Introduction

Bone and muscle are two deeply interconnected organs with integrated functions in growth and locomotion (1). A strong relationship exists between their development, maintenance and correct functioning, from which the concept of “bone-muscle unit” derives (2). Muscle and bone interactions take place at different levels (organ, cellular, and molecular level) with bidirectional pathways. Mechanical loading and muscle contraction are the most important determinants of bone mass and shape, and physical activity (PA) is known to exert an anabolic effect on the metabolism of both these tissues (2-4). A linear relationship exists between bone mineral content (BMC) and lean body mass (2,5,6). The rise in life expectancy in developed countries resulted in the progressive increase in the prevalence of chronic degenerative diseases, with particular emphasis on those affecting the musculoskeletal system. Osteoporosis and
sarcopenia are two widespread diseases of increasing public interest and represent an important area of investigation of geriatric and nutritional research. Recently, a rapid increase in the knowledge of the pathologic processes associated with the aging of the musculoskeletal system, together with important advances in the field of high resolution imaging techniques, have improved dramatically our understanding of osteoporosis and sarcopenia.

Different imaging modalities are available for a non-invasive evaluation of bone and skeletal muscle mass and quality in osteoporosis and sarcopenia. Dual energy X-ray absorptiometry (DXA), computed tomography (CT), magnetic resonance imaging (MRI) and ultrasound are of variable importance in the study of osteoporosis and sarcopenia and often play a complementary role, depicting different aspects of the same pathology (7,8).

This paper aims to review the role of different imaging modalities currently available for the non-invasive investigation of bone and skeletal muscle in osteoporosis and sarcopenia, highlighting the main limitations of conventional imaging techniques, the potential value of recently developed diagnostic tools and novel advances in the field of high resolution imaging, which provide useful insights into pathophysiology and fracture risk.

**Clinical implications**

Osteoporosis is defined as “a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture” (9). Bone strength, a term commonly used to describe the health of bone and its ability to resist fracture, is primarily affected by two parameters, namely, bone density and bone quality (10,11). Bone density expresses the amount of mineral per unit area or volume, while bone quality refers to the complex microarchitecture and peculiar composition of bone tissue (9). In 2010 osteoporosis affected about 27.6 million adults in Europe and 53.6 million adults in the United States were estimated to have osteoporosis or low bone mass at either the femoral neck or lumbar spine (12,13). Osteoporotic fractures represent a major cause of morbidity and mortality in elderly population and are associated with a significant increase in healthcare costs. In Europe the cost of osteoporosis (including cost of incident fractures, cost of long-term disability and cost of pharmacological fracture prevention) was estimated at approximately 37.4 billion euro in 2010 (12). In the United States the annual estimated cost associated with osteoporosis-related fractures amounted to 16.9 billion dollars in 2005 and it is expected to rise to 25.3 billion dollars in 2025 (14,15).

The term sarcopenia derives from the Greek term “sarx” or flesh + “penia” or loss and it was used for the first time in 1989 to describe the age-related loss of muscle mass (16). However, a broadly accepted definition of sarcopenia is not yet agreed upon. To address this gap, the European Working Group on Sarcopenia in Older People (EWGSOP, the Sarcopenia Working Group) was created in 2009. According to EWGSOP, sarcopenia is defined as a “syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength”, stressing the importance of the decrease of muscle function (i.e., muscle strength or performance) for the diagnosis (17). The estimated prevalence of sarcopenia varies extremely within different cohorts according to patients’ characteristics, ranging from 0.1% to 85.4% in adult subjects (18). The prevalence of this condition differs also depending on the definition used for its diagnosis. According to the EWGSOP definition, the prevalence of sarcopenia is 1–29% for older adults living in the community and 14–33% in long-term care populations (19). Sarcopenia is associated with many adverse outcomes like increases in morbidity and mortality, hospitalization, physical disability and loss of independence in activities of daily living (20,21). Data concerning the health-care costs of sarcopenia are largely lacking. The very few studies available in this field reported that the direct cost of sarcopenia (estimated on the basis of the medical expenditures associated with physical disability in older persons and attributable to sarcopenia) in the United States was approximately of 18.5 billion dollars in 2000 (22). To the best of our knowledge, no similar study has been performed in Europe.

The development and the maintenance of bone mass and muscle mass are deeply related, and both are affected by age. The peak of both bone and muscle mass is achieved in early adulthood, followed by a progressive decline after the age of 40 (20,23). After the age of 50, the decline of muscle mass becomes more substantial proceeding at a rate of 1% per year (23,24). The decline in the total amount of bone and muscle mass is accompanied by concomitant modifications of their cells population (25). In bone tissue, the aging process is characterized by a progressive accumulation of adipose cells within the bone marrow (BM). The role of marrow adipose tissue (MAT) as an important component of the BM microenvironment has been deeply investigated in the last few years and a growing body of evidence shows an inverse association between MAT content and both bone...
mineral density (BMD) and bone integrity (25-29). Estrogen deficiency, prolonged glucocorticoid treatment, altered leptin signaling, disuse and bed-rest are some of the most important conditions known to be associated with BM adipogenesis and, according to recent evidences, they are also responsible for the fatty infiltration of skeletal muscles (myosteatosis) (25). Two different pathways are implicated in the development of myosteatosis: (I) the accumulation of intracellular lipids within the myofibers (intramyocellular lipids), which is associated with decreased insulin sensitivity and inflammation; and (II) the disproportional differentiation to the “adipogenic lineage” of the mesenchymal stem cells population, which is responsible for the accumulation of inter-myofiber fat. Fatty infiltration in skeletal muscles is responsible for a decreased insulin sensitivity and has a detrimental effect on muscle health and function (25). The term “sarcopenic obesity” is currently used to describe the co-existence of loss of muscle mass and increased adiposity, resulting in a relative increase in fat mass (FM) compared to lean muscle mass, and it is currently recognized as an important aspect of frailty in older adults (20,25,30-32). The mean Hounsfield unit (HU) of the lean tissue within the mid-thigh muscle bundle, obtained by CT scan, was used by Lang et al. as an indicator of intramuscular fat infiltration. After adjustment for age, race, gender, height, body mass index (BMI), and total percentage of body fat (measured with DXA), a significant association was found between decreased thigh muscle attenuation and increased risk of hip fracture [relative risk/standard deviation (RR/SD) =1.58] and this association remained significant after further adjustment for total femur BMD (33). Muscle weakness is also the most important individual risk factor for falls in older people, being associated with an approximately 4.4-fold increase in mean attributed risk (34-36). This data is of particular clinical interest, considering that sarcopenia is a potentially treatable condition, if interventions are initiated early (37).

Conventional radiography

Findings suggestive of osteoporosis are frequently encountered on radiographs and it is extremely important for the radiologist to be aware of them. The main radiographic features of osteoporosis are: (I) increased bone radiolucency; (II) cortical thinning; (III) changes in the trabecular pattern (38,39). Increased bone radiolucency (I) results from the progressive decline in BMC and the concomitant impoverishment of the trabecular microarchitecture, resembling the appearance of an over-exposed radiograph. However, this feature is detectable only in the advanced stages of the disease, when the amount of bone loss reaches at least 30% (39). Cortical thinning (II) results from the reabsorption of the periosteal, intracortical and endosteal layers. The resorption process is very active in the endosteum because of its rich blood supply, resulting in a gradual widening of BM space. In the vertebral bodies, this feature together with the concomitant increase in bone radiolucency is responsible for the typical “picture frame” appearance, also known as “ghost vertebra” (Figure 1) (38,39). In the early-stages of osteoporosis, a scalloping in the inner margin of the cortex, known as “endosteal scalloping”, can be seen, but this finding is non-specific and can be found also in pathologic conditions characterized by rapid bone turn-over like hyperparathyroidism, renal osteodystrophy or reflex sympathetic dystrophy syndrome. Lastly, the alteration in trabecular pattern (III) is caused by the fact that trabeculae, which are more abundant in the axial skeleton and at the ends of long bones, offer a greater surface area for resorption processes and respond faster to metabolic changes than cortical bone (38,39). Early on, secondary trabeculae, which are not primarily involved in weight bearing, are lost first, while the primary trabeculae become more prominent and will disappear only at a later stage. Using these specific and predictable sequence of resorptive processes, some authors developed
semi-quantitative indexes for the diagnosis and grading of osteoporosis. Among these indexes, those proposed by Singh et al. considering the proximal femur and by Jhamaria et al. considering the calcaneus were perhaps the most widely used ones in the past (40-42). However, these indexes suffer from a great inter-observer variation and the quality of the radiographs itself and the superimposition of soft tissues (with particular regards to the Singh index) can also affect the results. An extended description of these indexes is beyond the purpose of this article, and for a more comprehensive discussion, the interested reader is referred to the referenced publications (38,40-42).

Currently DXA represents the most widely used technique for the assessment of BMD, thanks to its wide availability, the extremely low radiation dose and low costs. However, in the past, great efforts were undertaken to develop quantitative evaluation methods of osteoporosis from conventional radiography. Among these, radiographic absorptiometry (RA) and metacarpal radiogrammetry represent the oldest techniques (43).

The development of the principles behind RA first enabled BMD estimation (44). In this method, a radiograph of a peripheral site, typically metacarpals and phalanges, is obtained placing a reference wedge of hydroxyapatite or aluminum close to the bone. BMD is then expressed relative to the density of this wedge material (45). Even if RA was limited to peripheral skeletal sites, the composite volume density and the composite area density derived by RA at the phalanges showed a good correlation with the standard measurements of BMD derived by DXA (r=0.69 and 0.68 respectively, at lumbar spine; r=0.63 and 0.67 respectively, at the femoral neck) (45). However, RA was time consuming and results were strongly influenced by multiple factors including operator expertise, X-ray beam intensity and soft tissue thickness (43).

Radiogrammetry was introduced in 1960 and represents a simple and inexpensive method to evaluate cortical bone changes in tubular bones using plain radiographs. Radiogrammetry can be used in different bone segments. However, the second metacarpal of the non-dominant hand is the most common site of application (43). The metacarpal index (MCI), also known as Barnett-Nordin index, is expressed as the ratio between the combined cortical thickness (Ct.Th) (radial and the ulnar cortex) of the mid second metacarpal and the diameter itself of the shaft at this level, measured with a caliper (46,47). As medullary width and bone width (BW) both increase with aging due, respectively, to endosteal reabsorption and periosteal apposition, the MCI was used for a long time to obtain an objective evaluation of the degree of cortical bone loss in osteoporosis (43). The manual measurement technique represented the main limitation of radiogrammetry, with a high precision error (PE) and a great inter-observer variation (46). Recently, technological advances brought this old technique back into focus: in digital X-ray radiogrammetry (DXR) a computer automatically identifies the average Ct.Th and BW of the second, third and fourth metacarpal midshafts. The fully automated measurements obtained from three metacarpal bones, instead of one, are responsible for the enhanced accuracy and precision of DXR. From Ct.Th and BW measurements, a compound measurement called “bone volume per projected area” (VPA) is determined and BMD is then derived through a geometrical operation (43,48). In 1999 DXR was approved by the Food and Drug Administration (FDA) as a reliable method for estimating BMD. A significant correlation was proved to exist between DXR-derived BMD (DXR-BMD) at the three mid-metacarpals and DXA-derived BMD at the spine, total hip and distal radius (r=0.52, 0.55 and 0.76, respectively; P<0.0001 for all) (46). When five regions of interest (ROIs) were used to derive DXR-BMD (second, third, fourth mid-metacarpals and the distal radius and ulna) the level of correlation with DXA-derived BMD was even higher (r=0.86 at the distal forearm; r=0.79 at the ultradistal forearm; r=0.62 at the spine; r=0.69 at the total hip; r=0.73 at femoral neck; P<0.0001 for all) (48).

**DXA**

The role of DXA is central in the study of body composition (BC) thanks to its ability to differentiate FM, non-bone lean mass (LM) and BMC at both the regional and the whole-body level (Figure 2) (49-60). DXA relies on the use of two X-ray beams of different energy; the measurement of the “R value” is calculated as the ratio between the degree of attenuation of the lower energy and the higher energy beam. The R value is specific for each tissue and depends on the patient’s soft tissue composition. From the R value, using complex algorithms, it is possible to derive the amount of BMC in pixels containing bone. BMD is then calculated as the ratio BMC/area (in g/cm²). In pixels without bone, soft tissue is further characterized as FM and non-bone LM (61,62).

**DXA in osteoporosis**

Currently, DXA represents the gold standard for diagnosis
and monitoring of osteoporosis and low bone mass conditions (63). Several guidelines have been developed in recent years to ensure proper use of this densitometric technique, from appropriate indications for BMD testing to procedures for correct data analysis and reporting. In 2016, a panel of experts reviewed the current guidelines on DXA for adults: the overall quality of DXA guidelines was satisfactory and “Adult and Pediatric Official Positions” issued by the International Society for Clinical Densitometry (ISCD) achieved the highest total score according to the Appraisal of Guidelines for Research & Evaluation version II (AGREE II) (64). BMD is expressed in terms of SD comparing individual BMD measurement to a reference range derived from a population of healthy young adults (at peak bone mass) in the case of T-score, and from an age-matched population of the same gender and ethnic group in the case of Z-score. In post-menopausal women and in men older than 50 years old osteoporosis is defined as a T score of −2.5 or less at the lumbar spine (from L1 to L4), femoral neck or total hip. In pre-menopausal women and in men younger than 50 years old a Z-score of −2 or less is defined as “below the expected range for age” (63,65). In clinical practice, DXA generally represents the first exam performed in patients with suspected osteoporosis, thanks to its wide availability, low cost, and minimal radiation exposure [the effective dose to an adult from a spine and hip examination is approximately between 1 and 10 μSievert (μSv)] (66-68). Nonetheless, BMD measured by DXA accounts only for 60–70% of variation in bone strength and the majority of osteoporotic fractures occur in people whose BMD is in the non-osteoporotic range (69-71). This fact takes on particular importance in diabetes-related bone disease. Patients with diabetes mellitus (DM) show an increased risk of fractures, especially at the hip (summary RR for hip fractures according to two large meta-analyses: 6.3–6.9 in type 1 DM; 1.4–1.7 in type 2 DM) (72,73) that does not appear to be explained by the variation of BMD, which is just slightly reduced in type 1 DM and normal or even increased in type 2 DM in comparison with non-diabetic controls (29,74).

Today, other parameters beyond BMD can be derived from DXA for a better understanding of bone strength. Trabecular bone score (TBS) is a novel gray-scale textural analysis recently approved by the United States FDA that can be derived from a previously acquired lumbar spine DXA scan, providing information about bone microstructure and bone strength. The major advantages of TBS are its simplicity and low cost, since TBS is measured in the same ROIs of the lumbar spine BMD (77-79). Dedicated software is used to measure the gray level of variation among pixels within the 2D DXA image and differentiate between bone structures with similar areal BMD (a-BMD) but different bone microarchitecture (Figure 3) (80). TBS is not a direct measure of trabecular microarchitecture, but it has been proved to be associated with vertebral, hip and major osteoporotic fracture risk in postmenopausal women and with hip and major osteoporotic fracture risk in men over 50 years of age (81). Specifically, each SD decline in TBS corresponds to a 20–50% increase in the risk of vertebral, hip and major osteoporotic fractures in postmenopausal women and to a 30–40% increase in risk of hip and major osteoporotic fractures in patients with diabetes mellitus (66-67).
fractures in men over 50 years of age. However, according to ISCD guidelines, TBS should not be used alone in clinical practice but rather in combination with BMD and FRAX to enhance fracture prediction (81). For postmenopausal women the following range has been proposed: TBS >1.350, normal; TBS between 1.200 and 1.350, partially degraded microarchitecture; TBS <1.200 degraded microarchitecture; a similar range for men has not yet been proposed (79). TBS has also been proven to be associated with major osteoporotic fracture risk in postmenopausal women with type 2 DM, thus it may serve as an additional tool for fracture risk assessment in this selected class of patients (81-83). TBS can also be assessed in other conditions characterized by bone loss (as primary hyper-parathyroidism, rheumatoid arthritis, chronic kidney disease, etc.). However, further studies are needed to establish its use in these disorders (84,85).

Other non-BMD parameters can be derived from 2D DXA images in addition to TBS. Hip Structural Analysis (HSA; Hologic, Bedford, MA, USA) and Advanced Hip Assessment (AHA; GE-Lunar, Madison, WI, USA) are two recently developed software products that can be applied to a standard DXA scan to automatically derive various parameters related to bone geometry and bone strength, including hip axis length (HAL), cross sectional area (CSA), outer diameter (OD), section modulus (SM), buckling ratio (BR), cross-sectional moment of inertia (CSMI) and neck shaft angle (NSA) (86,87). According to the ISCD 2015 official positions, among hip geometry parameters derived from DXA, only the HAL is associated with hip fracture risk in postmenopausal women (88).

CT and MRI are considered the gold standard for the assessment of skeletal muscle mass; however, the significant complexity and the high cost strongly limit their use (7). DXA is an attractive opportunity in the study of muscle mass both at whole body and regional level and, according to the EWGSOP, it represents the preferred alternative method in research setting and clinical practice (17,54,55). Previous studies reported a good level of correlation between the measurements of skeletal muscle mass at lower limbs derived by DXA and those derived by CT and MRI, even if DXA tends to underestimate the extent of sarcopenia in comparison with the latter two techniques (92,93). DXA specific measures of LM include lean mass index (LMI: total LM/height²), appendicular lean mass (ALM: arms LM + legs LM) and appendicular lean mass index (ALMI: ALM/height²) (94). ALMI is of high clinical significance, since the maintenance of appendicular skeletal muscle mass is critical in the preservation of mobility and functional independence in advanced age (95). According to ISCD guidelines, “low lean mass” could be defined using ALMI with Z-scores derived from a young adult, race, and sex-matched population, however the threshold for the definition of low LM is yet to be validated (96). In recent years, age- and sex-specific data on ALM derived from general population have
been collected in different countries and could be useful in the future as a reference standard to monitor the wasting of muscle mass (97-101).

**CT**

In daily practice, CT represents the most widely used 3D imaging modality. In the last few years, CT technology has evolved quickly and new advanced quantitative imaging modalities are available to study bone and muscle structures, in addition to the “standard” CT scan. Quantitative CT (QCT) and peripheral quantitative CT (pQCT) were originally designed to assess bone parameters respectively at central and peripheral sites, but are currently also used for the quantification of muscle mass and fat distribution (7,38). In the field of high-resolution imaging, high-resolution peripheral quantitative CT (HR-pQCT) represents an exceptional tool for 3D in vivo investigation of bone and muscle at peripheral sites (102).

**CT in osteoporosis**

The usefulness of QCT in the study of osteoporosis is related to its ability to measure BMD in a chosen volume [volumetric-BMD (v-BMD)] without overlapping of other tissues and to the possibility of addressing cortical and trabecular bone separately (103,104). Compared to other imaging modalities, QCT has several advantages. QCT-derived v-BMD represents a true density measure expressed in g/cm$^3$, instead of an areal density as measured by DXA and may avoid the overestimation of BMD by DXA resulting from spinal degenerative changes, vascular calcifications and other sclerotic lesions in the surrounding soft tissues (105). QCT, compared to densitometric techniques, provides a measure of purely trabecular bone, which is more metabolically active and primarily affected by metabolic bone diseases, showing a better sensitivity for detecting osteoporosis than projectional methods (such as DXA) (103,106). For these reasons, QCT-derived T-scores cannot be used according to the WHO diagnostic classification since they are not equivalent to those obtained from DXA (105). QCT is generally applied to lumbar spine. Other measurement sites commonly evaluated include the proximal femur, forearm and tibia (105,107). In clinical application, spine and proximal femur are analyzed using standard whole-body CT scanners equipped with a

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**Figure 4** Hip scan with hip geometry analysis. HAL is automatically traced throughout femoral neck from the lower base of the greater trochanter to the inner pelvic brim. The colored map on the right shows the different distribution of BMD values in different femoral regions (from blue to red—from high to low). HAL, hip axis length; BMD, bone mineral density.
HR-pQCT specific measures of BMD include using HR-pQCT at an in-scan calibration phantom and the lack of a strictly opportunistic scan (of the abdomen and pelvis) for the assessment of BMD. In this context, the major advantages in comparison with QCT are the absence of any additional DXA scan. In clinical practice DXA should be preferred to limit radiation exposure, unless QCT can provide superior information.

In the last few years a large number of studies have evaluated the clinical utility of QCT in fracture prediction and longitudinal monitoring: according to the ISCD 2015 official positions, the ability of spinal trabecular BMD derived by QCT to predict spinal fractures in postmenopausal women is comparable to or better than that of lumbar spine BMD derived by DXA; sufficient evidence to support this statement in men is lacking (105). Total femur trabecular BMD derived by QCT has the capability to predict hip fractures as well as hip BMD derived by DXA in both menopausal woman and older men (110). QCT has also been extensively tested in monitoring age, disease and treatment related BMD changes (105,110). Despite this, for therapeutic decisions the role of DXA at the lumbar spine and femur remains central and in clinical practice DXA should be preferred to QCT to limit radiation exposure, unless QCT can provide superior information (110). Even QCT subjects the patients to a higher radiation exposure than DXA (radiation dose expressed as effective dose equivalent estimated for female QCT protocols: <1 mSv for single-slice QCT spine L1–L3; ~1.5 mSv for 3D QCT spine L1–L2; ~2.5–3.0 mSv for 3D proximal femur QCT; <0.01 mSv for pQCT forearm on clinical CT scanners) (105). Finally, the feasibility of opportunistic screening deserves a special mention. The term opportunistic denotes the use a pre-existing diagnostic CT scan (of the abdomen and pelvis) for the assessment of BMD to screen for patients at increased risk of fracture, without need for any additional DXA scan. In this context, the major disadvantages in comparison with QCT are the absence of an in-scan calibration phantom and the lack of a strictly standardized acquisition protocol. According to the ISCD 2015 official positions, the identification of patients with high fracture risk (according to low BMD or strength measures derived by CT at the spine or proximal femur) is possible with “standard” CT scan only if machine-specific cutoff values and scanner stability have been established (108).

Recently, dedicated high resolution pQCT scanners have been developed to evaluate bone microarchitecture in detail (104). HR-pQCT represents an attractive non-invasive technique to further investigate bone structure in vivo. Thanks to the high spatial resolution and the high signal-to-noise ratio (SNR) HR-pQCT can directly visualize trabecular microarchitecture at peripheral sites (normal isotropic voxel of 82 and 41 μm, respectively in older and newer scanner; trabecular dimension ~82 μm). HR-pQCT can assess BMD, microstructural and mechanical parameters of both cortical and trabecular bone separately at the distal radius and tibia with a very low effective dose (~4.2 μSv) and without the involvement of radiosensitive organs (102,111). Even though HR-pQCT is limited to the appendicular skeleton, a good correlation has been proven between density (a-BMD and v-BMD), geometry (CSA) and stiffness parameters measured peripherally and those derived by QCT at lumbar spine and proximal femur, the sites where the vast majority of osteoporotic fractures occur (112,113). HR-pQCT specific measures of BMD include whole bone v-BMD and, after the segmentation of the cortical and trabecular compartments, trabecular bone BMD (Tb.BMD) and cortical bone BMD (Ct.BMD); a-BMD of the ultradistal radius can be accurately estimated from 3D-HRpQCT image data with results that strongly correlate with DXA measurements (114,115). In addition, different microstructural bone parameters can be assessed, including bone volume ratio (BV/TV, %), average trabecular number (Tb.N, 1/mm), average trabecular thickness (Tb.Th, mm), average trabecular separation/spacing (Tb.Sp, mm) and individual trabecula segmentation (ITS) (114). In particular, ITS refers to trabecular orientation and the ratio of rod-like and plate-like trabeculae, which have been recognized as important determinants of bone strength (116). Recently, the use of HR-pQCT has allowed interesting insights into the investigation of bone deterioration in secondary osteoporosis and related bone diseases (114). Using HR-pQCT at the distal radius and tibia, Stein et al. documented that postmenopausal women with primary hyperparathyroidism (PHPT) have thinner cortices, reduced Tb.BMD and Ct.BMD in comparison with healthy controls; in the PHPT group, ITS analyses documented a large heterogeneity in the...
distribution of the trabeculae and a depletion of plate-like trabeculae, with a lower trabecular plate-to-rod ratio (117). In postmenopausal women with type 2 DM and history of fragility fractures HR-pQCT documented that both cortical bone porosity and pore volume are increased at the distal radius and distal tibia (significantly greater intra-cortical pore volume, relative porosity and endocortical bone surface at the ultra-distal and distal tibia; 4.7-fold greater cortical porosity at the distal radius; higher intra-cortical pore volume and larger trabecular heterogeneity at the ultra-distal radius) (118). The increased cortical porosity and the impaired trabecular microarchitecture among type 2 DM patients could explain, at least in part, the high incidence of fragility fractures, despite the normal or even elevated a-BMD derived from DXA examination (119).

**CT in sarcopenia**

Together with MRI, CT represents the gold standard for investigating BC and to assess quantitative and qualitative changes in muscle mass. Measuring the attenuation of an X-ray beam crossing different tissues, a CT scan can differentiate between fat and fat-free mass. Zero and 100 HU are respectively the upper and lower threshold values commonly used to identify skeletal muscle tissue; a reduced muscle attenuation reflects an increased amount of fat within muscle (120). Normal density muscles show attenuation values in the range of 31–100 HU (within 2 SD of the mean attenuation value observed in lean normal muscle) while low-density muscles show attenuation values in the range of 0–30 HU (121). Quantification of skeletal muscle composition and adipose tissue distribution from different body segments and individual muscle groups can be achieved with a high level of accuracy using dedicated mathematical reconstruction algorithms. Mitsiopoulos et al. reported an excellent level of correlation between CT-derived CSA (cm^2) values of arm and leg adipose tissue-free skeletal muscle (ATFSM), adipose tissue surrounding muscle and adipose tissue embed within muscle (interstitial adipose tissue) and those obtained from cadaveric studies (r=0.97, 0.99 and 0.96, respectively); similarly good results were observed between cadaveric and CT volume estimates for the same three compartments (122). The use of CT to assess skeletal muscle mass is however limited in clinical practice by the high radiation dose, high cost and operational complexity (7). Although it was originally designed to assess bone structure, pQCT can also be used for soft tissue area quantification, being increasingly used to investigate the complex relationships between bone, muscle and fat. Traditionally, pQCT analysis was limited to the peripheral limbs (generally at 66% of the length of the tibia, the area with the largest muscle CSA within the lower leg, and at the mid-forearm), however newer scanners with larger gantries also allow the study of the mid-thigh. As whole-body CT, pQCT can provide CSA of soft tissue and estimate muscle density, while having the important advantages of an extremely low effective dose and relatively low cost (7). Since with pQCT fat is calibrated to zero, typical muscle density ranges from 65 to 90 mg/cm^3 (120). In individuals with a great amount of fat within and between myofibers, density values are lower. Muscle CSA and muscle density can be derived with dedicated manufacturer software after the delineation of the muscle-subcutaneous fat boundary in a semi-automated manner, even if these boundaries may not be immediately apparent (requiring a greater manual intervention to delineate muscle from surrounding fat) (123). An important limitation of pQCT is the lack of homogeneity in the protocols for image acquisition and analysis: the large majority of the studies published in this field examined muscle mass at the 66% tibia and 65% radius sites, but this is not an absolute rule (other studies evaluated for example the 38% or 55% sites) (7).

**MRI**

Unlike CT which uses ionizing radiation to produce diagnostic images, MRI is based on the absorption and emission of radiofrequency energy by hydrogen nuclei (contained in large abundance in water and fat) under the influence of an applied external magnetic field. In the study of BC, variations in radiofrequency pulse sequence are used to differentiate between adipose tissue and fat-free mass; adipose tissue is characterized by a short T1 and a long T2 proton relaxation time (124). The lack of ionizing radiation is one of the major advantages of MRI, making it particularly suitable for long-term follow-up and for monitoring disease progression and treatment efficacy. The opportunity to study the trabecular network extended the use of MRI for the investigation of the mechanisms of age/disease-related bone weakening (38). T1- and T2-weighted imaging, chemical shift imaging, diffusion weighted imaging (DWI), dynamic contrast enhanced perfusion MRI and proton magnetic resonance spectroscopy (H-MRS) are just some of the MRI-imaging based approaches to investigate...
BC and bone status.

**MRI in osteoporosis**

Recent advances in MRI technology have brought significant progress to the study of osteoporotic bone, allowing the assessment of the features of trabecular bone and hence of its biomechanical proprieties (125). The investigation of trabecular network with MRI is however, perhaps, less intuitive than with QCT and HR-pQCT because the physical principles underlying the generation of diagnostic images are complex and completely different from those of X-ray based techniques (126). In MRI, cortical bone appears dark (or as a signal void) due to the very small number of mobile protons and the extremely short T2 relaxation time; the trabecular bone network also appears as a signal void but the surrounding BM gives rise to a high signal, which intensity depends on the amount of fatty content (vs. hematopoietic BM) (102,127). Differences in diamagnetic susceptibility at the interfaces between BM and trabecular bone structure cause a reduction of marrow relaxation time T2*, and the extent of this decay depends on density and microarchitecture of the surrounding trabeculae (126).

Today, high resolution MRI allows the depiction of trabecular bone density and structure in vitro and in vivo with a high spatial resolution (in-plane resolutions as high as 78 μm; slice thickness of approximately 300 μm) (8,102,128,129). If at the beginning, the study of trabecular microarchitecture and cortical bone was limited to peripheral sites (like the radius, tibia and calcaneus), the use of optimized pulse sequences, high magnetic field systems (3 Tesla) and phased array coils led to enhanced SNR, allowing the investigation of deep-seated skeletal sites, such as the proximal femur (130). The potential of MRI as a diagnostic modality for the assessment of trabecular bone without the use of ionizing radiation has to be considered, however some limitations must be taken into account. At resolutions similar to individual trabeculae dimension, partial volume effects may arise; additionally, MRI is time consuming and technically challenging and the MRI protocol best suited for the study of trabecular bone is still being debated (131). Since QCT and HR-pQCT can directly depict the trabecular network, in our opinion, they represent the most suitable techniques for the investigation of trabecular bone; however, comparing high-resolution MRI with QCT and HR-pQCT, recent studies have documented that MRI performs equally well with trabecular bone measurements (129,131,132). Three classes of structural parameters can be derived from MRI, namely, scale, topology and orientation. Scale refers to structural parameters analogous to standard histomorphometry, including apparent BV/TV, apparent Tb.Th, apparent Tb.Sp and apparent Tb.N; topology refers to the structure of the trabeculae themselves that can be “rod-like” or “plate-like”; finally, orientation refers to the degree of anisotropy of the structure (102). Analyzing femoral head specimens, Sell et al. found a high degree of correlation between trabecular bone structure measures obtained by 3T MRI and by micro-CT (μCT), as the standard of reference (r² for App. BV/TV =0.82; r² for App. Tb.Sp =0.84; r² for App. Tb.N =0.81; r² for App. Tb.Th =0.67) (133). MRI-derived measures of trabecular architecture were shown to be distinctively affected by osteoporosis (e.g., high Tb.Sp; low BV/TV, Tb.Th and Tb.N), and may be used, in combination with DXA measures, for an improved prediction of osteoporotic fractures (134-136). Wehrli et al. demonstrated that the conversion of trabecular structure in the distal radius from “plate-like” to “rod-like” and the disruption of rodlike trabecular elements were significantly associated with the extent of vertebral deformities, determined by a semi-automated approach on sagittal MRI sequences (137). MRI also has the ability to depict cortical bone three-dimensionally: among cortical parameters that can be investigated, cortical porosity and Ct.Th are of particular clinical importance, as among cortical parameters that can be investigated, cortical porosity and Ct.Th are of particular clinical importance, as stressed above (102). Recently, ultra high-field (7 Tesla) MRI has shown its potential in the field of research thanks to the high SNR and increased spatial resolution, that allows a more accurate depiction of trabecular bone microarchitecture (138). Using 7 Tesla MRI equipment, Guenoun et al. demonstrated a significant association between bone microarchitecture parameters from cadaveric lumbar spine specimens (e.g., BV/TV; Tb.Th and Tb.Sp measured in axial and sagittal planes) and biomechanical parameters (failure load and the failure stress determined with biomechanical compression test) (139).

Today MAT is consensually recognized as an important component of the BM microenvironment; in particular a negative association exists between MAT content and BMD (140). H-MRS is an interesting extension of diagnostic MRI that can be used not only to quantify MAT content but also to further investigate its composition (141,142). H-MRS-based analysis of MAT composition is an emerging field of research in diabetic patients,
who generally show a lower unsaturated lipid fraction in comparison with healthy controls (29).

**MRI in sarcopenia**

MRI represents the most advanced and most fascinating technique in the study of BC and skeletal muscle. MRI allows not only the quantification of muscle size (CSA, volume), but also the comprehensive assessment of muscle quality (143,144). Similarly to CT, Mitsiopoulos et al. reported an excellent level of correlation between MRI-derived CSA (cm^2) values of ATFSM, adipose tissue surrounding muscle and adipose tissue embed within muscle (interstitial adipose tissue), and those obtained from cadaveric studies (r=0.97, 0.99 and 0.92, respectively); similarly good results were observed between cadaveric and MRI volume estimates for the same three compartments (122). A key advantage of MRI, in comparison with other imaging techniques, is the capability to detect changes in the muscle structure occurring with aging and disease progression. In this regard, abnormal edema and the progressive accumulation of adipose tissue and fibrous connective tissue (both non-contractile tissues) within muscles contribute to loss of muscle strength and quality, which has increasingly been recognized as a critical aspect of aging and sarcopenia (143,144). Two-point Dixon-based technique and chemical shift-based water-fat separation are two quantitative MRI techniques that have been frequently applied to objectively measure muscle fat content (145-147). Finally, the amount of intramyocellular lipid, which is negatively correlated to insulin sensitivity, can be quantified by H-MRS (143,148-150). Over the last few years, many studies have focused on age-related changes in skeletal muscle mass. In a large cohort of healthy adults (men: n=268; women: n=200) Janssen et al. observed that the loss of skeletal muscle mass with advancing age was more relevant in the lower limbs in comparison with the upper limbs for both men and women (151). Using MRI, several studies documented substantial differences in the composition of muscular groups of the lower limbs in the elderly in comparison with young people. Kent-Braun et al. documented a larger contractile CSA of the tibialis anterior muscle in healthy young subjects (25–45 years old) in comparison with older (65–85 years old) adults; in addition, the amount of non-contractile tissue (expressed as absolute and relative non-contractile areas) was smaller in the first group (152). Similarly, Macaluso et al. documented a significantly lower amount of muscle contractile volume and a significantly greater amount of intramuscular non-contractile tissue in older women (mean age: 69.5±2.4 years) in comparison with young women (mean age: 22.8±5.7 years), in both quadriceps and hamstrings (153). In the study by Nilwik et al., quadriceps muscle CSA was 14% smaller in the elderly group compared with young men and this difference was largely explained by a reduction in type II muscle fiber size (29%; P<0.001), with a concomitant tendency for smaller type I muscle fibers (P=0.052) (154).

All these features make MRI (together with CT) the gold standard in investigating muscle mass and quality in a research setting, however high cost, limited access to the equipment and its complexity limit the use of MRI in routine clinical practice (17,50). The lack of a standardized assessment protocol in image analysis represents a strong methodological weakness, limiting comparison between the results of different studies; in particular, tissue segmentation algorithms can vary a lot ranging from a manual segmentation technique to a fully automated method (7).

**Ultrasound**

Thanks to its low cost, portability and the lack of ionizing radiation, ultrasound has gained increasing importance in clinical practice and currently represents an important tool to investigate osteoporosis and sarcopenia (155,156).

**Quantitative ultrasound (QUS)**

QUS represents an effective alternative method for non-invasive evaluation of bone status; this technique analyzes the interactions between ultrasounds (frequency range employed between 200 kHz and 1.5 MHz) and bone tissue and provides insight complementary to that provided by densitometric techniques (157). Whereas bone density methods measure the X-ray attenuation in a given volume of tissue, QUS is based on the interaction and the propagation of ultrasounds (i.e., mechanical waves) across cortical and trabecular bone; QUS parameters reflect the structural anisotropy of bone, allowing to deduce its mechanical proprieties (156). The low cost, the portability and the lack of ionizing radiation represent the major advantages of QUS. Unfortunately, reproducibility of the results is a major concern and comparing measurements is difficult and sometimes misleading (156). The commercially available devices show great technological diversity (larger than what is commonly found in DXA) and employ different methods for calibration. For these reasons, according to the ISCD official positions, results from different devices cannot be...
directly compared (158).

QUS specific measures of bone status include speed of sound (SoS), a parameter closely related to bone mineralization, and broadband ultrasound attenuation (BUA), which is closely related to structural characteristics of trabecular bone (104,157). A strong level of correlation has been found between heel trabecular transmission parameters (SoS and BUA) and BMD derived by DXA at lumbar spine and femoral neck (159,160). Other more complex parameters can be derived from the combination of SoS and BUA, such as amplitude dependent speed of sound (AD-SoS), stiffness and quantitative ultrasound index (QUI) (157,161). QUS is generally applied to peripheral skeletal sites, including the distal metaphysis of the phalanx, calcaneus, radius and tibia; however, the only validated measurement site in osteoporosis management is the heel. Validated heel QUS devices have been proved to predict fragility fractures in postmenopausal women (hip, vertebral, and global fracture risk) and in men over 65 years of age (hip and all non-VFs), independently of central DXA BMD (158).

QUS for the study of osteoporosis is completely validated and supported by scientific evidence. According to ISCD official positions, even though DXA remains the method of choice in clinical practice for therapeutic decisions, if a DXA scan cannot be performed, pharmacologic treatment can be initiated on the basis of a sufficient high fracture probability, assessed by heel QUS (using device specific thresholds) in conjunction with clinical risk factors (158). In particular, age over 75 years, BMI <20 kg/m², history of previous fractures after the age of fifty or falls within the last 12 months, maternal history of hip fracture, current smoking, DM, previous/current glucocorticoid treatment and use of arms to stand up from a chair are the clinical risk factors to take in account for decision making (158,162,163).

Ultrasound-derived measurements of muscle mass have shown a good-to-high level of correlation with those derived from reference methods (155,166-169). In 2005, Sanada et al. developed regression-based prediction equations for estimating total and regional skeletal muscle mass in healthy Japanese adults using ultrasound-derived muscle thickness taken at nine sites (lateral forearm, anterior and posterior upper arm, abdomen, subscapula, anterior and posterior thigh, anterior and posterior lower leg); a significant and strong site-matched correlation was found with MRI measured muscle mass (r=0.83–0.96 in men, r=0.53–0.91 in women, P<0.05) (170). Muscle thickness, CSA, echo intensity, fascicle length and pennation angle of the lower limbs (as sarcopenia preferentially affects postural muscles of the lower limbs such as the quadriceps femoris and gastrocnemius medialis) are the parameters most commonly evaluated by ultrasound examination; in penna muscles, the pennation angle (defined as the angle formed at the attachment site of the fibers into deep and superficial aponeurosis) can be evaluated in static and dynamic conditions and provides information about mechanical and contractile proprieties (171). All of these parameters are affected by aging to a different extent but need to be further validated before their inclusion in diagnostic algorithms (155). The large majority of the available studies were conducted with small samples of healthy patients and no validated site-specific cut-off points for the ultrasound-based assessment of low muscle mass in geriatric patients exist. Minetto et al. were the first to collect and report muscle specific cut-off values for the detection of low muscle mass in the elderly (enrolled from institution-dwelling subjects with ≥1 of Fried’s frailty criteria) (172).

**Quality assurance (QA) procedures**

QA is defined as “a programme for the systematic monitoring and assessment of the various aspects of a service or facility to ensure that standards of quality are being met” (173). A QA program consists of five different parts: (I) guidelines and requirements for the correct installation of the facilities and their clinical use; (II) standardized periodic instrument quality control (QC) and technologists’ QC; (III) preventive assessment of the condition of devices; (IV) training and education of technicians and radiologists performing and reporting the examination; (V) radiation safety policies and procedures (173,174). These aspects are of clinical importance in the management of osteoporotic patients and in the study of BC. QA procedures have been developed for...
DXA, QCT, pQCT, HR-pQCT, MRI and QUS to ensure that accuracy and precision of the devices are consistent with manufacture guidelines (174,175). Accuracy is by definition the ability of a measurement to match the actual value of the quantity being measured and it is expressed in terms of accuracy error; precision, expressed in terms of PE, refers to the degree of reproducibility of repeated measurements under unchanged conditions (176).

To determine the accuracy of a DXA densitometer, a scan of a phantom of known density (density range and size of normal human spine) should be performed as often as possible (optimally three times a week, in any case, no less than once a week) to assess system calibration; phantom scanning and calibration should be repeated after any service is performed on the densitometer (65,173,174). PE of a DXA examination derives from the summation of inherent machine fluctuations and inaccuracies of the technologists performing the examination; the least significant change (LSC) enables the identification of statistically significant changes in BMD over time and is essential to make sure that any change observed in BMD measurement is real and not due to machine or to operator variability (176); the LSC can be calculated from PE using the ISCD precision calculating tool. Since the PE supplied by the manufacturer is not representative in general of the PE in the clinical setting, the ISCD recommends that each DXA center should determine its PE and LSC in vivo; if two or more technologists work on the same DXA scan, an average PE should be determined, combining data from all technologists (65). According to the ISCD, the minimum acceptable PE for an individual technologist (expressed as coefficient of variations (CV%) is, 1.9% at the lumbar spine, 1.8% at the total hip and 2.5% at the femoral neck; if the PE of a technologist exceeds these thresholds, retraining is required. In order to compare quantitatively to prior machine results, a cross calibration of DXA systems is required when (I) the DXA hardware has been changed, (II) when replacing a system with the same technology and (III) when changing the entire system to one made by the same manufacturer or different manufacturer (65).

One important advantage of BMD estimation by digital DXR is the high precision, which makes the technique particularly feasible for longitudinal studies. The short-term DXR precision has been shown to be superior to those of DXA at the hip and spine in both pre- and post-menopausal women and in both normal and osteoporotic subjects (46,48,177,178); in particular for DXR-BMD derived directly from digital hand radiographs (direct DXR), in vitro precision (expressed as CV%) ranged from 0.14% to 0.30%, while the in vivo precision for mean values of both hands was 0.46% (179). This means that DXR has the ability to detect any significant change in BMD in a very short period of time (thus to identify the progression of the disease or the response to treatment) and this supports the role of DXR-BMD as an important clinical tool for assessment of disease activity in rheumatoid arthritis (43,179).

ISCD recommendations are also available for DXA total BC with regional analysis; in this case the QC program should include adherence to manufacturer guidelines for system maintenance and, if not recommended in the manufacturer protocol, a BC phantom scan should be performed at least once a week to assess system calibration. Each technologist should perform an in-vivo precision assessment using patient’s representative of the clinic’s patient population; the minimum acceptable PE for an individual technologist is 3% for total FM, 2% for total LM and 2% for percent FM. In-vivo cross-calibration and cross calibration with a whole-body phantom are necessary to compare in-vivo results across different manufacturers and systems of the same make and model, respectively (65).

According to the ISCD official positions the following general recommendations should be followed for QCT, pQCT and QUS: (I) bone density measurements from different devices should not be directly compared; (II) device specific training and education of technicians and radiologists performing and reporting the examination should be provided; (III) QC procedures should be performed regularly (65).

QA of QCT systems represents as a main topic of investigation and recommendations for equipment specifications, equipment QC tests and radiation safety have been developed (174,180). PE depends on many factors including scanner model, scanning method, operator’s skill, patient’s positioning, ROI’s positioning and collaboration of the patient during the examination; for these reasons, each QCT-center should perform a precision test to determine its PE and calculate LSC (174). In particular, the main source of error in determination of both Ct.BMD and Tb.BMD is represented by the partial volume effect. The presence of MAT results in an underestimation of bone density; the effect of marrow fat is more relevant at the spine and at the hip than at peripheral sites and is responsible for an accuracy error in the range of 5–15%, depending on age bands. This problem can be solved using age-related reference databases or, when available, using dual-energy QCT that recently gained popularity thanks
to the possibility to explore age, disease and drug-related effects on MAT (180). Finally, precision is enhanced with the use of 3D multidetector QCT in comparison with single slice 2D QCT technique, which is highly operator dependent (180).

Movement artifacts represent a significant source of error also in HR-pQCT examinations: although these errors can be minimized providing detailed instructions to the patients and by forearm or lower leg fixation, they can’t be completely removed. The reproducibility of repeated measurements is generally affected by errors in repositioning (181). Recently, MacNeil et al. performed cadaveric forearm scanning, with and without repositioning, to determine the inherent reproducibility of the HR-pQCT technique (standard evaluation protocol based on the 2D area matching method); in the best scenario (without repositioning) the reproducibility measured in percent root mean squared (RMS) coefficient of variance was found to be less than 0.3%, while the reproducibility with repositioning error was less than 1.5%. The in vivo reproducibility of density, morphological and stiffness measurements was found to be less than 1%, 4.5% and 3.5%, respectively. When the standard evaluation protocol was repeated on the 3D registered data, repositioning error was reduced on average by 23% and 8% at distal radius and tibia, respectively (181). Similarly to QCT, daily and weekly QC programs should be conducted to identify drift, which can result as a consequence of decreased X-ray emission (109).

The reliability of pQCT for the analysis of muscle mass has been well established. For DXA, the ISCD recommends to assess the PE using the RMS approach from an assessment with 30 degrees of freedom to ensure the calculated PE is statistically accurate and unbiased, in order to identify the LSC (182). The same procedure should be used to assess the PE for pQCT (183). In the study by Swinford et al., the overall relative PE calculated by the RMS method for pQCT measures (absolute and relative muscle and fat CSA, and muscle density) at 66% of tibial length was <1.5% and 3.0%, respectively for muscle and fat (184).

In ultrasound examinations, PE results from different factors, including incorrect positioning of the patient, coupling between the transducer and the skin and the effect of soft tissue properties (174). A constant check of the correct functioning of the probes and calibration of ultrasound signal emitted are mandatory to ensure the reliability of the technique (157,185). Each device has a specific calibration procedure and requires its own calibration phantom (generally composed of plastic material or Plexiglas) for QC (157). The ambient temperature itself can play a major role, influencing the coupling between probes and measurements sites. This is of particular importance in calcaneus devices that use water as coupling medium (186,187). In addition, the cross calibration of different devices represents an important concern in multicenter clinical studies and, even if many QUS models are currently available, their scientific validity was confirmed only in a small minority of cases (157). For all these reasons, the ISCD recommends not to use QUS to monitor the skeletal effects of treatments for osteoporosis (65). As stressed above, in the assessment of skeletal muscle mass and quality the lack of standardized protocols in image acquisition and image analysis and the operator dependence of ultrasound measures strongly limit the comparison of the results from different studies. Strasser et al. documented that ultrasound-derived measurements of muscle thickness assessed separately at the four heads of the quadriceps muscle in both young and elderly subjects were highly reproducible (intraclass correlation coefficients: 85–97%); on the contrary the reproducibility of echogenicity measurements differs between young (intraclass correlation coefficients: 57–65%) and old patients (intraclass correlation coefficients: 20–31%) (188).

MRI is affected by multiple technical and image quality problems and, even if several organizations suggested their QA recommendations and each manufacturer usually uses its own test protocols, the development of universally accepted guidelines would be desirable in MRI (175,189-191). To ensure high quality in radiology practice, the American College of Radiology (ACR) has proposed an accreditation program (i.e., a survey instrument to determine if standards are met) for MRI. The ACR method provides a comprehensive and easy to perform assessment of the performance of an MRI scanner and proved to be feasible in QA procedures (175,192). Its main advantages include the definition of a standardized image quality measurement protocol and the use of a standardized phantom developed specifically for the ACR to assess the technical quality of MRI sequences. The following technical parameters are evaluated for the assessments of MR image quality: high-contrast resolution, slice-thickness accuracy, geometric accuracy, slice position accuracy, signal uniformity, percent signal ghosting and low-contrast delectability (193).

**VFAs assessment**

As discussed, above the increased fracture risk represents the main clinical outcome of osteoporosis. In particular,
VFs represent the hallmark of osteoporosis, accounting with a prevalence of about 35% to 50% among women over 50 years of age (194). VFs frequently occur in absence of a major trauma and are often asymptomatic (195,196). Despite this, the occurrence of a VF is very important in the management of osteoporotic patients since it results in an increase in the risk of experiencing a new incident VF and other fragility fractures. Women with a pre-existing VF suffer a 4- to 5-fold increase risk of sustaining a new incident VF; this risk increases with the number of prevalent fractures at the baseline and was proved to be BMD-independent (194,197). Conventional radiography and DXA represent the techniques of choice for VFs detection (198-200). Radiologic methods for the identification and the scoring of VFs include quantitative morphometry (QM), the visual semiquantitative (SQ) method and the “algorithm-based qualitative” (ABQ) method (8). QM was introduced in order to obtain an objective and reproducible evaluation of vertebral deformities based on the measurement of vertebral body heights. QM may be performed on conventional lateral spinal radiographs or on lateral spine views acquired by DXA; the densitometric approach is commonly termed as vertebral fracture assessment (VFA) (Figure 5) (201). Typically, the center of the superior and the inferior end plates and the corner points of each vertebra from T4 to L4 are identified and then these six points are used to calculate the anterior, the middle and the posterior heights (Ha, Hm and Hp, respectively) of each vertebra and the three height ratios (Ha/Hp for wedge fracture; Hm/Hp for biconcavity fractures; ratio between Hp of adjacent vertebrae for compression fractures) (Figure 6) (38). Today, the six points placement is performed automatically with the use of a dedicated computer-assisted system to avoid inter observer variability, but manual corrections are possible at any time during the post processing of the digital images (202). Point placement may be challenging due to normal variation in the shape of vertebral bodies and projection variations; moreover, QM does not allow to distinguish between real VFs and vertebral deformities (e.g., Scheuermann’s disease, Schmorl’s node, Cupid’s bow deformity, etc.) (38,203).

Unfortunately, at the current state of investigation, there is no gold standard for the definition of VFs and the exact degree of deformity that constitutes a fracture is still a matter of debate (203,204). In clinical trials, prevalent VFs are typically defined as a reduction of 3 SD or more (below the normative reference values for that particular vertebra) in any of the ratios of anterior, middle and posterior heights. In serial radiographs an incident fracture (i.e., a new VF) is defined both as an absolute change in vertebral height of at least 4 mm or as a percentage of reduction (15% or 20% reduction, depending on different studies) in the anterior, middle, or posterior heights from the baseline measurements (203,205).

The predominant approach for diagnosis of VFs is represented by the visual SQ method proposed by Genant et al. (206). On lateral spine images, VFs are identified on the basis of the apparent degree of vertebral

**Figure 5** VFA on lateral DXA scan. The placement of six vertebral points to determine vertebral heights is automated and the operator only needs to check for their correct positioning. VFA, vertebral fracture assessment; DXA, dual energy X-ray absorptiometry.

**Figure 6** Morphometric analysis of DXA spine image with accompanying chart (on the top right). The magnified view (on the bottom right) illustrates moderate wedging of the T9 vertebral body. DXA, dual energy X-ray absorptiometry.
height loss (anterior, middle and/or posterior height) and/or projected area reduction estimated by visual inspection, without direct measurements (204,206). Vertebrae from T4 to L4 are graded as normal/not fractured (grade 0), mildly deformed (grade 1: 20–25% reduction in height; 10–20% reduction of projected vertebral area), moderately deformed (grade 2: 25–40% reduction in height and 20–40% reduction of projected vertebral area) and severely deformed (grade 3: >40% reduction in any height and projected vertebral area) (206). However over time, the area reduction requirement was excluded from the examination (205). Since a linear relationship exists between the number and severity of prevalent VFs and the risk of future VFs, a spinal deformity index (SDI) can be derived from this scoring system, resulting from the sum of all grades assigned to the vertebrae divided by the number of evaluated vertebrae (38,197). The SQ method has been extensively validated and according to the ISCD official positions it represents the technique of choice for diagnosing VFs with VFA (207,208,209). Its major strength relies on the visual approach that, in experienced hands, allows to discriminate fractures from non-fracture deformities (208,209). However, a critical point of this method is represented by the accurate estimation of vertebral height reduction, especially when the degree of this reduction is close to the thresholds of the grading system (204). In light of the above issues, we believe that an integrated approach may be preferable for defining and reporting VFs. In particular, the use of QM should be encouraged to objectively graduate VFs, previously identified by the SQ method, in epidemiological studies and follow up evaluations (202).

The ABQ is an alternative visual approach, recently developed and still waiting further validation for clinical practice. This method emphasizes the importance of visual inspection of the vertebral endplates to improve specificity in VFs detection (39). In the ABQ method the diagnosis of osteoporotic fractures is made on the basis of specific endplates abnormalities with depression of the central vertebral end-plate, regardless of vertebral height reduction; when a vertebra is reduced in height without endplate irregularities and texture changes below endplate (due to microfractures), this is classified as non-osteoporotic short vertebral height (SVH) (210). Using this method, the prevalence of morphometric VFs could drop approximately of 30% (39). Jiang et al. documented a relatively poor agreement between SQ and ABQ methods for the identification of VFs (κ =0.36) (211).

**“Incidental” identification of VFs**

A discussed above, VFs frequently occur in absence of a major trauma and often remain asymptomatic; thus the majority of VFs are not clinically recognized at the time of their occurrence (212). Incidental diagnosis of VFs refers to the fortuitously identification of VFs in imaging examinations performed for other clinical purposes, such as chest and abdominal radiographs, CT and MRI that include visualization of the spine in their field of view (213). This represents a massive opportunity to gain additional information without the need for further scanning or radiation exposure, improving the management of patients at high risk of future osteoporotic fractures; nonetheless, there is significant underreporting of such fractures by radiologists (214). A possible explanation of this underreporting could be that, in reviewing CT and MRI performed for other clinical indications, intra-thoracic and/or intra-abdominal pathologies represent the main focus of the observer’s attention; for this reason, a constant retraining of radiologists to emphasize the importance of VFs is required (214,215). Multidetector spiral CT of the thorax and abdomen is perhaps the most useful method for incidental VFs identification: on the midline sagittal CT reformations, the central area of the vertebral body end plate (the site where all VFs occur first) can be accurately analyzed allowing the detection of VFs not always evident on the transverse sections (214). Due to the growing number of CT and MRI examinations being routinely performed, the possible role of initial localizer views of CT (scout-CT) and MRI (MR-loc) for the detection of incidental VFs has recently been evaluated (195,213,216). The lateral scout CT view, preliminary acquired to define the anatomical area to be scanned, proved to be suitable to assess VFs (214,217). Intra-observer and inter-observer agreement based on a semiquantitative method are fair to good, with fractures of mild severity and fractures at the upper (T4–T9) thoracic levels representing the main sources of error (217,218). MR-loc images are a set of three-plane (axial, coronal and sagittal), low-resolution and large field-of-view images that serve to define the exact position and angulation of slices of MRI sequences; despite the limited image quality, MR-loc should be routinely scrutinized to search for incidental VFs that can be further confirmed by subsequently acquired T2-weighted sagittal images (213,214). MRI features also allow to discriminate between benign and malignant VFs and for the correct identification of recent and old vertebral compression fractures (219).
Conclusions

Bone and muscle are two organs deeply related both in function and development, that conceptually constitute a unit, with interactions that take place at different levels from molecular to organic in a bidirectional way. Mechanical loading and muscle contraction are the most important determinants of bone mass and shape, with PA known to exert an anabolic effect on the metabolism of both bone and muscle. The development and the maintenance of bone mass and muscle mass are deeply related and both are affected by age and metabolic diseases.

Osteoporosis and sarcopenia represent two widespread diseases of increasing public interest, especially in the context of the rise of life expectancy, and represent an important area of geriatric and nutritional research. This review presents the different imaging modalities available for a non-invasive investigation of bone and muscle mass and quality in osteoporosis and sarcopenia, with special emphasis on the clinical application and limitations of each technique, and their potential complementary roles. In this regard, DXA is the most frequently utilized quantitative technique to investigate bone and skeletal muscles status in clinical practice, thanks to its wide availability, cost-effectiveness and low dose of ionizing radiation. In the field of osteoporosis, additional parameters, beyond BMD, can be extracted from DXA images; TBS and HAL have been extensively evaluated in research setting and have the potential to improve fracture risk prediction accuracy. The potential value of ultrasound for the prediction of fracture risk (QUS at the heel) and for the evaluation of muscle tissue has been well documented; however, the low reproducibility and difficulties in comparing measurements strongly limit its use. At the current state of knowledge, none of the operative definitions of sarcopenia include ultrasound in its diagnostic algorithm. Even if currently technically challenging and not widely available, in the future high-resolution imaging techniques will continue to expand our knowledge of the age, disease and treatment related changes that occur in bone and skeletal muscles.

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Footnote

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