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Impacto da dieta hiperlipídica contendo óleo de canola ou de soja no desenvolvimento da adiposidade abdominal e estrutura óssea

Rio de Janeiro

2012

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Tese apresentada, como requisito parcial para obtenção do título de Doutor, ao Programa de Pós-graduação em Biociências, da Universidade do Estado do Rio de Janeiro.

Orientador: Prof. Dr. Egberto Gaspar de Moura

Coorientadora: Profª. Dra. Celly Cristina Alves do Nascimento-Saba

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Nikola Tesla

RESUMO

COSTA, Carlos Alberto Soares da. *Impacto da dieta hiperlipídica contendo óleo de canola ou de soja no desenvolvimento da adiposidade abdominal e estrutura óssea*. 2012. 44 f. Tese (Doutorado em Biociências) - Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2012.

A baixa relação de ômega-6/ômega-3 esta relacionada com propriedades benéficas para a saúde óssea. No entanto, a dieta rica nestes compostos pode levar a obesidade. Adipócitos e osteoblastos derivam de células progenitoras comuns, e o consumo de óleo de canola pode ter ação adipogênica e osteogênica. Nosso objetivo foi avaliar a adiposidade abdominal, insulina e estrutura óssea em ratos tratados com dieta contendo baixa relação ômega-6/ômega-3, proveniente do óleo de canola. Após desmame, os ratos foram divididos em grupos alimentados com dieta normocalórica: Controle (S) e experimental (C), contendo 7ml/100g de óleo de soja ou de canola e grupos tratados com dieta rica em lipídios: Controle (7S) ou hiperlipídico contendo 19ml/100g de óleo de soja (19S) ou de canola (19C), até completarem 60 dias de idade. Os dados foram significativos com $P<0,05$. No primeiro modelo, o grupo C apresentou redução de: Massa e área do adipócito intra-abdominal; Colesterol; Insulina; Densidade mineral (DMO) e massa óssea total e na coluna vertebral; Massa do fêmur; Espessura da diáfise; DMO do fêmur e das vértebras lombares e radiodensidade da cabeça do fêmur. No segundo modelo, os grupos 19S e 19C apresentaram maior ingestão calórica, densidade corporal, massa de gordura intra-abdominal, e maior massa e comprimento do fêmur e da coluna lombar. O grupo 19S apresentou maior área e menor número de adipócitos da região retroperitoneal. Glicose e a insulina foram aumentadas no grupo 19C *vs.* 7S. A tomografia do fêmur revelou maior radiodensidade na região proximal e da coluna lombar, no grupo 19C. Sugerimos que a quantidade e o tipo de lipídio consumido, após o desmame, induzem não somente o desenvolvimento corporal e os depósitos de gordura, além de afetarem a resistência insulínica e a saúde óssea.

Palavras-chave: Óleo de canola. Óleo de soja. DXA. Tomografia. Adipócito. Osso. Rato.

ABSTRACT

The lower ratio of omega-6 to omega-3 polyunsaturated fatty acids is associated with healthy bone properties. However, fat diets can induce obesity. Adipocytes and osteoblasts derive from a common progenitor, and canola oil intake may have an adipogenic and osteogenic effect. Our objective was to evaluate the intra-abdominal adiposity, insulin and bone growth in rats fed diet containing lower ratio of omega-6 to omega-3, provided in canola oil. After weaning, rats were divided into groups fed with normocaloric diet: control (S) and experimental (C), containing 7ml/100g soybean or canola oil, respectively and groups fed with fat diet: control (7S) or fat diets containing 19ml/100g soybean oil (19S) or canola oil (19C), until they 60 days old. Differences were considered significant with $P<0,05$. In normocaloric diet model, C group showed a significant reduction in: Intra-abdominal fat mass; Area of adipocyte; Cholesterol; Insulin; Total body and spine bone mineral content and bone area; Femur mass; Width of the diaphysis; Femur and lumbar vertebrae bone mineral density and radiodensity of femoral head. To high-fat diet model, 19S and 19C groups showed higher energy intake, body density growth, intra-abdominal fat mass and higher femur mass and, lumbar vertebrae mass and length. 19S showed higher area and lower number of retroperitoneal adipocytes. Glucose and insulin were significantly increased in 19C compared to 7S group. Computed tomography of femur revealed higher radiodensity in proximal femoral epiphysis and lumbar vertebrae of 19C. We suggest that the amount and the source of fat used in the diet, after weaning, induce not only the body and fat depots growth, besides affecting the insulin resistance and the bone health.

Keywords: Canola oil. Soybean oil. DXA. Tomography. Adipocyte. Bone. Rat.

LISTA DE ABREVIATURAS E SIGLAS

AA– Ácido araquidônico

AIN– American Institute of Nutrition

C– Grupo experimental alimentado com dieta contendo 7ml de óleo de canola

CMO– Conteúdo Mineral Ósseo

DHA– Ácido docosa-hexaenóico

DMO– Densidade Mineral Óssea

DXA– Dual-energy X-ray absorptiometry

EPA– Ácido eicosapentaenóico

HDL– Lipoproteína de alta densidade

HE– Hematoxilina-Eosina

HU– Unidades de Hounsfield

IMC– Índice de massa corporal

IRI– Índice de Resistência Insulínica

LDL– Lipoproteína de baixa densidade

TC– Tomografia Computadorizada

VL– Vértebra lombar

SUMÁRIO

INTRODUÇÃO

1 OBESIDADE, ALIMENTAÇÃO E SAÚDE ÓSSEA

A obesidade é considerada um problema de saúde pública mundial, que geralmente inicia nos primeiros anos de vida, persiste na idade adulta e aumenta significativamente o risco de morbidade por dislipidemia, diabetes mellitus tipo 2 e doença coronariana (FAGOT-CAMPAGNA, 2000; JAMES *et al.*, 2001; EKNOYAN, 2006). Estes distúrbios endócrino-metabólicos podem também interferir com a remodelação óssea (COBAYASHI *et al.*, 2005), pois embora a característica genética seja um dos principais contribuintes para o pico de massa óssea, fatores ambientais como os componentes da dieta também contribuem para a qualidade óssea (SIROIS *et al.*, 2003; HSU *et al.*, 2006). Mudanças no estilo de vida e nos hábitos alimentares, como a ingestão de excessiva de alimentos ricos em lipídios, vêm determinando a alta incidência de obesidade, não apenas em adultos, mas também em adolescentes e crianças. Em especial, o grande consumo de óleos vegetais, interfere no processo de remodelação óssea, além de contribuir para efeitos adversos sobre a saúde.

1.1 Tecido ósseo

A estrutura óssea possui múltiplas funções, dentre as quais, as mais importantes estão relacionadas à locomoção, proteção dos órgãos internos, hematopoese e reserva corporal de cálcio. O osso é composto por um substrato orgânico, que consiste de ampla quantidade de colágeno tipo I (~40% do volume), intercalado com cristais de hidroxiapatita (~45%). O restante do volume (~15%) é preenchido por água. Esta combinação permite ao osso apresentar resistência à compressão e elasticidade. Estruturalmente, é diferenciado em osso cortical ou trabecular (WEHRLI, 2007). O osso cortical é a camada compacta que forma a porção axial, ou externa, enquanto que o osso trabecular, constitui-se em uma série de finas placas (trabéculos), o qual forma o interior dos ossos. A estrutura óssea não é estática, sendo caracterizada pela contínua reestruturação adaptativa, na qual ocorre renovação de tecido pré-

existente, através da reabsorção óssea, pelos osteoclastos e, subsequente formação de tecido novo, pelos osteoblastos (FATORRE *et al.*, 2010; MARCU *et al.*, 2011).

A infância e adolescência são consideradas os períodos de vida mais relevantes para a aquisição do pico de massa óssea. Este é definido como o mais alto nível de massa óssea alcançado como resultado do crescimento normal, vinculado e influenciado por fatores genéticos, hormonais e ambientais como dieta e exercício físico (COBAYASHI *et al.*, 2005). No indivíduo adulto, o balanço entre a reabsorção e a formação óssea mantém uma massa óssea constante (KRUGER *et al.*, 2010). No caso das mulheres, a lactação representa um período de substancial aumento da remodelação óssea, com predominância da reabsorção e perda do conteúdo ósseo. Essa mudança no metabolismo mineral visa atender a demanda de cálcio para a produção de leite materno (VAJDA *et al.*, 2001; MILLER *et al.*, 2005). Imediatamente após a lactação, ocorre uma rápida reversão no metabolismo ósseo, denominado *postlactation recovery period*, no qual há um aumento da formação óssea. Esta fase anabólica tem como objetivo preparar o esqueleto materno para um próximo ciclo reprodutivo (MILLER & BOWMAN, 2007). Aproximadamente aos 40 anos de idade, começa a etapa de perda óssea, que é para as mulheres uma fase rápida. Em uma etapa de vida mais avançada, o processo de mineralização óssea se torna menos eficiente, com a possibilidade de desenvolvimento de osteopenia seguida da osteoporose (ORIMO, 2010).

1.2 **Relação entre os adipócitos e a estrutura óssea**

Os adipócitos e os osteoblastos são originados das células-tronco mesenquimais. O balanço na adipogênese apresenta crucial importância na manutenção de diversas funções fisiológicas. O estudo da relação adipócito/osteoblasto representa um novo olhar para o tratamento de distúrbios fisiológicos relacionados a adiposidade e a estrutura óssea (CHIELLINI *et al.*, 2008).

Apesar das repercussões metabólicas da obesidade, a elevação da massa corporal ou do índice de massa corporal (IMC) apresentam ação positiva sobre a estrutura óssea (REDDY *et al.*, 2009; RHIE *et al.*, 2010; UUSI-RASI *et al.*, 2010). A obesidade em períodos precoces da vida está associada com aumento da densidade mineral óssea (DMO) na vida adulta (UUSI-RASI *et al.*, 2010). Em indivíduos adultos com sobrepeso a DMO é maior, principalmente na região do quadril de (REDDY *et al.*, 2009) e, em mulheres menopausadas a

adiposidade representa um fator protetor contra a perda óssea (HO-PHAM *et al.*, 2010). Em modelos experimentais a mesma ação positiva da obesidade é relatada (IWANIEC *et al.*, 2008). Baseado nos resultados, os referido estudos sugerem que a obesidade atue diretamente, pela ação de carga mecânica, estimulando a osteogênese e o aumento da densidade mineral óssea. Por outro lado, outros estudos evidenciaram que a adiposidade tem ação direta, porém prejudicial à estrutura óssea. TAES *et al.*(2009) e AFGHANI & GORAN (2010) verificaram relação inversa entre a adiposidade intra-abdominal e DMO em crianças e homens adultos. Enquanto, GILSANZ *et al.*(2009) verificaram uma relação negativa entre gordura visceral e DMO em mulheres adultas.

Além de sua ação direta, a adiposidade atua na estrutura óssea de maneira indireta, através da secreção de adipocinas, onde a adiponectina apresenta ação protetora contra a osteoporose e a leptina promove a diferenciação dos osteoblastos. Já o tecido adiposo intra-abdominal está relacionado com a expressão de pró-inflamatórios, como o fator de necrose tumoral alfa (TNF-α) e a interleucina 6, que promovem a reabsorção óssea (HSU *et al.*, 2006; GILSANZ *et al.*, 2009; PAULA & ROSEN, 2010).

A obesidade está associada a distúrbios metabólicos, incluindo o aumento dos níveis séricos de insulina. A insulina é considerada um potente regulador do desenvolvimento ósseo, que atua diretamente na proliferação e na atividade dos osteoblastos, estimulando a formação óssea (GILSANZ *et al.*, 2009; ZILLIKENS *et al.*, 2010).

1.3 **Relação entre a dieta, estrutura óssea e a adiposidade**

Os componentes da dieta são fatores importantes para o desenvolvimento e manutenção da massa óssea. Os nutrientes de maior relevância para a saúde óssea são o cálcio e o fósforo, que representam de 80 a 90% do conteúdo mineral. As proteínas são essenciais para a manutenção do colágeno presente na matriz orgânica. Os micronutrientes, como a vitamina D e K, são cruciais para a realização de processos metabólicos (OKUBO *et al.*, 2006; KONTOGIANNI *et al.*, 2009). Dietas ricas em ácidos graxos poliinsaturados, contendo mais do que dezoito carbonos em sua cadeia, também interferem na saúde óssea (KRUGER *et al.*, 2010).

Os lipídios têm sido tradicionalmente considerados como um nutriente altamente calórico e fonte de ácidos graxos essenciais, cada vez mais reconhecidos como importantes

reguladores biológicos (DECKELBAUM *et al.*, 2008). Organizações, incluindo o *United States Department of Agriculture*, a *American Heart Association* e a *National Academy of Sciences/Institute of Medicine* têm feito recomendações dietéticas focando não somente na quantidade, mas também no tipo de lipídio presente na dieta (KRAUSS *et al.*, 2000). As recomendações dietéticas geralmente aconselham a reduzir a ingestão de ácidos graxos saturados e a manter ou aumentar a ingestão de ácidos graxos poliinsaturados (World Health Organization (WHO), 2003).

Os principais ácidos graxos poliinsaturados são o ácido graxo linoléico (ômega-6, C18: 2n-6) e o ácido alfa-linolênico (ômega-3, C18: 3n-3), essenciais, determinando a necessidade de ingestão diária, por não serem sintetizados no organismo humano (RUXTON *et al.*, 2004; ZEVENBERGEN *et al.*, 2009).

Dados epidemiológicos têm indicado que o consumo de alimentos ricos em gordura saturada presente, por exemplo, no óleo de coco e de palma, contribuem para a redução da densidade óssea e aumento do risco de fraturas. No entanto, os ácidos graxos poliinsaturados ômega-6 e ômega-3 podem ser benéficos quando consumidos em quantidades apropriadas (CORWIN, 2003).

O ácido linoléico é precursor do ácido araquidônico (AA, C20:4n-6), sendo este último necessário para síntese de prostaglandinas da série 2 (PG2) e leucotrienos da série 4, ambos com ação pró-inflamatória. Enquanto o ácido linolênico é precursor dos ácidos eicosapentaenóico (EPA, C20:5n-3) e docosa-hexaenóico (DHA, C22:6n-3), que reduzem a produção dos derivados de AA, e têm ação anti-inflamatória (SIMOPOULOS, 1991; BARHMAM *et al.*, 2000).

A maior proporção ômega-6/ômega-3 está associada a efeitos negativos sobre a fisiologia óssea, com maior estímulo para a reabsorção óssea, via osteoclastos. A excessiva produção de PG2 afeta a remodelação óssea, reduzindo a diferenciação das células mesenquimais em osteoblastos e, aumenta o recrutamento de pré-osteoclastos, sua maturação e retarda a apoptose dos osteoclastos (KRUGER *et al.*, 2010).

No entanto, a menor proporção ômega-6/ômega-3 está associada com melhora da formação óssea (WEISS *et al.*, 2005). O ácido eicosapentaenóico (EPA), derivado do ómega-3, é precursor de prostaglandinas da série 3 e de leucotrienos da série 5, que contribuem para a redução da síntese de PG2, possibilitando o recrutamento de pré-osteoblastos, sua diferenciação e maturação, o que consequentemente, contribui para a formação óssea. Paralelamente, EPA reduz o recrutamento de pré-osteoclastos e diminui o tempo de vida média dos osteoclastos, promovendo a apoptose destas células (KRUGER *et al.*, 2010).

O padrão alimentar, com elevada ingestão de ácidos graxos poliinsaturados, pode levar a obesidade. Dependendo do tipo de ácido graxo predominante na dieta pode ocorrer uma ação direta sobre pré-adipócitos, aumentando sua taxa de replicação e/ou diferenciação (GHIBAUDI *et al.*, 2002). Assim, quando uma dieta hiperlipídica contendo ômega-6 é consumida, o receptor ativado por proliferadores de peroxissoma gamma (PPAR-γ) é recrutado para converter pré-adipócitos em adipócitos, o que leva ao consequente acúmulo da gordura. Portanto, a redução na ingestão de alimentos ricos em ômega-6 está associada a menor adipogênese e a menor hipertrofia do adipócito (SHILLABEER & LAU, 1994). O ácido graxo ômega-3 limita o acúmulo de gordura e a hipertrofia dos adipócitos, com o estímulo de genes relacionados à oxidação dos ácidos graxos, através do receptor ativado por proliferadores de peroxissoma alfa (PPAR-α) e, com a supressão de genes lipogênicos, causando a redução da área dos adipócitos (RACLOT & GROSCOLAS, 1994; HSU & HUANG, 2006).

Embora a ingestão de uma dieta hiperlipídica e o consequente desenvolvimento da obesidade tenham sido extensivamente estudados, o papel dos ácidos graxos sobre o desenvolvimento ósseo têm emergido na última década, como uma nova área de pesquisa (HARROLD *et al.*, 2000; GHIBAUDI *et al.*, 2002; WEILER & FITZPATRICK-WONG, 2002; SIROIS *et al.*, 2003; HSU *et al.*, 2006; JO *et al.*, 2009;).

1.4 Óleo de soja e óleo de canola

No Brasil, dados relativos à disponibilidade domiciliar de alimentos (2002/2003) relatam que o óleo de soja é o mais consumido em todas as classes sociais. Já o óleo de canola (incluído no grupo dos outros óleos vegetais) é pouco consumido em todas as classes sociais (LEVY-COSTA *et al.*, 2006), correspondendo a menos de 10% do total de calorias, oriundas de óleos e gorduras vegetais, consumidas nos Estados Unidos e Brasil (RUXTON *et al.*, 2004; LEVY-COSTA *et al.*, 2006). No Brasil, o maior consumo de óleo de soja deve-se ao fato do país ser um dos maiores produtores mundiais de soja, o que torna seu preço o mais acessível para a população. Ao contrário, o óleo de canola é um dos óleos vegetais disponível no mercado de maior custo.

O óleo de soja é composto basicamente por 15% de ácido graxo saturado, 23% de ácidos graxos monoinsaturados, 8% de ácido graxo alfa-linolênico (C18:3n-3) e 54% de ácido

graxo linoléico (C18:2n-6), apresentando uma relação ômega-6/ômega-3 igual a 6,75 (MCDONALD 2005; LEVY-COSTA *et al.*, 2006). A ingestão do ácido graxo linoléico, através do óleo de soja, corresponde a uma média de 80% do total de calorias oriundas de óleos e gorduras vegetais nos Estados Unidos e no Brasil (RUXTON *et al.*, 2004; LEVY-COSTA *et al.*, 2006).

Por outro lado, o óleo de canola é composto basicamente por 7% de ácido graxo saturado, 11% de ácido graxo linolênico (C18:3n-3) e 21% de ácido graxo linoléico (C18:2n-6), ambos poliinsaturados e, 61% de ácidos graxos monoinsaturados (MCDONALD, 2005). A relação ômega-6/ômega-3 é igual a 1,90 e o ácido graxo oléico (ômega-9, C18:1n-9), corresponde a 98% do total de ácidos graxos monoinsaturados presentes no óleo de canola (PRZYBYLSKI, 2007).

Diversos relatos científicos têm estimulado o aumento da ingestão de alimentos ricos em ácido graxo ômega-3 e a redução na relação ômega-6/ômega-3, para a prevenção e terapêutica das doenças cardiovasculares, inflamatórias e metabólicas, na infância e na idade adulta, independente do sexo (SIMOPOULOS, 1991; RUXTON *et al.*, 2004; DECKELBAUM *et al.*, 2008). No entanto, na insuficiência de dados esclarecedores, nossa proposta foi comparar os efeitos da ingestão de uma dieta, contendo alta ou baixa relação ômega-6/ômega-3, provido pelo óleo de soja ou de canola, respectivamente, sobre a composição corporal, metabolismo lipídico e estrutura óssea de ratos Wistar.

2 **OBJETIVOS**

Avaliar o desenvolvimento corporal e a estrutura óssea em ratos Wistar alimentados com dietas normo ou hiperlipídicas, contendo óleo de soja ou de canola, após o desmame.

2.1 **Objetivos específicos**

Avaliar a ingestão alimentar.

Avaliar o desenvolvimento físico e composição corporal.

Avaliar a interação dos indicadores de desenvolvimento físico e adiposidade.

Avaliar a estrutura de fêmur e coluna lombar.

Quantificar a concentração sérica de albumina, glicose, cálcio, fósforo e o perfil lipídico.

Quantificar as concentrações séricas de insulina e leptina.

Realizar a avaliação morfológica do tecido adiposo branco retroperitoneal.

3 **ARTIGOS**

3.1 **Artigo 1**

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ORIGINAL CONTRIBUTION

Diet containing low n-6/n-3 polyunsaturated fatty acids ratio, provided by canola oil, alters body composition and bone quality in young rats

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Abstract
Purpose Adipocytes and osteoblasts were derived from a common progenitor, and canola oil intake may have an adipogenic and osteogenic effect. Thus, our objective was to evaluate the effect on adipocyte, lipid profile, glucose homeostasis, and bone of canola oil as main lipid source on the diet during development.
Methods After weaning, rats were divided into two groups ($n = 10$ per group): control (S) and experimental (C) diets containing 7 mL/100 g soybean or canola oil, respectively. At 60 days, body composition, liver and intra-abdominal fat mass, adipocyte morphology, serum analysis, femur and lumbar vertebras density by dual-energy X-ray absorptiometry and computed tomography were determined. Differences were considered significant with $P < 0.05$.
Results C group showed the following: lower liver (−12%) and intra-abdominal fat mass (−19%) area of adipocyte (−60%), cholesterol (−33%), insulin (−22%), lower total body (−9%) and spine (−33%) bone mineral content and bone area (−7 and −24%, respectively), femur mass (−9%), width of the diaphysis (−6%), femur (−10%) and lumbar vertebrae bone mineral density (−9%), and radiodensity of femoral head (−8%).
Conclusions The lower intra-abdominal adiposity could have more beneficial effects in a short term, since it can be associated with a better insulin sensitivity and lipid profile, than the small reduction in femur and lumbar vertebra density. However, it has to be considered the incremental effect of this reduction along the aging process.

Keywords Canola oil · Soybean oil · Adipocyte · Bone · Dual-energy X-ray absorptiometry · Computed tomography · Rats

Abbreviations

EFA	Essential fatty acids
SFA	Saturated fatty acids
PUFA	Polyunsaturated fatty acids
LA	Linoleic acid
ALA	Linolenic acid
S	Control group fed with diet containing 7 mL soybean oil/100 g
C	Experimental group fed with diet containing 7 mL canola oil/100 g
AIN	American Institute of Nutrition
CT	Computed tomography
DEXA	Dual-energy X-ray absorptiometry
BMD	Bone mineral density
BMC	Bone mineral content
HE	Hematoxylin–eosin
LV	Lumbar vertebra
CT	Computed tomography
HU	Hounsfield units
PPARα	Peroxisome proliferator-activated receptor alpha
SREBP-1c	Sterol regulatory element-binding protein
MUFA	Monounsaturated fatty acids

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Introduction

Fat has been traditionally regarded as an important calorie-dense nutrient and as a source of essential fatty acids (EFA). Fat and especially the EFA have been increasingly recognized as major biological regulators [1]. Organizations including the United States Department of Agriculture, American Heart Association, and National Academy of Sciences/Institute of Medicine have made dietary recommendations that focus not only on the quantity but also on the type of fats in the diet [2]. Dietary recommendations often advise to reduce the saturated fatty acids (SFA) intake and maintain or increase the intake of polyunsaturated fatty acids (PUFA) [3]. PUFA contains essential fatty acids, such as linoleic acid (LA, 18: 2n-6) and alpha linolenic acid (ALA, 18: 3n-3), and both are necessary every day, because they cannot be synthesized in the body [4, 5].

LA represents 80% of total PUFA energy in the United States and Brazil, by soybean oil intake, while ALA represents about 10%, and it is widely accepted that a large intake of ALA contributes to human health [4–9]. Canola oil, when compared to soybean oil, is characterized by a very low level of SFA (7% vs. 15%) and LA (21% vs. 54%). It contains 11% of ALA (vs. 8%) and a better balance of n-6 and n-3 fatty acid (n-6/n-3 1.90 vs. 6.75) [9]. However, canola oil represents less than 4% of the fat intake in American and Brazilian populations [6, 7].

Although the beneficial effects of polyunsaturated fatty acids intake, decreasing plasma cholesterol level and reducing the risk of coronary heart disease, have been extensively studied, the role of dietary fats on adipose tissue and bone growth has only emerged recently as an interesting area of research [10–17]. Adipocytes and osteoblasts were derived from a common progenitor—the mesenchymal stem cell. Extensive epidemiological data show that adipose tissue might influence bone density, through stresses caused by mechanical loading. And by indirect action, it influence bone through the production of leptin and adipokines and through the regulation of bone-active hormones as insulin [18, 19]. In addition, a higher ratio of n-6/n-3 fatty acids is associated with deleterious effects on bone health, while a lower ratio is associated with healthy bone properties [20]. These PUFA were acting directly on preadipocytes and increasing the rate of replication and/or differentiation, mainly in early stages of adipose tissue development [12, 21].

The positive action of adipose tissue on bone health has been related in several obesity or high-fat diet intake studies [22–25]. To the best of our knowledge, there are no studies about experimental model young rats fed with diet containing canola oil on bone metabolism. Than, the aim of this study was to compare the effects of feeding young adult animals, after weaning, with normal-fat diets containing higher or lower n-6/n-3 ratio, provided by soybean or canola oil, respectively, on body composition.

Materials and methods

Animals and diets

The protocol to use and handling the experimental animals was approved by the Ethical Committee of the Biology Institute of the State University of Rio de Janeiro, which based their analysis on the principles adopted and promulgated by the Brazilian Law that concerns the rearing and use of animals in teaching and research activities in Brazil [26].

Wistar rats were kept in a room with controlled temperature (23 ± 1 °C) and with an artificial dark–light cycle (lights on from 0700 to 1900 hours). Virgin female rats (3 months old) were caged with male rats, and after mating, each female was placed in an individual cage with free access to water and food. Within 24 h of birth (day 0), excess pups were removed, so that only six male pups were kept per dam. This procedure maximizes lactation performance [27]. During 21 days of lactation, rat dams were continued on an ad libitum diet of standard laboratory food (Nuvilab®, Paraná, Brazil).

Male Wistar rats were randomized and divided on postnatal day 21, from different litters, to receive a diet containing either 7 mL of soybean (S control group, $n = 10$) or canola oil (C group, $n = 10$), 20 g of casein, and 54 g of cornstarch/100 g. The groups received the same amounts of vitamins and minerals per gram of diet (Table 1). The diets were manufactured each 7 days and stored in pellets at 4 °C in agreement with American Institute of Nutrition (AIN-93G) recommendations [28, 29]. The animals had free access to diet and water during the course of experimental period. Food intake (g), body mass (g), and length (cm, measured as the distance from tip of the nose to the tip of the tail) were evaluated every 4 days.

Body composition analysis

At the end of the nutritional period, 60-days-old rats, after 8 h of fasting, were anesthetized with Avertin® (*Tribromoethanol*, 300 mg/kg) and subjected to dual-energy X-ray absorptiometry (DEXA) [30–32], using a Lunar DEXA 200368 GE instrument (Lunar, Wisconsin, USA) with specific software (encore 2008. Version 12.20 GE Healthcare). The evaluation was blind, since the DEXA technician did not know the experimental protocol. Total lean (g), fat mass (g), trunk fat mass (g), and bone analysis (bone mineral density—BMD (g/cm^2); bone mineral

Table 1 Composition of experimental diets

Ingredient (g/100 g)	S	C
Casein	20	20
Cornstarch	52.95	52.95
Sucrose	10	10
Soybean oil	7	–
Canola oil	–	7
Fiber	5	5
AIN-93G mineral mix	3.5	3.5
AIN-93 vitamin mix	1	1
L-Cystine	0.3	0.3
Choline bitartrate	0.25	0.25
Energy		
kJ/g	19.7	19.7
kcal/g	4.7	4.7
Protein (% of energy)	17	17
Carbohydrate (% of energy)	65	65
Fat (% of energy)	17	17

Formulated to meet the American Institute of Nutrition AIN-93G recommendation for rodent diets [28]

S control group fed with diet containing 7 mL/100 g soybean oil, and *C* experimental group fed with diet containing 7 mL/100 g canola oil

Casein; Mineral and Vitamin Mix; L-Cystine; Choline Bitartrate: Agroquímica®; Cornstarch: Cargill®; Fiber: Natural Pharma®; Soybean and Canola oil: Proquímios®; Commercial Sucrose: União®

content—BMC (g); and bone area (cm^2)) were measured for each rat.

The intra-abdominal fat depots and liver were dissected and weighed (g). For morphological analyses, samples of retroperitoneal fat were fixed in formaldehyde. The fixed tissues were embedded in paraffin, cut into 5-μm sections, and stained with hematoxylin–eosin (HE). The sectional area of the adipocytes (μm^2) was determined on digital images acquired at random (TIFF format, 36 bit color, 1,360 × 1,024 pixels) with an Optronics CCD video camera system and Olympus BX40 light microscope and analyzed with the software Image-Pro Plus version 5.0 (Media Cybernetics, Silver Spring, MD, USA).

Serum analysis

Blood was collected by cardiac puncture after DEXA procedures. Samples were centrifuged, and serum was stored at −20 °C for posterior analysis of glucose, triglycerides, cholesterol, HDL-cholesterol, calcium, phosphorus, and albumin by colorimetric method (Bioclin, Belo Horizonte, MG, Brazil). Serum hormones concentrations were analyzed by RIA. Insulin kit (Linco Research, Inc., St Charles, MO, USA) determined an assay sensitivity of 0.1 ng/mL and a range of detection from 0.1 to 1.0 ng/mL, and the intra-assay CV was 8.5%. Leptin kit (Millipore, Billerica, MA, USA) determined an assay sensitivity of 0.5 ng/mL and the intra-assay CV of 4.8%.

Bone analysis

Right femur and lumbar vertebra (LV2–LV6) were collected and cleaned of soft tissue and preserved in saline solution (0.9% of NaCl) until analyzed. Bone dimension: the distance between epiphysis, the distance between the greater and lesser trochanter [33], the length of the entire LV2–LV6 [10] and LV4, and the medial point width of the diaphysis were measured using calipers with a readability of 0.01 mm. After drying overnight at 95 °C, femur and LV4 were weighed [34]. Before, the bones were submitted to DEXA and computed tomography (CT) analyses. In the same way for body composition, the DEXA or CT technician did not know about the experimental protocol.

Bone mineral density (BMD) in femur, LV2–LV6, and LV4 were determined by DEXA using a modification of previously described procedure [10]: In order to mimic soft tissue conditions, excised bones were fixed on constant volume of rice in a plastic container. After DEXA, 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 femoral head and vertebral body regions (R1 and R2, respectively, Fig. 1) were measured with a computerized analyzer software system (eFilm Lite, 2.0, 2003, Milwaukee, USA), by manual measurement Tool-Ellipse.

Statistical analysis

Statistical analyses were carried out using the Graph Pad Prism statistical package version 5.00, 2007 (San Diego, CA, USA). Food intake, body mass, and length were analyzed by two-way ANOVA, followed by Bonferroni post-test. The other data were analyzed by Student's *t* test. All results are expressed as means ± SEM with significance level of 0.05.

Results

During the nutritional period, food intake did not differ between groups. After 60 days, S and C groups showed similar body mass and length, although C group showed a slightly but significant higher body mass and length between 33–41 and 25–53 days, respectively (Fig. 2). The intra-abdominal fat mass and area of adipocytes were lower in C group (C: 4.39 ± 0.35 g vs. S: 5.45 ± 0.23 g and C: 241.60 ± 19.01 μm^2 vs. S: 611.90 ± 20.94 μm^2, $P < 0.05$ and $P < 0.0001$, respectively) (Fig. 3).

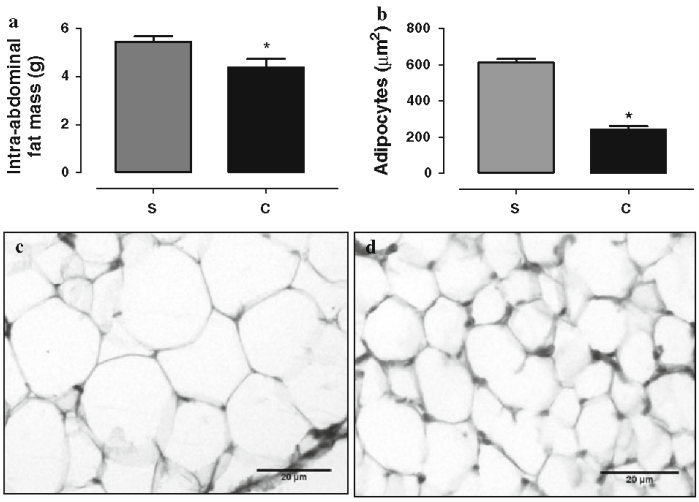

Fig. 3 Intra-abdominal fat mass (**a**) and adipocytes morphometry (sectional area, **b**), after 60 days. Control group, fed with diet containing 7 mL of soybean oil (S, $n = 10$) and experimental diet, containing 7 mL of canola oil (C, $n = 10$). *Significantly different from the control group (Student's t test, $P < 0.05$). Photomicrographs of the adipose tissue staining with HE (original magnification 200×): (**c**) S group, usual aspect of adipocyte and (**d**) C group, lower adipocyte area

Table 2 Serum analyses, at 60 days

	S (n10)		C (n10)	
	Mean	SEM	Mean	SEM
Glucose, mg/dL	122.90	13.30	145.40	4.98
Triglycerides, md/dL	34.40	2.49	29.46	4.36
Cholesterol, mg/dL	69.39	6.47	46.40*	5.68
HDL-cholesterol, mg/dL	23.40	3.88	24.12	2.54
Calcium, mg/dL	9.05	0.47	9.13	0.20
Phosphorus, mg/dL	5.18	0.57	6.16	0.71
Albumin, g/dL	3.26	0.65	3.46	0.42
Insulin, μUI/mL	35.42	3.52	27.55*	0.87
Leptin, ng/mL	1.68	0.13	1.56	0.08

Postweaning groups fed with control diet, containing 7 mL of soybean oil (S, $n = 10$) or experimental diet, containing 7 mL of canola oil (C, $n = 10$), until 60 days

* Significantly different from the control group (Student's t test, $P < 0.05$)

Table 3 Body compartments analyzed by DEXA, at 60 days

	S (n10)		C (n10)	
	Mean	SEM	Mean	SEM
Total lean, g	169.10	4.71	154.80	6.28
Fat mass, g	69.50	1.70	68.60	1.72
Trunk fat mass, g	45.80	2.03	42.67	3.43
Body BMD, g/cm^2	0.11	0.01	0.11	0.01
Body BMC, g	6.58	0.08	5.95*	0.16
Body bone area, cm^2	54.80	0.51	51.00*	0.73
Spine BMD, g/cm^2	0.11	0.01	0.10	0.03
Spine BMC, g	1.47	0.07	0.97*	0.10
Spine area, cm^2	12.70	0.70	9.67*	0.86

Postweaning groups fed with control diet, containing 7 mL of soybean oil (S, $n = 10$) or experimental diet, containing 7 mL of canola oil (C, $n = 10$), until 60 days

* Significantly different from the control group (Student's t test, $P < 0.05$)

Discussion

To our knowledge, this is the first study to evaluate the effect of a substitution of soybean per canola oil on the rat diet, after weaning. Our results showed that canola oil diet was associated with a leaner intra-abdominal adiposity, lower body BMC, normal body BMD, but lower mass, and BMD of femur and lumbar vertebras. But the groups had similar food intake and body growth. These results demonstrate that despite protein, calcium, phosphorus, fat, and energy intake constant in groups, the polyunsaturated fatty acids composition of diets was decisive for the outcomes observed in this experimental model.

There has been a considerable interest in the role of amount and nature of fatty acids in the diet and body fat accumulation and adipogenesis [35, 36]. In the present study, the intake of diet containing canola oil contributed to lower accumulation of intra-abdominal fat mass, associated with lower adipocytes area. The lowest n-6 intake prevents the formation of mature adipocytes and hypertrophy [37]. The n-3 intake inducing the fatty acid oxidation genes through PPARα (Peroxisome proliferator-activated receptor alpha) and the suppression of lipogenic genes through SREBP-1c

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3.2 **Artigo 2**

COSTA, C.A.S.; CARLOS, A.S.; SANTOS, A.S.; MONTEIRO, A.M.; MOURA, E.G.; NASCIMENTO-SABA, C.C. Abdominal adiposity, insulin and bone quality in Young male rats fed a high-fat diet containing soybean or canola oil. **CLINICS**, n. 66, p. 1811-1816, 2011.

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BASIC RESEARCH

Abdominal adiposity, insulin and bone quality in young male rats fed a high-fat diet containing soybean or canola oil

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OBJECTIVES: A low ratio of omega-6/omega-3 polyunsaturated fatty acids is associated with healthy bone properties. However, fatty diets can induce obesity. Our objective was to evaluate intra-abdominal adiposity, insulin, and bone growth in rats fed a high-fat diet containing low ratios of omega-6/omega-3 provided in canola oil.

METHODS: After weaning, rats were grouped and fed either a control diet (7S), a high-fat diet containing soybean oil (19S) or a high-fat diet of canola oil (19C) until they were 60 days old. Differences were considered to be significant if $p<0.05$.

RESULTS: After 60 days, the 19S and 19C groups showed more energy intake, body density growth and intra-abdominal fat mass. However, the 19S group had a higher area (200%) and a lower number (44%) of adipocytes, while the 7S and 19C groups did not differ. The serum concentrations of glucose and insulin and the insulin resistance index were significantly increased in the 19C group (15%, 56%, and 78%, respectively) compared to the 7S group. Bone measurements of the 19S and 19C groups showed a higher femur mass (25%) and a higher lumbar vertebrae mass (11%) and length (5%). Computed tomography analysis revealed more radiodensity in the proximal femoral epiphysis and lumbar vertebrae of 19C group compared to the 7S and 19S groups.

CONCLUSIONS: Our results suggest that the amount and source of fat used in the diet after weaning increase body growth and fat depots and affect insulin resistance and, consequently, bone health.

KEYWORDS: Canola oil; Soybean oil; Bone; Computed tomography; Rat.

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INTRODUCTION

Obesity, a worldwide public health problem, usually begins early in life, persists into adulthood and significantly increases the risk for morbidity from dyslipidemia, type-2 diabetes mellitus, and coronary heart disease.[1-3] These endocrine-metabolic disturbances can also interfere with bone remodeling.[4] Although genetic composition is a major contributor to peak bone mass, lifestyle factors (such as diet) also contribute to the attainment of peak bone mass.[5,6]

Despite the metabolic repercussions of obesity, extensive epidemiological data have shown that a high body weight or body mass index (BMI) is associated with increases in bone mass and a reduced risk for fractures.[7-9] This event occurs directly via mechanical loading and indirectly via hormonal production by adipocytes or insulin.[10,11] In contrast, investigators have suggested that fat mass may or may not be associated with bone mass.[12,13] Given these discrepancies, the effect of fat tissue on bone health is far from clear.

Adipocytes and osteoblasts share the same mesenchymal precursor; studying adipocyte/osteoblast balance represents a challenge to treating adipose tissue and bone disorders.[14] In recent years, a growing body of evidence has supported the notion that dietary long-chain polyunsaturated fatty acids (PUFAs) with a chain length longer than 18C are beneficial for bone health.[15] In addition, a higher ratio of omega-6 (linoleic acid, 18:2n-6) to omega-3 (alpha linolenic acid, 18:3n-3) fatty acids is associated with detrimental bone health effects, and a lower ratio is associated with healthy bone properties.[16] These PUFAs can induce obesity by acting directly on preadipocytes, mainly increasing the rate of replication and/or differentiation[17]

No potential conflict of interest was reported.

in the early stages of adipose tissue development.[18] Although the role of a high-fat diet in obesity development have been extensively studied, the role of dietary fats on bone development has only recently emerged as an interesting area of research.[5,6,17-20]

Previously, we reported the effects of a normocaloric diet containing canola oil on adiposity and bone growth in young rats.[21] Canola oil (7%) seems to beneficially decrease both abdominal adiposity and insulin resistance. However, it reduces bone density compared to 7% soybean oil. However, to the best of our knowledge, there have been no studies with an experimental model of bone metabolism in young rats fed a high-fat diet containing canola oil. In Brazil, the population consumption of soybean oil, which is rich in polyunsaturated fatty acids (ratio omega-6/omega-3 = 6.75), represents 82% of the calories originating from fat sources, while canola oil (ratio omega-6/omega-3 = 1.90) represents less than 4%.[22,23]

The aim of this study was to evaluate the body growth and bone health of young adult animals fed a high-fat diet containing soybean or canola oil after weaning.

MATERIALS AND METHODS

The protocol to use and handle the experimental animals was approved by the Ethics 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.[24]

Wistar rats were kept in a room under a controlled temperature (25±1℃) and an artificial dark-light cycle (lights on from 07:00 to 19:00 hours). Virgin female rats (three months old) were caged with male rats, and, after mating, each female was placed in an individual cage with free access to water and food until delivery.

Within 24 h of birth (day 0), any excess pups were removed, such that only six male pups were kept per dam. This procedure has been shown to maximize lactation performance.[25] During the 21 days of lactation, the rat dams were continued on an *ad libitum* diet of standard laboratory food (Agroceres®, São Paulo).

Male Wistar rats from six different litters were randomized and grouped on postnatal day 21 to receive either a control diet containing 7 ml of soybean oil and 54 g of cornstarch/100 g (7S group; n = 12) or a high-fat diet containing either 19 ml of soybean (19S group; n = 12) or canola oil (19C group; n = 12) along with 42 g of cornstarch/100 g. The 7S and high-fat diet groups received the same amounts of vitamins and minerals per gram of diet (Table 1). The diets were manufactured once a week and stored as pellets at 4℃ in agreement with American Institute of Nutrition (AIN-93G) recommendations.[26,27] The energy intake (kcal/day) and body density (body mass [g] divided by the length [cm, measured as the distance from tip of the nose to the tip of the tail])[28] were evaluated in all pups every three days. All groups had free access to diet and water during the course of experimental period.

At 59 days of age, the rats were deprived of food overnight, and, the next morning (at 60 days), the fasting rats were anesthetized with a lethal dose of pentobarbital. Blood was obtained by cardiac puncture. The abdominal fat mass, right femur and lumbar spine were excised. The blood samples were centrifuged to obtain the serum, which was stored at -20℃ for a posterior analysis of glucose, calcium, and phosphorus by the colorimetric method (Bioclin, Belo Horizonte, MG, Brazil). The serum insulin concentration was analyzed using an RIA kit in only one assay (Linco Research, Inc., St. Charles, MO, USA). To determine the insulin sensitivity of the animals, we used the insulin resistance index (IRI), defined as the fasting insulin (μUI/ml) × the fasting glucose (mmol/l).

Table 1 - The compositions of the experimental diets.

Ingredient (g/100 g)	7S	19S	19C
Casein	20	20	20
Corn starch	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-Cysteine	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

7S, the 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-cysteine; choline bitartrate: Agroquímica®; corn starch: Cargill®; fiber: Natural Pharma®; soybean and canola oil: Proquímios®; commercial sucrose: União®.
Formulated to meet the American Institute of Nutrition AIN-93G recommendations for rodent diets.[25]

The abdominal fat depots were dissected and weighed, and the values were expressed in grams (g). The samples of retroperitoneal fat were collected and fixed in buffered formaldehyde. The tissues were embedded in paraffin, cut into 5 μm sections and stained with hematoxylin-eosin. For the morphometric analyses, profiles with at least 100 adipocytes were randomly selected and captured for each animal. The cross-sectional area (μm^2) and number (per 100 μm^2) of adipocytes were determined from the digital images acquired (TIFF format, 36 bit color, 1360×1024 pixels) with an Optronics CCD video camera system and an Olympus BX40 microscope and analyzed with Image-Pro Plus version 5.0 software (Media Cybernetics, Silver Spring, MD, USA).[21] Two different observers independently evaluated the images and obtained similar results.

The right femur and lumbar vertebrae (LV1-LV6) were cleaned to remove any soft tissue. The distance between the epiphysis[29] and the LV1-LV6[5] and the medial-point diaphysis width (mm for both measurements) were measured using calipers (0.01-mm readability) and stored in saline solution at -20℃ until analysis. After a single scan by computed tomography (CT, Helicoidally model HISPEED, GE®), the images were obtained from 5-mm-thick axial slices. The radiodensity (expressed as Hounsfield units, HU) of the proximal epiphysis and the diaphysis of the femur and the mean cross-sectional areas of the lumbar vertebrae were measured using computerized analysis software (DicomWorks v1·3·5, 2002) by manual selection of the region of interest.[30] After the computed tomography analyses, the femur and LV1-LV6 were dried overnight at 95℃ and weighed (mg).[31]

Statistical analyses were performed using the GraphPad Prism statistical package (version 5.00, 2007, San Diego, CA, USA). The energy intake and body density were analyzed using two-way ANOVA followed by *post hoc* Bonferroni tests. The remaining results were analyzed using one-way ANOVA followed by *post hoc* Newman-Keuls tests. All of the results are expressed as means ± SEM with a significance level of 0.05.

RESULTS

After weaning, the 19S and 19C groups showed similar energy intake and body density growth, but these were significantly increased compared to the control group at 27 days of age (Figure 1).

The intra-abdominal fat mass did not differ between the 19S (12.0 ± 0.6 g) and 19C (11.4 ± 1.0 g) groups; however, it was significantly higher ($p<0.05$) compared to the 7S group (7.2 ± 0.7 g). The morphometric analyses of the adipocytes revealed larger cells in the 19S group (5048 ± 201.2 μm^2; $p<0.0001$) than in the 7S (2607 ± 186.3 μm^2) and 19C (2489 ± 322·7 μm^2) groups. However, the number of adipocytes in the 19S (20.17 ± 0.73) group was significantly less than in the 7S (36.33 ± 2.59) and 19C (36.18 ± 3.52) groups (Figure 2).

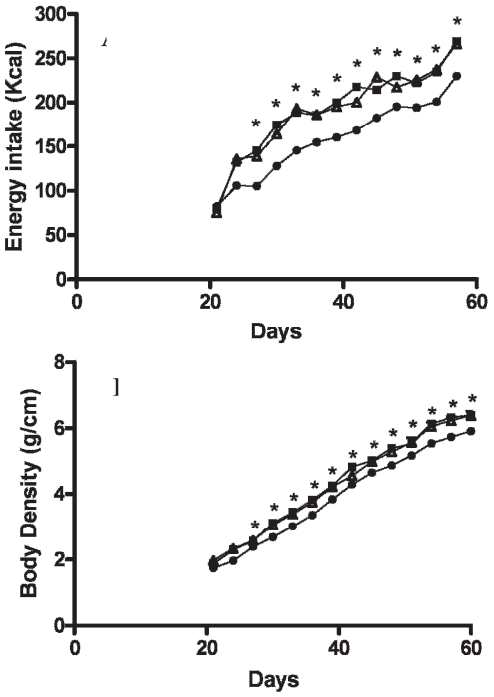

Figure 1 - Energy intake (A) and body density (B) post-weaning until 60 days-old. Control group, fed with diet containing 7ml of soybean oil /100g (●, 7S, n = 12) and experimental diets, containing 19ml of soybean (■, 19S, n = 12) or canola oil (△, 19C, n = 12) /100g. *$p<0.05$ (two-way ANOVA).

The serum analyses did not reveal any differences in the calcium and phosphorus concentrations. The 19C group showed significantly higher concentrations of glucose (+15%) and insulin (+56%) and an increased insulin resistance index (+78%) compared to the 7S group. The 19S group did not differ from 7S and 19C groups (Table 2).

Differences in the femur mass between the 19S and 19C groups were not found. However, they were higher (+25%; $p<0.05$) in these groups than in the C group. The femur measurements showed that the distance between the epiphyses was similar in all of the groups, and the width of the diaphysis was higher in the 19C group (+9%; $p<0.05$). The mass and the length of lumbar vertebrae did not differ between the 19S and 19C groups, but they were higher (+11% and +5%; $p<0.05$, respectively) in these groups than in the 7S group (Table 3).

Evaluating the femur using computed tomography (CT) showed that the radiodensity of the proximal epiphysis in the 19C group was significantly higher than in the 19S and control groups (+20% and +27%, respectively). The radiodensity of the diaphysis did not differ between the 19S and 19C groups, but these groups had significantly higher (+15%) diaphyseal radiodensities compared to the control group. The lumbar vertebrae analysis of the 19S and 19C groups using CT showed an increased radiodensity (+17% and +29%; $p<0.05$, respectively). Simultaneously, the 19C group was greater lumbar radiodensity (+9%; $p<0.05$) than the 19S group when comparing the high-fat groups (Table 4).

DISCUSSION

Dietary fat is calorie-dense and extremely palatable. It is easily overconsumed because it can cause less satiety than carbohydrates and protein,[17] causing "high-fat hyperphagia."[32] However, we did not verify hyperphagia when the rats were fed a high-fat diet containing soybean or canola oil after weaning.[33] Nevertheless, the increase in the diet lipids' caloric percentile from 17% to 39% contributed to increased body density growth and larger intra-abdominal fat depots in the 19S and 19C groups, independent of the vegetable oil type and absence of hyperphagia.

Hyperlipidic diets affect cell morphology, hormone sensitivity, and gene expression within the preexisting adipocytes in a complex manner. These lead to the recruitment of adipocyte precursor cells, initiating differentiation and producing the infrastructure required to sustain the new tissue.[34,35] In our experimental model, despite the similar gains in body density and intra-abdominal fat mass, the 19S group showed an increase in the size and a decrease in the number of retroperitoneal adipocytes compared to the 19C group. Other authors studying models of obesity induced by a high-fat diet intake have observed that the development of body fat compartments and the adipocyte fatty acid composition are affected by the type of fat contained in the diet.[36-39] When a high-fat diet containing omega-6 is consumed, peroxisome proliferator-activated receptor gamma (PPARγ) helps to convert unspecialized cells into adipocytes to store extra fat.[40] Interestingly, high-fat diets containing omega-3 (PUFA) limit post-intake fat storage and adipocyte hypertrophy.[41] In contrast, canola oil (when compared to soybean oil) is characterized by very low levels of omega-6 (21% *vs.* 54%, respectively) and high levels of omega-3 (11% *vs.* 8%,

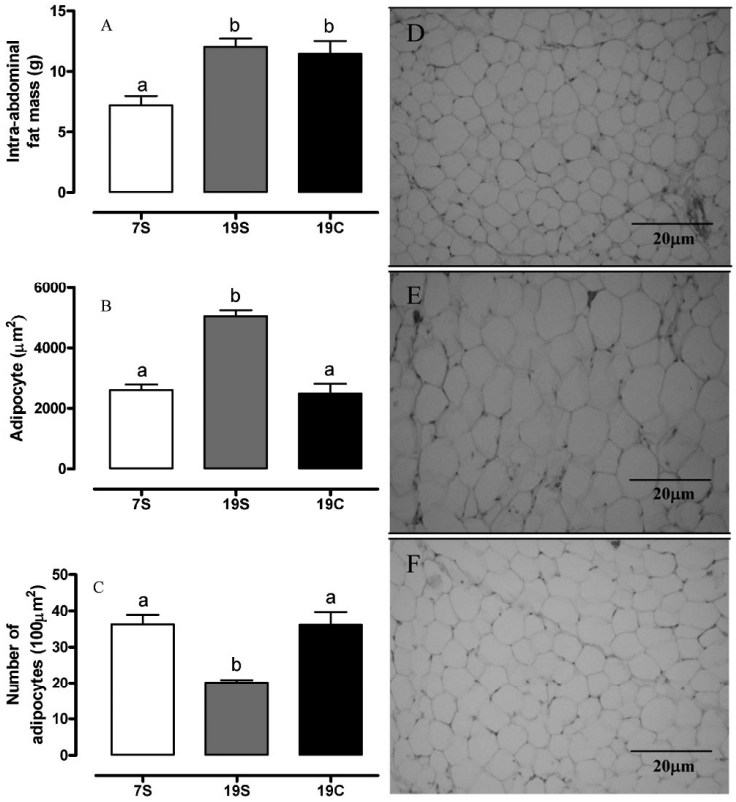

Figure 2 - (A) Intra-abdominal fat mass. (B) Adipocyte size and (C) number of retroperitoneal adipocytes. Groups fed with control diet (7S, n = 12) or with high fat diet containing soybean (19S, n = 12) or canola oil (19C, n = 12), at 60 days. [a,b]Values with different superscripts are significantly different (one-way ANOVA; $p<0.05$). Photomicrographs of the adipose tissue staining with Hematoxylin-Eosin (original magnification 200X): (D) 7S, (E) 19S and (F) 19C groups.

respectively).[23] Thus, these pathways help to explain the cell size distribution of the retroperitoneal adipocytes.

From the bone analysis, the increases in the body density and abdominal fat depots might be associated with the increases in the femur mass, the lumbar vertebrae mass and length, and the radiodensity of diaphysis and lumbar vertebrae in the groups fed a high-fat diet. Some human and experimental studies have revealed a positive relationship between body weight or body mass index (BMI) and bone mass. This is mediated by mass mechanical stress,

Table 2 - Serum analyses after 60 days.

	7S	19S	19C
Calcium, mg/dL	9.8±0.2	9.3±0.1	9.4±0.2
Phosphorus, mg/dL	9.5±0.4	10.4±0.4	8.8±0.3
Glucose, mg/dL	97.3±3.5[a]	104.7±2.8[a,b]	112.0±3.6[b]
Insulin, μUI/ml	37.6±4.3[a]	43.5±2.5[a,b]	58.4±7.3[b]
Insulin Resistance Index (IRI)	203.3±0.8 [a]	252.0±0.4[a,b]	363.0±1.4[b]

The post-weaning groups were fed a control diet (7S; n = 12) or a high-fat diet containing either soybean (19S; n = 12) or canola oil (19C; n = 12) until they were 60 days old.
[a,b]Mean values within a row with dissimilar superscript letters were significantly different (one-way ANOVA; $p<0.05$).

Table 3 - Femur and lumbar vertebrae (LV1-LV6) measurements after 60 days.

	7S	19S	19C
Femur:			
Mass, mg	355.1±22[a]	444.8±10.3[b]	444.2±9.4[b]
Distance between epiphysis, mm	31.5±0.6	31.9±0.3	31.9±0.3
Width of the diaphysis, mm	3.2±0.1[a]	3.25 ±0.1[a,b]	3.51±0.1[b]
Lumbar vertebrae:			
Mass, mg	613.8±24.8[a]	689.7±11[b]	681.9±17.4[b]
Maximum length, mm	37.7±0.4[a]	39.9±0.3[b]	39.5±0.4[b]

The post-weaning groups were fed a control diet (7S; n = 12) or a high-fat diet containing soybean (19S; n = 12) or canola oil (19C; n = 12) until they were 60 days old.
[a,b]The mean values within a row with dissimilar superscripts are significantly different (one-way ANOVA; $p<0.05$).

Table 4 - Computed tomography analyses of the femur and lumbar vertebrae (LV1-LV6) after 60 days.

	7S	19S	19C
Proximal epiphysis, Hu	546.0±37.5[a]	515.7±34.7[a]	658.1±24.5[b]
Diaphysis, Hu	492.6±21.2[a]	562.2±24.4[b]	579.4±22.1[b]
LV1-LV6, Hu	389.9±14.2[a]	459.0±7.1[b]	502.4±4[c]

The post-weaning groups were fed a control diet (7S; n=12) or a high-fat diet containing soybean (19S; n=12) or canola oil (19C, n=12) until they were 60 days old.
[a,b,c]The values within a row with dissimilar superscripts are significantly different (one-way ANOVA; $p<0.05$).

which is important for remodeling bone architecture[10,42-44] and providing stimuli for osteogenesis.[6] Our results agree with previous literature that reported positive effects of fat mass on bone density. Furthermore, adipose tissue might influence bone density by promoting bone-active hormone secretion from the pancreas (e.g., insulin).[11]

The serum analyses revealed high concentrations of insulin and glucose and, consequently, high insulin resistance in rats fed a high-fat diet containing canola oil. These results are surprising because omega-6 has been associated with the development of type-2 diabetes mellitus.[39] However, human and animal studies have revealed that excessive fat intake might induce metabolic disturbances independent of the diet lipid composition.[45-50] Thus, a high-fat diet containing 19% canola oil seems to promote insulin resistance. The fatty acid composition and the size and number of adipocytes are likely stronger contributors to insulin resistance than fat mass. Our data regarding fat cell morphology are based on retroperitoneal samples; therefore, further studies using mesenteric, epididymal and subcutaneous fat are required to elucidate the mechanisms that explain the association between canola oil intake and insulin sensitivity. Furthermore, increased bone density has been associated with hyperinsulinemia in non-diabetic models.[51,52] Insulin is a potent regulator of bone growth, acting directly on osteoblasts by stimulating their proliferation and, consequently, inducing bone formation.[11,53] Although this study has no data to confirm osteoblast activity, we hypothesized that the hyperinsulinemia observed in the 19C group may have had a positive effect on the bones, increasing their radiodensities.

Bone density increases more predominantly at the trabecular site (i.e., the vertebral body) than at the cortical site (i.e., the proximal epiphysis), and bone remodeling explains these regional differences.[53] When we evaluated each bone individually, we found that high canola oil intake promotes similar trends in the proximal epiphysis and in the lumbar vertebrae, increasing bone radiodensity. These analyses indicate that the use of computed tomography for bone analysis enables the differentiation between the 19S and 19C groups that is impossible using other bone measurements.

The results suggest that the amount and source of fat in the diet after weaning have differential effects on adiposity and bone. When the canola oil diet is normocaloric, a lower intra-abdominal adiposity and lower bone density result. [21] Thus, regardless of the source (soybean or canola oil), a high-fat diet ameliorates bone quality and induces adiposity. However, canola oil also causes hyperinsulinemia in this model. Thus, public policies to adopt various oils in the diet of the population must consider the deleterious effects of higher fat contents.

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4 **DISCUSSÃO**

Considerando que a sociedade moderna está em período de transição nutricional onde a desnutrição está dando lugar à obesidade, cada componente alimentar torna-se alvo de observação dadas as repercussões sobre a composição corporal. Nesse contexto, compreender a participação dos lipídios é fundamental, visto serem componentes básicos da alimentação e cujo consumo tem se tornado excessivo, perante as necessidades nutricionais. Dentre as fontes lipídicas utilizadas no Brasil se destaca o óleo de soja contrapondo-se a utilização do óleo de canola, por seu baixo custo.

Apesar do óleo de canola ser de alto consumo em países como Canadá e Austrália e ter suas propriedades físico-químicas e seus efeitos clínicos intensamente estudados (SIMOPOULOS, 1991; MCDONALD, 2005), para nosso conhecimento essa é a primeira vez que um grupo de pesquisa avalia o óleo de canola como a fonte lipídica na dieta em substituição ao óleo de soja, em ratos jovens.

Em relação à ingestão alimentar, massa e comprimento corporais, dos ratos alimentados com dieta normolipídica do desmame até a idade de 60 dias (Artigo 1), fica evidente que independe da fonte lipídica, o resultado não se altera ao final do período experimental. Por outro lado, quando para o mesmo período de vida são oferecidas dietas hiperlipídicas, contendo óleo de soja ou de canola (Artigo 2), a ingestão calórica é maior, independente de hiperfagia (COSTA *et al.*, 2009); apesar deste tipo de dieta ser mais palatável e estar associada a menor saciedade (GHIBAUDI *et al*., 2002).

A substituição do óleo de soja pelo óleo de canola e o aumento da quantidade deste óleo na dieta proporcionou alterações na composição corporal, na estrutura e distribuição dos adipócitos intra-abdominais e na integridade óssea. O conjunto dos resultados demonstra que a composição de ácidos graxos de cada dieta é decisiva para definir as alterações da composição corporal.

A natureza e a quantidade de ácidos graxos são fundamentais para o desenvolvimento e a morfologia do tecido adiposo abdominal (LOSSA *et al.*, 2001; JANG *et al.*, 2003). A dieta normolipídica, contendo óleo de canola, contribuiu para o menor acúmulo de gordura intra-abdominal e área dos adipócitos. Enquanto, os animais tratados com a dieta hiperlipídica apesar de apresentarem similaridade quanto a massa de gordura e densidade corporal, não mantiveram o mesmo padrão para a morfologia dos adipócitos da região retroperitoneal. Animais alimentados com dieta hiperlipídica contendo óleo de soja têm maior área de adipócitos, que os alimentados com dieta hiperlipídica contendo canola. Em relatos anteriores,

nosso grupo (COSTA *et al.*, 2007) e outros (SHILLABEER & LAU, 1994; DULLO *et al.*, 1995; HEREDIA *et al.*, 2008) observaram que o desenvolvimento dos adipócitos no compartimento intra-abdominal é afetado pelo tipo de lipídio presente na dieta. Com a elevada ingestão de ômega-6 ocorre o recrutamento de pré-adipócitos, com o intuito de estocar a gordura extra, através da ação de PPAR-γ. Logo, a redução da ingestão deste ácido graxo determina a diminuição da formação e da hipertrofia dos adipócitos (MASSIERA *et al.*, 2003). Com o aumento da ingestão de fontes ricas em ômega-3, ocorre através de PPAR-α, maior estímulo à expressão de genes relacionados à oxidação dos ácidos graxos e à supressão de genes lipogênicos, utilizando para isso fatores de transcrição como SREBP (*sterol regulatory element-binding protein*), com consequente limitação ao acúmulo de gordura ingerida e à hipertrofia dos adipócitos (HSU & HUANG, 2006; RACLOT & GROSCOLAS, 1994). O óleo de canola quando comparado ao óleo de soja é caracterizado pela baixa concentração de ômega-6 (21% *vs.* 54%) e, maior concentração de ômega-3 (11% *vs.* 8%). Estas diferenças nas relações entre os ácidos graxos nos óleos vegetais ajudam a explicar a quantidade de tecido adiposo e a área dos adipócitos, nos grupos estudados.

Apesar da falta de dados sobre a massa de tecido adiposo subcutâneo, através da utilização do DXA, foi possível obter uma perspectiva quanto a esse compartimento corporal. No modelo de alimentação com dieta normolipídica, a massa de gordura da região do tronco, nos grupos S e C, são similares. Associado à menor massa de gordura intra-abdominal é provável que a ingestão de dieta a base de óleo de canola tenha um aumento compensatório da massa de gordura subcutânea. A análise dos dados resultantes do DXA na região do tronco ajuda a explicar a semelhança encontrada entre os grupos, quanto às concentrações séricas de leptina, corroborando com estudos anteriores, clínicos e experimentais, nos quais este hormônio está positivamente correlacionado com a adiposidade corporal (JANG *et al.*, 2003; HAVEL, 2000).

A remodelação e a massa óssea são influenciadas pelo estresse mecânico, através das cargas impostas pela musculatura e pelo tecido adiposo, o que estimula a osteogênese (IWANIEC *et al.*, 2009; TAES *et al.*, 2009; HSU *et al.*, 2006). Nos animais alimentados com a dieta normolipídica contendo óleo de canola, a menor adiposidade intra-abdominal implicou em menor ação mecânica e redução dos parâmetros ósseos. De forma contrária, nos animais alimentados com dieta hiperlipídica ocorreu maior adiposidade abdominal e aumento da massa do fêmur, da massa e do comprimento da coluna lombar e da radiodensidade da diáfise do fêmur e da coluna lombar. Estes resultados corroboram estudos clínicos e experimentais que reportam os efeitos positivos da ação direta do tecido adiposo sobre a estrutura óssea

(GILSANZ *et al.*, 2008; IWANIEC *et al.*, 2009, HO-PHAM *et al.*, 2010; PAULA & ROSEN, 2010).

Além da ação direta, o tecido adiposo pode afetar a densidade óssea, estimulando a secreção de hormônios que atuem no sistema esquelético, como a insulina (HSU *et al.*, 2006).

Os osteoblastos são células alvo de insulina e, quando ligado ao seu receptor, o hormônio estimula esta proliferação celular (ABRAHAMSEN *et al.*, 2000). Assim, a deficiência insulínica pode ser associada à redução da formação, da massa e da densidade mineral óssea (REID, 2008). Animais alimentados com a dieta normolipídica, contendo óleo de canola, apresentaram menores concentrações séricas de insulina, sugerindo ser este um dos fatores determinantes da redução da massa e da densidade mineral ósseas de fêmures e vértebras. Já a dieta hiperlipídica contendo óleo de canola, elevou as concentrações séricas de insulina, glicose, causando resistência insulínica. O diabetes mellitus tipo 2 têm sido associado a elevada ingestão de alimentos ricos em ômega-6 (HEREDIA *et al.*, 2008). No entanto, estudos evidenciaram que a excessiva ingestão lipídica pode levar a distúrbios metabólicos, independente da composição de ácidos graxos presentes na dieta de animais ou humanos (BAKER & GIBBONS, 2000; GRAVENA *et al.*, 2002; LOVEJOY *et al.*, 2002; WINZELL *et al.*, 2006). Pode-se ainda, associar o aumento da densidade óssea na região da epífise proximal e na coluna lombar, no grupo 19C, com a hiperinsulinemia.

O aumento da massa de gordura intra-abdominal é associado com resistência insulínica e dislipidemia (VÁZQUES-VELA *et al.*, 2008; WREE *et al.*, 2011). A alimentação com dieta normolipídica, contendo óleo de canola, determinou menores concentrações séricas de colesterol. Anteriormente, no entanto verificamos que a dieta hiperlipídica induziu alterações lipídicas levando a diminuição de triglicerídeos e aumento do HDL-colesterol no soro (COSTA *et al.*, 2009). Nosso conjunto de resultados corrobora estudos anteriores nos quais ficou evidenciado que uma dieta enriquecida com ômega-3 é benéfica para redução sérica de colesterol, triglicerídeos e para a manutenção do HDL-colesterol (MAZIÈRE *et al.*, 1998; VOGEL *et al.*, 2000; LIEN *et al.*, 2001; MORI *et al.*, 2003).

O óleo de canola, além de ser uma importante fonte de ômega-3, apresenta elevada concentração de ômega-9, quando comparado ao óleo de soja (61% *vs.* 23%, respectivamente) (MCDONALD, 2005). Segundo o estudo de SMITH *et al*. (2003), o ômega-9 tem um importante papel na redução da concentração sérica de colesterol e manutenção de HDL-colesterol e triglicerídeo. Assim, sugerimos que a composição de ácidos graxos de uma dieta contendo óleo de canola contribui para um perfil lipídico saudável.

O uso do DXA como meio de avaliação da composição corporal é considerado "padrão ouro" em estudos clínicos, e tem sido usado com sucesso para análise da estrutura óssea em modelos animais (LUKASKI *et al.*, 2001; GLICKMAN *et al.*, 2004; TSUJIO *et al.*, 2009). Assim, no artigo 1, o uso dessa técnica, permitiu verificar que a dieta normolipídica, contendo óleo de canola levou a menor área e conteúdo mineral ósseo total e na região da coluna vertebral. Após a análise de corpo inteiro, foi necessário a coleta das peças ósseas, fêmur e coluna lombar, para posterior análise, tendo em vista as diferenças regionais quanto à proporção de osso trabecular e cortical (SIROIS *et al.*, 2003; GREEN *et al.*, 2004; CAO *et al.*, 2009; RAHMAN *et al.*, 2009).

Apesar da ingestão de dietas normo (grupos S *vs*. C) ou hiperlipídicas (19S *vs*. 19C) não diferenciarem o desenvolvimento corporal, ao final do período experimental, ambas exerceram distinta influência sobre as medidas das peças ósseas, quando estas foram avaliadas individualmente. Ou seja, os animais do grupo C, apresentaram menores massas de fêmur e da quarta vértebra lombar além da menor espessura do ponto médio da diáfise. No entanto, quando os animais foram alimentados com dietas hiperlipídicas (19S e 19C), nenhuma alteração nesses parâmetros ósseos foi constatada.

Complementando o estudo das peças ósseas, foram realizadas análise radiológicas. Por meio da tomografia computadorizada (TC), foi evidenciado o aumento da radiodensidade na região da cabeça do fêmur e da coluna lombar nos animais do grupo 19C. Já no modelo de alimentação com dieta normolipídica foi constatado a partir dos resultados do DXA que os animais do grupo canola tinham menor DMO de fêmur e coluna lombar. No entanto, através da TC, enquanto a região da cabeça do fêmur apresentou menor radiodensidade, no corpo vertebral na quarta vértebra lombar, nenhuma diferença foi detectada.

Sabendo-se que a região proximal do fêmur é composta predominantemente de osso cortical, enquanto o corpo vertebral é composto de osso trabecular, e por isso, a taxa de remodelação óssea nessas regiões difere (REID, 2008), uso da TC permitiu uma avaliação mais sensível dos compartimentos de uma mesma peça óssea. Essa premissa fica fundamentada quando comparamos a TC aos demais métodos empregados no estudo da vértebra lombar. Em adição, os dados obtidos na TC corroboram a definição de que ossos trabeculares, como a vértebra lombar, apresentam maior taxa de remodelação óssea, quando comparados a ossos corticais, como o fêmur (SEEMAN, 2007).

5 CONCLUSÂO

Classificar um alimento como benéfico ou prejudicial à saúde requer cautela. Dependendo do compartimento corporal de interesse, a ingestão de um nutriente pode gerar desfechos positivos ou negativos em longo prazo. Na dieta normolipídica, contendo óleo de canola, ocorreu menor massa de tecido adiposo intra-abdominal, no entanto, com prejuízo aos parâmetros ósseos. De maneira contrária, na dieta hiperlipídica, o óleo de canola causou a melhora da qualidade óssea, porém, na presença de maior adiposidade e resistência insulínica. Portanto, além do tipo e quantidade do óleo vegetal, as práticas dietoterápicas necessitam considerar o compartimento corporal de interesse, antes do manejo nutricional.

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