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Impact of a high-fat diet containing canola or soybean oil on body development and bone; parameters in adult male rats

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Abstract

Introduction: **The role of the fatty acid in the prevention or progression of chronic diseases has generated significant interest on the part of researchers. Thus, our objective was to evaluate the long-term effects of high-fat diet containing soybean or canola oil on body development and bone parameters of male rats.**

Methods: **After weaning, rats were grouped and fed either a control diet (7S) or a high-fat diet containing soybean (19S) or canola oil (19C). Femur and lumbar vertebra (LV4) structure were determined at 180 days by dual-energy X-ray absorptiometry and computed tomography.**

Results: **The groups showed similar food intake, body mass and length development. The bone parameters of the 19C were similar to the control group, while the 19S showed lower bone parameters when compared to the other groups.**

Conclusions: **The high-fat diet containing soybean oil was unfavorable to bone structure, while the canola oil contributed bone health during the adult stage of life.**

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Keywords: *Canola oil. Bone. Rat. Dual-energy X-ray absorptiometry. Computed tomography.*

IMPACTO DE UNA DIETA ALTA EN GRASA QUE CONTIENE ACEITE DE CANOLA O ACEITE DE SOJA EN EL DESARROLLO DEL CUERPO Y LOS HUESOS; PARÁMETROS EN RATAS MACHO ADULTAS

Resumen

Introducción: **El papel del ácido graso en la prevención o la progresión de las enfermedades crónicas ha generado un interés significativo por parte de los investigadores. Por lo tanto, nuestro objetivo fue evaluar los efectos a largo plazo de la dieta alta en grasas que contienen soja o aceite de canola en los parámetros de desarrollo del cuerpo y los huesos de ratas macho.**

Métodos: **Después del destete, las ratas se agruparon y se alimentaron con una dieta control (7S) o una dieta alta en grasa que contiene soja (19S) o aceite de canola (19C). Fémur y vértebras lumbares (LV4) estructura se determinaron a los 180 días por absorciometría dual de rayos X y tomografía computarizada.**

Resultados: **Los grupos mostraron similares ingesta de alimentos, la masa corporal y el desarrollo de longitud. Los parámetros óseos de la 19C fueron similares al grupo control, mientras que los 19S mostró parámetros óseos inferiores en comparación con los otros grupos.**

Conclusiones: **La dieta alta en grasas que contiene aceite de soja fue desfavorable a la estructura ósea, mientras que el aceite de canola contribuyó salud de los huesos en la etapa adulta de la vida.**

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Palabras clave: *El aceite de canola. Hueso. Rata. Densitometría osea. La tomografía computarizada.*

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Introduction

Obesity is a multifactorial disorder that can be associated to environmental factors, especially diet. Individuals fed a diet containing a high proportion of fat as calories tend to a positive fat balance and consequently to adipose mass accumulation[1].The type of fatty acids plays a far pivotal role more effects in health and disease than the absolute amount[2]. Dietary recommendations often advise to reduce saturated fatty acids (SFA) intake and maintain or increase the intake of polyunsaturated fatty acids (PUFA), such as linoleic (LA) and alpha linolenic fatty acid (ALA)[3]. Although the effect of fat diet in obesity has been studied, the role of fats on bone physiology has emerged as an interesting area of research[4].

The identification of mechanisms linking skeletal and metabolic homeostasis, suggest that obesity and osteoporosis may be related disorders[5]. In this context, the ALA has received attention for its favorable role in regulation of bone metabolism. The LA was associated with lower bone density. Thus, the fatty acids affect bone cells affecting bone formation, resorption and bone density[6].

Canola oil, when compared to soybean oil, contains a very low level of SFA (7% vs. 15%) and LA (21% vs. 54%), and slightly higher levels of ALA (11% vs. 8%). This oil represents less than 4% of fat intake in Brazilian populations, while soybean oil is the main energy source[7,8]. High-fat diets, over long-term feeding regimes can affect the bone structure and bone health[9]. However, there are insufficient data regarding the bone structure after the intake of canola oil during the adult stage of life. The aim of this study was to evaluate the body development and bone structure of male rats fed a high-fat diet containing canola oil (vs. soybean oil), after weaning up to 180 days-old.

Materials and methods

The protocol of the use and handling of the experimental animals was approved by the Ethical Committee of the Biology Institute of the State University of Rio de Janeiro, based on the principles adopted and promulgated by Brazilian Law concerning the rearing and use of animals in teaching and research activities in Brazil[10].

Wistar rats were kept in a room under a controlled temperature (23 ± 1ºC) and an artificial dark-light cycle (lights on from 07·00 to 19·00 hours). Virgin female rats (three months old), after mating, was placed in an individual cage with free access to water and food.

Within 24h of birth, excess pups were removed, such that only six male pups were kept per dam. This procedure has been shown to maximize lactation per-

Table I
Composition of experimental diets

Ingredient (g/100g)	*7S*	*19S*	*19C*
Casein	20	20	20
Cornstarch	52·95	40·63	40·63
Sucrose	10	10	10
Soybean oil	7	19·32	---
Canola oil	---	---	19·32
Fiber	5	5	5
AIN-93G Mineral Mix	3·5	3·5	3·5
AIN-93 Vitamin Mix	1	1	1
L-Cystine	0·3	0·3	0·3
Choline Bitartrate	0·25	0·25	0·25
Energy			
Kcal/g	4·7	5·8	5·8
Protein (% of energy)	17	14	14
Carbohydrate (% of energy)	65	45	45
Fat (% of energy)	17	39	39

Formulated to meet the American Institute of Nutrition AIN-93G recommendations for rodent diets. 7S, control group fed a diet containing 7 ml/ 100 g soybean oil; 19S and 19C, the experimental groups fed diets containing 19 ml/ 100 g soybean or canola oil, respectively. Casein; Mineral and Vitamin Mix; L-Cystine; Choline Bitartrate: Agroquímica®; Cornstarch: Cargill®; Fiber: Natural Pharma®; Soybean and Canola oil: Proquímios®; Commercial Sucrose: União®.

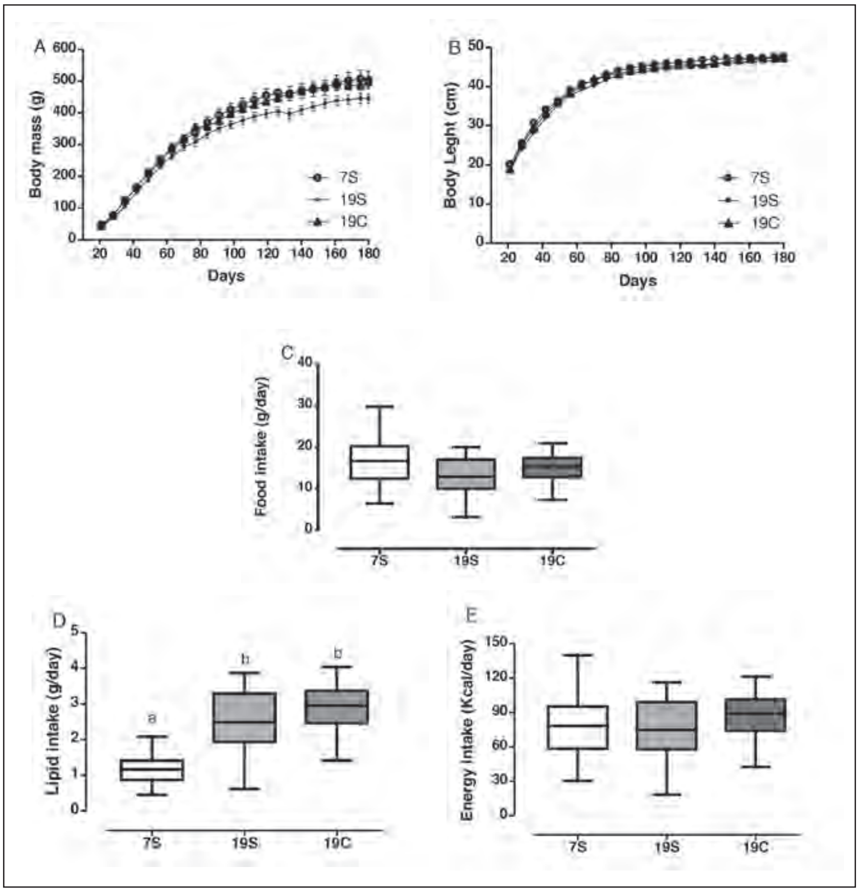

Fig. 1.—Body mass (a), length (b), food intake (c), lipid intake (d) and energy intake (e) postweaning until 180 days. Control group, fed with diet containing 7 ml of soybean oil (7S, n=10) and experimental diets, containing 19 ml of soybean (19S, n=10) or canola oil (19C, n=10). [a,b]Values with different superscripts are significantly (p<0·0001) different (A and B, two-way ANOVA. C, D and E, one-way ANOVA).

Table II
Body composition analyzed by DXA and intra-abdominal fat mass at 180 days

	7S		*19S*		*19S*	
	Mean	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
Total lean mass (g)	283·10	7·22	275·40	5·41	281·00	10·76
Total fat mass (g)	176·20	19·02	153·10	10·20	180·00	9·92
Trunk lean mass (g)	189·80	9·35	200·70	9·31	187·30	10·45
Trunk fat mass (g)	120·00	11·32	115·60	8·09	121·60	8·93
Body BMD (g/cm^2)	0·157^{a}	0·001	0·150^{a,b}	0·002	0·162^{a,c}	0·003
Body BMC (g)	12·88^{a}	0·51	10·75^{b}	0·39	13·85^{a}	0·58
Body bone area (cm^2)	81·67^{a}	2·47	72·25^{b}	2·49	85·25^{a}	2·17
Retroperitoneal fat mass (g)	16·07^{a}	2·87	7·65^{b}	0·75	14·72^{a}	1·81
Mesenteric fat mass (g)	6·51^{a}	0·79	3·85^{b}	0·54	7·23^{a}	1·01
Epididymal fat mass (g)	8·79^{a}	0·98	5·36^{b}	0·67	8·62^{a}	0·93
Intra-abdominal fat mass (g)	30·05^{a}	4·45	17·16^{b}	1·63	28·35^{a}	2·90

Post-weaning groups fed with control diet, containing 7 ml of soybean oil (7S, n=10) or experimental diets, containing 19 ml of soybean (19S, n=10) or canola oil (19C, n=10), until 180 days a,b,cMean values within a row dissimilar superscripts letters were significantly different (one-way ANOVA; P < 0·05) formance. During the 21 days of lactation, the rat dams were continued on an *ad libitum* diet of standard laboratory food (Nuvilab®, Paraná, Brazil).

Male Wistar rats were randomly chosen on postnatal day 21, to receive either a control diet containing 7 ml of soybean oil (7S group; n=10) or a high-fat diet containing either 19 ml of soybean (19S group; n=10) or canola oil (19C group; n=10). The diets were manufactured once a week and stored as pellets at 4°C in agreement with American Institute of Nutrition (AIN-93G) recommendations (Table I)[11]. All groups had free access to diet and water during the course of experimental period. Body mass (g), length (cm, measured as the distance from tip of the nose to the tip of the tail) and food (g/day), lipid (g/day) and energy intake (Kcal/day) were evaluated weekly.

The 180-days-old rats, after 8 h of fasting, were anesthetized with Avertin® (*Tribromoethanol*, 300 mg/Kg) and subjected to dual-energy X-ray absorptiometry (DXA), using a Lunar DXA 200368 GE instrument (Lunar, Wisconsin, USA) with specific software (encore 2008. Version 12.20 GE Healthcare). Total and trunk lean and fat mass, and bone analysis (bone mineral density- BMD; bone mineral content - BMC; and bone area) were measured for each rat.

Blood was collected by cardiac puncture after DXA procedures. Samples were centrifuged, and serum was stored at -20ºC for posterior analyze of calcium and phosphorus by colorimetric method (Bioclin, Belo Horizonte, MG, Brazil). Insulin (kit Linco Research, Inc., St Charles, MO, USA), leptin and osteocalcin were analyzed (kit Millipore, Billerica, MA, USA, respectively) by RIA.

The intra-abdominal fat depots were dissected and weighed (g).

Right femur and lumbar vertebra (LV4) were collected and cleaned of soft tissue and preserved in saline solution (0.9% of NaCl) until analyzed. Bone dimension: the distance between epiphysis and the medial point width of the diaphysis were measured using calipers with a readability of 0·01mm. Femur and LV4 were weighed (g). BMD and BMC in femur and LV4 were determined by DXA. After DXA, bones were analyzed by a single-scan computed tomography (CT Helicoidally model HISPEED, GE®). The images of femur and LV4 were obtained through axial cuts of thickness of 1 mm. The radiodensity (expressed as Hounsfield units, HU) of proximal epiphysis, distal epiphysis and lumbar vertebra were measured with a computerized analyzes software system (eFilm Lite, 2.0, 2003, Milwaukee, USA), by measurement Tool-Ellipse[12].The regions of interest were the femoral head, distal epiphyseal plate and vertebral body.

Statistical analyses were performed using the GraphPad Prism statistical package (version 5.0, 2007, San Diego, CA, USA). Body mass and length were analyzed using two-way ANOVA, followed by *post hoc* Bonferroni post-test. The remaining results were analyzed using one-way ANOVA followed by *post hoc* Newman-Keuls tests. All results are expressed as means ± SEM with significance level of 0.05.

Results

The groups showed similar body mass, length, food and energy intake. The fat intake was increased ($P<0{\cdot}0001$) in the 19S and 19C (Figure 1).

In regard to body composition, the groups showed similar total, trunk lean and fat mass. The 19S showed lower body BMD ($P<0{\cdot}05$, -7% *vs*. 19C), body BMC ($P<0{\cdot}05$, -16% *vs*. 7S and -22% *vs*. 19C) and body bone area ($P<0{\cdot}05$, -11% *vs*. 7S and -15% *vs*. 19C). The group 19S showed lower ($P<0{\cdot}05$) retroperitoneal, mesenteric and epididymal fat mass compared to the 7S and 19C. The intra-abdominal fat mass was significantly lower in the 19S group ($P<0{\cdot}05$, -42% *vs*. 7S and -39% *vs*. 19C) (Table II).

Serum analyses showed calcium, phosphorus, insulin, leptin and osteocalcin similar between groups (Table III).

Table III
Serum analyzed at 180 days

	7S		*19S*		*19S*	
	Mean	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
Calcium (mg/dL)	8·91	0·14	8·41	0·30	9·05	0·36
Phosphorus (mg/dL)	5·73	0·40	5·17	0·25	5·11	0·40
Insulin (μUI/mL)	44·19	4·66	49·14	4·84	42·70	2·47
Leptin (ng/mL)	6·71	1·97	4·22	1·15	6·53	1·39
Osteocalcin (ng/mL)	21·64	2·10	24·96	1·26	24·36	2·01

Post-weaning groups fed with control diet, containing 7 ml of soybean oil (7S, n=10) or experimental diets, containing 19 ml of soybean (19S, n=10) or canola oil (19C, n=10), until 180 days.
Values are means (one-way ANOVA)

Table IV
Femur and lumbar vertebra (LV4) analyzed by DXA and CT at 180 days

	7S		*19S*		*19S*	
	Mean	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
Femur mass (g)	1·32	0·10	1·33	0·01	1·33	0·04
Distance between epiphysis (mm)	40·27	0·26	39·98	0·31	39·63	0·36
Width of the diaphysis (mm)	5·14	0·20	4·90	0·07	4·87	0·16
Femur BMD (g/cm²)	0·20[a]	0·01	0·18[b]	0·01	0·20[a]	0·01
Femur BMC (g)	0·61[a]	0·01	0·55[b]	0·01	0·60[a]	0·01
Proximal epiphysis (Hu)	1547[a]	49·87	1376[b]	58·45	1620[a]	51·26
Distal epiphysis (Hu)	1290 [a]	35·34	1078[b]	70·00	1382[a]	90·85
LV4 mass (g)	0·53	0·02	0·49	0·02	0·49	0·01
LV4 BMD (g/cm²)	0·18[a]	0·01	0·16[b]	0·01	0·17[a]	0·01
LV4 BMC (g)	0·20[a]	0·01	0·15[b]	0·01	0·20[a]	0·01
Vertebral body (Hu)	1085[a]	49·23	866[b]	79·76	1171[a]	69·89

Post-weaning groups fed with control diet, containing 7 ml of soybean oil (7S, n=10) or experimental diets, containing 19 ml of soybean (19S, n=10) or canola oil (19C, n=10), until 180 days a,bMean values within a row dissimilar superscripts letters were significantly different (one-way ANOVA; P < 0·05)

Bone measures showed no differences to the femur, LV4 mass, distance between epiphysis and width of diaphysis between the groups. However, the femur analyses showed lower (*P<0·05*) femur BMD, BMC, and radiodensity of proximal and distal epiphysis in the 19S group compared to the 7S and 19C groups. The lumbar vertebra (LV4) analyses showed lower (*P<0·05*) BMD, BMC and radiodensity of vertebral body in the 19S compared to the other groups. The DXA and CT results did not differ between the control and the 19C groups (Table IV).

Discussion

Our results showed that a 19% canola oil diet was associated with maintaining the femur and LV4 parameters. However, the 19% soybean oil diet was unfavorable to bone parameters. The fat type was decisive for the outcomes observed in this experimental model.

Subsequent rodent models have validated the use of the high-fat diet to study the pathogenesis of the metabolic syndrome[13]. However, the groups treated with 19% of soybean or canola oil diet did not show consistent results in regard to obesity development. The reason for this discrepancy may be due to the composition of the fat diet. The study used a fat diet providing 39% energy as fat, while other experimental models have employed diets containing an average of 50 to 60%[14,15]. Jang[16] related that rats treated with a diet containing 32% energy as fat showed body development not characteristic to models of obesity. Although diets containing 19% of soybean or canola oil did not affect body weight, the fatty acid composition as well as amount of vegetable oil was a relevant factor to the intra-abdominal adiposity and bone parameters.

Genetic and environmental factors, such as fat diet composition, are crucial for the quantity and distribution of white adipose tissue in mammals[16,17]. The 19S and 19C showed higher fat intake, however, the intake of diet containing 19% soybean oil resulted in lower intra-abdominal fat depots. These results are surprising because LA is associated with adipogenesis and increasing expression of lipogenic genes. Meantime, ALA is common in soybean and canola oil[8,18]. Raclot and Groscolas[19] observed that diets containing high concentration of ALA limit post-intake fat storage and adipocytes hypertrophy. ALA is elongated and desaturated to eicosapentanoic acid and further to decosahexanoic acid, however the efficiency of this conversion has been debated[18]. Casey[20] and Gibson[21] related that oleic acid (OA) has potential substrates for desaturase that can compete with ALA for binding. The increased concentration of these alternate substrates can subsequently reduce ALA conversion even further. Soybean oil compared to canola oil contains a low concentration of OA (23% vs. 61%, respectively). Thus, these pathways help to explain the lower intra-abdominal fat mass observed in the group fed diet containing 19% soybean oil.

Although the effect of fat distribution on BMD is far from clear[22], the lower loads imposed by intra-abdominal fat mass, in group fed with a diet containing 19% soybean oil, could be related to verified lower bone parameters. Furthermore, by indirect action, the adipose tissue influences the bone structure through the production of adipokines, such as leptin[23]. Despite the limited clinical evidence[24], experimental models relate leptin administration improved bone formation and BMD[25]. In the present study, the lower intra-abdominal fat mass and serum concentration of leptin (-22% vs. 7S and -19% vs. 19C) may be associated with the bone parameters observed in the 19S group.

Life-style factor, such as composition of diets, are also important determinants to bone health[26,27]. LA is associated with extended osteoclast lifespan by through the inhibition of apoptosis, possibly leading to enhanced bone resorption[6,28]. However, the diets containing high levels of ALA are associated with lower maturation of the osteoclast. In regard to osteoblasts, such diets preserve bone mass increasing expression of key transcription factors that enhance differentiation of pre-osteoblasts into mature osteoblasts[6,29]. In the present study, the amount of ALA is very close in the experimental diets, while there is the higher concentration of LA in the 19% soybean oil diet. The 7% soybean oil diet contains the amount of LA recommended for growing rats[11]. However, the 19% soybean oil was unfavorable to bone structure. Its oil is the main source of lipid energy in Brazil[7]. Thus, the excessive consumption of this oil requires caution.

DXA has been used in studies of the whole body and regional bone in rats[30,31]. We verified that 19% soybean diet induced lower femur and LV4 BMD and BMC. On the other hand, there are regional differences in the mineral density in trabecular and cortical site that determine the modelling and the remodelling of bone[32]. The 19% soybean oil diet promotes a similar trend in bone structure, with low radiodensity of femoral head, distal epiphyseal plate and vertebral body regions. These analyses indicate the use of CT as a method for measuring differentiation between regional bone compartments of femur and lumbar vertebra, which is not possible for DXA technique in experimental models. Although CT has a greater diagnostic sensitivity than DXA[12], both techniques showed compatible data in the present study.

In summary, the amount and source of fat in the diet have differential effects on bone health. Moreover, lower concentrations of linoleic acid, present in canola oil seem to contribute to maintain bone health during the adult stage of life.

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References

1. Youngson NA, Morris MJ. What obesity research tells us about epigenetic mechanisms. *Phil Trans R Soc B* 2012; 368: 20110337.
2. Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA, Franklin B, Kris-etherton P, Harris WS, Howard B, Karanja N, Lefevre M, Rudel L, Sacks F, Van Horn L, Winston M, Wylie-Rosett, J. Diet and lifestyle recommendations revision 2006: a scientific statement from the american heart association nutrition committee. *Circulation* 2006; 114: 82-96.
3. World Health Organization. Diet, nutrition and the prevention of chronic diseases. *WHO Technical Report Series* 2003; 916, Geneva: WHO.
4. Weiler HA, Fitzpatrick-Wong SC. Modulation of essential (n-6):(n-3) fatty acid ratios alters fatty acid status but not bone mass in piglets. *J Nutr* 2002; 132: 2667-2672.
5. Maia LA, Lisboa PC, Oliveira E, Lima NS, Costa CAS, Moura EG. Two models of early weaning decreases bone structure by different changes in hormonal regulation of bone metabolism in neonate rat. *Horm Metab Res* 2012; 44: 1-6.
6. Kruger MC, Coetzee M, Haag M, Weiler H. Long-chain polyunsaturated fatty acids: selected mechanisms of action on bone. *Prog Lipid Res* 2010; 49: 438-449.
7. Levy-Costa RB, Sichieri R, Pontes NS, Monteiro CA. Household food availability in brazil: distribution and trends (1974-2003). *Cad Saude Publica* 2006; 39: 530-540.
8. McDonald BE. Canola oil: Nutritional properties. Canada: Canola Council of Canada. http://www.canolacouncil.org/health_nutritional.aspx.
9. Lau BY, Fajardo VA, Mcmeekin L, Sacco SM, Ward WE, Roy BD, Peters SJ, Leblanc PJ. Influence of high-fat diet from differential dietary sources on bone mineral density, bone strength, and bone fatty acid composition in rats. *Appl Physiol Nutr Metab* 2010; 35: 598-606.
10. Marques RG, Morales MM, Petroianu A. Brazilian law for scientific use of animals. *Acta Cir Bras* 2009; 24: 69-74.
11. Reeves PG. Components of the AIN-93 diets as improvements in the AIN-76A diet. *J Nutr* 1997; 127: 838-841.
12. Maia LA, Lisboa PC, Oliveira E, Lima NS, Lima ICB, Lopes RT, Ruffoni LDG, Nonaka KO, Moura EG. Bone metabolism in obese rats programmed by early weaning. *Metabolism* 2014; 63: 352-364.
13. Buettner R, Parhofer KG, Woenckhaus M, Wrede CE, Kunz-Schughart LA. Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. *J Mol Endocrinol* 2006; 36: 485-501.
14. Park S, Kim Y, Kim J, Jang E, Doh K, Lee S. Effect of high fat diet on insulin resistance: dietary fat versus visceral fat mass. *J Korean Med Sci* 2001; 16: 386-390.
15. Axen KV, Dikeakos A, Sclafani A. High dietary fat promotes syndrome X in nonobese rats. *J Nutr* 2003; 133: 2244-2249.
16. Jang IS, Hwang DY, Chae KR, Lee JE, Kim YK, Kang TS, Hwang JH, Lim CH, Huh YB, Cho JS. Role of dietary fat type in the development of adiposity from dietary obesity-susceptible Sprague-dawley rats. *Br J Nutr* 2003; 89: 429-437.
17. Lopes PA, Martins SV, Pinho MS, Alfaia CM, Fontes CM, Rodrigues PO, Morais GS, Castro MF, Pinto R, Prates JA. Diet supplementation with the cis-9, trans-11 conjugated linoleic acid isomer affects the size of adipocytes in wistar rats. *Nutr Res* 2008; 28: 408-486.

18. Joffe YT, Collins M, Goedecke JH. The relationship between dietary fatty acids and inflammatory genes on the obese phenotype and serum lipids. *Nutrients* 2013; 5: 1672-1705.
19. Raclot T, Groscolas R. Individual fish-oil n-3 polyunsaturated fatty acid deposition and mobilization rates for adipose tissue of rats in a nutritional steady state. *Am J Clin Nutr* 1994; 60: 72-78.
20. Casey JM, Banz WJ, Krul ES, Butteiger DN, Goldstein DA, Davis JE. Effect of stearidonic acid-enriched soybean oil on fatty acid profile and metabolic parameters in lean and obese zucker rats. *Lipids Health Dis* 2013; 12: 147.
21. Gibson RA, Neumann MA, Lien EL, Boyd KA, Tu WC. Docosahexaenoic acid synthesis from alpha-linolenic acid is inhibited by diets high in polyunsaturated fatty acids. *Prostaglandins Leukot Essent Fatty Acids* 2013; 88: 139-146.
22. Zillikens MC, Uitterlinden AG, van Leeuwen JPTM. The role of body mass index, insulin, and adiponectin in the relation between fat distribution and bone mineral density. *Calcif Tissue Int* 2010; 86: 116-125.
23. Watkins BA, Li Y, Lippman HE, Feng S. Modulatory effect of omega-3 polyunsaturated fatty acids on osteoblast function and bone metabolism. *Prostaglandins Leukot Essent Fatty Acids* 2003; 68: 387-398.
24. Motyl KJ, Rosen CJ. Understanding leptin-dependent regulation of skeletal homeostasis. *Biochimie* 2012; 94: 2089-2096.
25. Hamrick MW, Della-Fera MA, Choi Y. Leptin treatment induces loss of bone marrow adipocytes and increases bone formation in leptin-deficient ob/ob mice. *J Bone Miner Res* 2005; 20: 994-1001.
26. Boyle W, Simonet WS, Lacey DL. Osteoclast differentiation and activation. *Nature* 2003; 423: 337-342.
27. Farquharson C, Staines K. The skeleton: no bones about it. *J Endocrinol* 2011; 211: 107-108.
28. Yuan, J., Akiyama, M., Nakahama, K., Sato, T., Uematsu, H., Morita, I. The effects of polyunsaturated fatty acids and their metabolites on osteoclastogenesis in vitro. *Prostaglandins Other Lipid Mediat* 2010; 92: 85-90.
29. Rahman, M., Bhattacharya, A., Banu, J., Kang, J., Fernandes, G. Endogenous n-3 fatty acids protect ovariectomy induced bone loss by attenuating osteoclastogenesis. *J Cell Mol Med* 2009; 13: 1833-1844.
30. Lukaski, H.C., Hall, C.B., Marchello, M.J., Siders, W.A. Validation of dual x-ray absorptiometry for body-composition assessment of rats exposed to dietary stressors. *Nutrition* 2001; 17: 607-613.
31. Tsujio, M., Mizorogi, T., Kitamura, I., Maeda, Y., Nishijima, K., Kuwahara, S., Ohno, T., Niida, S., Nagaya, M., Saito, R., Tanaka, S. Bone mineral analysis through dual energy x-ray absorptiometry in laboratory animals. *J Vet Med Sci* 2009; 71: 1493-1497.
32. Seeman, E. Is a change in bone mineral density a sensitive and specific surrogate of anti-fracture efficacy. *Bone* 2007; 41: 308-317.