Original Investigation

Bone Marrow Lipid Profiles from Peripheral Skeleton as Potential Biomarkers for Osteoporosis: A ^{1}H-MR Spectroscopy Study

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Rationale and Objectives: To characterize the lipidic profile of bone marrow in the calcaneus and femoral neck of healthy, osteopenic, and osteoporotic women, by using magnetic resonance spectroscopy (MRS) at 3T. The final goal was to identify specific metabolites with the potential ability to discriminate between healthy, osteopenic, and osteoporotic subjects.

Materials and Methods: Sixty-two and thirty three postmenopausal women recruited to investigate calcaneus and femoral neck, respectively, underwent a bone mineral density (BMD) measurement to be classified as healthy subjects ($n = 22$), osteopenic ($n = 45$), or osteoporotic ($n = 28$) patients.

MRS spectra were used to quantify and compare bone marrow fat resonances between the three BMD groups. Between-group differences were tested using a Welch analysis of variance. Multiple comparisons were made with the Games–Howell correction. Relationships between pairs of variables were assessed with linear correlation analysis. Reproducibility analysis was performed for all the lipid resonances in both sites.

Results: The reproducibility was satisfactory. In femoral neck, methylene (L13), glycerol (L41, L43), and total lipid resonances were significantly lower in healthy as compared to osteoporotic subjects. On the other hand, in calcaneus, L13/glycerol significantly discriminated between osteopenic and osteoporotic subjects whereas L13/(unsaturated lipid) discriminated between healthy and osteopenic group. However, the reproducibility of both unsaturated lipid and glycerol resonances were less optimal.

Conclusions: MRS of bone marrow lipid profiles from peripheral skeletal sites may be a promising tool for screening of large population to identify individuals with or at risk for developing osteoporosis. Moreover, it provides information about the metabolic changes occurring in bone marrow with the development of osteoporosis, which are skeletal site dependent.

Key Words: Osteoporosis; fatty acids; bone marrow; femoral neck; calcaneus; glycerol.

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INTRODUCTION

To date, several studies performed in different populations by using distinct measurement techniques have established that higher bone marrow fat is associated with lower bone mineral density (BMD) and prevalent vertebral fracture (1). This has led to a growing interest in the study of the interplay between marrow fat and bone mineral metabolism in connection to the development of osteoporosis.

In particular, bone marrow adiposity plays an important role in affecting bone quality. Osteoblasts and adipocytes share a common precursor cell originating from the bone marrow stroma, namely, the mesenchymal stem cell (SMCs) (2). Several studies underlined that SMCs differentiation is a mutually exclusive process with commitment to either fat or bone lineage. Some in vivo studies performed in the elderly individuals, where bone loss was found to be associated with an increase in bone

marrow fat content (FC), suggested a preferential differentiation of stem cell into marrow fat at the expense of bone-forming osteoblast during aging (3). Adipogenic transdifferentiation contributes to age-related diseases, such as osteoporosis and osteopenia, which are accompanied by increased adipose tissue and a decreased number of osteoblast in the bone marrow (4). Justesen et al. (5) examined the composition of bone marrow in iliac crest bone biopsies obtained from a large population of males and females with different ages, and from patients with osteoporosis. Their results showed that adipogenesis is enhanced over aging, and this associates with decreased osteogenesis. Moreover, these changes were found to be remarkably more pronounced in patients with osteoporosis.

Following these results, FC quantification in bone marrow has gained increasing attention as a potential tool to identify biomarkers for bone quality.

Recently, it has been suggested that the composition as well as the quantity of marrow fat may be relevant for skeletal health (6). The fatty acid composition of biological samples is commonly quantified by gas chromatography. Mass spectrometry is an alternative technique which is commonly used in lipid research, especially in combination with gas chromatography (7). However, both these techniques are based on destructive methods, and, for this reason, they are hardly applicable to clinical routine (8).

Magnetic resonance spectroscopy (MRS) is a completely non-invasive technique, which represents a promising candidate to quantify, in vivo, different components in bone marrow. It allows investigation of the adipogenic differentiation of MSCs, by monitoring changes in cellular metabolites and metabolite profiles, specifically those derived from the signal of fatty acids (9). Moreover, MRS is already available as a routine examination in most clinical scanners (10–13). After some preliminary works (14–16), more recently, Ren et al. performed a detailed analysis of the tibial marrow fat in healthy humans by using high resolution MRS at 7T (17). Some other MRS studies identified a clear relationship between FC and BMD. In particular, subjects with osteopenia and osteoporosis are characterized by significantly higher FC than those with normal bone density, in vertebral bone marrow (18–21). Moreover, marrow lipid unsaturation index (UI) is lower in osteoporotic and osteopenic patients as compared to individuals with normal bone density (19).

All these studies have focused their investigation on a single skeletal site: the vertebrae. In this site, because of the presence of strong water resonance, only a limited selection of resonances can be easily detected for the investigation of bone marrow. Typically, the quantified resonances include olefinic protons (-CH=CH), located around 5.3 ppm; water, located around 4.65 ppm; methylene protons (-CH_2-CH=CH-CH_2-), located around 2.03 ppm; bulk metylene protons (-$(CH_2)_n$-), located around 1.30 ppm (14). However, there are up to 22 different fatty acids in marrow fat, each of which could potentially be a more specific biomarker, compared to the increase of total fat, for detecting osteoporosis. Moreover, there are other peripheral skeletal locations, such as the femur and the calcaneus, that are of interest for the analysis of bone marrow, as well as for the diagnosis of osteoporosis. Indeed, proximal femur fractures have become the international barometer of osteoporosis because they are strongly associated with low BMD, cost more to repair, and cause more disability than any other type of osteoporotic fracture (22).

On the other hand, some authors established, in vivo, correlation between vertebral body fracture and poor trabecular bone density in calcaneus cancellous bone (23,24). Moreover, correlation among vertebral body, proximal femur fractures, and trabecular bone microstructures of calcanei have been obtained in vitro (25). As a consequence calcaneus analysis, performed without wholly placing patients inside the magnetic resonance (MR) scanner, thus avoiding common problems of claustrophobia, could be a useful procedure to obtain data from a larger population.

Thus, the specific aims of this study were (1) to quantify all bone marrow resonances in vivo at 3T on two peripheral skeletal sites not previously investigated in detail, that is, the femoral neck and the calcaneus; (2) to assess their reproducibility; (3) to determine whether the analysis of FC in these sites is able to discriminate between healthy subjects and patients with and at risk for developing osteoporosis.

The final goal was to identify useful markers for potential diagnostic usage in clinical settings.

MATERIALS AND METHODS

Subjects

The current MR study was designed to investigate two specific skeletal sites, namely, the calcaneus and the femoral neck. To increase the tolerability for MR scanning, we maintained the acquisition time relatively short by dividing our investigation in two distinct experiments, one for each skeletal location. Two different groups of subjects were therefore recruited to investigate either the calcaneus (group I) or the femoral neck (group II): group I involved 62 Caucasian postmenopausal women (mean ± SD age: 62.1 ± 5.8 years; range: 53–75), whereas group II involved 33 Caucasian postmenopausal women (mean ± SD age: 68.1 ± 9.5 years; range: 55–80). Subjects were excluded in the following cases: (1) clinical or imaging evidence for metabolic bone disease or metastasis; (2) previous history of lumbar spinal surgery or irradiation; (3) current use of steroids or hormone replacement therapy or any medication for osteoporosis; (4) any contraindication to MR examination.

The study was approved by the Local Ethics Committee. Written informed consent was collected from each subject before BMD and MR examination.

As no reference data is available to classify subjects on the basis of their BMD directly evaluated in calcaneus, each volunteer of group I underwent BMD evaluation by quantitative