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High-refined carbohydrate diet promotes detrimental effects on alveolar bone and femur microarchitecture

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ABSTRACT

The impact of high-refined carbohydrate (HC) diet on fat accumulation, adipokines secretion and systemic inflammation is well described. However, it remains unclear whether these processes affect bone remodeling.
Objective: To investigate the effects of HC diet in the alveolar bone and femur parameters.
Methods: BalbC mice were fed with conventional chow or HC diet for 12 weeks. After experimental time maxillae, femur, blood and white adipose tissue samples were collected.
Results: The animals feed with HC diet exhibited considerable increase of adiposity index and adipose tissue levels of TNF-α, IL-6, IL-10, IL-1β, TGF-β and leptin. Microtomography analysis of maxillary bone revealed horizontal alveolar bone loss and disruption of trabecular bone in mice feed with HC diet. These deleterious effects were correlated with a disturbance in bone cells and an augmented expression of *Rankl/Opg* ratio. Consistently, similar effects were observed in femurs, which also exhibited a reduction in bone maximum load and stiffness.
Conclusion: Our data indicates that HC diet consumption disrupts bone remodeling process, favoring bone loss. Underlying mechanisms relies on fat tissue accumulation and also in systemic and local inflammation.

1. Introduction

Obesity is a condition characterized by excess body fat accumulation as a result of a chronic imbalance between energy expenditure and energy intake (Rosen & Spiegelman, 2014; Ahlma & Filler, 2004). The overconsumption of a high- refined carbohydrate diet (HC), causes adipose tissue expansion, which increases glucose utilization, lipoprotein lipase activity and lipogenesis rate (Ferreira, Menezes-Garcia, Viana, Mário, & Botion, 2014; Dneton & Martin, 1970; Shafrir, Gutman, Gorin, & Orevi, 1970). Excessive body fat storage is associated with several health complications such as insulin resistance, type 2 diabetes, cardiovascular disease, metabolic syndrome and bone remodeling-related disorders (Houssain, Kawar, & Nahas, 2007; Marchetti et al., 2012; Zhao et al., 2007).

Previous studies have linked adipose tissue accumulation with altered secretion of the certain adipokines, such as leptin and adiponectin. These adipokines play an important role in a series of systemic events, including bone remodeling process (Marcus et al., 1994; Rosen & Spiegelman, 2014; Schaffler, Choi, & Milgrom, 1995). *In vitro* studies have shown that adipocytes, driven from bone morrow, directly regulate the function and formation of osteoblasts and stimulate osteoclastogenesis (Cooke, Heine, Taylor, & Lubahn, 2001; Jankowska, Rogucka, & Medras, 2011; Reid, 1996; Wade, Gray, & Bartness, 1985). Receptors for both, leptin and adiponectin are expressed in osteoblasts (Baldock et al., 2002; De Laet et al., 2005), osteoclasts precursors (Kontogianni, Dafni, Routsias, & Skopouli, 2004) and osteoclasts (Goulding, Jones, Taylor, Williams, & Manning, 2001) indicating that these adipokines can directly influence bone cells formation and differentiation. However, the influence of HC diet consumption on bone remodeling process, especially in alveolar bone, remains unclear. Herein, we investigated the morphological and molecular effects of HC diet on maxillary alveolar bone and femur.

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2. Materials and methods

2.1. Experimental animals and diet

6–8 weeks male BALB/C mice were obtained from animal care center of Universidade Federal de Minas Gerais (CEBIO-UFMG) and treated under Institutional Ethics Committee regulations (257/2014). Animals (n = 10 per group) were fed with standard laboratory chow (Labina) or experimental diet for 12 weeks (Oliveira et al., 2013). Chow diet composition was 65.8% of carbohydrate, 3.1% fat and 31.1% protein (4.0 Kcal/g) (Oliveira et al., 2013). HC Diet was composed of 72.2% of carbohydrate, 5.8% of fat and 20% of protein (4.4 Kcal/g) (Oliveira et al., 2013). HC diet contains at least 30% of refined sugars, mostly sucrose. Mice were weighted once a week and the food intake was recorded twice a week. At the end of dietary treatment, animals were euthanized and samples of maxillae, femur, blood, liver, epididymal (EAT), retroperitoneal (RAT) and mesenteric (MAT) white adipose tissues were collected and weighed. Then, the adiposity index was calculated using the following formula: [(EAT + RAT + MAT)/body weight in grams] ×100 (Oliveira et al., 2013).

2.2. Determination of serum parameters

Fasting triglycerides, cholesterol and glucose levels were analyzed using enzymatic kits (KATAL Belo Horizonte, Brazil). Adiponectin, resistin, leptin and chemerin serum levels were determined by ELISA, using commercially available kits (all R&D systems Europe, Abington, UK).

2.3. ELISA

Quantification of IL-6, TNF-α, IL-10, IL-1β and TGF-β was performed in EAT samples using DouSet ELISA kits according to manufactures instructions. (R&D System, Inc., Mineapolis, USA).

2.4. Micro CT analysis

Maxillary alveolar bone and femurs were fixed in 10% neutral buffered formalin for 48 h and scanned using a microCT system (Skyscan 1172 X-Ray microtomograph; Skyscan, Aartselaar, Belgium). The calibration was carried out with known density calcium hydroxyapatite phantoms (Skyscan). High-resolutions scans with an isotropic voxel size of 18 μm were acquired (50 kv, 0.5 mm aluminum filter, 0.5° rotation angle). Contouring methods were used to delineate the region of interest to be analyzed as described (Macari et al., 2015). Trabecular morphometry was measured within the furcation area of the first molar root or in the metaphyseal region of proximal femurs. The tissue was analyzed to determine bone mineral density (BMD g/cm^{-3}), bone volume (BV mm^3), percent bone volume/tissue volume (BV/TV%), trabecular thickness (Tb Thmm), trabecular number (Tb nmm^{-1}), trabecular separation (Tb spmm) and trabecular bone pattern factor (Tb $Pfmm^{-1}$). Alveolar bone crest (ABC) loss was measured by determining the area between the cemento-enamel junction (CEJ) and the ABC (CEJ-ACB) in three dimension images (Fiji – National Institute of Health, USA) of the first, second and third molars (Macari et al., 2015).

2.5. Histopathological analysis

The maxillae were decalcified in 14% ethylenediaminetetraacetic acid (pH7.4) for 21 days and embedded in paraffin. Samples were cut into sagittal sections of 5 μm thickness. For osteoblasts identification sections were stained with Masson's trichrome in order to count cells on the distal side of distal-buccal root of the first molar. Osteoclasts were identified as tartrate-resistant acid phosphatase (TRAP)–positive multinucleated cells, stained for TRAP (Sigma-Aldrich, St. Louis, MO, USA) and counted on the mesial side of the first molar distal-buccal root.

2.6. mRNA extraction and qPCR analysis

For qPCR analysis, the surrounding alveolar bone was taken from the upper first molars. Total RNA was extracted using Trizol reagent followed by column purification (RNeasy Mini Kit; Qiagen, Valencia, CA, USA). The integrity of RNA samples was checked by analyzing 1 μg total RNA on a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions. Complementary DNA was synthe-sized from 2 μL RNA using Quanti TectRT kit (Qiagen). The targets analyzed were: *Tnf-α* (Tnf), receptor activator of nuclear factor κB (*Rank,* Tfnrsf11a), *Rankl* (Tnfsf11) and *Opg* (Tnfrsf11b).

2.7. Mechanical test

Mechanical properties as maximum load (Lmax) and stiffness (St) were determined by testing right femurs to fracture in a universal testing machine (EMICs, DL 10000, Ribeirão Preto, Brazil) equipped with a load cell of 500 N, and TESC software, version 13.4 (EMIC). Bones were tested by the three-point bending flexural test, with force applied at a speed of 1.0 mm/min in the anterior-posterior direction. The gap between the two points was 8 mm and a 2 N preload was used for 30 s (Yanagihara et al., 2016).

2.8. Statistical analyses

Data are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed using Student's *t*-test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of HC diet on adiposity and serum biochemistry parameters

Weakly food intake and body weight gain were similar in control and HC diet animals (Fig. 1A, B) but final body weight was significantly augmented in HC diet group (Fig. 1C). The HC group exhibited considerable increase in epididymal, mesenteric, retroperitoneal and inguinal adipose tissue mass, as demonstrated by a higher adiposity index (Fig. 1D). The serum levels of glucose, total cholesterol and triglycerides were significantly elevated in animals fed with HC diet (Fig. 1E–G).

3.2. Inflammatory markers and adipokines changes in serum and adipose tissue

The HC group showed a significant increase of leptin (Fig. 2A) and a decrease of adiponectin in the serum (Fig. 2C). The serum concentrations of resistin and chemerin were similar in both groups (Fig. 2B, D).

In order to evaluate the influence of HC on local production of cytokines, concentration of TNF-α, IL-1β, IL-10,TGF-β and IL-6 in epididymal adipose tissue was analyzed (Fig. 2E–I). Concentration of all cytokines evaluated were significantly increased in mice feed with HC diet (Fig. 2E–I).

3.3. HC diet induced detrimental effects on maxillary alveolar bone

HC consumption caused osteopenic effects on maxillae, as demonstrated by a decrease in multiple bone parameters: BMD, BV/TV, BV, Tb.Th, Tb.pf and Tb.n. Significant differences in relation to controls were observed in BMD, Tb.Th and Tb.pf (Fig. 3A–I). No significant changes were observed in Tb.sp between groups (Fig. 3H). Moreover, CEJ-ABC area was significantly increased in HC animals (Fig. 3J–L). These results indicates that HC deteriorates alveolar bone microarchitecture and induces significant horizontal loss of alveolar bone crest.