.70	3.26	3.30	12.30
Monounsaturated(g)	1.85	5.30	6.94	5.10	6.78
Polyunsaturated (g)	3.85	11.00	9.80	11.60	0.92
P/S ratio	3.00	3.00	3.00	3.50	1.10
18:2 (n-6)/18:3 (n-3) ratio	13.70	13.70	67.90	0.40	2.40
Vitamins, minerals, fiber			AIN-93G		

Percentage of calories from fat was 16 in C and 40 in SO, CO, LO, and BT. To convert kcal to kJ multiply kcal by 4.184. Oils were provided by Molinos Rio de la Plata. Argentina. Protein: as Potassium Caseinate, containing 87% of protein mesh 90, provided by Inmobal Nutrer SA. Argentina. Carbohydrate: corn dextrin from corn refinery, provided by Food SA Argentina. Vitamins: mixture to meet rat requirements during growth, according to AIN 93G, manufactured by the Department of Food Science School of Biochemistry, University of Buenos Aires. Minerals: mixture of minerals to meet rat requirements during growth, according to AIN 93G, manufactured by the Department of Food Science, School of Biochemistry, University of Buenos Aires. Choline chloride salt (C-6556), Anedra SA. Buenos Aires. Argentina. Fiber to meet rat requirements during growth, according to AIN 93G

C control diet, *SO* soybean oil diet, *CO* corn oil diet, *LO* linseed oil diet, *BT* beef tallow diet

food consumption was measured with a Mettler scale PC 4000 (accuracy ±1 mg). Daily food intake was recorded as kcal per 100 g of body weight and per day (kcal/100 g W/day).

Zoometrics

Body weight and length (L) were measured every 4 days, after a fasting period of 2–4 h. A Mettler PC 4000 scale was used to measure W with an accuracy of ±1 mg. L was determined in slightly anesthetized rats with a scaled ruler in mm from the nose tip to the hairline of the tail.

DXA measurements

At the end of the study, total skeleton bone mineral density (BMD) and bone mineral content (BMC) were determined "in vivo" under light anesthesia using 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 [19]. The anesthesia was given by intra-muscular injection of ketamine hydrochloride (0.1 mL/100 g W) (Holliday Lab. Buenos Aires. Argentina) and xilasyn (0.02 mL) (Konig Lab. Buenos Aires. Argentina).

All rats were scanned using an identical procedure. The precision of the scan software for the determination of total body BMD was assessed by measuring one rat five times after repositioning between scans, both on the same and on different days. The coefficients of variation (CV) were 3.0 and 0.9% for total skeleton BMC and BMD, respectively. BMC was expressed per body weight (BMC/W).

Analysis of spine BMD was carried out on the image of the animal on the screen using a region of interest for each segment. The CV for spine BMD was 1.8%. All DXA measurements were performed by the same operator to minimize inter-assay variation.

Biochemical determinations

At the end of the experimental period, blood was obtained by cardiac puncture and serum was removed and stored at −20 °C to determine total (t-AP) and bone (b-AP) alkaline phosphatase. Serum concentrations of b-AP were measured using a colorimetric method (Boehringer Mannheim, Germany) after bone enzyme isoform precipitation with wheat-germ lectin [20].

Body composition

Percentage of body fat was quantified at the end of the experimental period; animals were killed under ether anesthesia, and carcasses were dried at 100 °C until a constant weight was achieved. Body fat was determined by Soxhlet's method [21]. Total body fat was expressed as percentages of total weight (g/100 g W).

Bone histomorphometry

After rats were killed, tibiae were removed and cleaned of soft tissue, fixed in 10% neutral formalin, decalcified in EDTA (pH = 7.2), and processed to be embedded in paraffin. Oriented sections were stained with hematoxylin-eosin (H-E). The bone volume as a percentage of total bone volume (BV %) of trabecular bone in the tibiae was determined on the central area of metaphyseal bone [22].

Statistical analysis

The results were expressed as mean values with their standard deviations (SD). One-way analysis of variance (ANOVA) was used to compare data among groups. When a statistically significant difference was encountered, a Student-Newman-Keul's test was performed. In all analyses, Bartlett's test for homogeneous variances was done. Significance was set at the $P < 0.05$ level. The Statistical Product and Service Solutions for Windows 9.0 (SPSS, Inc., Chicago, IL) were used for statistical analyses.

Results

Dietary intake and energy metabolism

The body weight, length, and body fat, as well as the energy intake, absorption, and digestibility, are shown in Table 2. Also shown are the lipid intake, lipid absorption and digestibility, and the efficiency of energy utilization for body weight gain.

There was a significant effect of the type of dietary fat ingested on body weight. In the beginning of the experiment, all the animals in the five groups studied had similar body weights (day 21 of life/g, C: 38.46 ± 6.05; SO: 38.74 ± 5.04; CO: 38.75 ± 4.43; LO: 38.83 ± 6.42; and BT: 38.75 ± 6.01; $P > 0.05$). However, at the end of the experimental period, the W was significantly lower in the BT rats as compared with C group.

The high-fat vegetable oil diets with different (n-6)/(n-3) PUFA ratios showed similar effects on the zoometric measurements. The body weights of SO, CO, and LO rats did not differ among themselves, and their weights were similar to the C group.

The type and amount of fat in the diets did not alter linear growth. The body length of the rats fed high-fat diets did not differ with dietary lipid source. The percentage of body fat differed among the rats fed the various diets. Rats fed LO achieved the lowest body fat content, while the CO group showed the highest levels.

Energy intake and energy absorption did not differ among the five treatment groups, despite the difference in energy content among them. Apparent diet digestibility was significantly lower with the ingestion of the BT diet. Whereas the other types of diets did not detect any differences among them and/or with the C intake. In addition, consumption of BT diet resulted in significantly lower apparent lipid digestibility compared with the other diets. Daily efficiency of energy utilization for W gain

Table 2 Zoometrics and body composition of rats fed various fat diets for 8 weeks

Parameters	Groups					
	C	SO	CO	LO	BT	ANOVA
Final body weight (g)	345.5 ± 20.8^{a}	324.7 ± 40.9$^{a.b}$	320.8 ± 48.3$^{a.b}$	318.5 ± 36.9$^{a.b}$	285.5 ± 25.3^{b}	0.02
Final body length (cm)	24.1 ± 0.5	23.9 ± 0.7	24.1 ± 0.6	23.8 ± 0.6	23.5 ± 0.3	NS
Final body fat (% body weight)	11.1 ± 1.9^{a}	12.5 ± 1.7$^{a.b}$	13.6 ± 1.2^{b}	11.1 ± 2.0^{a}	11.8 ± 1.2$^{a.b}$	0.01
Energy intake (Kcal/100 g rat/day)	43.7 ± 13.8	46.0 ± 13.8	46.1 ± 15.8	44.3 ± 16.1	50.7 ± 18.1	NS
Energy absorption (Kcal/100 g rat/day)	37.6 ± 13.8	39.1 ± 13.7	39.3 ± 15.7	37.4 ± 16.0	38.7 ± 18.0	NS
Diet digestibility	0.84 ± 0.06^{a}	0.84 ± 0.05^{a}	0.84 ± 0.07^{a}	0.83 ± 0.07^{a}	0.73 ± 0.10^{b}	0.001
Lipid energy intake (Kcal/100 g rat/day)	8.73 ± 2.77	9.11 ± 2.75	9.15 ± 3.14	8.78 ± 3.20	10.13 ± 3.60	NS
Lipid energy absorption (Kcal/100 g rat/day)	8.61 ± 2.77	9.02 ± 2.75	9.05 ± 3.14	8.66 ± 3.20	9.89 ± 3.60	NS
Lipid digestibility	0.981 ± 0.005^{a}	0.985 ± 0.005^{a}	0.983 ± 0.007^{a}	0.979 ± 0.007^{a}	0.965 ± 0.009^{b}	0.001
Energy utilization for daily body weight gain (kcal/g)	12.67 ± 1.30^{a}	13.28 ± 2.3$^{a.b}$	13.37 ± 2.38$^{a.b}$	12.68 ± 2.70$^{a.b}$	16.15 ± 4.18^{b}	0.037

The food and lipid digestibility, intake, and utilization are also shown

Data are means ± SD after 8 weeks on the respective diets. Values with different superscript letters are significantly different ($P < 0.05$) from diets. *NS* not significant difference between diets

C control diet, *SO* soybean oil diet, *CO* corn oil diet, *LO* linseed oil diet, *BT* beef tallow diet for 8 weeks

also declined for rats fed BT diet (Table 2). The BT fed rats exhibited an 18% increase in energy intake for daily weight gain over the SO and CO diets and a 22% over the C rats.

Dietary intake and bone metabolism

The results of total skeleton BMC, BMC related to body weight (BMC/W), total skeleton BMD, spine BMD, t-AP, b-AP, and BV % are shown in Fig. 1a–f, respectively.

No significant differences among rats fed high-fat vegetable oils diets were found for BMC, BMC/W, BMD, spine BMD, t-AP, and BV %, regardless of the different (n-6)/(n-3) PUFA ratios. An increase in b-AP was only found in the LO group as shown in Fig. 1e. There were no differences between C and SO groups.

Rats fed the BT diet had lower total skeleton BMC, similar BMC/W, total skeleton BMD, and spine BMD but higher t-AP as compared with those consuming high-fat vegetable oils diets. However, BT group had a similar BV % as compared with the CO group. Rats fed the BT diet showed significantly diminished total skeleton BMC (Fig. 1a), BMC/W (b), spine BMD (d), and the BV % (Figs. 1f, 2) as compared with the C group.

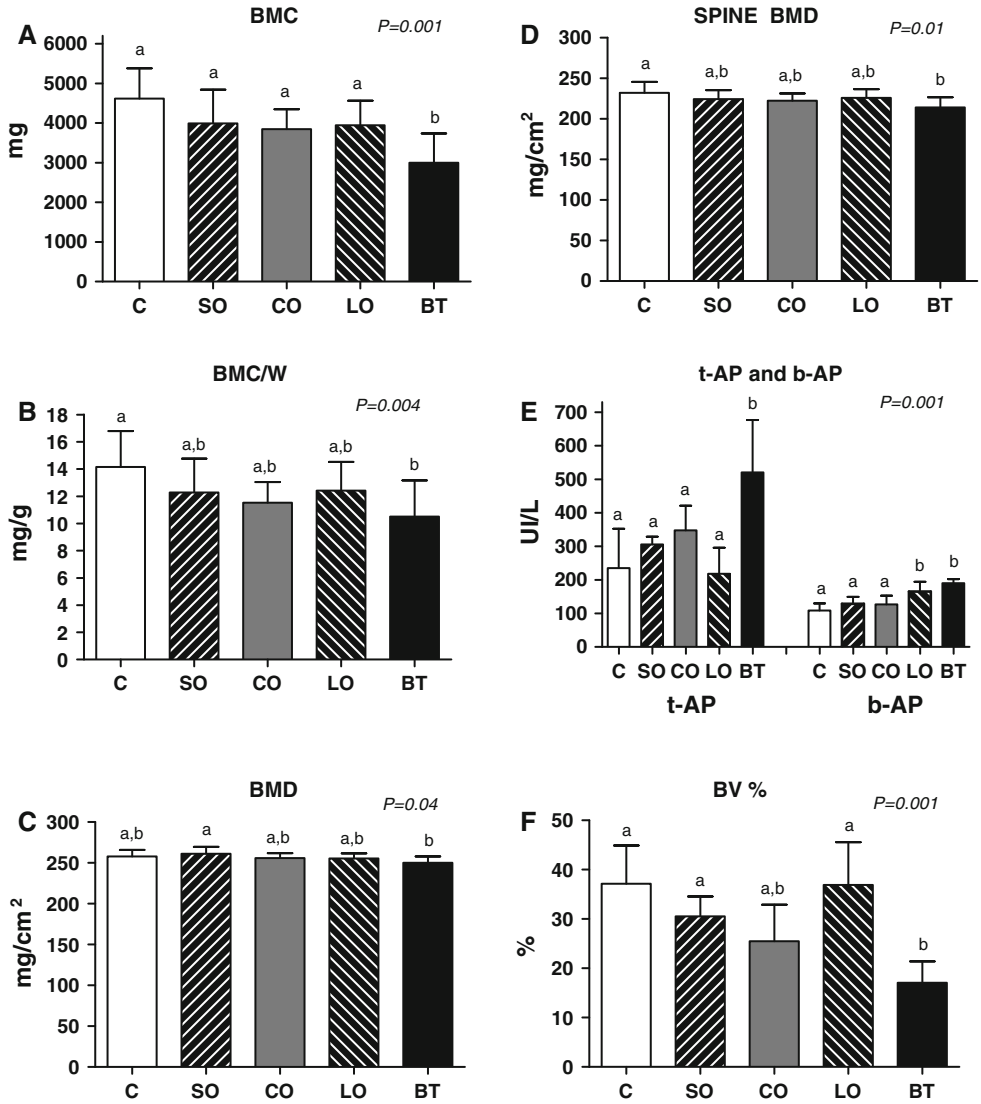

Fig. 1 Total skeleton body mineral content (*BMC*) (**a**), total skeleton body mineral content per body weight (*BMC/W*) (**b**), total skeleton body mineral density (*BMD*) (**c**), *spine BMD* (**d**), total and bone alkaline phosphatase (*t-AP* and *b-AP*) (**e**), and bone volume as a percentage of total bone volume, *BV (%)* (**f**) of trabecular bone in the tibiae of healthy weaning rats fed for 8 weeks with a diet containing vegetable or animal fat. *C* control diet, *SO* soybean oil diet, *CO* corn oil diet, *LO* linseed oil diet, and *BT* beef tallow diet. Values with different superscript letters are significantly different ($P < 0.05$) from diets

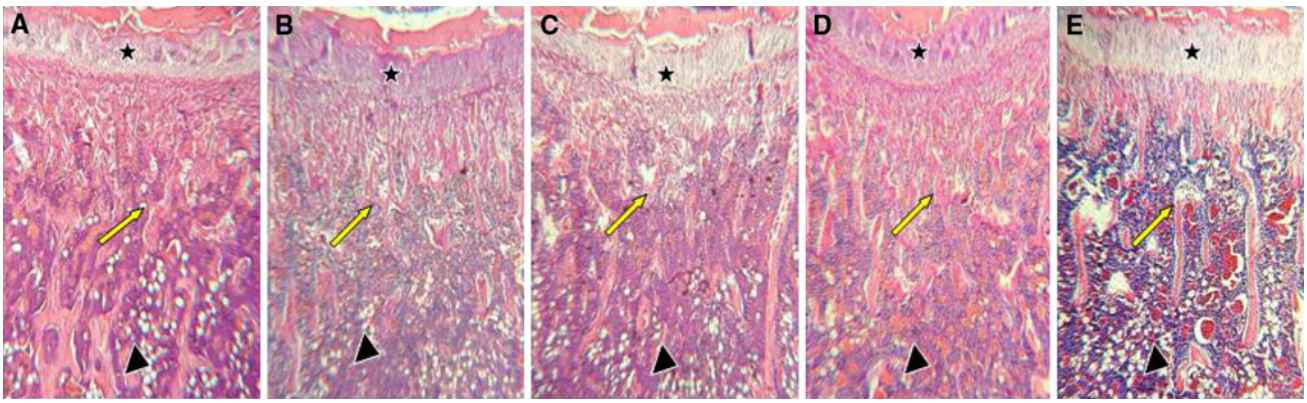

Fig. 2 Histology of decalcified oriented sections of the proximal of the tibiae of healthy weaning rats fed diets containing vegetable or animal fat for 8 weeks. C (**a**), SO (**b**), CO (**c**), LO (**d**), and BT (**e**) groups. Note the decrease in the BV % in BT group as compared with C, SO, and LO groups, while CO shows a tendency to a lower value (H&E 50×), $P < 0.05$. *C* control diet, *SO* soybean oil diet, *CO* corn oil diet, *LO* linseed oil diet, *BT* beef tallow diet, and *BV* bone volume. *Arrows* show the trabecular bone; *stars* indicate metaphyseal growth cartilage and *arrowheads*, the bone marrow

Discussion

This study was performed on rapidly growing rats, from weaning through puberty and early-adolescence, the period that the risk of adult onset diseases may be established. We showed that the intake of a high-fat saturated diet did not lead to increased body weight gain. Indeed, the reverse occurred; the average body weight of rats fed BT diet was lower than those fed the other diets. The body weight difference was present even though the BT group had an increased energy intake of about 20% as compared with the other groups. The BT rats gained less weight than those receiving high-fat vegetable oil diets, since the food and lipid digestibility of BT were decreased. These data are in agreement with the negative effects on nutrient absorption shown by others [23, 24].

However, the energy efficiency of the food fed may determine the final body weight independently of dietary composition [25]. Also, the composition of diet may produce changes in energy utilization that lead to excess fat deposition [26]. Rats gain weight rapidly and can become obese when they can select the food. Studies demonstrated that rats fed cafeteria diets (similar to western diets plus sedentary life style) become more obese than with high-fat diets, probably due to greater hyperphagia arising from the food variety. Additionally, the duration of exposure to high-fat diets could increase body fat content [7]. On the other hand in obese humans, high-fat (20% from saturated fat) and low-carbohydrate diets induce weight loss; several mechanisms for this effect have been postulated, with most relating to spontaneous reductions in energy intake [26].

In our experiments, there was no difference in body fat content observed in the rats fed a BT diet, suggesting that the lower body weight was not necessarily due to decreased body fat. However, body fat content was affected by the type of lipids present in the diets. The highest body fat value in CO group could be due to the high content of linoleic acid (LA) in the CO diet. This diet could enhance the storage of an excess of LA in adipose tissue and inhibit its metabolic conversion [24]. In contrast, diets rich in linolenic acid (ALA) are more prone to oxidation than to storage and might be expected to result in less fat deposition. If so, the rates of oxidation of individual fatty acids should be related to differences in weight gain observed when different types of fat are fed during long-term periods. [27].

Dietary saturated fats appeared to impair bone health in growing animals. In the present study, rats fed high-saturated animal fat diet achieved the lowest total skeleton BMC and cancellous bone (spine BMD). The bone mineral alterations may be the result of the formation of intestinal soaps with calcium that reduce its absorption [28]. There may also be alterations of membrane fluidity that diminish calcium uptake by brush border membrane vesicles, reducing mineral content [23] compared with the vegetable oil diets providing PUFA. Bone with faster rate of turnover (modeling) could reveal the potential adverse effects of high-saturated animal fat diet on the skeletal of a growing animal; in fact, studies performed in vitro showed that saturated fatty acids promoted osteoclast survival by preventing apoptosis [29].

On the other hand, the animals fed the high-fat vegetable oils diets, regardless the n-6/n-3 fatty acids ratio, achieved similar total skeleton BMC, BMD, and BMC/W compared with the C group which consumed a diet based on the AIN 93G formulation. The absence of differences on bone formation or resorption between high-fat vegetable oil diets could be due to the fulfillments of the requirements of n-6

and n-3 fatty acids by each one of the diets studied. Healthy growing rats were not susceptible to n-6/n-3 fatty acids ratios, whereas the response would be different in animals with skeletal/bone disorders [13].

Since the BT group showed pronounced reductions in densitometric bone data, the existence of possible structural tibiae alterations was analyzed by static-histomorphometry and a biochemical marker of bone formation. Histologic sections of tibiae showed low BV % and high t-AP and b-AP. These data appear to indicate that AP failed to achieve an adequate subendochondral ossification and mineralization despite increased levels of both the total and the bone AP. The changes in the architecture of trabeculae could be possible due to the decreased bioavailability of the calcium. In contrast, the groups fed vegetable oil diets showed a bone strengthened structure. This finding is of importance since trabecular bone quality is associated with the biomechanical bone properties [30].

The excess of n-6 PUFA in the vegetable CO oil may explain the BV % detected as this diet met the nutrient needs of growing rats [17]. The unsaturated dietary fatty acids are important because both n-6 and n-3 PUFA are precursors of prostaglandins E (PGE); n-6 for PGE_2, from the arachidonic acid (AA), and n-3 for PGE_3, from Eicosapentaenoic acid (EPA). PGE_2 has a dual effect that depends on the amount; at low concentrations, it stimulates bone formation, but inhibited it at high concentrations. It is known that the rate of synthesis is more rapid for PGE_2 than PGE_3 [31, 32], which could be enhanced by the great amount of formed AA [15] as expected in the CO group.

Our data suggested that LO diet did not positively or negatively affect the bone parameters studied. Others showed that the exposure to a flaxseed oil diet in mice resulted in higher serum ALA, EPA, and DHA, and lower LA and AA compared with those exposed to the CO diet [33]. LO oil diet rich in n-3 PUFA could maintain lower levels of AA in bone and cartilage [13]. Taken into account the potential adverse effects of fish contamination [34], as well as food allergies [35], LO could be used instead of fish-oil. The LO group would resemble fish oil as a source of n-3 PUFA; however, the fish oil is a direct source of EPA [36]. The large proportion of ALA in LO diet is the most rapidly oxidized unsaturated fatty acid [37]. Another possible reason why we did not observe differences may be because we chose a LO that probably contained linatine (a dipeptide of *N*-(gamma-L-glutamyl) amino-D-proline), an antagonist of vitamin B6 [38].

Weanling Wistar rats have been demonstrated to be an adequate animal model to evaluate suboptimal nutrition [22, 39] and insulin resistance [37, 40]. However, the type, quantity, and time fed a given diet are important factors to consider in animal studies [28].

In summary, these data suggest that the BT diet rich in saturated fatty acids had decreased digestibility and adversely affected energy and bone metabolism, in the growing healthy male rats. Although, no changes in zoometric and bone parameters with high vegetable oil diets were found, further long-term studies should be performed to determine possible adverse effects.

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

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