High-fat diet disrupts bone remodeling by inducing local and systemic alterations

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Abstract

A high-fat (HF) diet leads to detrimental effects on alveolar bone (AB); however, the mechanisms linking adiposity to bone loss are poorly understood. This study investigated if AB resorption induced by an HF diet is associated with the regulation of inflammatory gene expression and if adipocytes can directly interfere with osteoclastogenesis. We also evaluated the effects of diet restriction (DR) on bone phenotype. C57BL6/J mice were fed normal chow or an HF diet for 12 weeks. Samples of maxillae, femur, blood and white adipose tissue were analyzed.

In vitro co-culture of bone marrow-derived osteoclasts and mature adipocytes was carried out. The results revealed an increased number of osteoclasts and fewer osteoblasts in animals fed the HF diet, which led to the disruption of trabecular bone and horizontal AB loss. Similar effects were observed in the femur. The metabolic parameters and the deleterious effects of the HF diet on AB and the femur were reversed after DR. The HF diet modulated the expression of 30 inflammatory genes in AB such as Fam3c, InhBa, Tnfs11, Ackr2, Pxmp2 and Chl3, which are related to the inflammatory response and bone remodeling. In vitro, mature adipocytes produced increased levels of adipokines, and co-culture with osteoclasts resulted in augmented osteoclastogenesis. The results indicate that the mechanisms by which an HF diet affects bone involve induction of osteoclastogenesis and inflammatory gene expression. Adipokines apparently are key molecules in this process. Strategies to control diet-induced bone loss might be beneficial in patients with preexisting bone inflammatory conditions.

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1. Introduction

Adipose tissue expansion generated by a high-fat (HF) diet is known to cause several systemic disorders such as cardiovascular disease, type 2 diabetes, insulin resistance and metabolic syndrome [1–3]. However, the effects of an HF diet and obesity in bone remodeling are less defined [4–6]. While some results suggested that mechanical loading caused by excess weight has a positive impact on bone mass maintenance [7], recent studies showed that body fat accumulation increases bone resorption [5,6].

Bone remodeling disorders caused by HF consumption have been studied in different experimental models of steady-state conditions [8], periodontal disease [9], ovariectomy [10] and bone healing [11]. The majority of the reports investigated the effects of HF on femurs, tibiae and vertebrae [12–14], while few studies evaluated the impact of HF consumption on alveolar bone (AB) [8,15,16].

AB preservation is essential for tooth support. AB loss can be induced or aggravated by systemic inflammatory conditions as demonstrated by experimental [17,18] and clinical studies [19,20]. Several potential mechanisms have linked adipose tissue accumulation and AB deterioration, including (1) increased gingival oxidative stress [21], (2) adipocyte differentiation in detrimental osteoblast formation, (3) local dysbiosis favoring periodontopathogens [22], (4) local and/or systemic increase of receptor activation of NF-kappaB ligand (RankL) [23], (5) release of proinflammatory cytokines and adipokines [24], and (6) the direct influence of bone marrow-driven adipocytes on osteoclast formation via RankL cell-to-cell contact [25]. However, the pathways linking obesity/adipose tissue expansion with AB alteration have not yet been defined [26]. Furthermore, there is little evidence that osteoclast differentiation and activity can be influenced by mature adipocytes. The aims of this study were to investigate the effects of HF diet and diet restriction on AB remodeling and to explore the mechanisms by which

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adiposity negatively impacts bone quality. Herein, we hypothesized that HF intake causes AB and femur deterioration by activating local and systemic pathways that favor bone loss.

2. Materials and methods

2.1. Experimental animals and diet

C57BL6/J mice, at 6–8 weeks of age, were obtained from the animal care center of the Universidade Federal de Minas Gerais (CEBIO-UFMG) and were treated following Institutional Ethics Committee regulations (257/2014). Animals were placed on either a regular diet (n = 10) consisting of 60% carbohydrate, 11% fat and 23% protein (Labina) or an HF diet (n = 10) consisting of 37.06% carbohydrate, 41.1% fat and 21.83% protein. Mice were weighed once each week and food intake was measured twice each week. After 12 weeks, animals were euthanized and samples of maxilla, femur, blood, liver, epididymal tissue (EAT), retroperitoneal tissue (RAT), inguinal adipose tissue (IAT) and mesenteric white adipose tissue (MAT) were collected. Visceral adipose tissue samples (EAT, RAT and MAT) were weighed. The adiposity index was calculated using the following formula: [(EAT+RAT+MAT)/(body weight in grams)] × 100 [27].

2.2. Diet restriction

Animals were fed an HF diet for 8 weeks as described above. For caloric restriction, daily food intake was calculated. During caloric restriction, each mouse from the HF diet group received 70% of its ad libitum consumption (70% of average food intake/average weight) for 7 (n = 5) or 42 days (n = 5). Mice were weighed once each week and food intake was measured daily. At the end of the experiment, samples of adipose tissue, serum, maxilla and femur were collected.

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

2.4. Micro-CT analysis

Maxillary AB and femurs were fixed in 10% neutral-buffered formalin for 48 h and scanned using a micro-CT system (Skyscan 1172 X-ray microtomography; Skyscan, Aartselaar, Belgium). Calibration was carried out with known-density calcium hydroxyapatite phantom (Skyscan). High-resolution scans with an isotropic voxel size of 18 μm were acquired (50 kV, 0.5 mm aluminum filter, 0.5° rotation angle). Scan time was approximately 30 min per bone. The scans were reconstructed using NRecon software (Skyscan, Belgium), Contouring methods were used to delineate the region of interest to be analyzed [17]. Trabecular morphometry was measured within the fusion area of the first molar root or in the metaphyseal region of proximal femurs. The contouring method on femurs was used to delineate the trabecular bone region with an irregular, anatomic region of interest drawn manually a few voxels away from the endocortical surface. The base of the growth plate was used as a standard reference point. The region of interest was defined just after the growth plate with a further 40 slices below in the direction of the diaphysis. The tissue was analyzed to determine bone mineral density (BMD; g/cm²), bone volume (BV; mm³), percent BV/tissue volume (BV/TV; %), trabecular thickness (Tb.Th; mm), trabecular number (Tb.n; mm⁻¹) and trabecular separation (Tb.Sp; mm). Alveolar bone crest (ABC) loss was measured by determining the area between the cemento-enamel junction (CEJ) and the ABC (CEJ–ABC) in three-dimensional images (Fiji—National Institutes of Health, Bethesda, MD, USA) of the first, second and third molars [28].

2.5. Histopathological analysis

Maxillae were decalcified in 14% ethylenediaminetetraacetic acid (pH 7.4) for 21 days and embedded in paraffin. The sections were cut into sagittal sections (5 μm thick). The sections were stained with Mason’s trichrome, and the osteoblasts were counted on the distal side of the first molar distal-buccal root. Osteoclasts were identified as tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells and counted on the mesial side of the first molar distal-buccal root by immunohistochemistry (Santa Cruz Biotechnology Inc., Dallas, TX, USA).

2.6. Mechanical analysis

Mechanical properties of maximum load (Lmax) and stiffness (Sf) were determined by testing right femoral fractures in a universal testing machine (EMICs, DL 10000, Brazil) equipped with a load cell of 500 N using TESC software, version 13.4 (EMIC). The 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 [29].

2.7. RNA extraction

Total RNA was isolated from 16 samples of periodontal ligaments and surrounding AB from control (N = 8) and HF diet animals (N = 8) using Trizol followed by column purification (RNasey Mini Kit; Qiagen Inc., Valencia, CA, USA). RNA was treated for complete digestion of DNA using the TURBO DNA-free Kit (Invitrogen Life Technologies, Carlsbad, CA, USA). Subsequently, 2 μg of RNA from each sample was used for the reverse transcription reaction using SuperScript VILO cDNA Synthesis Master Mix (Invitrogen Life Technologies).

2.8. Polymerase chain reaction array

The TaqMan OpenArray Mouse Inflammation panel (Applied Biosystems, Foster City, CA, USA) was used to evaluate the expression of 632 genes associated with inflammatory diseases and 16 endogenous control genes. Real-time polymerase chain reaction (PCR) reactions were conducted on the QuantStudio 12K Flex Real-Time PCR System (Applied Biosystems). cDNA was added to the TaqMan OpenArray Real-Time PCR Master Mix (Applied Biosystems) and distributed into 384-well plates. The samples with the master mix from the 384-well sample plates were loaded onto the OpenArray Plate using the OpenArray AccuFill System (Applied Biosystems). PCR was run on the QuantStudio 12K Flex Real-Time PCR System (Applied Biosystems). We conducted the analysis with the Applied Biosystems analysis software, v. 1.0, using global normalization to determine the amount of mRNA in each total RNA sample. We considered differentially expressed genes to be significantly different when P < 0.05, adopting the correction of Benjamini and Hochberg for false-positive findings [30].

2.9. Adipocyte and osteoclast co-culture

Bone marrow cells (BMCs) were obtained from the femur and tibia. For osteoclast differentiation, BMCs were incubated in minimum essential medium alpha (with no nucleosides; Thermo Fisher Scientific, Wallingford, MA, USA) supplemented with 10% heat-inactivated fetal bovine serum (GIBCO, Carlsbad, CA, USA) and soluble macrophage colony-stimulating factor (30 ng/ml; R&D Systems, Minneapolis, MN, USA) for 3 days. RANKL (10 ng/ml; R&D Systems) was then added, as well as mature adipocytes (0.2–10³), which were isolated from epididymal fat pads, as previously described [31]. Briefly, digestion with collagenase (1 mg/ml) was carried out at 37°C with constant shaking (140 cycles/min) for 40 min. Cells were filtered through nylon mesh and washed three times with buffer plus 1× bovine fatty-acid-free serum albumin. A concentration of 4.4×10⁶ adipocytes was obtained. Adipocytes were incubated with osteoclasts (2×10³ cells/well) for 20 h at 37°C. At the end of the incubation period, an aliquot of the infranatant was removed for adipokine and cytokine quantification. The medium was replaced and osteoclast cell culture continued for 3 days without adipocytes (Fig. 6A). Cells were then fixed with acetic, citrate and formaldehyde 3% and stained with TRAP (Sigma-Aldrich) after 7 days of total cell culture. TRAP-positive cells were counted and their areas were measured (Fiji—National Institutes of Health). To analyze the possible modifications of osteoclast function in the presence of adipocytes, the cells were also seeded in an osteoassembly surface 96-well plate (Sigma–Aldrich) for a resorption pit assay. The same protocol described above was carried out. To analyze the surface of the pit formation, the media was aspirated from wells after 10 days of osteoclast differentiation, and a concentration of 4.4×10³ adipocytes was obtained. Adipocytes were incubated with osteoclasts (2×10³ cells/well) for 20 h at 37°C. At the end of the incubation period, an aliquot of the infranatant was removed for adipokine and cytokine quantification. The medium was replaced and osteoclast cell culture continued for 3 days without adipocytes (Fig. 6A). Cells were then fixed with acetic, citrate and formaldehyde 3% and stained with TRAP (Sigma-Aldrich) after 7 days of total cell culture. TRAP-positive cells were counted and their areas were measured (Fiji—National Institutes of Health).

2.10. Statistical analyses

Data are presented as the mean±standard error of the mean. Statistical analyses were performed using Student’s t test or one-way analysis of variance followed by Newman–Keuls posttest for multiple comparisons. P < 0.05 was considered statistically significant.

3. Results

3.1. HF diet affects metabolic parameters and adipokine release

To explore the mechanisms by which an HF diet could affect bone metabolism, we fed male C57BL6/J mice with regular chow or HF diet. As reported previously [27], mice fed with an HF diet gained significantly more body weight than control mice after 12 weeks (Fig. 1A). HF animals also presented increased food intake (kcal/d) compared with control group (Fig. 1B). Animals receiving the HF diet exhibited a considerable increase in the adiposity index and cholesterol levels (Fig. 1C, E). Glucose and triglyceride levels were similar for both groups (Fig. 1D, F). Serum levels of leptin increased significantly (Fig. 1I), whereas adiponectin and resistin levels were reduced in the HF diet group (Fig. 1C, H). No significant difference was observed in serum levels of chemerin between the groups (Fig. 1J).
3.2. HF diet induces detrimental effects in maxillary AB

Disruption of maxillary bone architecture, as demonstrated by the reduction of multiple bone parameters including BMD, BV/TV, Tb.Th, Tb.n and Tb.Sp (Fig. 2A–K), was observed in the animals fed the HF diet. These results showed that the HF diet reduced BMD, Tb.Th and Tb.n and consequently increased Tb.sp (Fig. 2A–H). Moreover, the CEJ/ABC distance was significantly increased in the HF diet mice when compared to controls (Fig. 2I–K), showing that the HF diet spontaneously induced horizontal AB loss (Fig. 2I–K).

3.3. HF diet reduces the number of osteoblasts and increases osteoclastogenesis in maxillary AB

In parallel with the AB loss observed in the micro-CT, we noticed in microscope analyses a reduction in osteoblasts (Fig. 2L–N) and an increase in osteoclasts in the animals fed the HF diet. These cell populations (Fig. 2O–Q) were identified at the distal (osteoblasts) and mesial (osteoclasts) sites of the AB that surrounds the distal–buccal root of the first molar.

3.4. HF diet promotes deterioration of femoral microarchitecture and impairs mechanical properties

Consistent with the phenotype observed in AB, the animals on the HF diet displayed a marked decrease of BMD and greater space between bone trabeculae (Fig. 3A–H). No differences were detected in BV, BV/TV, Tb.n and Tb.Th when comparing both groups (Fig. 3A–H). In agreement with these findings, a significant reduction of femoral stiffness and maximum load mechanical properties were seen in the HF diet group compared with controls (Fig. 3I, J).

3.5. Diet restriction partially reverses deleterious effects of HF diet on bone

Seven days of diet restriction (7d DR) resulted in a significant reduction of body weight, adiposity index and resistin levels (Fig. 4A, B, D). No significant difference was observed in adiponectin production (Fig. 4C). Regarding AB quality, diet restriction led to
Fig. 2. Alveolar bone changes induced by HF diet. (A–H) Alveolar bone parameters: representative images of the analyzed area in control and HF diet groups (A, B), bone mineral density (C), bone volume (D), bone volume per tissue volume (E), trabecular thickness (F), trabecular number (G), trabecular separation (H), alveolar bone loss (I–K), and bone cell counts in AB (L–Q). (L–O) Osteoblasts (per bone perimeter) were counted on the distal side of the distal–buccal root of the upper right first molar. (O–Q) Osteoclasts were identified immunohistochemically as TRAP-positive cells on the medial side of the distal–buccal root of the upper right first molar. Red squares indicate the analyzed area in maxillary AB. Yellow squares represent the analyzed area in histological views. Small inserts indicate the analyzed area in histological sections. Black arrows represent osteoblasts or osteoclasts on the bone surface. Yellow arrows indicate the amount of AB loss. MB, mesial bone; DB, distal bone; r, root; PL, periodontal ligament. The bar represents 100 μM. *P≤0.05 (n=10 for each group).
recovery of the maxilla BMD comparing with control (chow diet) and HF groups (Fig. 4E–I). There was a significant reduction of Tb.sp between the control and 7d DR groups (Fig. 4K). No significant was observed for BV/TV parameter (Fig. 4J). Accordingly, 42 days of diet restriction (42d DR) resulted in significant improvement of AB parameters BMD, BV/TV and Tb.Th (Fig. 4E–K). Comparing the control, HF, 7d DR and 42d DR groups, we observed that 42d DR improved BMD parameter and BV/TV parameters (Fig. 4I, J).

On femurs, 7d DR consistently resulted in a positive recovery of BV/TV and Tb.Sp values (Fig. 4L–R). However, no significant difference was observed for BMD parameter when comparing 7d DR with the HF diet group (Fig. 4P). Similarly, 42d DR promoted significant augment of BMD, BV/TV and Tb.Th (Fig. 4L–R). Significant differences between 7d and 42d DR on femurs were observed only in BMD parameter (Fig. 4P). Overall, the results showed that diet restriction recovered important parameters of AB and femoral microarchitecture.

3.6. HF diet promoted marked changes in inflammatory gene expression

AB was analyzed with OpenArray PCR. The HF diet group showed an increased expression of the cytokine Tnfsf11 (tumor necrosis factor ligand superfamily member 11), the cytokine-like-protein Fam3c (family with sequence similarity 3, member C), the chemokine Ackr2 (atypical chemokine receptor 2), the enzymes Sod1 (superoxide dismutase 1, soluble) and Hmox1 (heme oxygenase 1), the chemotactic Chil 3 (chitinase-like 3), the receptor Cd40 (CD40 antigen) and the integral membrane protein Pxmp2 (peroxisomal membrane protein 2) (Fig. 5A).

On the other hand, the HF diet showed a reduced expression of 22 genes: the receptors Tnfrsf14 (tumor necrosis factor receptor superfamily, member 14), Tnfrsf1b (tumor necrosis factor receptor superfamily, member 1b), Ifnr2 (interferon alpha and beta receptor subunit 2), Cnfr (ciliary neutrophic factor receptor), Siglec1 (sialic acid binding Ig-like lectin 1, sialoadhesin) and C3ar1 (complement component 3a receptor 1); the chemokines Cxc16 (C-X-C motif chemokine ligand 16), Ccl11 (chemokine (C-C motif) ligand 11) and
Cxc12 (C-X-C motif chemokine ligand 12); the adaptor protein Tirap (TIR domain containing adaptor protein); the transmembrane protein Stab1 (stabilin 1); the peptidase inhibitor Serpina3c (serine proteinase inhibitor, clade A, member 3c); the dimer subunit RelA (RELA proto-oncogene, Nf-kB subunit); the enzyme encoded by Ndsf1 (N-deacetylase/N-sulfotransferase [heparan glucosaminyl]); the extracellular matrix protein Ltbp4 (latent transforming growth factor beta binding protein 4); the aminopeptidase Erap1 (endoplasmic reticulum aminopeptidase 1); the elastase Elane (elastase, neutrophil expressed); the cluster of differentiation Cd14 (CD14 antigen); the ABC transporter family protein Abcf1 (ATP binding cassette subfamily F member); the member of transforming growth factor beta superfamily Lnh8a (inhibin beta A); the putative transforming gene Jun (jun proto-oncogene, AP-1 transcription factor subunit); and the ring finger protein Lirf (ring finger protein 39–Lirf) (Fig. 5B).

3.7. Adipocytes stimulate osteoclast differentiation in vitro

Co-cultures were carried out to investigate if mature adipocytes can directly affect osteoclast differentiation (Fig. 6A). An increased number of TRAP-positive multinucleated osteoclasts were obtained when preosteoclasts were co-cultured with adipocytes (Fig. 6B–D). Osteoclasts with increased size and number of nuclei were formed in the presence of adipocytes (Fig. 6B–D). No significant difference was observed in osteoclast activity between the control (OCL) and co-culture groups (Fig. 6E–G).

An analysis of the culture infranatant showed that adipocytes produce higher concentrations of IL-6, resistin, adiponectin, leptin and chemerin compared to osteoclast controls (OCL) (Fig. 6I–M). The co-culture group presented higher levels of IL-6 and adipokines compared with the OCL group (Fig. 6I–M). No significant changes were observed in TNF-α production between the groups (Fig. 6H).

4. Discussion

The main findings of this study can be summarized as follows: (1) an HF diet promoted significant deterioration in AB and femoral microarchitecture; (2) diet restriction partially reversed deleterious bone effects induced by an HF diet, confirming the bone phenotype induced by HF consumption; (3) an HF diet impacted bone cell
Fig. 5. Inflammatory gene expression plot showing differentially expressed genes induced by HF consumption. The mean fold change (logarithmic scale) of differentially expressed genes in HF diet and controls is shown, considering a control sample as a reference. Error bars represent standard error of the mean expression level calculated from samples of the two groups (n=8 for each group).
population in AB by reducing the number of osteoblasts and by increasing osteoclastogenesis in association with significant changes in the expression of proinflammatory-related genes such as Chil3, Ackr2, Tnfsf11 and Fam3c; (4) effects of HF diet on bone may be linked to increased body adiposity, disturbances in adipokine production and adipocytes stimulation of osteoclastogenesis.

Previous data demonstrated a significant increase in horizontal AB loss in animals fed an HF diet with different concentrations, 60% [8], 20% [32], 32% [33] or 72% [34]. Moreover, in a 60% HF diet model, an increase of porosity and Tb.sp and a decrease in Tb.Th and BV/TV were observed. Meanwhile, when periodontal disease (PD) was induced, controversial results were obtained [9,35,36]. While some studies observed increased AB loss in PD animals fed an HF diet [9], others found no significant difference [9,35,36]. Our data clearly demonstrated that HF consumption exerted detrimental effects in maxillary AB by decreasing important bone parameters such as BMD, Tb.Th, BV/TV and Tb.n. Furthermore, a significant increase in spontaneous horizontal ABL was observed in the HF diet group. Some studies attributed these effects to disturbances in osteoblast and osteoclast differentiation [5]. In accordance, we observed that the HF diet promoted a reduction in the osteoblasts and an increase in the amount of osteoclasts, which might have contributed to the observed bone phenotype.

In parallel with AB changes, the femoral parameters were also affected by HF consumption. In contrast to the dogmatic view that obesity is protective against osteoporosis and increases in BMD [7], recent data demonstrate that an HF diet exerts detrimental effects on the femur [6,7,37–39]. In agreement with our findings, previous studies showed a reduction in BMD [5,38,39], BV/TV [5,39], Tb.n [5,39], Tb.sp [39] and Tb.Th [6] utilizing either 45% or 20% HF diets. Regarding mechanical properties, it was demonstrated that mice fed with a 20% HF diet presented a reduction in maximum stress [6] and elastic modulus [37]. Interestingly, these studies reported no difference in the maximum load and stiffness [6,37]. However, when lipoprotein receptor-deficient mice (LDL−/−) were fed a 20% HF diet, a significant reduction in the femur mechanical properties [6,37] was observed. In agreement with these findings, we observed a reduction in bone stiffness and maximum load in the HF diet group. These results confirm the concept that consumption of an HF diet impairs bone microarchitecture with a negative impact on mechanical properties, thereby increasing susceptibility to bone fracture.
To confirm that bone loss was induced by an HF diet, we performed a diet restriction experiment. We chose to evaluate bone phenotype changes after 7 and 42 days of DR, as our group and others [40] proved that DR animals presented improvements in body weight and biochemical markers at both time points. Previous data have demonstrated negative effects of caloric restriction on bone remodeling. These effects were related to the reduction of bone mineral content [41] or decreased BMD, bone mass, bone size and strength [42]. Our results revealed that when an HF diet restriction was implemented, there was a significant improvement represented by increases in BV/TV and Tb.Sp of femurs, as well as BMD in AB. The improvement of bone quality was associated with decreased levels of resistin in diet restriction animals. Accordingly, resistin is negatively correlated with BMD in osteoporosis patients [43]. However, it is the first study showing the potential benefits of DR on bone and further investigation of underlying mechanisms should be conducted. Although DR protocol has already been tested [40] and knowing that the minimum concentration of micronutrients has been assured in our experimental conditions, we cannot exclude that minor changes in vitamins and minerals contents may influence bone turnover and be a limitation of this study.

Since the HF diet disrupted AB microarchitecture, this study investigated the possible local mechanisms involved in this process. HF intake was associated with altered expression of 30 inflammatory genes, including the upregulation of Tnfsf11, Fam3c and Ackr2. These molecules have inflammatory actions and are also involved in the bone remodeling process. The Tnfsf11 gene encodes a member of the TNF family, which acts synergistically to inhibit mesenchymal stem cell differentiation into osteoblasts and to increase osteoclast formation and activation via augmentation of RankL expression [44,45]. Likewise, increased expression of Fam3c is related to the reduction of Runx2 expression and, consequently, with osteoblast formation [46]. In addition, the literature shows that Ackr2 regulates the local availability of chemokine and, consequently, the inflammatory response [47]. Recently, Ackr2 was shown to play an important role in mechanically induced bone remodeling in AB by affecting differentiation and activity of bone cells [48]. Accordingly, our results revealed that an increased expression of Tnfsf11, Fam3c and Ackr2 on AB might contribute to the imbalance between bone resorption and formation in the HF diet group.

We also observed a reduction in the expression of the gene IhnBa. Existing data highlight the role of IhnBa in bone metabolism [49,50]. IhnBa encodes a member of the Tgf-B protein superfamily and is known to be involved in the disruption of bone remodeling in osteoarthritis patients [49]. Moreover, IhnBa generates a subunit of the dimeric activin A [50]. Activin A inhibits Rankl-induced osteoclast differentiation, and its reduced levels are associated with palate and tooth defects [50]. Accordingly, an HF diet might favor Rankl-induced osteoclastogenesis via reduction of the IhnBa pathway.

There is a large body of evidence showing that Ccl11 [51], Tnfsf1B polymorphism [52] and Tirap activation [53] can influence the inflammatory process during fracture healing, osteoporosis and the production of Tnf-α and IL-1, respectively. In addition, several studies evaluated Jun, RelA and Cxcl12 roles in Rankl activity and bone remodeling [54,55]. In this context, both genes (Jun and RelA) were reported to increase osteoclastogenesis, either by mediating Rankl effects [54] or by suppressing Rankl–induced apoptosis in osteoclasts [55]. Interestingly, there was decreased expression of Ccl11, Jun, RelA, Tnfsf1B and Tirap. These results may indicate that these pathways are not associated with the observed bone phenotype in the HF diet mice. However, further investigation is needed for clarification.

Negative impact of HF diet on bone may be associated with local and systemic alterations. Therefore, we evaluated systemic effects induced by diet as adipose tissue expansion, biochemical, metabolic and inflammatory changes [45,56–59]. Our results showed that an HF diet promoted a significant gain of white adipose tissue and body weight, and also augmented cholesterol levels as previously reported [60,61]. Mice under diet restriction consistently presented a reduction in body weight, adiposity index and systemic levels of biochemical markers. Accordingly, it was suggested that moderate diet restriction gradually reduces body weight and fat mass without significantly affecting lean mass [40].

Fat tissue produces serologically active substances called adipokines [62]. In obesity, adipokine levels are altered, leaving individuals more susceptible to insulin resistance and metabolic syndrome [26,63–65]. Accordingly, we observed that HF consumption induced a reduction in adiponectin serum levels as well as promoted an increase in leptins, showing that our model was sufficient to induce obesity in mice. In diet restriction animals, we observed a reduction in resistin serum levels and no significant alterations in adiponectin levels. Similarly, others have found a decrease in resistin transcript levels in the epididymal adipose tissue of diet restriction rats [63].

Adipokines also interfere with bone quality [66–68]; thus, we investigated, for the first time, whether mature adipocytes would affect osteoclast differentiation and activity independently of cell–cell interaction. We demonstrated that adipocyte stimulus increased not only the number but also the cell area of differentiated osteoclasts. Moreover, co-cultures exhibited augmented concentrations of the adipokines adiponectin, resistin, chemerin and leptin, which were mainly produced by adipocytes. Surprisingly, no difference was observed in osteoclast function between the groups. As previously reported, increased levels of resistin and chemerin are able to induce osteoclastogenesis [16,69]. In contrast, adiponectin and leptin seem to present dual roles in bone cell differentiation [70]. Recent data demonstrated that leptin suppressed osteoclast differentiation in RAW264.7 cells by inhibiting PPARy expression [71]. Overall, the increased expression of adipokines has a positive impact on osteoclast differentiation accounting for increased osteoclastogenesis and bone resorption.

In conclusion, we demonstrated that an HF diet promotes detrimental changes in AB microarchitecture by reducing osteoblasts, increasing osteoclastogenesis and regulating inflammatory gene expression. HF-induced AB loss occurred with similar detrimental effects on the femur. Furthermore, HF diet restriction resulted in the recovery of AB and femur bone parameters. We also showed that mature adipocytes can directly modulate osteoclast differentiation and that adipokines seem to be a key part of these processes. Despite the limitation to apply findings obtained in animals to humans, strategies to control diet-induced bone loss might be considered, especially in patients with preexisting bone inflammatory conditions and in situations in which bone repair/remodeling is expected for the success of medical or dental treatment.

References


