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RESEARCH TOPICS

PHYSIOLOGY AND PATHOPHYSIOLOGY OF MUSCULOSKELETAL AGING

Topic Editors
Ali Mobasheri and
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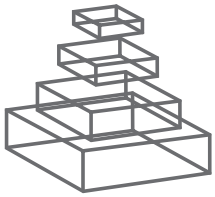

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PHYSIOLOGY AND PATHOPHYSIOLOGY OF MUSCULOSKELETAL AGING

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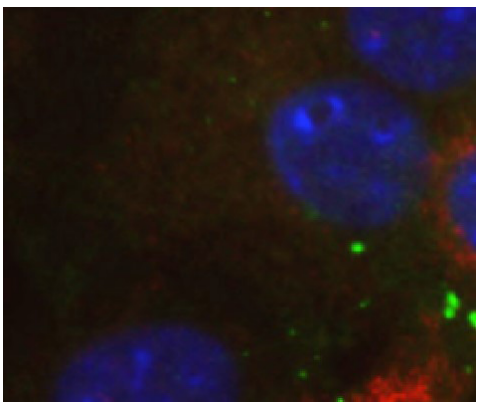

Immunofluorescence of Chondrocytes in Culture. Two cultured OA chondrocytes showing the Gap Junction Connexin 43. Connexin 43 is involved in chondrocyte communication and its expression is increased in OA chondrocytes and cartilage. Credit: Francisco J Blanco/Instituto de Investigacion Biomedica da Coruña, Hospital Universitario A Coruña, A Coruña, Spain

We live in a world with an ever-increasing aging population. This aging population is predicted to place a huge financial burden on healthcare systems around the world. Understanding healthy ageing is a key research priority, along with a better understanding of the pathophysiology of ageing that occurs in a number of age related diseases, such as arthritis. By gaining a better understanding of healthy musculoskeletal ageing we can provide better care and new therapies for common musculoskeletal problems. This Research Topic is intended to bring together basic researchers and clinicians working in the broad area of musculoskeletal ageing. The topic includes mechanisms of healthy ageing in the musculoskeletal system, which we define as skeletal muscle and the synovial joint, particularly constituent structures including articular cartiwlage, subchondral bone tendon and ligament. A particular focus of this Research Topic is dietary modulation of musculoskeletal ageing.

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Application for proteomic techniques in studying osteoarthritis: a review

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After the genomic era, proteomic corresponds to a wide variety of techniques that study the protein content of cells, tissue, or organism and that allow the isolation of protein of interest. It offers the choice between gel-based and gel-free methods or shotgun proteomics. Applications of proteomic technology may concern three principal objectives in several biomedical or clinical domains of research as in osteoarthritis: (i) to understand the physiopathology or underlying mechanisms leading to a disease or associated with a particular model, (ii), to find disease-specific biomarker, and (iii) to identify new therapeutic targets. This review aimed at gathering most of the data regarding the proteomic techniques and their applications to osteoarthritis research. It also reported technical limitations and solutions, as for example for sample preparation. Proteomics open wide perspectives in biochemical research but many technical matters still remain to be solved.

Keywords: proteomic, osteoarthritis

INTRODUCTION

OSTEOARTHRITIS

Osteoarthritis (OA) is a progressive disorder characterized by the destruction of articular cartilage accompanied by subchondral bone sclerosis and synovial inflammation. Pain is the main symptom of OA. In the early stages of disease, pain occurs only when the joint is required. In the later stages of disease, pain is felt not only at every movement and but also at rest. The movements of the involved joints are limited. It installs a very debilitating functional impairment for the patient. Physical inactivity is settled down with its own set of physiological and psychological impacts. OA is the major cause of physical disability in people over 50 years of age, excluding traumatic causes. After 65 years, 70–80% of individuals show radiological signs of OA in at least one joint.

Multiple causes lead to OA development. Genetic predispositions were identified in specific hand or hip localization of OA (Hunter et al., 2004; MacGregor et al., 2009) or in generalized OA (Miyamoto et al., 2007; Evangelou et al., 2009) and could be implicated in 15% of OA (Felson, 2010). Aging, obesity, and being a female are well-known risk factors of OA but others parameters implicated in the physiopathology of the disease remain to be detailed. In recent years, an association between diabetes mellitus, metabolic syndrome, and other conditions characterized by impaired glucose metabolism have also been postulated to be associated with OA (Burner and Rosenthal, 2009; Rosa et al., 2009, 2011; Berenbaum, 2011).

Currently, the diagnosis of OA is based on symptoms and radiological signs which occur late during disease progression. More specifically, diagnosis is based on cartilage integrity. However, articular cartilage is invisible on radiographs and must be assessed indirectly by the joint space width corresponding to the spacing between subchondral bone ends in a joint. This method does not allow detection of early structural damage, and its use in follow-up of the disease is not recommended. Moreover, the diagnosis of OA occurs with the appearance of pain, when cartilage degradation is often already advanced. Patients are therefore treated for symptoms with anti-inflammatory drugs and analgesics because no disease-modifying drugs (DMOAD) are currently available.

PROTEOMICS

Proteome was originally defined as the complete protein content of a cell, a tissue or an organism. The term was proposed by Wilkins in 1994 (Wilkins et al., 1996). Because protein expression is dependent on environmental conditions, thus making the proteome a

Abbreviations: 1DE, one-dimensional electrophoresis; 2DE, two-dimensional electrophoresis; 2D-DIGE, two-dimensional difference gel electrophoresis; CIA, collagen-induced arthritis; COMP, cartilage oligomeric matrix protein; CRP, C-reactive protein; DMOAD, Disease-modifying OA drug; HPLC, high performance liquid chromatography; ICAT, isotope-coded affinity tag; IL, interleukin; iTRAQ, isobaric tag for relative and absolute quantitation; LC, liquid chromatography; LRG, leucine-rich alpha 2 glycoprotein; MALDI, matrix-assisted laser desorption/ionization; MIAs, miscellaneous inflammatory arthritides; MMP, matrix metalloproteinase; MS, mass spectrometry; MRM, multiple-reaction monitoring; OA, osteoarthritis; RA, rheumatoid arthritis; ReaA, reactive arthritis; ROS, reactive oxygen species; RT–PCR, reverse transcriptase and polymerase chain reaction; SELDI, surface-enhancer laser desorption/ionization; SOD, superoxide dismutase; TIINE, new epitope of type II collagen; TLR, toll-like receptor; TNF, tumor necrosis factor; WB, western blot.

very dynamic structure, the original definition was specified: the proteome is the complete protein set of a cell, tissue, or organism considered at a particular moment, in a specific environment. Succeeding to the genomic era, this direct evaluation of protein expression is essential for the analysis of biological system. Indeed, there is no correlation between protein abundance and mRNA levels (Gygi et al., 1999a). This was illustrated by Lorenz et al. (2003a) who have shown a poor correlation between the transcriptome and the proteome modifications in synovial tissue from OA and rheumatoid arthritis (RA) patients. Furthermore, post-translational modifications bring supplementary variations in the proteome that cannot be analyzed at the gene level.

The principle of the method relies on the separation of proteins and their further analysis using either gel-based or gel-free methods. Protein separation methods are coupled to spectrometer for identification by mass spectrometry (MS; **Figure 1**). Several modes of analysis are available in MS. They differ markedly by the ionization source of the sample. The main sources used in proteomic analysis are matrix-assisted laser desorption/ionization (MALDI) and surface-enhancer laser desorption/ionization (SELDI). These techniques allow a soft ionization of molecules without excessive fragmentation, making the analysis of proteins possible. In the MALDI, the sample is co-crystallized with the matrix and then deposited on a metal support. The source of ionization is a nitrogen laser that bombards the sample. The energy transmitted by the laser is absorbed by the matrix and the input of energy causing it expands in the gas phase with the molecules contained in the sample. MALDI ion source is mainly coupled to an analyzer or time-of-flight (TOF). Its speed, sensitivity, simplicity, and reproducibility make it a very powerful technique for the detection and identification of proteins. However, in many diseases, molecules of interest are often present in very small quantities making them difficult to detect. The SELDI-TOF technology is based on the retention of proteins to small chromatographic surfaces treated to selectively adsorb proteins based on their physicochemical properties. This technique simplifies the protein mixture to be analyzed. Many types of samples such as biological fluids and cell extracts, or cell lysates and histological sections can be analyzed. The proteins of interest are coated to allow their crystallization and facilitate the desorption and ionization generated by the laser in the drive. In single MS mode, the spectrometer quantifies a protein/peptide and determines its structure and its molecular mass. In tandem mode (MS/MS), ions from the first fragmentation are selected and fragmented, allowing identification of the protein/peptide present in the sample.

Applications of proteomic technology may concern three principal objectives in several biomedical or clinical domains of research as in OA: (i) to understand the physiopathology and underlying mechanisms leading to a disease or associated to a particular model, (ii), to find disease-specific biomarker, and (iii) to identify the targets of new therapeutic and their mechanisms of action. Different technologies are available and appropriate to be used in these aspects of investigation. Here, we present different technologies used in proteomic analysis and their main applications and scientific contributions in the field of arthritis research (**Table 1**).

GEL-BASED METHODS

TWO-DIMENSIONAL ELECTROPHORESIS-BASED METHODS

Two-dimensional electrophoresis (2DE) is the standard proteomic method. It is widely used to compare the proteome of two samples or more. In this technique, proteins extracted from various samples are separated according to their isoelectric point in first dimension and depending on their molecular mass in the second

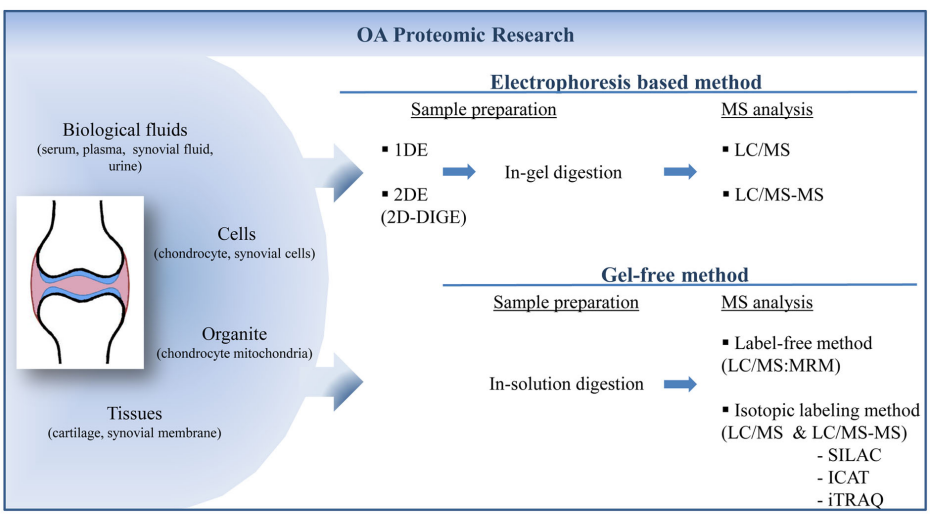

FIGURE 1 | Proteomic strategies to quantify and identify biomarkers of osteoarthritis from different kind of samples. Proteins extracted from samples could be separated by electrophoresis in one (1DE) or two (2DE) dimensions. Proteins are labeled before or after gel migration to perform quantification. After in-gel digestion proteins are identified by mass spectrometry. Gel-free methods involve the separation of digested peptides which could be quantified directly by mass spectrometry analysis. 1DE, one-dimensional electrophoresis; 2DE, two-dimensional electrophoresis; 2D-DIGE, two-dimensional-differential in-gel electrophoresis; LC, liquid chromatography; MS, mass spectrometry; MRM, multiple-reaction monitoring; SILAC, stable isotope labeling by amino acid; ICAT, Isotope-coded affinity tag; iTRAQ, isobaric tag for relative and absolute quantitation.

Table 1 | Identification of protein in OA samples with proteomic techniques.

Reference	Sample	Technique	Results
Sinz et al. (2002)	Plasma and synovial fluid	2DE	Identification of fibrinogen beta chain degradation products in synovial fluid of RA, OA, and ReaA patients; identification of calgranulin B and C as biomarker of RA in synovial fluid; identification of serum amyloid A as biomarker of RA in both plasma and synovial fluids
Hermansson et al. (2004)	Cartilage explant	2DE	Increase in type II collagen synthesis, presence of regulatory proteins as activin A, connective tissue growth factor and cytokine-like protein C17
Catterall et al. (2006)	Chondrocytes in monolayer	2DE	Beta-2-microglobulin, S100A11, matrix MMP-1 and -3, peroxidin 1, YKL40, cyclophilin A, transthyretin, and cofilin
Ruiz-Romero et al. (2008)	Human OA chondrocytes in monolayer	2DE	Twenty-eight proteins altered by OA, 19 of them increased and 9 of them decreased Increase of four proteins (GRP78, HSP90β, GSTO1, and ANXA1) confirmed by immunoblotting and immunohistochemistry
Ruiz-Romero et al. (2009)	Human OA chondrocytes in monolayer	2DE–DIGE	Specific pattern of expression of mitochondria
Lambrecht et al. (2008)	OA chondrocytes in alginate beads	2DE, Sypro ruby staining	Differential expression of proteins in the intact and damaged zones of cartilage. Identification of vimentin and cofilin
Wilson et al. (2008)	Mouse cartilage explants	1DE + 2DE–DIGE	Identification of differentially abundant proteins in media of explants control or treated with interleukin-1 alpha or all-trans-retinoic acid
Xiang et al. (2004)	Chondrocyte lysate	2DE	Triosephosphate isomerase in 25% of OA samples
Stevens et al. (2008)	Cartilage explants	1DE	Identification of new cartilage proteins: CD109, platelet-derived growth factor receptor-like, angiopoietin-like 7, and adipocyte enhancer binding protein 1. Release of type VI collagen, COMP, and fibronectin after compression
Haglund et al. (2008)	Rat chondrocytes	1D-LC–MS/MS	TLR activation after LPS stimulation
de Seny et al. (2011)	OA serum	SELDI	Identification of 4 potential biomarkers: V65 vitronectin fragment, C3f peptide, CTAP-III, and m/z 3762 protein
Kamphorst et al. (2007)	OA synovial fluid	NanoLC–MS	Peptide profiling
Nemirovskiy et al. (2007)	Cartilage explant	LC–MS/MS	TIINE identification
Nemirovskiy et al. (2010)	Synovial fluid and serum	LC–MS/MS	TIINE measurement
Ji et al. (2010)	Model of mesenchymal stem cell differentiation	iTRAQ	Identification of 1756 proteins 100 of them were modified in abundance between chondrogenic differentiated and undifferentiated stem cells. Validation of six modifications by western-blotting
Dean and Overall (2007)	Fibroblasts	iTRAQ + ICAT	MMP-2 degradome
Polacek et al. (2010a)	Cartilage explants and chondrocytes	SILAC	Identification of the secretome
Henrotin et al. (submitted)	Urine	2D-DIGE	Thirteen proteins identified. Focus on fibulin-3 specific sequences

dimension on a polyacrylamide gel. Traditionally, proteins were stained by silver nitrate, Coomassie blue, or fluorescent dye. After an in-gel trypsin digestion, the identity of proteins is determined by MS/MS.

Several studies described below used 2DE in the context of OA research. However, this traditional method showed some limitations in term of reproducibility. Indeed, gel to gel variation leading to significant variability is classically observed. This issue makes difficult to distinguish between the system variations and the induced biological changes. Moreover, available staining methods such as silver nitrate or Coomassie blue, lack of sensitivity and have a poor dynamic range, then limiting the quantitative performance of the technique.

To overcome these technical limitations, 2DE has recently been improved by the introduction of labeling before the migration of proteins, allowing a simultaneous migration of different samples on a single polyacrylamide gel. Two-dimensional difference gel electrophoresis (2D-DIGE) methodology is a powerful tool for the investigation of protein expression profiles in multiple sets of samples (Unlu et al., 1997; Marouga et al., 2005). Samples can be individually labeled with Cy3 or Cy5 CyDye DIGE Fluors, whereas Cy2 CyDye DIGE Fluor is used to label a pooled sample comprising equal amounts of each sample, then acting as an internal standard (**Figure 2**). Interesting spots with differential fluorescent intensity between Cy3 and Cy5 are removed from the preparative gel after post-staining with Coomassie Blue in order to allow proteins identification by MS analysis.

In the context of OA research, 2DE was used to find disease-specific proteins by comparing the protein content of biological fluids, cells (chondrocytes), or other biological material such as tissues (cartilage, synovial membrane) collected from patients and healthy controls. Descriptive applications were also performed to gain insights into arthritic diseases.

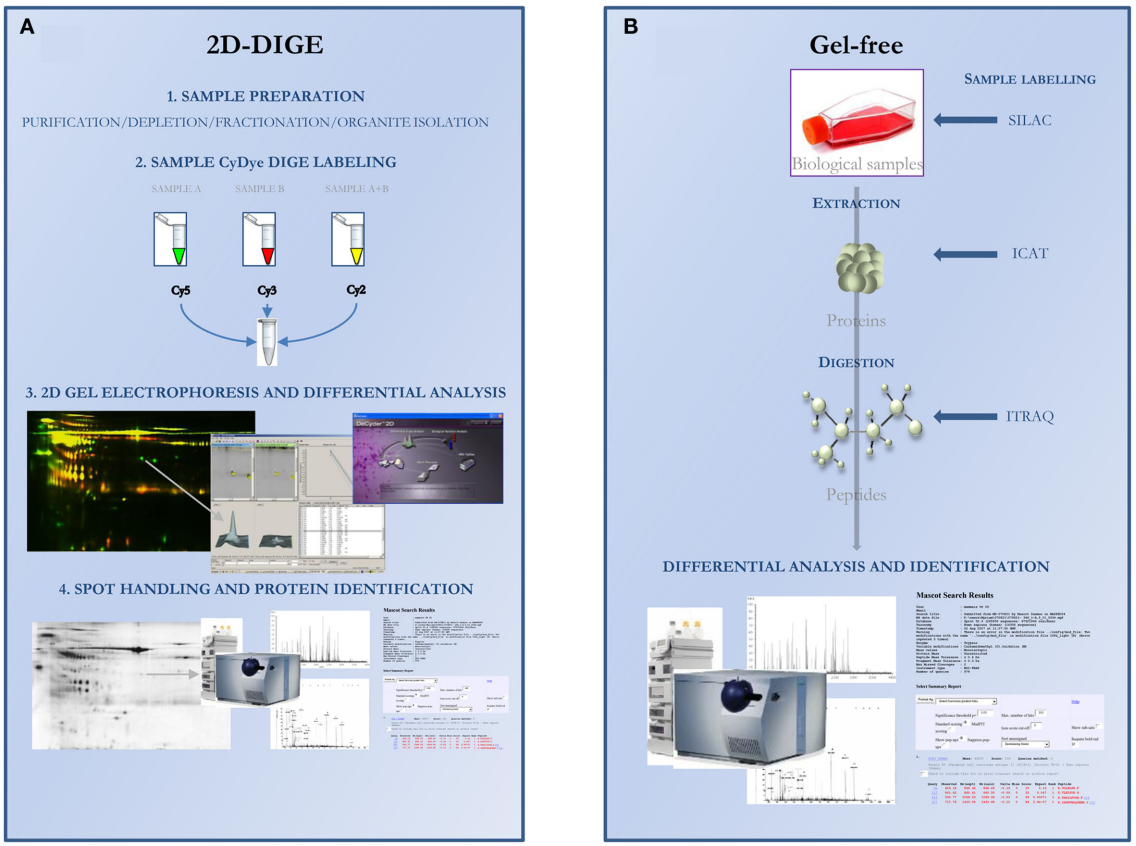

FIGURE 2 | Overview of the proteomic workflow using 2D-DIGE (A) or gel-free (B) approaches for differential analysis. (A) 2D-DIGE approach (1) Proteins are extracted from sample which could be optimized for complexity reduction or particular protein identification; (2) Three fluorescent dyes were used. Samples A and B were fluorescently labeled with either Cy3 or Cy5, and a pooled internal standard is labeled with Cy2; (3) Samples are mixed and resolved in the same 2DE gel. Protein spot pattern could be visualized for each dye by selection of specific wavelength. 2D images are analyzed by specific software and internal standard is used for normalization; (4) Differentially expressed protein spots are excised from a preparative gel and identified by mass spectrometry. **(B)** Gel-free approach. Proteins or peptides could be labeled at different stages of sample preparation depending on SILAC, ICAT, or iTRAQ technology. Light and heavy forms of isotopic analogs are resolved by 2D-LC and then quantified and identified by mass spectrometry. SILAC: stable isotope labeling by amino acid in cell culture; ICAT: isotope-coded affinity tag; iTRAQ: Isobaric tag for relative and absolute quantitation.

2DE applied to biological fluids

By their easy access and their abundant availability, serum and plasma are samples of choice for the identification of new biomarkers. Synovial fluid is in direct contact with the cartilage and may reflect the metabolism of chondrocytes but synovial fluid sampling is difficult. Exchanges between the serum and synovial fluid allow the nutrition of cells but also the release of substances from the joint into the peripheral blood. So, synovial fluid and plasma are often investigated to discover new biomarkers.

Plasma and synovial fluid from patients with OA, RA, or reactive RA (ReA) were studied (Sinz et al., 2002). The authors compared the composition of plasma and synovial fluid from each patient. They showed that the products of degradation of fibrinogen were abundant in synovial fluid with a variable ratio depending on the disease. This study revealed the presence of specific spots containing calgranulin B (S100A9) in the synovial fluid of RA patients. They found the presence of serum amyloid A in synovial fluid and plasma of RA patients, but not in samples from OA patients (Sinz et al., 2002). The modification of the protein S100A9 was also observed in synovial fluid by other team showing a correlation between the level of S100A8/S100A9 complex in plasma and synovial fluid and its capability to discriminate RA from other inflammatory diseases (Drynda et al., 2004).

In urine, we used 2D-DIGE technology to compare the proteome of young healthy volunteers and late-stage OA patients (Henrotin et al., submitted). We identified 13 proteins from spots that exhibited an abundance ratio greater than 1.5 between groups. We also focused on specific sequences of fibulin-3, the only extracellular matrix protein found to be significantly modified in the proteome of urine of OA patients.

The sample preparation represents an important issue for 2DE. High-abundant proteins are present in milligram (mg/ml) quantities particularly in biological fluids and represent more than 95% of the total proteins in plasma. Proteins of interest as potential biomarkers are usually present in samples at levels as low as nanogram (ng/ml) to picogram (pg/ml), making them difficult to detect among abundant proteins. Strategies to remove them consist in the use of immunoaffinity columns retaining the two

more abundant proteins, i.e., albumin and immunoglobulins or up to 20 of the most abundant proteins. Some problems linked to these commercial disposable columns reside in their high cost or in their employment. A new method was recently proposed for the depletion of immunoglobulins in serum by thiophilic chromatography (Salgado et al., 2010). The authors applied this technique to compare RA with healthy control sera.

2DE applied to cells and tissues

Articular cartilage is a poorly cellular structure with chondrocyte as the only one cell type. The poor cellularity of human cartilage requires an expansion of cells in culture to obtain enough material to proceed to the proteomic analysis. Another technical challenge is that over 90% of articular volume is majorly composed of large proteins such as collagens or proteoglycans. These proteins have to be removed from sample before 2DE because, as in biological fluids, they hide minor proteins and interfere with the migration of proteins during isoelectric focusing.

A method to analyze the protein secreted by cartilage explants has been developed (Hermansson et al., 2004). They are easily resolved on gels after aggrecan depletion by precipitation with cetylpyridinium chloride. By incorporation of ^{35}Sulfur in culture, the authors identified newly synthesized proteins. They compared the protein pattern of OA and healthy adult cartilage. The results of this study concerned an increase in type II collagen synthesis in OA and the presence of regulatory proteins as activin A, connective tissue growth factor, and cytokine-like protein C17.

In the same way, the proteins secreted by chondrocytes in monolayer culture stimulated by cytokines as interleukin (IL)-1 and oncostatin were analyzed (Catterall et al., 2006). 2DE allowed the identification of low molecular proteins or fragments: beta-2-microglobulin, S100A11, matrix metalloproteinases (MMP)-1 and -3, peroxidin 1, a member of the "mammalian chitinase-like proteins" (YKL40), cyclophilin A, transthyretin, and cofilin. As with the previous investigation, the depletion of abundant proteins was required to improve the resolution in the upper part of the gels. This was achieved using cetylpyridinium chloride precipitation and anion exchange.

The 2DE technique was used to compare the chondrocytic proteome from control and OA patients (Ruiz-Romero et al., 2008). Twenty-eight proteins were showed to be altered by OA, 19 of them were increased and 9 of them were decreased. The increase of four proteins (GRP78, HSP90β, GSTO1, and ANXA1) was confirmed by immunoblotting and immunochemistry. In addition, the authors showed that IL-1 increased not only the gene expression but also the content of the cited proteins by chondrocytes. The same authors further showed by 2D-DIGE technology that a larger number of proteins were concerned by a statistically significant modification in quantity in the analyzed samples. Moreover, the authors improved their investigations by focusing on a particular organite of the cell, i.e., the mitochondria (Ruiz-Romero et al., 2009). The expression of numerous proteins was found to vary in mitochondria of OA cells. Among them, three forms of superoxide dismutase (SOD)-2 were decreased. SOD is an important actor of the oxidative protection pathway. This decrease means that during OA, the cell defense against oxidative stress is impaired. The authors confirmed the observed modifications by western blot (WB), reverse transcription and polymerase chain reaction (RT–PCR), and immunohistochemistry on cartilage tissues. On the contrary, TRAP1 was found to be increased by 2D-DIGE analysis of proteome modification in OA. This protein is related to the protective mechanism against reactive oxygen species (ROS) injury by protecting the cell against oxidative stress-induced apoptosis. The different expression patterns between OA and control and between the different damaged zones of cartilage were further studied. Lambrecht et al. (2008) compared the intracellular content in proteins extracted from healthy and OA chondrocytes cultured in alginate beads. Moreover, they compared intact and damaged zones of cartilage from OA patients. Proteins were extracted from cultured chondrocytes in two different fractions: membrane (hydrophobic) or cytosol. They performed 2DE and stained proteins with Sypro ruby. They identified 16 proteins differentially expressed between control and intact zone of OA patients and 28 proteins between control and damaged zones of OA patients. Finally, 17 proteins were differentially expressed between intact and damaged zones of OA. Among the identified proteins, authors focused on vimentine and confirmed the observed modification by WB. In addition, the authors analyzed the phosphorylation of differentially expressed proteins and focused on cofilin which is involved in severing and depolymerization of actin filaments (DesMarais et al., 2005).

Some investigations were also made in a mouse model of cartilage explants to respond to the increase use of genetically modified mouse model in arthritis research (Wilson et al., 2008). The authors compared effect of retinoic acid (retA) and IL-1 on femoral head cartilage extracts or conditioned media from explant culture. Using one-dimensional electrophoresis (1DE) and 2DE, they showed no modification of protein pattern in cartilage extract while the effect of tested substances was marked on proteins from conditioned media. To confirm and quantify the observed modifications, they used 2D-DIGE technology. The results of this study reported an increase in MMP3, CH3L1, neutrophil gelatinase-associated lipocalin, and haptoglobin content induced by the addition of IL-1; an increase in aggrecan G1 domain, serotransferrin, cartilage oligomeric matrix protein (COMP), matrilin 3, and link protein induced by the addition of retA and a decrease in gelsolin content in both IL-1 and retA conditioned media compared to control. Gelsolin is an actin-capping protein with important roles in extracellular actin scavenging (Vasilopoulos et al., 2007). It is also involved in the regulation of cytoskeletal architecture and cell–matrix interactions in many cell types, including osteoblasts, fibroblasts, and developing chondrocytes (Chellaiah et al., 2000; Djouad et al., 2007; El Sayegh et al., 2007).

Another group has identified 76 proteins in cartilage from collagen-induced arthritis (CIA) mice using 2DE (Lorenz et al., 2003b). In this study, mice were immunized with bovine collagen II which corresponded to a RA model. Five proteins were found significantly changed in expression and three of them have been identified: lymphoid enhancer binding factor 1 was decreased while the ferritin light chain and antioxidant protein 2 were increased.

A novel approach was applied to chondrocytes proteins for the identification of OA-specific antibodies (Xiang et al., 2004). Proteins from chondrocyte lysates were transferred onto a nitrocellulose membrane after 2DE separation and blotted with serum from OA or RA patients in order to investigate autoimmunity

profiles. Auto-antigens were found in both OA and RA sera. Several proteins were found to be recognized only by OA serum. Among them, triosephosphate isomerase appeared in almost 25% of the tested samples but only in few controls and in about 6% of RA samples.

The use of chondrocytes as source of proteins for cartilage proteomic analysis requires a cellular expansion *in vitro*. It is noteworthy to keep in mind that the chondrocyte culture leads to a gradual shift of the cells from chondrocytes to fibroblastic phenotype known as the dedifferentiation process, and to the modification of matrix component synthesis. In fact, chondrocyte dedifferentiation occurs upon the proliferation which can be circumvented by setting up high density non-proliferating cultures. Some authors have compared the protein content in cartilage from healthy and OA knee joint cartilage directly – after removal of collagens and proteoglycans – without the intermediate step of culture (Guo et al., 2008). They used traditional 2DE technology with silver nitrate staining and found 16 proteins of interest. Eight of them were increased while eight others were decreased as annexin A1 whose modification was confirmed by WB analysis on cartilage samples.

Apart from chondrocytes and synovial cells, other teams were interested in other cell types that could probably be involved in the disease pathophysiology. Others cells of interest were bone marrow mesenchymal stem cells. Using 2D-DIGE technology, Rollin et al. (2008) found 38 differentially expressed spots of proteins between OA and control isolated mesenchymal cells. Again, DIGE technology revealed a large number of modifications. This observation was probably due to its sensitivity.

Another approach using 2DE consists in the complete description of most of the proteins found in cells, tissues, or biological fluids in order to obtain a proteomic profile of reference to compare with unknown samples. 2DE of total proteins provides a visual representation of proteome and allows the detection of post-translational modifications of proteins. The proteome of human OA cartilage have been revealed (Vincourt et al., 2006). The authors resolved more than 500 spots on 2D-gel and identified 191 proteins. The proteome of normal chondrocytes isolated from healthy cartilage was also investigated (Ruiz-Romero et al., 2005). The reference map obtained by 2DE was compared with the one obtained of Jurkat cells. They identified some specific proteins more abundant in chondrocytes like cathepsin D, heat shock protein (HSP) 47, mitochondrial superoxide dismutase (SOD), cytoskeleton-related proteins, or members of annexin family.

ONE-DIMENSIONAL ELECTROPHORESIS

When proteins are too hard to resolve by isoelectrofocusing, as insoluble proteins, some authors explored protein content using 1DE. Proteins are only resolved on polyacrylamide gel according to their molecular weight. This technique could also be useful to prefractionate the sample before liquid chromatography (LC) and MS analysis. Thereby, Gobezie et al. (2007) determined 342 gel slices from 1D-gels to identify proteins modified in OA synovial fluid in comparison to control. They found 18 distinct proteins between control and OA and interestingly two specific subsets of proteins profile in OA group not linked to age, sex, ethnicity, medication, or stage of the disease.

The 1DE investigation was further pursued with cartilage from OA patients or control (Wu et al., 2007). Proteins were directly extracted from cartilage without any culture step. After depletion of abundant matrix proteins like collagens and aggrecans, the authors identified 59 proteins differently expressed between the normal and OA tissues then providing important information on protein expression.

Two approaches of proteomic analysis using MS were compared (Garcia et al., 2006). One the one hand, the proteins from cartilage were digested by trypsin in solution and directly analyzed by MS. On the other hand, proteins were fractionated on 1D-gel and further in-gel-digested by trypsin. Interestingly, the authors showed the significant amelioration of analysis by the introduction of gel fractionation. This technique allowed the detection of more proteins by the simplification of protein mixture. This method was further used on bovine cartilage in order to compare the response of chondrocytes and cartilage matrix to injurious mechanical compression and treatment with IL-1beta and tumor necrosis factor (TNF)-alpha (Stevens et al., 2008). Analyses were made on secreted proteins in culture media of cartilage explants. This analysis retrieved 250 proteins. Among them, new cartilage proteins were identified, i.e., CD109, platelet-derived growth factor receptor–like, angiopoietin-like 7, and adipocyte enhancer binding protein 1. The authors also identified the protein expression induced by the stimulation with IL-1beta and TNF-alpha. They demonstrated that the compression was responsible for the release and proteolysis of type VI collagen, COMP, and fibronectin.

Finally, the analysis of the proteins secreted by chondrocytes after stimulation with lipopolysaccharide soluble (LPS) and the comparison of the obtained pattern with that one of unstimulated cells were performed in rat cells by 1D-LC–MS/MS (Haglund et al., 2008). This study showed the capability of articular chondrocytes to respond to the activation of the toll-like receptor (TLR).

TECHNICAL IMPROVEMENTS

As seen with these different studies, the sensitivity of the techniques was often limited by the composition of sample itself. Indeed, very few proteins were found on gels from thousands of different proteins contained in a cell or in biological fluid. In order to improve the sensitivity of proteomic analyses, modifications in the methodology were applied to reduce sample complexity. For example, one approach when working on cells was to focus on one particular organite. Purification of this organite prior to protein extraction strongly improved gel resolution and enhanced proteomic analysis by targeting specific proteins of interest. Ruiz-Romero et al. (2006) established with this technique the mitochondrial proteome of chondrocytes.

Other technical modifications as passive rehydration loading without voltage application have been developed before the first dimension of electrophoresis. It reduces the entry of large proteins in-gel strips and was tested to improve the direct separation of protein from cartilage extract without sample pretreatment (Sanchez et al., 1997; Pecora et al., 2007). Molecular weight cut-off was also evaluated to improve protein load, optimize resolution, and enhance detection of low-abundance proteins (Wilson et al., 2005). In addition, the authors described a method of joint micro-dissection.

CONCLUSION

Although 2DE-based methods are still widely used in proteomic analysis, this technology has drawbacks that are difficult to circumvent. Limitations concern problems in the detection of low-abundant proteins and in the dynamic range of the protein detection dependent on gel staining. Proteins with extreme characteristics such as very low or high-molecular weight or very acidic or basic isoelectric point like membrane proteins are difficult to resolve on polyacrylamide gel. Moreover, 2DE requires extensive sample handling without easy automation, increasing the risk of bias induced by the manipulation.

GEL-FREE METHODS OR SHOTGUN PROTEOMICS

To overcome problems inherent to electrophoresis-based methods, others separation methods associated to MS were developed. These techniques were grouped under the general term of shotgun proteomics.

In shotgun proteomics, the proteins are separated by high performance LC (HPLC) before being analyzed by MS. To reduce sample complexity, after digestion of proteins in solution, usually with trypsin, multi-dimensional chromatography is often performed coupling cation exchange with reversed-phase chromatography. Unlike 2DE, this technology is fully automated – separation interfaced directly with the mass spectrometer – and covers a large scale of proteins. An overview of the gel-free methods is presented **Figure 2** and the main advantages and disadvantages of gel-free methods in comparison to 2D-DIGE are described in **Table 2**.

LABEL-FREE METHODS

In differential analysis, the peptides may be marked with stable isotope at various stages of the analysis process, depending on the used technique (**Figure 2**). This methodology is applied to identify new potential biomarkers in samples. Label-free methods could be performed to qualify, verify, and quantify a previously discovered biomarker by isotopic-label method with known fragmentation properties in a complex sample. In MS/MS mode, one pair of precursor characteristic of a single peptide is monitored using multiple-reaction monitoring (MRM). This technique provides highly selective, sensitive, and reproductive detection of peptides. MRM was used to quantify level of C-reactive protein (CRP) in serum of RA patients (Kuhn et al., 2004). They used labeled tryptic peptide of CRP as internal standard. They showed that MRM was a useful tool to directly qualify and quantify potential biomarker in clinical samples without a step of antiserum production.

Label-free methods in MS or MS/MS mode were used in OA samples to compare proteomic profile of different populations. The protein profiles of synovial fluid from OA and RA patients were compared using SELDI-MS technique (Uchida et al., 2002). This work reported the presence of specific proteins as myeloid related protein 8 in synovial fluid from RA patient. The concentration of proteins was estimated from the area under the peak of the chromatogram corresponding to the precursor peptide (Bondarenko et al., 2002; Chelius and Bondarenko, 2002). Proteins of interest were identified and their potential as marker was evaluated using MRM technology which allows a quantification using ^{13}C labeled peptide as internal standard (Liao et al., 2004). A methodology has been validated for peptide profiling in synovial fluid (Kamphorst et al., 2007). About 500 peptides from 40 distinct proteins were identified. Hyaluronic acid was removed by ultracentrifugation and solid-phase extraction in order to enrich the sample in low molecular weight peptides. Proteins and peptides were analyzed by shotgun proteomics without trypsin digestion. This approach allowed the evaluation of proteolytic activity by the analysis of cleavage sites. Several chip arrays in SELDI-MS technology were used in order to identify biomarkers of RA (de Seny et al., 2005). They compared the protein content of serum from RA patients with other inflammatory disease such as Crohn's disease, asthma, psoriatic arthritis, or non-inflammatory control group as OA patients or healthy subjects. Peak corresponding to myeloid related protein 8 appeared discriminant in RA group compared to controls (OA and healthy patients). This technique was recently applied to the serum of OA patients (de Seny et al., 2011). It provided interesting results. It allowed the identification of four potential biomarkers, i.e., V65 vitronectin fragment, C3f peptide,

Table 2 | Advantages and disadvantages of in-gel and gel-free methods.

Technology	Advantages	Disadvantages	Example of use in OA field (reference)
1DE, 2DE, 2D-DIGE	High resolution Direct detection of post-translational modifications Information about MW and pI of proteins	Low throughput Low dynamic range Limited number of experiments that can be compared	Sinz et al. (2002), Drynda et al. (2004), Henrotin et al. (submitted), Hermansson et al. (2004), Catterall et al. (2006), Ruiz-Romero et al. (2008), Ruiz-Romero et al. (2009), Lambrecht et al. (2008), Wilson et al. (2008), Xiang et al. (2004), Guo et al. (2008), Rollin et al. (2008), Vincourt et al. (2006), Ruiz-Romero et al. (2005), Gobezie et al. (2007), Wu et al. (2007), Garcia et al. (2006), Stevens et al. (2008), Haglund et al. (2008), Ruiz-Romero et al. (2006)
Gel-free LC–MS/MS	High resolution Easy to perform due to automation		
Label-free	Unlimited number that can be compared	Lower accuracy of quantifications than labeling-based methods	Uchida et al. (2002), Kamphorst et al. (2007), de Seny et al. (2005), de Seny et al. (2011), Li et al. (2007), Baillet et al. (2010), Lambrecht et al. (2010)
Differential labeling	Higher accuracy of quantification than label-free based methods	Limited number of experiments that can be compared High costs	Ji et al. (2010), Dean and Overall (2007), Polacek et al. (2010a), Calamia et al. (2011)

CTAP-III, and m/z 3762 protein. All of them could be involved in OA pathophysiology and could be relevant to reflect inflammation and cartilage and bone turn-over. MS-based technology is also used in targeted approaches. Indeed, shotgun methodology could be applied to the characterization of peptides. More specifically, LC–MS/MS is used to profile directly a peptide or protein suspected to be a useful biomarker. Thus, new epitope of type II collagen (TIINE) degradation by MMP was identified by working on explants and collagen (Nemirovskiy et al., 2007). The authors identified by immunoaffinity a peptide released by MMP13 in human urine and synovial fluid (Nemirovskiy et al., 2010) and developed a method of quantification by immunoaffinity-LC–MS/MS using a deuterated internal standard. The method was clinically validated by the quantification by MRM of TIINE in urine of patients with RA, OA, or polychondritis (Li et al., 2007). TIINE measure in urine was then shown as a potential biomarker of OA. The comparison of synovial fluid and serum content from OA and RA patients and from miscellaneous inflammatory arthritides (MIAs) and RA patients was performed in order to identify new biomarkers of RA. The authors found an overexpression of proteins S100A8, S100A9, and S100A12 in RA synovial fluid compared to OA samples. Versus MIAs, S100A8, S100A9, and alpha defensins -1, -2, -3 discriminates RA populations but with a weakly altered sensitivity and specificity. In serum, none of these markers were found to be modified with disease (Baillet et al., 2010). To improve the discovery of biomarkers in serum, researchers have developed methods for sample preparation before their analysis by shotgun proteomics. As in 2DE, strategies consist in removing abundant proteins to enrich the sample in less abundant proteins and peptides. To this aim, different methods such as membrane filtration cut-off and immunoaffinity are available. A third method using hollow-fiber membrane to remove high-molecular weight proteins and affinity columns to deplete high-abundant proteins has been developed (Tanaka et al., 2006). Moreover, the authors used 3D-LC with the introduction of reverse-phase-chromatographic preparation of sample before trypsin digestion circumventing the overload of MS profiles.

Another application for MS-based technology is the structural analyses of proteins and the study of the post-translational modifications. Zaia et al. (2000) defined the structure of proteins from extracellular matrix and analyzed fragments from matrix turnover by MALDI. Proteins as COMP, aggrecan, decorin, and biglycan were studied with specific experimental approaches. More recently, 2D-LC–MS/MS was applied to the characterization of the proteome of chondrocyte cultured in alginate beads. They identified 779 unique proteins (Lambrecht et al., 2010). Finally, the culture medium of equine cartilage was studied using 1D-LC–MS/MS after digestion with trypsin (Clutterbuck et al., 2011). Tryptic peptides were analyzed with the aim of identifying biomarker of the early cartilage disease.

ISOTOPIC LABELING-BASED METHODS

The main developed strategies consist in using isotopic tag for sample labeling. All these methods use the difference of mass as the basis of the quantitation with the measurement of relative peak areas of mass spectra. Quantitative analyses of proteins and peptides are achieved by comparing isotopic light and heavy forms contained in two samples. Peak ratios for isotopic analogs are highly accurate in a same experiment. Methods using stable isotope circumvent the problem of the variation in sample recovery. Different methods were described depending on samples and incorporation method. Stable isotope labeling by amino acid (SILAC) in cell culture was developed by Ong et al. (2002). It consists in the biological incorporation of labeled essential amino acids (e.g., L-leucine or deuterated L-leucine) in amino acid deficient cell culture medium resulting in labeling of all newly synthesized proteins and virtually all proteins after cell doubling population. Precocity of labeling provides a definite advantage to this method. Moreover, there is no requirement of additional purification and it is applicable to living samples on the contrary to Isotope-coded affinity tag (ICAT) method that will be further describe. Labeled isotopic tag can be added to cell culture or directly coupled to protein or peptide according to the moment of enzymatic digestion. ICAT technology was developed in that sense (Gygi et al., 1999b). ICAT reagent is composed of three functional elements: a reactive group, an isotopic coded light or heavy linker group and a biotin affinity tag. In addition to the quantitation of proteins, this method reduces the complexity of samples by selecting labeled proteins using affinity purification. The main limitation of this technique is that ICAT only labels cysteine-containing peptides. Hence, the proteome coverage and the number of peptides labeled per protein are reduced. To circumvent this drawback, isobaric tag for relative and absolute quantitation (iTRAQ) methodology was developed. This method is using multiplexed set of reagents that integrate isobaric mass labels at the N-terminus and lysine side chain of peptides in a digest mixture (Ross et al., 2004). This amine-specific reagent can label all peptides in up to four samples simultaneously.

The different methods using isotopic labeling were applied to OA and RA research. iTRAQ methodology was used in order to find proteins specific of chondrogenesis in a model of mesenchymal stem cell differentiation (Ji et al., 2010). Among the 1756 identified proteins, 17 appeared to be increased and 83 to be decreased. The 17 proteins and enzymes that were increased were involved in the synthesis of cartilage matrix in mature chondrocytes. The ones that were decreased were involved in energy metabolism, chromatin organization, transcription, mRNA processing, signaling transduction, and cytoskeleton. Most of them were newly found to be involved in the chondrogenic process and BTF3l4 and fibulin-5, two novel chondrogenesis-related proteins were also identified in the present study. iTRAQ and ICAT technologies were used in an original work to analyze the degradome of MMP-2 substrates (Dean and Overall, 2007). MMP-2 is known to be involved in OA as its expression is increased in cartilage during the disease process (Kevorkian et al., 2004). The peptides released in culture medium were labeled and identified by shotgun proteomics. New substrates for MMP-2, such as CX(3)CL1 chemokine fractalkine, osteopontin, galectin-1, and HSP90 alpha were identified. In addition, the authors showed a clear difference between iTRAQ and ICAT-labeling and quantitation systems. iTRAQ allowed the identification of eightfold to ninefold more proteins and the precise localization of the cleavage site.

Stable isotope technique also provided information on OA. This technique was recently applied to investigate the secretome of

chondrocytes cultured on monolayer or in explants (Polacek et al., 2010a). The authors showed differences between newly synthesized or cell-released proteins depending on the culture conditions. The same group further compared the secretome of articular chondrocytes with the one of mesenchymal stem cells (Polacek et al., 2010b) in order to consider the potential of autologous cell transplant. The same technique was also recently applied to chondrocytes in order to confirm the key role of mitochondria in OA (Calamia et al., 2011). Finally, stable isotope standards and capture by anti-peptide antibodies (SISCAPA) represents another recently developed method for quantitation of peptides in complex mixtures (Anderson et al., 2004). This combines an enrichment of sample in specific peptide by immunoaffinity with the use of internal labeled standard. To date, no application was published in the field of OA research.

CONCLUSION

Shotgun proteomics offer a set of technologies available for detailed analysis of various samples. They have significant application prospects. They permit the finding of new molecules of interest, the characterization of peptides that can be used in clinical practice or the deepening into the fundamental knowledge. Either one can prefer one technique to another to a specific purpose or one can use the complementary information from several techniques. Isotope labeling methods were used in global profiling strategies to identify biomarker. However, the need of simplification and enrichment of samples in low-abundant proteins and/or peptides have guided new experimental targeted strategies.

GENERAL CONCLUSION

In the recent years, proteomics have faced many challenges that have allowed its technical development and improvement. The identified problems often concerned the reproducibility and repeatability of experiments, particularly with gel electrophoresis. New technologies using more precise standards have then emerged. Another recurrent difficulty encountered in proteomic analysis of complex samples is the step of sample preparation. This is *a fortiori* the case in the field of osteoarthritis research which involves the study of samples such as cartilage and synovial fluid loaded with bulk proteins. Proteins of interest could be difficult to extract directly in that case. Several methods to overcome this issue or, at least, to reduce this concern have been proposed in the recent years and many proteins of interest have thus been highlighted by different research teams in the field of osteoarthritis.

Recent technological advances allow the exploration in depth of the proteome but also bring a wealth of information. MS-based technologies are able to identify and quantify thousands of proteins and/or peptides and its variants comprising post-translational modifications. In these conditions, the strategies for referencing a complete proteome may appear unrealistic. The examples cited above show that only one proteomic analysis can highlight a multitude of proteins and/or peptides of interest, then opening many possibilities for further investigation. Furthermore, proteomics and advanced technologies are valuable techniques that can be crucial tools to be included in a broader technological scheme in the context of targeted analysis to respond to a research hypothesis. Otherwise, recent technological advances propose new experimental strategies for proteome investigation. They are just beginning to be applied in the study of OA and still offer many opportunities for research application in this area. One still has to learn a lot from these techniques.

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Applications of proteomics to osteoarthritis, a musculoskeletal disease characterized by aging

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The incidence of age-related musculoskeletal impairment is steadily rising throughout the world. Musculoskeletal conditions are closely linked with aging and inflammation. They are leading causes of morbidity and disability in man and beast. Aging is a major contributor to musculoskeletal degeneration and the development of osteoarthritis (OA). OA is a degenerative disease that involves structural changes to joint tissues including synovial inflammation, catabolic destruction of articular cartilage and alterations in subchondral bone. Cartilage degradation and structural changes in subchondral bone result in the production of fragments of extracellular matrix molecules. Some of these biochemical markers or "biomarkers" can be detected in blood, serum, synovial fluid, and urine and may be useful markers of disease progression. The ability to detect biomarkers of cartilage degradation in body fluids may enable clinicians to diagnose sub-clinical OA as well as determining the course of disease progression. New biomarkers that indicate early responses of the joint cartilage to degeneration will be useful in detecting early, pre-radiographic changes. Systems biology is increasingly applied in basic cartilage biology and OA research. Proteomic techniques have the potential to improve our understanding of OA physiopathology and its underlying mechanisms. Proteomics can also facilitate the discovery of disease-specific biomarkers and help identify new therapeutic targets. Proteomic studies of cartilage and other joint tissues may be particularly relevant in diagnostic orthopedics and therapeutic research. This perspective article discusses the relevance and potential of proteomics for studying age-related musculoskeletal diseases such as OA and reviews the contributions of key investigators in the field.

Keywords: musculoskeletal aging, proteomics, osteoarthritis

THE GLOBAL CHALLENGE OF MUSCULOSKELETAL DISEASES CHARACTERIZED AND EXACERBATED BY AGING

The incidence of age-related diseases is rising, seriously affecting the health of millions of people around the world. According to the United Nations (UN)[1] and the World Health Organization (WHO)[2] musculoskeletal, rheumatic, and arthritic conditions are leading causes of morbidity and disability throughout the world, giving rise to enormous healthcare expenditures and loss of work (Woolf and Pfleger, 2003; source: http://www.arthritis.org/)[3,4]. Many types of rheumatic diseases and arthritic conditions are essentially age-related "inflammatory" disorders where the inflammation facilitates disease progression. The term "arthritis" characterizes a group of conditions involving inflammatory damage to synovial joints (Di Paola and Cuzzocrea, 2008). Arthritis literally means inflammation (*itis*) of the joints (*arthr*). It involves pain, redness, heat, swelling, and other harmful effects of inflammation within the joint. There are over 200 different forms of arthritis. However, the most common and important form of arthritis is osteoarthritis (OA), also known as osteoarthrosis or degenerative joint disease (DJD). OA is the most prevalent of the chronic diseases affecting the elderly (Aigner et al., 2004). The majority of the population over 65 years of age demonstrate radiographic evidence of OA in at least one joint. Although OA is rare in people under 40, it becomes much more common with age. More than 20 million Americans are estimated to have OA[5]. A 2005 study in the USA estimated that OA is one of the top five causes of disability amongst non-hospitalized adults [source: Center for Disease Control (CDC[6]), USA]. In 2006 it was estimated that around 35 million to 40 million Europeans suffer from OA and nearly 25% of people aged 60 and above suffer from OA induced disability. It is also anticipated that by the year 2030, 20% of adults will have developed OA in Western Europe and North America. Therefore, OA is expected to place a heavy economic burden on healthcare systems and community services throughout the world. The risk factors for OA are well known and include age, overweight/obesity, underlying metabolic or endocrine disease, genetics, and joint trauma (Lotz and Kraus, 2010). With increasing life expectancy, growth in the elderly population and an alarming escalation of

[1]http://www.un.org/
[2]http://www.who.int/en/
[3]http://www.who.int/healthinfo/statistics/bod_osteoarthritis.pdf
[4]http://whqlibdoc.who.int/bulletin/2003/Vol81-No9/bulletin_2003_81(9)_630.pdf
[5]http://www.niams.nih.gov/
[6]http://www.cdc.gov/

chronic, inflammatory, and age-related conditions (such as OA), there is increased demand for new treatments and preventative approaches.

ARTICULAR CARTILAGE STRUCTURE AND FUNCTION

Articular cartilage is the main tissue involved in OA. It is a mechanically unique and resilient connective tissue responsible for load-bearing and low-friction movement in the synovial joints of all vertebrates (Buckwalter et al., 2005). Cartilage is avascular and as a consequence it has a very limited capacity for intrinsic repair (Brittberg, 1999; Tew et al., 2001). It highly prone to structural degradation making it particularly difficult to restore once it is damaged or lost. The extracellular matrix (ECM) of cartilage gives the tissue resilience and elasticity. The ECM consists of three classes of molecules: collagens, aggregating proteoglycans, and non-collagenous proteins. Type II, IX, and XI collagens form a fibrillar framework of macromolecules that give the tissue form, tensile stiffness, and mechanical strength (Buckwalter and Mankin, 1998b; Eyre, 2004). Large aggregating proteoglycans (predominantly aggrecan) allow cartilage to swell and resist compressive forces (Hardingham and Fosang, 1992; Kuettner, 1992). Small proteoglycans including decorin, biglycan, and fibromodulin, bind to other matrix macromolecules and help to stabilize the ECM. Other collagenous and non-collagenous macromolecules present within the ECM perform a variety of structural and informational roles, facilitate cell–cell and cell-matrix interactions, and bind growth factors (Hardingham and Fosang, 1992; Feng et al., 2006). The chondrocyte is the only cell type present in articular cartilage (Archer and Francis-West, 2003). During embryonic development chondrocytes synthesize a cartilaginous template for endochondral ossification and skeletal development and in postnatal life they maintain the ECM by regulating the turnover of matrix components in response to biomechanical, biochemical, and endocrine signals (Goldring and Marcu, 2009). Chondrocytes actively synthesize new ECM components as well as the proteolytic enzymes such as matrix metalloproteinases (MMPs), a disintegrin, and metalloproteinase (ADAMs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs) are responsible for tissue remodeling during development. These enzymes are also involved in the catabolic breakdown of cartilage in OA (Aigner et al., 2006).

CARTILAGE DEGRADATION IN OSTEOARTHRITIS

Osteoarthritis is a degenerative disease that involves joint inflammation, bone remodeling, and catabolic destruction of the articular cartilage component (Goldring and Goldring, 2007; Samuels et al., 2008). In OA there is an imbalance between the synthesis and degradation of ECM macromolecules (Felson, 2004). This can be due to increased enzymatic activity of MMPs (Okada et al., 1992), and pro-inflammatory mediators such as cytokines (Goldring and Goldring, 2004), prostaglandins, and nitric oxide (Goldring and Berenbaum, 2004), coupled with the reduced anabolic capacity of chondrocytes (Aigner et al., 1997) and the tissue's inherently poor reparative capacity due to its avascular nature (Archer and Francis-West, 2003). Consequently OA is characterized by the loss of structural constituents from the ECM. The degradation and release of proteins and glycoproteins from cartilage in OA can vary according to the stage of the disease process. For example, elevated serum cartilage oligomeric matrix protein (COMP) is correlated with the presence of OA and disease severity (Clark et al., 1999).

AGING AND OSTEOARTHRITIS

Aging is a major contributor to musculoskeletal degeneration and the development of OA (Hamerman, 1998; Lotz and Carames, 2011). Age-related changes in articular cartilage contribute to the development and progression of OA. Although the degeneration of articular cartilage is not simply the result of aging and mechanical wear, aging nevertheless modifies the articular joint including cartilage, subchondral bone, muscle, soft tissues, synovial membrane, and synovial fluid (Buckwalter and Mankin, 1998a; Hamerman, 1998). Although older age is the greatest risk factor for OA, OA is not an inevitable consequence of growing old (Shane Anderson and Loeser, 2010). The mechanisms for the link between aging and OA are incompletely understood. Cell stress and oxidative damage contribute to chronic inflammation that promotes age-related diseases. In OA this results in senescence-associated secretory phenotype, which has many of the characteristics of an osteoarthritic chondrocyte in terms of the cytokines, chemokines, and proteases produced (Loeser, 2011).

BIOMARKERS OF OSTEOARTHRITIS

A major focus of clinical research in recent years has been the identification of new disease markers that can facilitate early diagnosis and optimize individualized treatments. Such markers can also facilitate the drug discovery process by reducing the high levels of attrition in clinical trials. A biomarker is classically defined as a biochemical entity that is used to measure the progress of a disease or the effects of treatment on clinical outcome. Biochemical markers can be measured in blood, serum, and urine or a variety of other body fluids and tissues. The National Cancer Institute (NCI)[7] defines a biomarker as "a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease," and the terms "molecular markers" or "signature molecules" have also been used to describe such markers. The term biomarker is all encompassing and can include proteins, protein fragments, metabolites, carbohydrates, nucleic acids (RNA and DNA), cellular features, and images.

Osteoarthritis is unambiguously diagnosed when it is "detected" by the best available test. Thus far the best test for this purpose has been radiography, the so-called "gold-standard." This process also requires clinical signs in the patient, which often occur well into the progression of the disease. However, there is often early, pre-clinical evidence of disease provided by various biomarkers, which if detected, may facilitate earlier diagnosis and treatment. Such an approach is particularly pertinent in the case of OA, a disease often characterized by a prolonged pre-clinical "molecular" phase, a "pre-radiographic" phase, and a "recalcitrant radiographic" phase by which time there are structural changes to joints along with pain and loss of function. Biomarkers have the potential to provide an early warning of joint degeneration

[7] http://www.cancer.gov/

which could prompt earlier, more targeted treatment to prevent the tissue destruction that results in the characteristic chronic disability associated with OA. In this context, biomarkers could make a significant contribution to the early diagnosis of OA, as well as informing key aspects of disease prognosis, monitoring, and therapy.

The National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) established the Osteoarthritis Biomarkers Network[8] to develop and validate standardized, sensitive biomarker assays in blood and urine to facilitate the diagnosis of the pre-radiologic stage of OA in humans and in animal models. Such markers can help us understand the biological processes involved in disease progression and allow us to monitor the effects of surgical or pharmacological treatment, thus accelerating the pace of drug discovery. Such biomarkers could also potentially be used to identify patients at increased risk of developing OA. Existing biomarkers of OA have major limitations: they do not "flag" the pre-radiographic phase of the disease; they are not specific for the various stages of OA, and in some cases, may not even be specific for OA.

Considering these challenges, the Osteoarthritis Research Society International (OARSI)[9] and the US Food and Drug Administration (FDA)[10] have recently established a new OA biomarkers working group, which has proposed the division of potential markers into two major groups: the so-called soluble or "wet" biomarkers, which typically reflect a modulation in an endogenous substance in body fluids such blood, serum, plasma, urine, or synovial fluid); and the "dry" biomarkers, which usually consist of visual analog scales, performed tasks, or images of joints (Kraus et al., 2011).

Therefore, the ability to detect biomarkers of cartilage degradation and/or inflammation in biological samples, such as serum, urine, or synovial fluid, may enable clinicians to diagnose subclinical OA as well as determining the disease stage in both human and companion animals. Identifying these biomarkers will also aid drug discovery and drug safety/efficacy monitoring in patients and in animal models. Using combinations of biomarkers may be more effective in achieving these goals, thus having a panel of biomarkers will help researchers and the pharmaceutical industry to monitor disease progression as well as to assess responses to treatment in experimental models of OA (Rousseau and Delmas, 2007; Williams, 2009).

SYSTEMS BIOLOGY AND PROTEOMIC APPROACHES FOR THE DISCOVERY OF OSTEOARTHRITIS BIOMARKERS

Systems biology is increasingly applied in orthopedics and rheumatology to cartilage and synovium in arthritis. These techniques include genomics, transcriptomics, proteomics, metabolomics, glycomics, and bioinformatics and can be applied to the study of cartilage, synovium, synovial fluid, and even blood (serum) or urine from OA patients. Proteomics involves the application of specialized analytical techniques that allow the evaluation of the protein composition of tissues, cells, and culture supernatants. Proteomics is being increasingly applied in basic cartilage biology (Polacek et al., 2010) and OA research (Ruiz-Romero et al., 2010). Characterization of cell lysates from isolated chondrocytes has yielded valuable information regarding the intracellular proteins of the chondrocyte proteome, and paved the way for future studies on cartilage pathologies such as OA (Ruiz-Romero et al., 2005; Ruiz-Romero and Blanco, 2010). Studies of soluble proteins in cartilage tissue from OA patients has increased the knowledge of the proteins contained within the ECM of diseased versus normal tissue (Wu et al., 2007). A number of papers have reported on proteins secreted from the cartilage ECM in response to pathological insults such as interleukin (IL)-1α and all-trans-retinoic acid (Wilson et al., 2008a,b; Ruiz-Romero and Blanco, 2010), IL-1β and TNF-α (Cillero-Pastor et al., 2010) and mechanical compression (Stevens et al., 2008; Zhang and Wang, 2009; Li et al., 2010). Identifying proteins released from cartilage has the potential to give an indication of disease biomarkers likely to be present in the synovial fluid or blood of patients in the early stages of OA.

RELEVANCE OF BIOMARKERS AND PROTEOMIC TECHNIQUES TO "PHYSIOLOGY AND PATHOPHYSIOLOGY OF MUSCULOSKELETAL AGING"

Understanding healthy aging is a key research priority, along with a better understanding of the pathophysiology of aging that occurs in a number of age-related diseases, such as arthritis. By gaining a better understanding of healthy musculoskeletal aging we can provide better care and new therapies for common musculoskeletal problems. "Physiology and Pathophysiology of Musculoskeletal Aging" is a Research Topic that is intended to bring together basic researchers and clinicians working in the broad area of musculoskeletal aging. The topic includes mechanisms of healthy aging in tissues of the musculoskeletal system (i.e., skeletal muscle, articular cartilage, subchondral bone, tendon, and ligament).

The discovery and validation for biomarkers of OA has accelerated significantly as our understanding of joint tissue molecules and their complex interactions have increased (Kraus, 2005). One of the main drivers in this context has been the urgent need for improved OA "outcome measures" in clinical trials (Kraus, 2005; Hunter et al., 2010). In particular there is a pressing need for new biomarkers that indicate early responses of the joint cartilage to degeneration that will be useful in detecting early, pre-radiographic changes. Novel markers that characterize the status and prognosis of OA, and that can be used to monitor response to therapy are also required (Mobasheri and Henrotin, 2010). Current "omics-based" research aims to develop an "analytical toolbox" which is hoped will contribute to the clinical development process (Bay-Jensen et al., 2010; Qvist et al., 2010). Combinations of existing biomarkers may improve their prognostic accuracy and help identify at-risk patients (Williams, 2009). The challenge is to use proteomics and other "omics-based" technologies in order to identify sensitive and reliable pre-radiographic biomarkers that can be accurately and reproducibly measured in body fluids. Biomarkers that "flag" early stage OA will be particularly useful in curbing disease progression by identifying patients that would benefit from early therapeutic intervention.

In this Research Topic Gharbi and co-workers (Gharbi et al., 2011) review the applications of proteomic techniques for studying OA. Their aim is to improve our understanding of the

[8] http://www.nih.gov/niams/
[9] http://www.oarsi.org/
[10] http://www.fda.gov/

physiopathology of the disease its underlying mechanisms and to discover disease-specific biomarkers and identify new therapeutic targets. This timely and focused review summarizes the currently available data regarding proteomic techniques and their applications to OA research. The authors discuss technical limitations and solutions to real and practical problems including sample preparation. Although proteomics has many potential applications in this area, there are technical challenges that still remain. This elegant and original article highlights the major issues facing researchers in this area.

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Proteomic profiling of fast-to-slow muscle transitions during aging

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Old age is associated with a large spectrum of physical ailments, including muscle wasting. Skeletal muscle degeneration drastically increases the risk of poor balance, frequent falling and impaired mobility in the elderly. In order to identify new therapeutic targets to halt or even reverse age-dependent muscle weakness and improve diagnostic methods to properly evaluate sarcopenia as a common geriatric syndrome, there is an urgent need to establish a reliable biomarker signature of muscle aging. In this respect, mass spectrometry-based proteomics has been successfully applied for studying crude extracts and subcellular fractions from aged animal and human muscle tissues to identify novel aging marker proteins. This review focuses on key physiological and metabolic aspects of sarcopenia, i.e., age-related muscle fiber transitions and metabolic shifts in aging muscle as revealed by proteomics. Over the last decade, proteomic profiling studies have clearly confirmed the idea that sarcopenia is based on a multi-factorial pathophysiology and that a glycolytic-to-oxidative shift occurs in slower-twitching senescent muscles. Both, newly identified protein factors and confirmed alterations in crucial metabolic and contractile elements can now be employed to establish a sarcopenia-specific biomarker signature.

Keywords: biomarker, mass spectrometry, muscle aging, muscle transitions, proteomics, sarcopenia

SARCOPENIA OF OLD AGE

Although inter-individual differences exist in the onset and severity of the natural aging process of the neuromuscular system, most humans experience an age-dependent loss in skeletal muscle mass accompanied by a considerable decline in contractile strength (Faulkner et al., 2007). In addition to a sedentary lifestyle and an unbalanced diet, other factors can complicate the pathophysiology of progressive muscle wasting in the senescent organism, such as unrelated co-morbidities. Obesity, diabetes, cardiovascular disease, or poor recovery from traumatic injury may affect muscle performance and/or be influenced by changes in the musculature. Epidemiological studies of sarcopenia suggest that nearly half the population over 75 years of age is suffering from muscular weakness leading in severe cases to loss of independence (Berger and Doherty, 2010). The findings from a large number of detailed histological, biochemical, and physiological studies of muscle aging strongly suggest that sarcopenia is due to a multi-factorial pathology. The loss of spinal motor neurons due to apoptosis probably presents one of the most crucial events that eventually leads to a drastic reduction in muscle fiber numbers and size during skeletal muscle aging (Aagaard et al., 2010). The decline in neural organization and an impaired capacity for axonal reinnervation of deinnervated muscle fibers was shown for both aged animals (Edstrom et al., 2007) and senescent humans (Vandervoort, 2002).

In addition to cycles of denervation and faulty reinnervation, age-dependent muscle wasting is associated with the pathophysiological uncoupling between excitation and muscle contraction, impaired muscle protein synthesis, abnormal levels of growth factors and hormones, impaired metabolic pathways, an increased susceptibility to apoptosis, disturbed ion homeostasis, a blunted cellular stress response, and a reduced regenerative capacity (Edstrom et al., 2007). In order to determine a potential hierarchy within these different pathological factors, global genomic, and proteomic investigations have been carried out over the last few years. Molecular genetic studies of sarcopenia have revealed a large number of differentially expressed genes in aged muscle tissue (Tan et al., 2011). It will now be crucial to determine how these age-related gene expression changes translate into an altered abundance and/or post-translational modifications in skeletal muscle proteins. This review outlines the main findings of recent proteomic studies that have focused on physiological and metabolic aspects of sarcopenia, i.e., fast-to-slow transitions and glycolytic-to-oxidative shifts in aging muscle.

PROTEOMICS IN SKELETAL MUSCLE PHYSIOLOGY

Investigations into the molecular basis of physiological and pathophysiological changes in skeletal muscle tissues have traditionally focused on single or small groups of genes, proteins, or metabolites. With the advance of high-throughput approaches such as genomics, proteomics, and metabolomics, it is now possible to carry out large-scale studies that determine global changes in biomolecules. Mass spectrometry-based proteomics presents an unbiased analytical tool for studying cell biological and physiological phenomena. Proteomics combines standardized biochemical methods in a streamlined approach for tissue extraction, protein separation, protein characterization, and protein identification (Walther and Mann, 2010). In the case of skeletal muscle proteomics, the mass spectrometric identification of altered protein expression patterns has established a large cohort of novel biomarkers associated with myogenesis, physical exercise,

denervation, stimulation-induced muscle transformation, disuse atrophy, and mechanical unloading, as well as a variety of neuromuscular pathologies (Ohlendieck, 2011).

In addition, an abnormal abundance and/or altered post-translational modifications were shown to exist in a large number of proteins in senescent skeletal muscle, including components involved in the regulation and maintenance of the excitation–contraction–relaxation cycle, ion homeostasis, and the cellular stress response, as well as both anaerobic and oxidative bioenergetic processes. Since an extensive review of the impact of muscle proteomics on the field of biogerontology has previously been published (Doran et al., 2009), this article will instead focus on a specific aspect of senescent fibers that is highly relevant for skeletal muscle physiology, i.e., the proteomics of muscle plasticity during aging. Relevant proteomic studies on age-related muscle plasticity are listed in **Table 1**. Since proteomic surveys documenting small abundance changes in individual subspecies of muscle proteins may be at variance between different reports on muscle aging, the listed findings in marker proteins do not represent results from specific studies but present a summary of major trends in proteome-wide alterations. This includes mostly regulatory proteins, contractile proteins, metabolic enzymes, and metabolite transporters. Since fiber type shifting and metabolic adaptations represent most likely secondary events during muscle aging, the combination of these novel protein markers can now possibly be used to differentiate early and late stages of sarcopenia for improved diagnostic procedures.

FAST-TO-SLOW TRANSITIONS IN AGED SKELETAL MUSCLE

Individual skeletal muscles consist of three main types of fibers, slow-oxidative type I fibers, fast oxidative–glycolytic type IIa fibers and fast glycolytic type IIb/x fibers, as well as a variety of hybrid fibers (Schiaffino, 2010). Considerable changes in the fiber type ratio occur as a result of physiological adaptations, in association with many muscular disorders and during the natural aging process. Molecular and cellular modifications in skeletal muscle tissues are reflected by major alterations in protein expression patterns (Gelfi et al., 2011). Slow-to-fast muscle transitions can be typically observed in disuse atrophy, microgravity and extended periods of bed rest. In contrast, endurance exercise, chronic low-frequency stimulation, hyper-excitability, and aging usually trigger fast-to-slow muscle transformation (Canepari et al., 2010). That proteomics technology is capable of detecting minute changes in the isoform expression pattern of skeletal muscle proteins has previously been demonstrated by the application of fluorescence difference in-gel electrophoresis for the analysis of muscle transitions following chronic electro-stimulation (Donoghue et al., 2007). The same methodology was applied for evaluating potential protein changes in sarcopenia.

During aging, specific force and maximum shortening velocity of muscles are reduced, which is believed to be mostly due to an altered density and property of myosin molecules (Prochniewicz et al., 2007). The pathophysiological shift to a slower muscle phenotype was clearly confirmed by the sub proteomic profiling of the contractile apparatus of aged rat muscle, which revealed a drastic increase in both abundance and phosphorylation levels of slow myosin light chain MLC2 (Gannon et al., 2009). Comprehensive proteomic surveys of crude extracts from aged human and animal muscle agree with the idea of a slower-contracting mode in senescent fiber populations. Key regulatory and contractile elements were shown to exhibit a switch to slower isoforms during aging, including myosin heavy chains, myosin light chains, actin, tropomyosin, and various subunits of the troponin complex (Piec et al., 2005; Gelfi et al., 2006; O'Connell et al., 2007; Doran et al., 2008; Donoghue et al., 2010). **Figure 1** outlines the proposed fast-to-slow transformation process in aged muscle with respect to contractile proteins. Muscle transitions are probably a secondary occurrence as a consequence of an apoptosis-triggered loss of

Table 1 | Major trends in protein changes during skeletal muscle aging as revealed by mass spectrometry-based proteomics*.

Proteomic approach	Muscle tissue	Fast-to-slow transitions	Glycolytic-to-oxidative shift	References
Proteomic profiling of urea-soluble proteome	Human vastus lateralis	Slow MLC2 ↑; fast MLC2 ↓; cardiac α-actin ↑; fast TnT ↓; TM-α ↓	ATP synthase ↑; ACO ↑; GAPDH ↓; ENO ↓; TPI ↓	Gelfi et al. (2006)
Proteomic profiling of urea-soluble proteome	Rat gastrocnemius	MHC IIB ↓; MHC I ↑; fast MLC2 ↓; slow MLC2 ↑; cardiac α-actin ↑; TM-α ↓	ATP synthase ↑; ICDH ↑; ACO ↑; SDH ↑; Cyt-c RED ↑; GAPDH ↓; ENO ↓; ALD ↓; TPI ↓; PGM ↓; PFK ↓; PK ↓; ALB ↑; MYO ↑; FABP3 ↑	Capitanio et al. (2009), O'Connell and Ohlendieck (2009), Lombardi et al. (2009), Doran et al. (2008), Piec et al. (2005)
Sub proteomic screening of contractile fraction	Rat gastrocnemius	Slow MLC2 ↑; various fast MLC ↓; fast TnT ↓; MHC I ↑; MHC II ↓	ATP synthase ↑; ENO ↓	Gannon et al. (2009)
Analysis of protein nitration, phosphorylation and glycosylation	Various rat muscles	Differential effects on PTMs; slow MLC2-P ↑	Differential effects on PTMs; various glycolytic enzymes ↓	Kanski et al. (2005), Gannon et al. (2008), O'Connell et al. (2008)

**The table lists markers of the contractile apparatus (MHC, myosin heavy chain; MLC, myosin light chain; TM, tropomyosin; TnT, troponin subunit T) and key enzymes of glycolysis (GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ENO, enolase; ALD, aldolase; TPI, triosephosphate isomerase; PGM, phosphoglucomutase; PFK, phosphofructokinase; PK, pyruvate kinase) and oxidative metabolism (ICDH, isocitrate dehydrogenase; ACO, aconitase; SDH, succinate dehydrogenase; Cyt-c RED, cytochrome-c reductase; ALB, albumin; MYO, myoglobin; FABP, fatty acid binding-protein).*

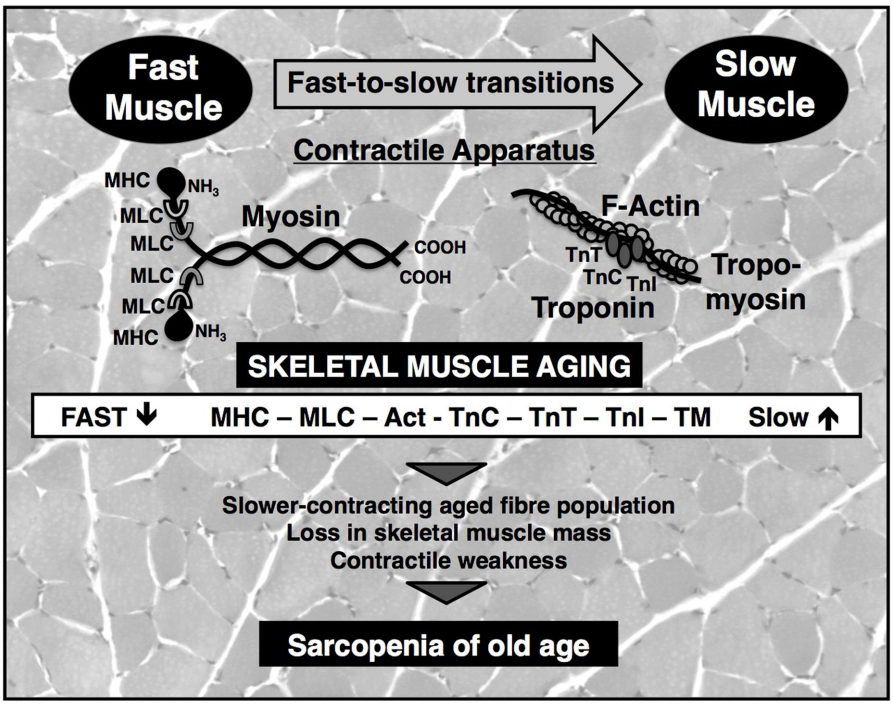

FIGURE 1 | Fast-to-slow muscle transitions during muscle aging as revealed by proteomics. Shown is a diagram of the effect of aging on the main contractile and regulatory proteins of the actomyosin apparatus. Proteomic profiling has clearly established an age-related shift to slower protein isoforms of myosin heavy chain (MHC), myosin light chain (MLC), actin (Act), and tropomyosin (TM), as well as troponin subunits (TnC, TnT, TnI). Besides the slowing of contractile force, the progressive loss of muscle mass and resulting contractile weakness are the main cause of sarcopenia of old age. The background shows a transverse section of gastrocnemius muscle stained with hematoxylin and eosin.

spinal motor neurons, faulty reinnervation mechanisms following denervation and selective atrophy of type II fibers (Vandervoort, 2002; Edstrom et al., 2007; Aagaard et al., 2010).

GLYCOLYTIC-TO-OXIDATIVE SHIFT IN SENESCENT MUSCLE

Energy for the regulation and maintenance of the excitation–contraction–relaxation cycle is supplied by ATP via anaerobic glycolysis, the phosphocreatine shuttle, the citric acid cycle and oxidative phosphorylation. Carbohydrate and fatty acids constitute major energy substrates during physical exercise and the amino acid pool also interacts with the citric acid cycle in contracting fibers. Under anaerobic conditions pyruvate is converted into lactate, and under aerobic conditions the glycolytic conversion of glucose to pyruvate is followed by the enzymatic reactions of the citric acid cycle and oxidative phosphorylation (Wells et al., 2009). The density of enzymes that are associated with glycolysis, the phosphocreatine shuttle, the citric acid cycle, and oxidative phosphorylation reflect the metabolic status of individual muscles. Although gel electrophoresis-based proteomics is afflicted with various biological and technical problems, it is an ideal analytical tool for studying the abundant and mostly soluble enzymes that constitute the glycolytic system (Ohlendieck, 2010). Proteomics has been successfully applied for studying the catalytic elements associated with glycolysis and shown that their density is drastically altered during development, muscle differentiation, physiological adaptations, and many pathological mechanisms, such as muscular dystrophy or diabetes mellitus. In analogy, proteomic profiling of senescent muscle tissue has confirmed that slower-contracting aged muscle exhibit a glycolytic-to-oxidative shift. This phenomenon is comparable to fast-to-slow transitions in chronic low-frequency stimulated fast muscles, which are characterized by a drastic decrease in glycolytic enzymes and a concomitant increase in mitochondrial markers of oxidative metabolism (Donoghue et al., 2007).

While the expression of glycolytic enzymes such as enolase, triosephosphate isomerase and pyruvate kinase is lower in senescent muscle (Gelfi et al., 2006; Capitanio et al., 2009), mitochondrial enzymes such as succinate dehydrogenase and NADH dehydrogenase are clearly elevated during aging (O'Connell and Ohlendieck, 2009). However, variances in the differential expression of certain glycolytic enzymes exist between human and rodent muscle during aging (Gelfi et al., 2006; Doran et al., 2008; Donoghue et al., 2010). Interestingly, the supramolecular organization of mitochondrial complexes involved in oxidative phosphorylation was shown to be disturbed. Aged mitochondria exhibited lower levels of complex I, complex III, and complex V, but increased amounts of complex II and an unchanged expression of complex IV (Lombardi et al., 2009). A recent proteomic study of calpain-interacting proteins has shown an association between the Ca^{2+}-dependent proteolytic system and ATP synthase and actinin, suggesting a role of calpains in mitochondrial and cytoskeletal dysfunction in sarcopenia (Brule et al., 2010). Age-related

muscle degeneration was also shown to have a drastic effect on post-translational modifications in numerous metabolic proteins, affecting especially glycosylation, phosphorylation, and tyrosine nitration in glycolytic enzymes (Kanski et al., 2005; Gannon et al., 2008; O'Connell et al., 2008). The diagrammatic presentation in **Figure 2** summarizes the involvement of cytosolic and mitochondrial pathways in the proposed glycolytic-to-oxidative shift in aged muscle metabolism. An age-related adaptation of metabolism is most likely a result of lost spinal motor neurons and subsequent faulty reinnervation of denervated muscle fibers, yielding a higher percentage of oxidative type I fibers (Vandervoort, 2002; Edstrom et al., 2007; Aagaard et al., 2010).

CONCLUSION

Skeletal muscle tissue is highly abundant in the body and plays a central role in metabolism and body movement. Hence, the age-dependent loss in muscle mass and function has a severe impact on overall body homeostasis and causes frailty in aged individuals. The frailty syndrome is increasingly recognized as an extreme risk indicator of adverse health outcomes in the elderly (Evans et al., 2010). This warrants detailed molecular and cellular studies into the molecular pathogenesis of metabolic and contractile dysregulation in the aged neuromuscular system. Mass spectrometry-based proteomics has clearly confirmed a fast-to-slow contractile transformation process and a glycolytic-to-oxidative metabolic shift during skeletal muscle aging. From a pathophysiological point of view, it is unlikely that fiber type shifting or bioenergetic changes are causative factors of sarcopenia, but rather a consequence of muscle wasting. Primary factors with an unknown pathological hierarchy are proposed to be loss of motor neurons, chronic inflammation, insulin resistance, disuse-related muscular atrophy, decreased levels of essential growth hormones, a reduced regenerative capacity, and various nutritional deficiencies with advancing age (Evans, 2010). Many histological studies indicate that a transition in fiber composition occurs during aging with a higher fiber type I to fiber type II ratio in senescent muscle tissue, probably based on selective atrophy of fast-twitching fiber populations (Vandervoort, 2002). Although there is no consensus on this aspect of a fiber-selective degradation process during the molecular pathogenesis of sarcopenia, proteomic findings clearly agree with a general reduction in the fast fiber population in the elderly.

As reviewed by Berger and Doherty (2010), after the fifth decade a 2% reduction in muscle mass per year is observed in many humans, which has a serious impact on the steadily increasing number of aged members of society. Thus, to prevent the loss of independence due to severe age-related impairments of the neuromuscular system, the urgent implementation of resistance training programs (Mangione et al., 2010) combined with a protein-rich and balanced diet (Rolland et al., 2011) is needed to promote healthy aging. For the proper differential diagnosis of sarcopenia of old age and the swift evaluation of novel treatment regimes, the

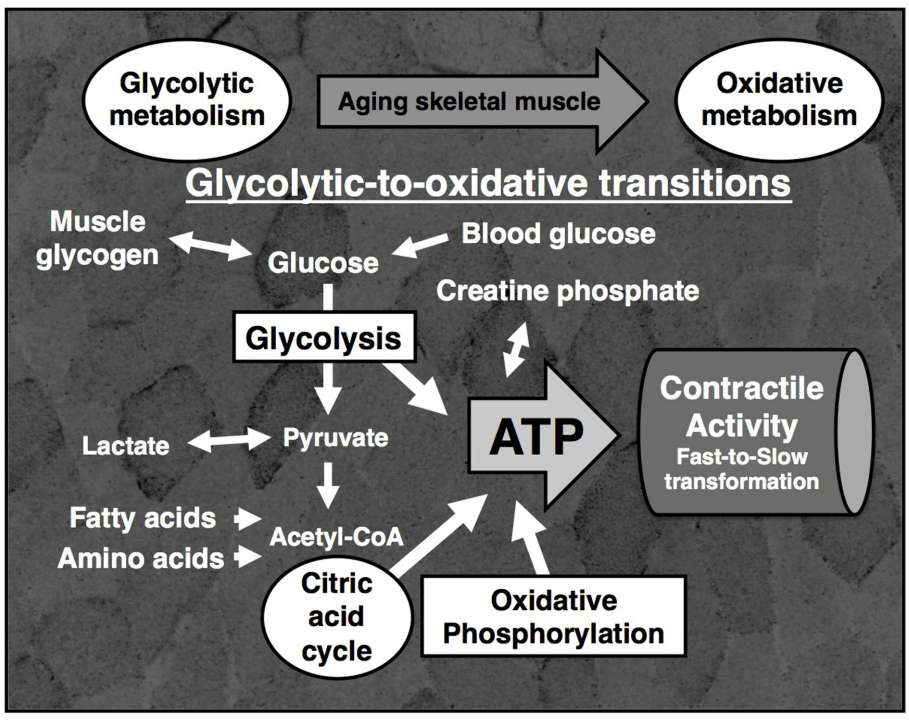

FIGURE 2 | Glycolytic-to-oxidative shift during muscle aging as revealed by proteomics. Shown is a diagram of the main bioenergetic pathways for the provision of adenosine triphosphate (ATP) for the contractile activity of skeletal muscle fibers including glycolysis, the creatine phosphate shuttle, the citric acid cycle and oxidative phosphorylation. Proteomic profiling has clearly established an increase in mitochondrial enzymes and concomitant decrease in glycolytic enzymes during the fast-to-slow transformation process in aging skeletal muscle tissue. The background shows a transverse section of gastrocnemius muscle that was histochemically stained for the presence of the mitochondrial marker enzyme succinate dehydrogenase.

availability of reliable biomarkers is essential. In order to catalog and characterize as many new indicators as possible, future biochemical studies should be more comprehensive with respect to integral membrane proteins and low-abundance proteins, which are currently underestimated in proteome-wide surveys. This will require the application of modified proteomic techniques involving improved protein separation methods, enhanced protein digestion approaches and more sensitive mass spectrometric analyses.

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Proteomic profiling and its applications to muscle aging and sarcopenia

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A commentary on

Proteomic profiling of fast-to-slow muscle transitions during aging
by Ohlendieck, K. (2011). Front. Physio. 2:105. doi: 10.3389/fphys.2011.00105

Aging causes dramatic changes in the musculoskeletal system. Muscle, bone, cartilage, and tendon are all affected. For example, bone mass is reduced as people age, especially in women after menopause. The bones become lighter and less dense as they lose calcium and other minerals. Intervertebral disks lose fluid and become thinner, compromising their gel-like properties and their ability to withstand load. Synovial joints become stiff and lose their flexibility. Articular cartilage becomes drier, thinner, and much more fragile and synovial fluid loses some of its lubricating properties. This eventually results in the erosion of cartilage, formation of osteophytes, and changes in subchondral bone.

Sarcopenia is the degenerative loss of skeletal muscle structure and function associated with the aging process. Age-related changes in muscle mass, structure, and function can be particularly dramatic. The gradual loss of muscle tissue (atrophy) reduces total body mass. The age-related alterations in muscle involve age-related muscle fiber transitions and metabolic shifts in aging muscle. These alterations collectively affect posture and gait and these changes are particularly evident in our seventies, eighties, and nineties. These changes involve anatomical, morphological, and enzymatic alterations in muscle (Grimby et al., 1982). In older individuals the normal physiological turnover of muscle is compromised and older muscle is replaced much more slowly, and the lost muscle may be replaced with adipose tissue or a tough fibrous connective tissue. The deposition of lipofuscin (also known as the aging pigment and by-product of lysosomal degradation) and lipids in muscle affects function and there a change in relative muscle fiber composition with age where muscle fibers effectively shrink (Grimby et al., 1984). These changes in muscle, combined with normal aging changes in the nervous system, reduce muscular tone and contraction. Muscles become rigid like joints and do not recover their tone, even with regular physical exercise. Aging is therefore a complex process but in muscle it is usually associated with a decrease in mass, strength, and efficiency of contraction. However, in essence, there is a generalized decline in muscle protein synthesis (Grimby and Saltin, 1983). The underlying mechanism of functional changes in aging muscle has yet to be fully understood.

"Physiology and Pathophysiology of Musculoskeletal Aging" is a Frontiers Research Topic aimed at increasing our understanding of musculoskeletal aging, placing special emphasis on healthy aging as a key research priority. Proteomics and mass spectrometry have been successfully applied to crude extracts and subcellular fractions of skeletal muscle to identify novel aging marker proteins in animal models of aging and human muscle tissues from elderly subjects. In this Research Topic Kay Ohlendieck reviews the applications of proteomic profiling to aging muscle and the "fast-to-slow" muscle transitions that are thought to occur during aging. The author discusses proteomic profiling approaches that have helped to established an age-related shift to slower protein isoforms of myosin heavy chain, myosin light chain, actin, and tropomyosin (TM), as well as subunits of troponin (Ohlendieck, 2011). His mini-review also discusses the "glycolytic-to-oxidative" shift that occurs in slower-twitching senescent muscles and the newly identified proteins that are altered in aging muscle using proteomic profiling (Ohlendieck, 2011). Proteomic profiling has also revealed an increase in mitochondrial enzymes and concomitant decrease in glycolytic enzymes during the fast-to-slow transformation process in aging skeletal muscle. This timely mini-review also discusses alterations in metabolic and contractile elements that can be used to define a "sarcopenia-specific" biomarker signature.

The preservation of skeletal muscle mass is central to maintaining mobility and quality of life with aging and also impacts on our capacity to recover from illness and injury (Murton and Greenhaff, 2010). Muscle mass loss accompanies periods of bedrest and limb immobilization in humans (Marimuthu et al., 2011). This is something that elderly human patients may frequently experience especially if they undergo major operations and are hospitalized for other age-related diseases. Studies investigating processes underlying disuse-induced muscle atrophy provide us with an opportunity to study temporal changes in cellular and molecular processes in muscle (Murton and Greenhaff, 2010). Proteomic profiling has many potential applications in this area. This area of research raises some important questions and paves the way forward for future areas of investigation. A substantial body of evidence has accumulated over the past 35 years in support of a role for reactive oxygen species (ROS) oxidative damage to the mitochondrial respiratory chain and mitochondrial DNA in aging (Jang and Remmen, 2009). More studies are clearly required to fully understand the role of mitochondria in age related disease and aging. These mitochondrial changes are thought to contribute to sarcopenia and muscle aging (Fulle et al., 2004). This article gives hope to muscle researchers who have adopted (or are planning to employ) proteomic profiling as tool for exploring the bioenergetic changes that occur in aging muscle and the determining potential relevance of the mitochondrial theory of aging to sarcopenia. This approach may also be useful for studies aimed at unrave-

ling the role of satellite cells and innervation in skeletal repair and adaptation and the potential for reversing skeletal muscle atrophy (Carlson, 1995), especially in the context of ROS imbalance induced by an increased oxidative metabolism (Celegato et al., 2006). Clearly there is a need for more dialog and discussion between researchers in closely related fields (e.g., endocrinology, skeletal muscle, physiology, exercise physiology, gerontology) and for multidisciplinary approaches in order to gain greater insight into sarcopenia. This is one of the overarching objectives of this Frontiers Research Topic.

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Chiropractic treatment as a primary care intervention for better musculoskeletal health in the aging population in the United Kingdom: an opinion and positioning paper

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INTRODUCTION

Many countries, including the UK, find themselves with an increasingly aging population [World Health Organisation (WHO), 2011]. The number of people in the UK aged over 75 is set to double over the next 25 years (ONS, 2011). However there is particular concern regarding the impact on health care provision and demand with debate in the literature concerning the strategies designed to cope with the increased demand such as improved health service performance, redesign, and investment in new treatments (Saltman et al., 2006).

Aging is associated with an increased incidence and risk of developing a wide variety of disease conditions, the most prevalent being the decline in musculoskeletal function. There is a debate around whether the musculoskeletal decline is an aging phenomenon *per se,* or whether it is a distinct pathological process increasing in risk as the individual ages (Lawrence et al., 1989; Dieppe, 1993).

In addition, muscle mass and function are also reduced with age (Minaker, 2007). Together, the muscle and bone factors are important in the observed increase in frailty and falls risk in the elderly population.

The elderly are generally encouraged to take more exercise to ensure, and maintain, musculoskeletal health, and many healthcare strategies build upon this principle. This paper aims to outline how chiropractic intervention and maintenance programs can play a pivotal role in the health promotion and continued musculoskeletal functioning of the elderly population.

TREATMENT OF THE ELDERLY POPULATION

Currently, geriatric medicine focuses on developing detailed histories, identifying co-morbidities, current use of medication, and treatment plans (Hulbert et al., 2005). With musculoskeletal problems management, rather than cure, is considered a more realistic strategy (Waxman et al., 2000).

The aim of chiropractic care in the elderly population is to restore function where possible and to arrest, or at least slow down, the degenerative processes that occur with aging (Souza, 2009). In order to maintain a state of independent living, it is vitally important for the individual to be able to maintain general function for as long as possible. This has a significant effect on perceived quality of life, particularly in the much older age groups (Fleming et al., 1995; Xavier et al., 2003). Additionally, elderly patients who receive chiropractic care in addition to medical care, have fewer hospitalization events and use fewer medications than those receiving traditional medical care alone (Coulter et al., 1996).

CHIROPRACTIC AS AN INTERVENTION

Chiropractic is an holistic intervention with chiropractic adjustments as the core concept of the treatment (Mootz and Haldeman, 1995). It focuses on relationships between structure and function of the musculoskeletal and nervous systems and how these preserve and restore health. The profession is regulated by statute (Chiropractors Act, 1994) under the auspices of the General Chiropractic Council[1].

Interventions, such as chiropractic, have become increasingly popular as patients seek options available to them outside traditional medicine (Neuberger, 1998). There is ample evidence that chiropractic is both effective and safe in musculoskeletal conditions, particular in patients with low back pain (Waddell, 1998; UK BEAM Trial Team, 2004). It enjoys high patient satisfaction – important as satisfaction may be related to clinical outcomes (Gemmel and Hayes, 2001; Gaumer, 2006).

Preventive, or maintenance, care is even more important as one grows older (Leboeuf-Yde and Hestbaek, 2008). Since musculoskeletal complaints are generally chronic in nature, maintenance programs seek to avoid acute exacerbations and maintain optimal function (Rupert et al., 2000). Consequently this age-group has a culture of "health promotion and preservation" that makes maintenance alternative health programs a popular adjunct to conventional medicine.

There is controversy surrounding treatment availability to the elderly (Leboeuf-Yde and Hestbaek, 2008). In the UK, chiropractic is a private treatment not available on the National Health Service (NHS) and, therefore, a chargeable service to patients. Lower income groups and the elderly are deterred from chiropractic treatment due to the costs (Manga et al., 1993).

INTEGRATION OF CHIROPRACTIC INTO THE UK NHS

In the United Kingdom, the NHS is the largest health service that relies on public funding and is government controlled through the Department of Health (DoH). The first point of NHS contact for the majority of the UK population is their General Medical Practitioner (GP), the primary care "gatekeepers" to onward referral into the NHS system.

The health service provision in the UK is publicly funded by the Government through the general taxation system rather than individual personal health insurances. A comprehensive range of health services are provided and the vast majority is free at the point of delivery to residents of the United Kingdom. Subject to residency regulations, healthcare is also provided free of charge to foreign nationals in the UK. Reciprocal services are

[1]http://www.gcc-uk.org

provided throughout the European Union through the European Health Insurance Card system (Gorsky, 2008). This is in contrast to the general provision in countries such as Belgium, France, and Germany where healthcare is paid through a mixture of insurances and government subsidy. In the USA, healthcare is totally funded by private individual healthcare insurance policies with no state subsidy.

This difference in healthcare provision also provides for an element of choice. In a totally private system, the patient is free to choose the type of treatment they may have for a particular condition. This also means that many complementary therapies are well established treatment modalities in countries where healthcare does not have such a high level of government subsidy and gives more patient choice (Cassileth, 1996). This is not so in the UK NHS. Many complementary and alternative therapies are not included in NHS provision and are provided on a privately funded basis with the patient having to pay. This cost element can be a barrier to the integration of certain therapies into the general primary care arena.

The popularity of complementary and alternative medicine (CAM), including chiropractic, has grown over the past few decades. However, despite this growth, chiropractic has failed to lay a foundation of authority and value with the general public preventing integration and acceptance of the chiropractic profession within primary care (Murphy et al., 2008). Since the GP is first contact for most patients, the opinion the GP can have a strong influence over acceptance of chiropractic and also how they deal with the patient referral pathways.

There are three key factors that may facilitate the integration of chiropractic more firmly into primary care and the NHS, namely a proper mechanism for inclusion of chiropractic into "mainstream" NHS, awareness of chiropractic and referral process and, finally, better communication between the medical and chiropractic professions.

MECHANISM OF INCLUSION FOR CHIROPRACTIC

Over recent years there has been a drive toward primary care services outside a traditional hospital setting (Breen et al., 2000). In response to a finding that almost a third of all GP consultations were for musculoskeletal complaints, the DoH produced the "Musculoskeletal Services Framework" (DoH, 2006). This clearly states "*the management of musculoskeletal conditions is multidisciplinary.*" Chiropractic is specified as a route that patients with joint pain can access without referral from a GP (DoH, 2006).

The NHS is currently undergoing further reorganization with new primary care commissioning groups made up of local GPs. Chiropractic would be in a position is now able to compete directly for referral business from the NHS and ensure professional viability and sustainability from both NHS funded and privately funded sources. Chiropractors would be well positioned to act as independent service providers, just like GPs, and develop appropriate service level agreements with local commissioning groups to provide musculoskeletal treatment services.

The increase in quality of evidence to support chiropractic and other manual therapies has been acknowledged and appraised by the National Institute for Clinical Excellence (NICE)[2]. NICE issued a specific guidance document for the early management of persistent, non-specific low back pain [NICE, 2009], commending the developments in research for low back pain. It also recognized that non-specific low back pain can be helped by spinal manipulation therapies, specifically chiropractic. NICE emphasizes that "manual therapies" are both clinically effective and cost–effective in the early management of non-specific lower back pain.

The most recent development is the release of the Government White Paper "*Equity and Excellence: Liberating the NHS*" (DoH, 2010a) and its consultation document "*Liberating the NHS: Greater Choice and Control*" (DoH, 2010b). Following this, the DoH has released "*Operational Guidance to the NHS on Extending Patient Choice of Provider*" and this sets out the guidance for the phased implementation of the "*Any Qualified Provider*" (AQP) initiative (DoH, 2011).

Musculoskeletal services have been identified as one of eight priority service lines for the AQP model, enabling patients to choose, through commissioning routes, AQP where this will result in improved and better care. Chiropractic is one such provider and qualified chiropractors may tender for services under the musculoskeletal service line to provide professional services for neck and back pain. This is crucially good news for the chiropractic profession and provides the most promising route to date for the inclusion of chiropractic into the NHS as an accepted intervention for musculoskeletal health across the whole population, including the elderly.

[2]www.nice.org.uk

AWARENESS AND REFERRAL PATTERNS BY GPs

Previous research and recent guidelines suggest that the knowledge and perceptions of chiropractic held by GPs are changing [British Medical Association General Practitioners Committee (BMA GPC), 2009]. A significant proportion of GPs have either already referred or will, at some point in their careers, refer to chiropractors and other forms of CAM and that a better understanding of the value of research by chiropractors will cause a rise in evidence-based practice (Murphy et al., 2008).

In an analysis of GP non-medical referral patterns, the majority of recommendations (63%) were for chiropractic or osteopathy (White et al., 1997). It was also concluded that GPs believed that acupuncture, chiropractic, and osteopathy were effective and should be available within the NHS. A further study postulated that the culture of evidence-based medicine has influenced the considerable proportion of GPs who consider the lack of scientific evidence is a reason that CAM therapies should not be offered to patients (Poynton et al., 2006). These and other studies (Greene et al., 2006; Smith et al., 2006) highlight the need for further research into the facilitators and barriers to developing more positive relationships between the professions.

COMMUNICATION BETWEEN PROFESSIONS

An investigation of drivers and barriers to inter-professional relationships between GPs and chiropractors found that good communication, openness to discussion by providers, and patient interest were identified as key factors for developing positive relationships. Lack of good communication, bias toward alternative medicine, lack of knowledge, or understanding of chiropractic care as well as economic and

geographic constraints were identified as barriers to good inter-professional relationships (Allareddy et al., 2007). A previous study showed substantial support of referral of musculoskeletal complaints (Jamison, 1995). This study also noted an increased range of conditions deemed suitable for chiropractic referral, suggesting that feedback from the chiropractor on chiropractic care improves GP perception of the scope of practice of chiropractic.

Awareness of chiropractic and it's achievements is essential for the improved inter-professional relationships between GPs and chiropractors. There is a clear lack of education on complementary medicine and chiropractic which needs to be addressed in the undergraduate medical training programs. Patients have an increased awareness of complementary medicine due to media and the internet and it is now in the interest of modern doctors to be informed. A survey of GPs in the South of England showed preference on the part of GPs to refer to physiotherapy, only because they had a better understanding of the treatment involved. Also, the majority of practitioners favored receiving a report when a patient completes treatment, outlining the nature of the treatment, examination findings, and the treatment involved (Breen et al., 2000).

CONCLUSION

How does chiropractic continue to position itself within a health care system? This is a question that It is not possible to definitively answer here, but there is no doubt that improving communication processes leads to better and more efficient referral practices, regardless of how chiropractic seeks to position itself outside or inside traditional medical practice.

Chiropractors are themselves well positioned to play a pivotal role in the health promotion, disease intervention, and geriatric care strands of primary healthcare. Chiropractic services are safe, health-effective, and cost–effective (Carey et al., 1995) and enjoy high patient satisfaction (Smith and Stano, 1996). Time pressures may preclude allopathic practitioners from spending the time to discuss health promotion and prevention programs adequately, but this is not so with chiropractic. In combination with the hands-on manipulative nature of the care, a strong practitioner–patient relationship is formed in which appropriate health and lifestyle recommendations can be discussed in a comfortable, supportive, and effective way.

A new and exciting mechanism for the advancement of chiropractic into mainstream NHS treatment is emerging. The chiropractic profession is now in one of its best positions to capitalize on this by offering patient-centered care for the management of musculoskeletal disorders. If there is to be equity of health provision, the chiropractic profession should now embrace these current initiatives.

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Mitochondrial haplogroups define two phenotypes of osteoarthritis

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Objective: To assess a mitochondrion-related phenotype in patients with osteoarthritis (OA). **Methods:** Serum levels of the following OA-related biomarkers: matrix metalloproteinase-1 (MMP-1); MMP-3; MMP-13; myeloperoxidase (MPO); a peptide of the alpha-helical region of type II collagen, Coll2-1, and its nitrated form Coll2-1NO_2; a C-terminal neoepitope generated by the collagenase-mediated cleavage of collagen type II triple helix, C2C; the C-propeptide of collagen type II, CPII; hyaluronic acid (HA); human cartilage glycoprotein 39, YKL-40; cartilage oligomeric matrix protein; and cathepsin K were analyzed in 48 OA patients and 52 healthy controls carrying the haplogroups H and J. Logistic regression models and receiver operating characteristic (ROC) curves were performed to predict the onset of OA. **Results:** MMP-13 was the only biomarker significantly increased in OA patients compared to healthy controls in both haplogroups H and J. The collagen type II biomarkers, Coll2-1, Coll2-1NO_2, the Coll2-1NO_2/Coll2-1 ratio, C2C, CPII, and the C2C:CPII ratio were significantly increased in OA patients carrying haplogroup H compared to OA carriers of the haplogroup J. Two logistic regression models for diagnosis were constructed and adjusted for age, gender, and body mass index. For haplogroup H, the biomarkers significantly associated with OA were MMP-13 and Coll2-1; the area under the curve (AUC) of the ROC curve for this model was 0.952 (95% CI = 0.892–1.012). For haplogroup J, the only biomarker significantly associated with OA was MMP-13; the AUC for this model was 0.895 (95% CI = 0.801–0.989). **Conclusion:** The mitochondrial DNA haplogroups are potential complementary candidates for biomarkers of OA; their genotyping in conjunction with the assessment of classical protein molecular markers is recommended.

Keywords: mitochondria, biomarkers, osteoarthritis, cartilage, arthritis

INTRODUCTION

Osteoarthritis (OA), the most common form of joint disease and cause of musculoskeletal disability in elderly people, is a disease affecting articular cartilage, bone, and soft tissue leading to joint destruction and severe impairment of mobility (Felson and Zhang, 1998). It is the main cause of work incapacity and one of the most common reasons for visiting primary care physicians. Among the risk factors that play a role in OA, gender, age, behavioral influences, obesity, estrogen loss in women and genetic contribution, are notable (Felson and Zhang, 1998; Valdes et al., 2004).

OA is traditionally associated with radiographic signs of joint space narrowing, osteophyte formation, and subchondral sclerosis. Currently, the diagnosis of OA relies on the description of pain symptoms, stiffness in the affected joints and the use of radiography as the reference technique for determining the grade of joint destruction. However, radiographic signs and clinical symptoms only develop in late-stage OA when significant joint damage has already occurred (Cibere et al., 2009). Current treatment strategies are limited, being mainly based on analgesia and, in some cases, surgical procedures (Spil van et al., 2010).

Because OA affects mainly bone, cartilage, and the synovium, structural molecules derived from these tissues could be candidate biological markers for OA. Biomarkers are molecules released into biological fluids during the process of tissue biosynthesis and turnover. The target of the use of biomarkers is to detect changes arising from OA with more reliability, sensitivity, and preferably at an earlier stage of the disease (Garnero et al., 2001, 2002).

With this in mind, several groups have studied potential biomarkers for OA, with sufficiently controversial results that some authors express some limitations about their use as molecular markers for OA (Felson and Lohmander, 2009). Our group proposes new candidate genetic biomarkers, the mitochondrial DNA (mtDNA) haplogroups, which we suggest can be useful as complementary factors when the classical OA-related molecular markers are analyzed.

The mtDNA haplogroups have been associated with several multifactorial diseases, including Alzheimer's (van der Walt et al., 2003), Parkinson's (van der Walt et al., 2004), or Leber hereditary optic neuropathy (LHON; Torroni et al., 1997). Interestingly, we

detected a lower risk for developing knee and hip OA in carriers of the mtDNA haplogroup J in a Spanish population (Rego-Pérez et al., 2008; Rego et al., 2010) and reported that some of the mtDNA haplogroups are associated with changes in serum levels of several classical OA-related molecular markers (Rego-Pérez et al., 2010, 2011). The proposed mechanism relies on the different metabolic characteristics of these haplogroups, reflected by the performance of the mitochondrial oxidative phosphorylation system (OXPHOS) of each haplogroup (Ruiz-Pesini et al., 2000; Gómez-Durán et al., 2010).

These findings and others strengthen the role of mitochondria in the pathogenesis of OA. A significant decrease in complex II and III activity in OA chondrocytes compared with normal chondrocytes has been demonstrated, and mitochondrial mass was also shown to be increased in OA chondrocytes (Maneiro et al., 2003). The apoptotic mitochondrial pathway has been implicated as one of the major cellular pathways of apoptosis in OA chondrocytes (Kim and Blanco, 2007). In addition, the inhibition of complexes III and V of the mitochondrial respiratory chain (MRC) causes an increased inflammatory response, which is potentially relevant to the production of prostaglandin E_2 (PGE_2) and reactive oxygen species (ROS; Cillero-Pastor et al., 2008). Mitochondrial free radical production has been shown to compromise chondrocyte function (Blanco et al., 2004; Henrotin and Kurz, 2007), causing mtDNA damage and reduced mtDNA capacity for repair (Grishko et al., 2009).

This study aims to assess a mitochondrion-related phenotype in OA patients. Logistic regression models that include the analyses of different OA-related biomarkers, mtDNA haplogroups and other clinical variables OA-related, such as gender, age, and body mass index (BMI) were performed in a population from Northern Spain.

MATERIALS AND METHODS

SUBJECTS

The population analyzed in this study has been previously described (Rego-Pérez et al., 2010). A total of 48 unrelated patients diagnosed with knee or hip OA were included in the present study, 25 carrying haplogroup J and 23 carrying haplogroup H. Patients meeting the inclusion criteria for this study included 32 females and 16 males older than 41 years-old (mean age: 68.48 ± 7.64 years-old; range: 52–95), and diagnosed with OA following the American College of Rheumatology (ACR) criteria (Altman et al., 1986). Of the 52 subjects who met the inclusion criteria for normal subjects, 25 carried haplogroup J and 27 haplogroup H. These control subjects included 27 females and 25 males older than 41 years-old (mean age: 66.5 ± 11.25 years-old; range: 42–91), who did not meet the ACR criteria for knee or hip OA. Knee and hip radiographs from all 100 subjects were classified according to the Kellgren and Lawrence (K/L) scoring system, which ranges from Grade 0 to Grade IV (Kellgren and Lawrence, 1957). The clinical variables gender, age, and BMI were collected for all subjects. In all cases, informed consent and the agreement of the ethical committee from Galician Health Administration were obtained.

mtDNA HAPLOGROUPS GENOTYPING

The samples obtained for the study were haplogroup-typed using a previously described assay (Rego-Pérez et al., 2008). For this study, only subjects carrying mtDNA haplogroups H and J were included.

MOLECULAR BIOMARKERS

Fasting blood samples were collected from each subject in plain tubes containing separation gel. These were allowed to stand for 20 min, then centrifuged for 10 min at $800 \times g$. The serum was then divided into aliquots and stored at −80°C pending assay.

For this study the following 12 OA-related molecular markers were measured: metalloproteinase-1 (MMP-1; interstitial collagenase, pro-enzyme), MMP-3 (stromelysin 1, active enzyme), MMP-13 (collagenase 3, active enzyme), myeloperoxidase (MPO), a denaturation epitope of the triple helical domain of collagen type II (Coll2-1) and its nitrated form (Coll2-$1NO_2$), a C-terminal neoepitope generated by the collagenase-mediated cleavage of collagen type II triple helix (C2C), the procollagen type II C-terminal propeptide (CPII), hyaluronic acid (HA), cartilage glycoprotein 39 (YKL-40), cartilage oligomeric matrix protein (COMP), and cathepsin K, a cysteine protease that also cleaves the triple helix of collagen type II. The determination of MPO, Coll2-1, and Coll2-$1NO_2$ was performed at the Bone and Cartilage Research Unit of the University of Liege (Belgium), using a previously described assay (Deberg et al., 2008). The remaining biomarkers were measured in our laboratory using enzyme-linked immunosorbent assays (ELISAs) according to the manufacturer's recommendations. Serum MMP-1 and MMP-3 levels were measured using kits from R&D Systems (Minneapolis, MN, USA), MMP-13 was measured using a kit from Bender MedSystems (Vienna, Austria), C2C, and CPII were measured using kits from Ibex Technologies (Montreal, QC, Canada), HA was measured using a kit from Corgenix Medical Corporation (Denver, CO, USA), YKL-40 was measured using a kit from Quidel Corporation (San Diego, CA, USA), COMP was measured using a kit from Abnova (Taipei City, Taiwan) and cathepsin K was measured using a kit from Biomedica Medizinprodukte (GmbH & Co KG Vienna, Austria).

The Coll2-$1NO_2$/Coll2-1 ratio was utilized as an additional measure of oxidative stress-mediated cartilage degradation, and the C2C/CPII ratio as a further index of cartilage breakdown.

The determination of the serum levels of all the biomarkers analyzed in this study was performed by simultaneously assaying OA patients and healthy controls regardless of mtDNA haplogroup.

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS software, release 17 (Chicago, IL, USA), and EPIDAT 3.1 (Dirección Xeral de Saúde Pública. Consellería de Sanidade. Xunta de Galicia. Santiago de Compostela, España). Haplogroups H and J were analyzed separately. A univariate analysis was performed on each of these haplogroups to compare serum levels of the biomarkers between OA patients and healthy controls using the Mann–Whitney *U*-test.

Receiver operating characteristic (ROC) curves were used to analyze the ability of different molecular markers to discriminate between OA patients and healthy controls in haplogroups H and J. The area under the curve (AUC) and its 95% confidence interval

were calculated, and only those curves with an AUC ≥ 0.8 were considered as discriminative. The ROC curves for each of the biomarkers were compared using the method of DeLong et al. (1988).

Multiple logistic regression models adjusted for age, gender, and BMI were used for each haplogroup studied to determine which biomarkers are significantly associated with OA patients (diagnostic model). A forward stepwise approach was followed to construct the final models. The optimal probability cut-off was assessed by maximizing the Youden index (sensitivity + specificity-1). For this cut-off, sensitivity, specificity, and positive likelihood ratio (LR) values were also calculated. The ROC curves resulting from the regression models were developed taking into account only significant variables.

RESULTS

A total cohort of 100 subjects, 48 OA patients, and 52 healthy controls, were included in this study; 50 were carriers of haplogroup H and 50 of haplogroup J.

UNIVARIATE ANALYSIS FOR COMPARISON OF THE TESTED MOLECULAR MARKERS

The mean values for the different molecular markers in each haplogroup, comparing OA patients and healthy controls with their corresponding *p*-values, are shown in **Table 1**. The results show that only MMP-13 is significantly increased in OA patients in either haplogroup. Most of the catabolic markers are increased in OA patients who carry haplogroup H, even when compared to OA patients that carry haplogroup J (**Table 1**; **Figure 1**). Interestingly, healthy controls carrying haplogroup J show significantly higher levels of MMP-3 and C2C than OA patients with this haplogroup. In summary, these results clearly show a different profile for some of the biomarkers in these two haplogroups.

ROC CURVES FOR THE DIAGNOSIS OF THE MOLECULAR MARKERS

The ROC curves and their corresponding AUC values for all the biomarkers were calculated. ROC curves were performed for each biomarker in each haplogroup. For carriers of haplogroup H, the most discriminative biomarkers were MMP-13, AUC value 0.869 (95% CI = 0.758–0.979), Coll2-1NO_2, 0.863 (95% CI = 0.756–0.970), Colll2-1NO_2/Coll2-1, 0.836 (95% CI = 0.719–0.953), C2C:CPII, 0.831 (95% CI = 0.718–0.944), and HA, 0.841 (95% CI = 0.727–0.955; **Table 2**). Carriers of haplogroup J had only the MMP-13 biomarker, with an AUC of 0.867 (95% CI = 0.749–0.985), able to discriminate between OA patients and healthy controls (**Table 2**).

Because MMP-13 was the most consistent biomarker in all the analyses, a comparison of the ROC curve for MMP-13 and the ROC curves for each of the other biomarkers was performed. For carriers of haplogroup H, the AUC of MMP-13 was statistically different from those of MMP-1 ($p = 0.0008$), C2C ($p = 0.0004$), YKL-40 ($p = 0.0017$), and COMP ($p = 0.0040$). For carriers of haplogroup J, the AUC of MMP-13 was statistically different from MMP-1 ($p = 0.0005$), MPO ($p = 0.0104$), Colll2-1NO_2/Coll2-1 ($p = 0.0039$), C2C:CPII ($p = 0.0081$), HA ($p = 0.0049$), and COMP ($p = 0.0015$; **Table 2**).

MULTIVARIATE LOGISTIC REGRESSION ANALYSIS FOR DIAGNOSIS OF OA

A model to discriminate between OA patients and healthy controls was developed for each of the two haplogroups to include the clinical variables gender, age, and BMI as well as the tested biomarkers.

After adjusting for age, gender, and BMI, haplogroup H carriers had biomarkers MMP-13 and Coll2-1 significantly associated with OA (**Table 3**). The AUC for this model was 0.952 (95% CI = 0.892–1.012). The optimal probability cut-off for discrimination between OA and healthy subjects was 0.536, with a sensitivity of 90%, a specificity of 95%, and a positive LR of 18.095; thus, the model discriminated very well between OA patients and healthy controls (**Figure 2A**). In summary, OA patients with haplogroup H showed higher serum levels of both MMP-13 and Coll2-1 than healthy controls with this haplogroup, as well as an increased BMI and age, regardless of gender.

After adjusting for age, gender, and BMI, patients carrying haplogroup J showed that biomarker MMP-13 was significantly associated with OA (**Table 3**). The AUC for this model was 0.895 (95% CI = 0.801–0.989). The optimal probability cut-off for discriminating between OA patients and healthy controls was 0.503, with a sensitivity of 88%, a specificity of 86%, and a positive LR of 6.125 (**Figure 2B**). In summary, OA patients with haplogroup J showed higher serum levels of MMP-13 than healthy controls with this haplogroup, as well as an increased BMI, regardless of age. This model also showed an increased risk of OA in females (**Table 3**).

DISCUSSION

This study was performed to find a mitochondrion-related phenotype in OA patients using the analysis of 12 OA-related molecular markers and two additional ratios, C2C:CPII and Coll2-1NO_2/Coll2-1. As described above, the choice of haplogroups H and J was because of the different relationships that our group previously found between the haplogroup J and the prevalence and severity of knee (Rego-Pérez et al., 2008) and hip OA (Rego et al., 2010), and because of the association of haplogroups H and J with the serum levels of collagen type II markers (Rego-Pérez et al., 2010).

In previous studies, some of the molecular markers found to be increased in OA appeared to show no differences between cases and controls (Rego-Pérez et al., 2010, 2011). This apparent discrepancy may be accounted for by the fact that the serum levels of some of these markers between cases and controls differed depending on the haplogroup carried. In this sense, we found that serum levels of Coll2-1 were (not statistically) increased in healthy controls (Rego-Pérez et al., 2010), contrarily to the findings of other investigators (Deberg et al., 2008); however, the serum levels of Coll2-1 were increased in OA patients with haplogroup H. Even OA patients that carry this haplogroup had also significantly higher serum levels of Coll2-1NO_2 than healthy controls carrying either of the haplogroups H or J, and also higher than OA patients with haplogroup J (Rego-Pérez et al., 2010). These results were strengthened by measuring the production of Nitric oxide (NO) in articular chondrocytes, showing that carriers of the mtDNA haplogroup J have lower production of NO

Table 1 | Univariate analysis of the biomarkers for haplogroups H and J.

Molecular marker	Haplogroup H			Haplogroup J		
	Healthy Mean ± SD ($n = 23$)	OA Mean ± SD ($n = 27$)	p*	Healthy Mean ± SD ($n = 23$)	OA Mean ± SD ($n = 27$)	p*
MMP-1 (ng/mL)	5.59 ± 4.25	5.47 ± 3.89	0.915	5.31 ± 4.30	5.13 ± 3.4	0.961
MMP-3 (ng/mL)	18.90 ± 10.79	24.89 ± 19.23	0.170	25.98 ± 13.70	15.98 ± 8.89	0.003**
MMP-13 (ng/mL)	0.56 ± 0.31	0.72 ± 0.15	<0.001**	0.48 ± 0.15	0.65 ± 0.15	<0.001**
MPO (ng/mL)	112.93 ± 85.40	181.49 ± 118.74	0.015	141.17 ± 78.73	134.06 ± 143.09	0.201
Coll2-1 (nM)	143.66 ± 33.14	161.31 ± 25.25	0.122	137.40 ± 50.05	107.78 ± 54.90	0.015
Coll2-1NO_2 (nM)	0.33 ± 0.10	0.52 ± 0.16	<0.001**	0.34 ± 0.17	0.28 ± 0.26	0.079
Coll2-1NO_2/Coll2-1 (nM)	0.23 ± 0.06	0.32 ± 0.07	<0.001**	0.25 ± 0.13	0.25 ± 0.24	0.281
C2C (ng/mL)	129.29 ± 43.52	122.55 ± 37.76	0.884	72.57 ± 39.97	42.92 ± 30.72	0.006**
CPII (ng/mL)	472.48 ± 249.94	610.60 ± 211.74	0.015	393.06 ± 213.03	275.75 ± 178.57	0.067
C2C:CPII (ng/mL)	0.32 ± 0.14	0.21 ± 0.06	<0.001**	0.25 ± 0.37	0.16 ± 0.04	0.388
HA (ng/mL)	41.00 ± 32.52	86.13 ± 45.71	<0.001**	59.56 ± 39.37	66.48 ± 35.56	0.438
YKL-40 (ng/mL)	110.55 ± 89.62	99.57 ± 73.54	0.793	116.63 ± 103.20	71.00 ± 38.21	0.073
COMP (ng/mL)	992.68 ± 540.79	963.67 ± 266.49	0.490	1040.34 ± 562.51	959.64 ± 484.14	0.808
Cathepsin K (pmol/L)	13.24 ± 22.44	15.31 ± 16.85	0.026	14.14 ± 21.94	14.60 ± 22.53	0.123

SD, Standard deviation.

**Mann–Whitney non-parametric U-test.*

***Statistical significance declared at $p \leq 0.01$ due to multiple comparisons.*

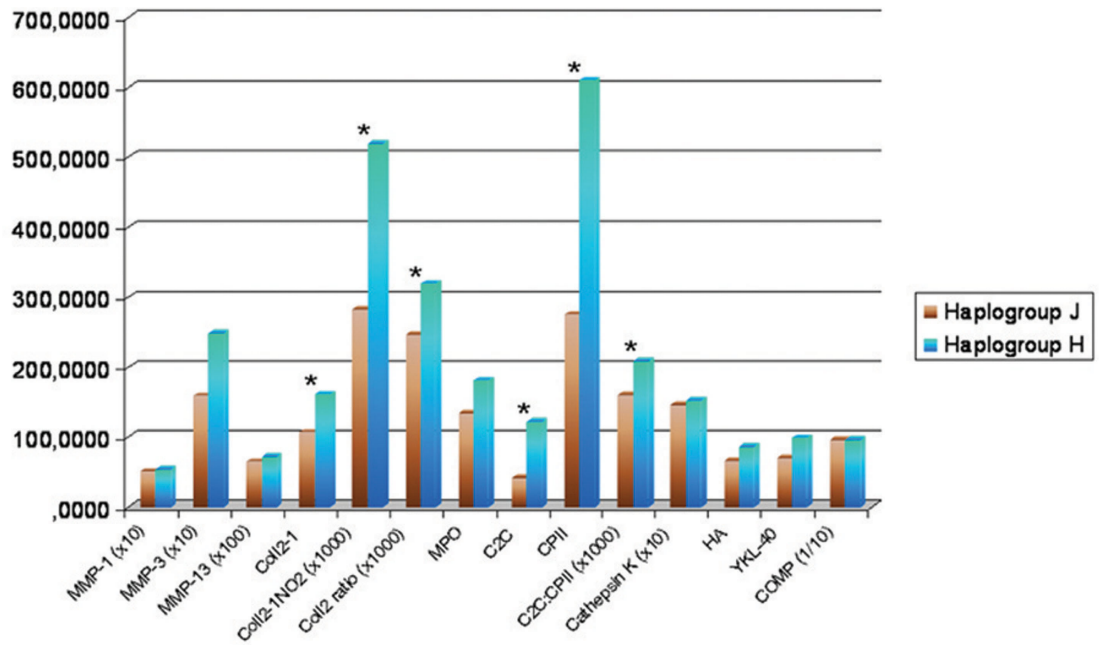

FIGURE 1 | Mean serum levels of osteoarthritis (OA)-related molecular markers in OA patients carrying haplogroup H and OA patients carrying the haplogroup J. (*$p \leq 0.01$); MMP, matrix metalloproteinase; MPO, myeloperoxidase; Coll2-1, denaturation epitope of the triple helical domain of collagen type II and Coll2-1NO_2 = its nitrated form; C2C, a C-terminal neoepitope generated by the collagenase-mediated cleavage of collagen type II triple helix; CPII, the procollagen type II C-terminal propeptide; HA, hyaluronic acid; YKL-40, cartilage glycoprotein 39; COMP, cartilage oligomeric matrix protein.

than non-J carriers (Fernández-Moreno et al., 2011). It is known that OA chondrocytes produce NO (Henrotin et al., 1993; Karan et al., 2003) and that the effect of NO on chondrocyte survival is mediated by its effect on the MRC (Maneiro et al., 2005). It is also known that Coll2-1NO_2 is an indicator of the oxidative stress status of the chondrocyte (Deberg et al., 2008), thereby suggesting that chondrocytes of haplogroup J carriers may have less oxidative stress.

Table 2 | Comparative analysis of the receiver operating characteristic (ROC) curves of biomarkers for haplogroups H and J.

Molecular marker	Haplogroup H			Haplogroup J		
	AUC	95% CI	*p**	AUC	95% CI	*p**
MMP-1 (ng/mL)	0.509	0.345–0.673	**0.0008****	0.504	0.340–0.961	**0.0005****
MMP-3 (ng/mL)	0.614	0.457–0.770	0.0185	0.747#	0.609–0.885	0.2001
MMP-13 (ng/mL)	**0.869**	0.758–0,979		**0.867**	0.749–0.985	
MPO (ng/mL)	0.705	0.558–0.851	0.0505	0.607#	0.446–0.767	**0.0104****
Coll2-1 (nM)	0.628	0.472–0.784	0.0155	0.701#	0.548–0.854	0.0905
Coll2-1NO_2 (nM)	**0.863**	0.756–0.970	0.9465	0.645#	0.484–0.806	0.0279
Coll2-1NO_2/Coll2-1 (nM)	**0.836**	0.719–0.953	0.6711	0.589#	0.425–0.753	**0.0039****
C2C (ng/mL)	0.488	0.324–0.651	**0.0004****	0.726#	0.579–0.874	0.1258
CPII (ng/mL)	0.700	0.552–0.849	0.0662	0.651#	0.497–0.806	0.0162
C2C:CPII (ng/mL)	**0.831#**	0.718–0.944	0.0895	0.571#	0.409–0.733	**0.0081****
HA (ng/mL)	**0.841**	0.727–0.955	0.7668	0.564	0.401–0.727	**0.0049****
YKL-40 (ng/mL)	0.522	0.359–0,684	**0.0017****	0.648#	0.494–0.802	0.0253
COMP (ng/mL)	0.557	0.395–0.719	**0.0040****	0.520#	0.357–0.683	**0.0015****
Cathepsin K (pmol/L)	0.684	0.535–0.834	0.0443	0.627	0.466–0.788	0.0132

AUC, area under the curve. Values in bold indicate the most discriminative (AUC ≥ 0.8).

CI, confidence interval.

#Lower values of the biomarker are associated with OA in carriers of this haplogroup.

**Test for homogeneity of areas comparing the AUC from the MMP-13 from those of the other molecular markers.*

***Statistical significance declared at p ≤ 0.01 due to multiple comparisons.*

Table 3 | Multivariate logistic regression analysis for diagnosis of osteoarthritis (OA) in haplogroup H and J carriers.

Variables	*B*	OR	95% CI	*p**
HAPLOGROUP H				
Age	0.263	1.301	1.026–1.649	0.030**
BMI	0.647	1.910	1.124–3.244	0.017**
MMP-13	0.430	1.537	1.023–2.310	0.038**
Coll2-1	0.104	1.110	1.013–1.217	0.026**
HAPLOGROUP J				
BMI	0.404	1.499	1.062–2.115	0.021**
Gender	−1.930	0.145	0.026–0.816	0.028**
MMP-13	0.551	1.735	1.017–2.959	0.043**

B = regression coefficient.

OR, odd ratio.

CI, confidence interval.

**p-Value from the logistic regression model.*

***Statistical significance declared at p ≤ 0.05.*

We believe that the explanation for these discrepant results is related to mtDNA haplogroups. The frequency of distribution of haplogroups in our cohort is substantially different from that observed in typical cohorts, which will follow the distribution of these haplogroups in the general population: haplogroup H 40–47% and haplogroup J 7–12% (Torroni et al., 1996; Dahmany et al., 2006). The frequency distribution in our cohort was the same for each haplogroup. We see that the results derived from the analysis of carriers of haplogroup H, the most common haplogroup in Caucasian populations, are more in line with those obtained by other authors, and reflect the fact that serum levels of most of the catabolic OA-related biomarkers are increased in haplogroup H carriers, as shown in **Figure 1**.

In this study, pursuant to the analyses of the ROC curves and univariate analysis, MMP-13 was the only molecular marker that differentiated between OA patients and healthy controls in carriers of both haplogroups H and J, as previously described by others (Reboul et al., 1996; Rego-Pérez et al., 2011). In this sense, a role for the mitochondrial dysfunction in the expression of the MMPs has also been proposed (Cillero-Pastor et al., 2008, 2010).

Other molecular markers that have been proposed for differentiating between OA patients and healthy controls, such as HA (Elliott et al., 2005; Mazières et al., 2006), appear to be increased in OA patients, but only significantly increased in haplogroup H carriers with OA. HA has been reported to be increased in OA patients in some studies and was proposed as a surrogate marker with predictive value for radiographic progression of OA (Mazières et al., 2006; Filková et al., 2009). Our study shows that this association is stronger in haplogroup H carriers (**Table 1**). Because the pro-inflammatory cytokine interleukin-1 (IL-1) is one of the main cytokines involved in the mitochondrial dysfunction and in the OA disease, we speculate that this association could be due to different effects of the IL-1, which is an important factor in the synovial production of HA (Nishida et al., 2000), on cells with different mitochondrial backgrounds.

The regression model of diagnosis for haplogroup H carriers shows that, together with both the BMI and age, those subjects with higher serum levels of MMP-13 and Coll2-1 are more likely to be diagnosed with OA. This model resulted in extraordinary LR and AUC values, hence discriminating very well between OA patients and healthy controls. On the other hand, the model for haplogroup J carriers shows that, together with both the BMI and gender, subjects with higher serum levels of MMP-13 only are more

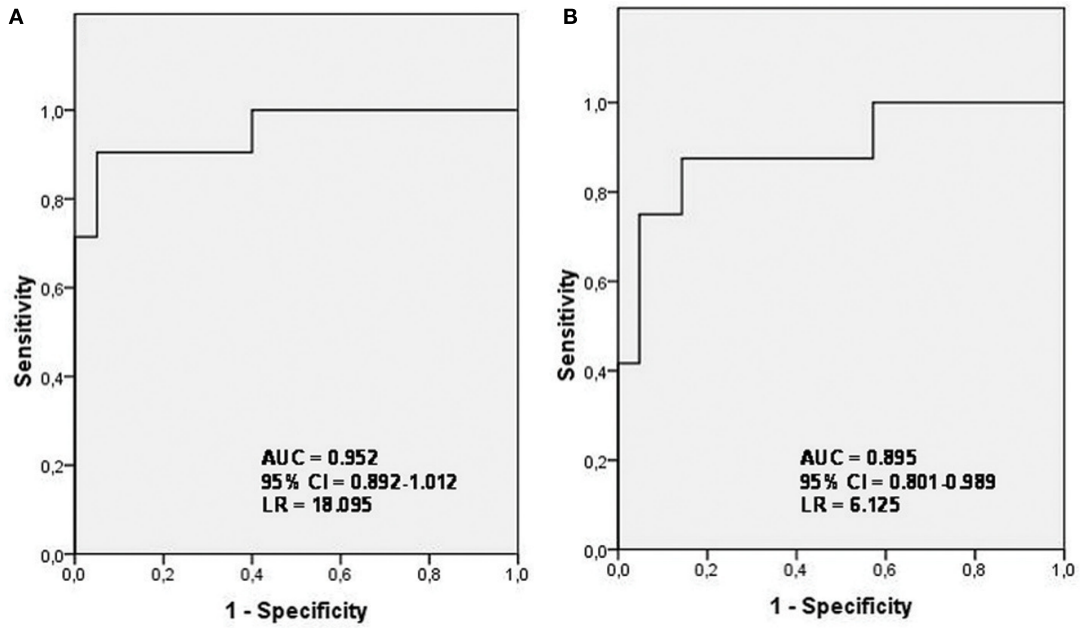

FIGURE 2 | Receiver operating characteristic (ROC) curves derived from the logistic regression models for haplogroups H and J. (A) ROC curve from the regression model for diagnosis in haplogroup H carriers; **(B)** ROC curve from the regression model for diagnosis in haplogroup J carriers. (AUC, area under the curve; CI, confidence interval; LR, likelihood ratio).

likely to be diagnosed as having OA, therefore do not appearing any other biomarker in this model significantly associated to discriminate between OA patients and healthy controls. In summary, these findings highlight the importance of the mitochondrial background in the OA disease, since Coll2-1 appeared significantly increased in OA patients with the mtDNA haplogroup H, but not in patients with the haplogroup J, probably by the fact that haplogroup H carriers have more active collagen type II metabolism than haplogroup J carriers do, as proposed in our previous work (Rego-Pérez et al., 2010). Because mtDNA haplogroups H and J are clearly biochemically different (Wallace, 1999), the possible differences in OXPHOS performance between them (Ruiz-Pesini et al., 2000; Martínez-Redondo et al., 2010) could explain the more active metabolism in haplogroup H carriers; hence CPII levels in OA patients with haplogroup H are higher than in healthy controls or OA patients with haplogroup J (Rego-Pérez et al., 2010), which may represent an attempt to repair OA damaged cartilage by increasing the synthesis of collagen type II, as previously described (Aigner et al., 1992; Lohmander et al., 1996; Nelson et al., 1998). Similarly, metabolic differences between these two haplogroups could account for our finding that most of the catabolic collagen type II biomarkers are increased in OA patients who carry haplogroup H, when compared with OA patients who carry haplogroup J.

CONCLUSION

In summary, the results obtained in this work are of special interest because they strengthen the role of the mitochondrion in the OA process. Either mtDNA haplogroups or an altered mitochondrial function could explain some of the controversial results reported in different studies when analyzing the same biomarkers in different populations of patients. A particular benefit of this study is the notable finding that patients that carry haplogroup H show higher levels of all the catabolic markers of OA than those OA patients that carry the mtDNA haplogroup J; two different mitochondrion-related OA phenotypes were clearly defined. Understanding the influence of the mitochondrial background will enable us to detect biomarkers most related to OA, allowing us to design models for haplogroup-based diagnoses.

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Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength; a quantitative review

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Changing demographics make it ever more important to understand the modifiable risk factors for disability and loss of independence with advancing age. For more than two decades there has been increasing interest in the role of *sarcopenia*, the age-related loss of muscle or lean mass, in curtailing active and healthy aging. There is now evidence to suggest that lack of strength, or *dynapenia*, is a more constant factor in compromised wellbeing in old age and it is apparent that the decline in muscle mass and the decline in strength can take quite different trajectories. This demands recognition of the concept of *muscle quality*; that is the force generating per capacity per unit cross-sectional area (CSA). An understanding of the impact of aging on skeletal muscle will require attention to both the changes in muscle size and the changes in muscle quality. The aim of this review is to present current knowledge of the decline in human muscle mass and strength with advancing age and the associated risk to health and survival and to review the underlying changes in muscle characteristics and the etiology of sarcopenia. Cross-sectional studies comparing young (18–45 years) and old (>65 years) samples show dramatic variation based on the technique used and population studied. The median of values of rate of loss reported across studies is 0.47% per year in men and 0.37% per year in women. Longitudinal studies show that in people aged 75 years, muscle mass is lost at a rate of 0.64–0.70% per year in women and 0.80–00.98% per year in men. Strength is lost more rapidly. Longitudinal studies show that at age 75 years, strength is lost at a rate of 3–4% per year in men and 2.5–3% per year in women. Studies that assessed changes in mass and strength in the same sample report a loss of strength 2–5 times faster than loss of mass. Loss of strength is a more consistent risk for disability and death than is loss of muscle mass.

Keywords: sarcopenia, dynapenia, skeletal muscle, aging, muscle atrophy, muscle aging, muscle quality, strength

INTRODUCTION

We rely upon skeletal muscle for every interaction with our environment and every activity of daily life. The physical challenges of rising from a chair, dressing and walking, bringing food to the open mouth, chewing and swallowing, clearing respiratory secretions, and managing personal hygiene are taken for granted by most, but these are the very activities which, when compromised due to weakness, necessitate institutional care for a significant proportion of the population. Whilst in some cases a specific cause of weakness such as a neurological disease may be identifiable, an almost inevitable contributing factor will be old age. With old age we see at best compromised physical prowess (Moore, 1975; Meltzer, 1994; Ojanen et al., 2007) and at worse a disabling loss in independence and mobility with approximately a quarter of those over 90 years of age requiring long term residential, nursing, or hospital care in the UK (Office of Fair Trading, 2005; Bajekal et al., 2006). Compromised muscle function has been identified as an independent predictor of hospitalization, disability, and death (Newman et al., 2006).

In this review we outline current understanding of the changes that occur in human skeletal muscles with age. We describe changes in size and changes in function and structure and then describe etiologies and potential interventions.

QUANTITATIVE CHANGES IN MUSCLE

SIZE

Historic observations of the loss of muscle bulk seen in old age, from Aristotle to Shakespeare, have been quoted in recent scholarly works on the topic (Evans, 1995; Narici and Maffulli, 2010). In the last half-century a range of elaborate techniques have confirmed a reduction in size of muscle mass with age. This is especially evident in the comparison of those aged 20–30 years to those over 70 years.

Sarcopenia as a concept

This phenomenon was given the name "sarcopenia," derived from the Greek "sarcos" referring to flesh and "penia," a lack of, by Rosenberg (1989). The purpose of giving it a title was to strengthen the concept of loss of skeletal muscle with old age, independent of

disease process, as an entity, and to stimulate scientific and clinical interest in the area. The term is now in widespread use with thousands of peer-reviewed articles identifying it as a keyword. Whilst originally it referred just to the loss of lean mass, it has also been used to refer to the loss of both strength and size, as discussed below (Morley et al., 2001; Cruz-Jentoft et al., 2010).

It has been suggested that sarcopenia should be considered a "geriatric syndrome" (Cruz-Jentoft et al., 2010). The term "geriatric syndrome" is used to capture those complex but common clinical situations seen in old age, which do not fit into discrete disease categories. Examples include delirium, falls, incontinence, and frailty. Dysfunctions in multiple systems, often at distant sites, contribute synergistically to these syndromes; the relative contributions can be difficult to establish (Inouye et al., 2007). The argument put forward for recognizing sarcopenia as a geriatric syndrome is to promote its identification and treatment even when the exact cause remains unknown.

Definition and classification of sarcopenia

No definition of sarcopenia has received universal acceptance. The term was initially used to describe the loss of lean mass with "healthy" aging (Rosenberg, 1989). One widely used definition of sarcopenia proposed in 1998 by Richard Baumgartner was based on a measure of relative muscle mass obtained by dividing absolute muscle mass, evaluated by dual-energy X-ray absorptiometry (DXA), by height squared. In a fashion analogous to the approach used to define underweight, overweight, and obese from BMI, sarcopenia was defined as relative muscle mass lower than two standard deviations below the mean of a large sex-specific reference population aged 18–40 years (Baumgartner et al., 1998). Another definition with a classification of severity, proposed by Ian Janssen in 2002, was based on a skeletal muscle index (SMI) calculated by dividing total muscle mass by total body mass. Muscle mass was evaluated by bioelectrical impedance analysis (BIA). Subjects were considered to have normal SMI if it was within one standard deviation of the sex-specific mean for young adults. Class I sarcopenia was considered present when a subject's SMI was between one and two standard deviations below the young adult values and class II sarcopenia was present in those subjects more than two standard deviations below the young adult reference (Janssen et al., 2002). This approach was considered comparable to the use of bone mineral density of a young reference group in the classification of normal bone density, osteopenia, and osteoporosis (Kanis, 1994).

Subsequently it has been suggested that the term sarcopenia should also encompass weakness and loss of function (Morley et al., 2001). Rosenberg himself, at a symposium in 1996, declared that the term actually describes important changes in body composition *and* function. The United States National Institutes of Health now recognize this broader definition (National Institutes of Health, 2004). In 2010 the European Working Group on Sarcopenia in Older People published a consensus document which proposed a diagnosis of sarcopenia to require "low muscle mass" accompanied by either "low muscle strength" or "low physical performance." This group suggested that stages of sarcopenia be recognized; presarcopenia with loss of muscle mass; sarcopenia when this is accompanied by either loss of strength or physical performance; and severe sarcopenia when all three aspects are present (Cruz-Jentoft et al., 2010).

Some reject this use because it implies a proportionality between loss of muscle bulk and loss of strength which, as discussed below, is not the case as with aging the decline in strength exceeds that of muscle size (Narici and Maffulli, 2010). The term *dynapenia* has been proposed to refer to the functional compromise of the entire neuromuscular apparatus (Clark and Manini, 2008) and although there is good evidence that this concept is of clinical significance (Clark and Manini, 2010) the term is yet to achieve widespread usage. Some writers argue against the separation of dynapenia and sarcopenia due to the risk of nomenclature introducing confusion (Cruz-Jentoft et al., 2010).

It has also been proposed that the term sarcopenia need not be reserved for muscle loss with old age and that the phenomenon may be seen, albeit less frequently, in the young; as is the case with dementia or osteoporosis. Similarly, sometimes the term is used to encompass any kind of muscle loss even that caused by a single identifiable disease ("secondary sarcopenia" as opposed to "primary sarcopenia" in an otherwise well individual; Cruz-Jentoft et al., 2010). Other writers reserve the use of sarcopenia for its origin use, considering secondary sarcopenia to be an aspect of cachexia (Thomas, 2007). A global consensus on the use of the term sarcopenia has not yet been achieved.

Clinical impact of sarcopenia

Two decades ago when the concept of sarcopenia entered vogue it was envisaged that loss of muscle mass was the major determinant of the decline in physical function with age and a modifiable risk factor in disease and disability (Frontera et al., 1991; Evans, 1995). Sarcopenia, as described above by Baumgartner, was independently associated with use of a frame or walker, with falls, and, in both sexes, with physical disability even when adjusted for age, obesity, and comorbidities (Baumgartner et al., 1998). Janssen's definition of sarcopenia was also used to associate low muscle mass with functional impairment and disability (Janssen et al., 2002). In the ilSIRENTE study, individuals in the low tertile of mid-arm muscle circumference, a simple anthropometric index of muscle mass, had a significantly greater mortality than individuals in the high tertile (Landi et al., 2005).

However other data do not support the strong association between low fat free or muscle mass and disability and death. Low fat free mass failed to demonstrate association with self-reported physical disability in both the Cardiovascular Health Study (Visser et al., 1998b) and the Framingham Heart Study (Visser et al., 1998a). The HABC Study has collected prospective body composition, strength, function, and health and survival data for more than 3000 older people over several years. It failed to demonstrate increased compromise in lower limb function in sarcopenic men when sarcopenia was defined as low appendicular lean mass/height^2 (Delmonico et al., 2007). In this study the incidence of mobility limitation does increase with low muscle size (mid-thigh muscle cross-sectional area; CSA by CT) and low muscle strength (maximal isokinetic knee extension strength) but after muscle strength was taken into account, muscle area did not remain a significant factor associated with incident mobility limitations. This suggests muscle strength mediates any

relationship between muscle mass and mobility limitation (Visser et al., 2005). Moreover, this study did not show upper or lower limb muscle mass to be associated with mortality (Newman et al., 2006).

Lean mass, measured by DXA, and thigh muscle CSA, measured by CT, in 3011 adults aged 70–80 years failed to show association with hospitalization rates in the subsequent 4.7 years (Cawthon et al., 2009).

Epidemiology of sarcopenia

Using the definition described above; muscle mass measure by DXA/height^2 less than an index of two standard deviations below the mean of a sex matched young reference population; the NMEHS study showed that in a New Mexico population c. 15% of males and c. 24% of females aged 65–70 years were sarcopenic. This rose to >50% in both sexes among the over 80s. Sarcopenia was generally more prevalent in Hispanics than non-Hispanic whites (Baumgartner et al., 1998). When similarly measured in a Caucasian New England population 53% of men and 31% of women aged over 80 years were sarcopenic (Iannuzzi-Sucich et al., 2002). A much lower prevalence of sarcopenia was seen amongst Danish women with sarcopenia diagnosed in 12% of >70-year olds (Tanko et al., 2002) and in Taiwan, with 26% of men and 19% of women over 80 sarcopenic (Chien et al., 2008).

According to the definition of Janssen et al. (2002) described above, in a study using a nationally representative cohort of Americans, 50% of men and 72% of women over 80 years were sarcopenic; with 7 and 11% suffering Class II sarcopenia.

Quantifying age-related changes in muscle mass

Numerous studies have aimed at quantifying the decrement in skeletal muscle bulk, either volume or mass. Attempts have been made to mathematically describe the decline in function of the musculoskeletal system seen with advancing years as if decline is a uniform process that starts at completion of growth (Sehl, 2001). However there is little consensus on the rate of decline. Estimates of loss of muscle mass by age 18–80 years range from 8 to 49% (Novak, 1972; Tzankoff and Norris, 1977). Studies done over the last five decades are summarized in **Tables 1–3**. **Table 1** summarizes those studies that compare cohorts in or near peak muscle bulk to those in their seventh, eighth, or ninth decades (Lexell et al., 1983; Young et al., 1985). An approximation of percentage loss per decade has been calculated for each study. The median value reported across studies for the rate of loss in men is 4.7% of peak mass per decade and in women it is 3.7% per decade. **Table 2** summarizes those studies that specifically look at rate of loss after the seventh decade (Baumgartner et al., 1995). In many studies a failure to report actual age characteristics of groups prevents calculation of loss per decade.

Some studies demonstrate an ever-accelerating wastage with the rate of loss expressed as a factor of age^2 (Kehayias et al., 1997; Janssen et al., 2000). Others describe a linear loss in later years following a plateau or muscle gaining phase (Gallagher et al., 1997; Kyle et al., 2001; Silva et al., 2009). A large study with a robust technique for measuring summed four-limb (appendicular) skeletal muscle mass (DXA, see below) has shown it to be almost static from ages 18 to 60 years, with a slight gain of muscle throughout this period in men and a slight loss in women (Kyle et al., 2001). The age at which decline starts has been reported as 27 years (Silva et al., 2009), 45 years (Janssen et al., 2000), and 60 years (Kyle et al., 2001).

Several reasons contribute to the differences between studies. Diverse techniques are employed to provide estimate skeletal muscle mass. Fat free mass or fat free cell mass have been used as indices of muscle mass (Novak, 1972; Cohn et al., 1980; Borkan et al., 1983). Total body potassium (TBK) and nitrogen (TBN) estimations of fat free mass may overestimate losses as K^+/gram skeletal muscle itself, a measure assumed constant in these studies, has been shown to decrease with age (Kehayias et al., 1997). Similarly, creatinine excretion per gram of muscle has been shown to be lower in old men compared to young yet the assumption of lifelong constant excretion rates has been used in the calculation of muscle mass estimates in non-agenarians (Tzankoff and Norris, 1977). Non-invasive imaging techniques (computed tomography; CT and magnetic resonance; MRI) are now regarded as the gold standard in measurement of whole body and limb specific muscle mass and these modalities estimate a loss of peak muscle mass of around 20% even in those aged 70–88 years (Janssen et al., 2000). In the last two decades DXA has been widely used in assessing body composition including measurement of lean tissue mass. It can provide an estimate of appendicular and total skeletal muscle mass which correlates closely to that measured by CT but at a much lower cost and exposure to a fraction of the ionizing radiation (Wang et al., 1996).

All cross-sectional studies make the assumptions that a contemporary young sample of the population is a fair proxy for their aged sample at a time in the past. Such studies are influenced by secular changes, i.e., intergenerational differences representing changes in the population characteristics rather than age-related changes that would be evident within a cohort over time.

It could be argued that some cross-sectional studies are particularly vulnerable to differences in selection criteria between young and old. For example studies looking at cadavers of those who died as a result of trauma assume that amount of muscle mass *per se* does not influence the probability of a traumatic death. If, indeed, larger muscle mass in young men was a risk factor *for* traumatic death (for example, due to association with manual laboring employment) whilst larger muscle mass *reduced* the chance of traumatic death in old age (for example, protective against falls), then a selection bias would contribute to the marked loss seen in such studies (Lexell et al., 1988).

Whilst all cross-sectional studies are at risk of these systematic influences, large studies with robust techniques will provide a good measure of variability between individuals in each age group as well as giving an estimate of changes with age. **Figure 1** demonstrates the subtlety of the downward trend in muscle mass with aging when it is seen in the context of the high degree of variability between individuals. This concept has not previously been highlighted in reviews of the subject.

Age-related changes are not uniform across the body

The rate of loss is not uniform across muscles. In an MRI-based study of 200 women and 268 men, the rate of loss of lower limb muscle was more than twice the rate of loss of upper limb

Table 1 | Summary of cross-sectional studies of changing skeletal muscle mass, comparing those in age groups considered to represent "peak muscle mass" versus elderly.

Study	Technique	Estimate	Sex	Young (years)	Aged (years)	*n*	Change	% Change	% Change/ year
Novak (1972)	Total body potassium	Fat free mass	M	18–25	65–85	27, 18	−13 kg	−22	−0.44
		Cell mass					−7.3 kg	−22	−0.44
		Fat free mass	F			89, 13	−3.0 kg	−8.0	−0.16
		Cell mass					−1.7 kg	−8.0	−0.16
Tzankoff and Norris (1977)	24 h urinary creatinine excretion	Muscle mass	M	20	90	14, 12	−965 mg/24 h	−49	−0.7
				20	80	14, 103	−745 mg/24 h	−38	−0.63
Cohn et al. (1980)	Total body nitrogen (Prompt gamma neutron-activation technique)	Fat free mass	M	20–29	70–79	24, 9	−9.0 kg	−14	−0.28
		SMM					−10.9 kg	−45	−0.9
		Fat free mass	F	20–29	70–79	10, 8	−7.8 kg	−18	−0.36
		SMM					−4.0 kg	−40	−0.8
Borkan et al. (1983)	Computed tomography scan	Upper leg muscle CSA	M	46.3 ± 2.6	69.4 ± 4.1	21, 20	−18.2 cm^2	−12	−0.52
		Upper arm muscle CSA					−6.4 cm^2	−11	−0.48
	Total body potassium	Fat free mass					−6.6 kg	−11	−0.48
Lexell et al. (1983)	Cadaveric dissection	Vastus lateralis CSA	M	30 ± 6	72 ± 2	6, 6	−576 mm^2	−17.6	−0.42
Young et al. (1985)	Ultrasound scan	CSA of quadriceps muscles (mid-thigh)	M	20–30	70–80	12, 12		−25	−0.5
Lexell et al. (1988)	Cadaveric dissection	Vastus lateralis CSA	M	19 ± 3	73 ± 2	9, 9	−960 mm^2	−26	−0.48
				19 ± 3	82 ± 1	9, 8	−1584 mm^2	−43	−0.68
Janssen et al. (2000)	Magnetic resonance imaging	SMM	M	18–29	>70	66, 11	−5.9 kg	−18	−0.36
		Lower body SMM					−4.7 kg	−25	−0.5
		Upper body SMM					−0.8 kg	−5.6	−0.11
		SMM	F	18–29	>70	40, 19	−3.8 kg	−17	−0.34
		Lower body SMM					−2.8 kg	−22	−0.44
		Upper body SMM					−1.0 kg	−11	−0.22
Kyle et al. (2001)	Dual-energy X-ray absorptiometry	ASMM	M	18–34	>80	68, 26	−5.4 kg	−19.9%	−3.3
			F	18–34	>80	40, 30	−2.6 kg	−14.1%	−2.3
Silva et al. (2009)	Dual-energy X-ray absorptiometry	SMM	M	18–80 mean 40 ± 14.4		468	−1.58 kg/decade after 27	N/A	−0.46
			F	18–80 mean 44.5 ± 15.9		1280	−0.81 kg/decade after 27	N/A	−0.46
Wroblewski et al. (2011)	Air displacement plethysmography and MRI in high-level recreational athletes	SMM	M	44.8 ± 3.2	65.4 ± 2.2	5, 5	−4.1 kg	−6.7	−0.32
				44.8 ± 3.2	76.3 ± 3.3	5, 5	−7.3 kg	−12	−0.38
			F	47.0 ± 2.8	65.0 ± 3.0	5, 5	−4.3 kg	−9.8	−0.54
				47.0 ± 2.8	74.8 ± 3.7	5, 5	−7.0 kg	−16	−0.57

Table 2 | Summary of cross-sectional studies of changing skeletal muscle mass, comparing between groups within the aged population.

Study	Technique	Estimate	Sex	Young (years)	Aged (years)	*n*	Change	% Change	% Change/year
Novak (1972)	Total body potassium	Fat free mass	M	55–65	65–85	42, 18	−6.5 kg	−12	
		Cell mass		55–65	65–85	42, 18	−3.6 kg	−12	
		Fat free mass	F	55–65	65–85	54, 13	−0.8 kg	−2	
		Cell mass		55–65	65–85	54, 13	−0.4 kg	−2	
Tzankoff and Norris (1977)	24 h urinary creatinine excretion	Muscle mass	M	80	90	103, 12	−220 mg/24 h	−18	−1.8
Cohn et al. (1980)	Total body nitrogen (prompt gamma neutron-activation technique)	SMM	M	60–69	70–79	10, 9	−4 kg	−23	
			F	60–69	70–79	14, 8	−0.9 kg	−13	
Lexell et al. (1988)	Cadaveric dissection	Vastus lateralis CSA	M	73 ± 3	82 ± 1	9, 8	−624 mm^2	−23	−2.6
Frontera et al. (1991)	Hydrostatic weighing	Fat free mass	M	50.5 ± 2.8	68.5 ± 2.8	24, 34	−4.8 kg	−8.0	−0.43
			F	50.2 ± 2.6	69.0 ± 3.8	28, 34	−4.3 kg	−11	−0.58
	24 h urinary creatinine excretion	Muscle mass	M	50.5 ± 2.8	68.5 ± 2.8	17, 29	−2.7 kg	−9.7	−0.52
			F	50.2 ± 2.6	69.0 ± 3.8	19, 20	−3.5 kg	−19	−1
Baumgartner et al. (1995)	Dual-energy X-ray absorptiometry	Appendicular SMM	M	60–70	>80	17, 32	−2.9 kg	−12	
			F	60–70	>80	50, 56	−1.6 kg	−10	
		Fat free mass	M	60–70	>80	17, 32	−4.8 kg	−8.2	
			F	60–70	>80	50, 56	−2.7 kg	−6.8	
	Anthropometrics	Bone free mid-arm muscle CSA	M	60–70	>80	17, 32	−7.0 cm^2	−13	
			F	60–70	>80	50, 56	+0.6 cm^2	+1.7	
Janssen et al. (2000)	Magnetic resonance imaging	SMM	M	60–69	>70	14, 11	−0.4 kg	−2.2	
		Lower body SMM		60–69	>70	14, 11	–	−25	
		Upper body SMM		60–69	>70	14, 11	–	−5.6	
		SMM	F	60–69	>70	11, 19	−0.4 kg	−2.2	
		Lower body SMM		60–69	>70	11, 19	−0.8 kg	−7.6	
		Upper body SMM		60–69	>70	11, 19	+0.2 kg	+2.7	
Kyle et al. (2001)	Dual-energy X-ray absorptiometry		M	60–69	70–79	25, 40	−0.5 kg	−2.1	
				70–79	>80	40, 26	−1.7 kg	−7.2	
			F	60–69	70–79	22, 48	−0.3 kg	−1.8	
				70–79	>80	48, 30	−0.7 kg	−4.2	
Wroblewski et al. (2011)	Air displacement plethysmography and MRI in high-level recreational athletes	SMM	M	65.4 ± 2.2	76.3 ± 3.3	5, 5	−3.2 kg	−5.6	−0.51
			F	65.0 ± 3.0	74.8 ± 3.7	5, 5	−2.7 kg	−6.9	−0.7

Table 3 | Summary of longitudinal studies of change in human muscle mass.

Study	Technique	Estimate	Sex	Baseline (years)	FU (years)	*n*	Change	% Change	% Change/year
Frontera et al. (2000)	Computed tomography	Thigh CSA	M	65.4	12.2	7	−24.4 cm^2	−12.5	−1.0
		Thigh muscle CSA					−19.8 cm^2	−14.7	−1.2
		Thigh extensor CSA					−10.3 cm^2	−16.1	−1.3
		Thigh flexor CSA					−5.2 cm^2	−14.9	−1.2
Hughes et al. (2002)	Hydro-densiometry	Fat free mass	M	61.1	9.5	53	−1.1 kg	−1.9	−0.2
			F	60.0	9.9	78	−0.1 kg	−0.24	−0.024
Dey et al. (2009)	Bioelectrical impedance	Fat free mass	M	70	5	38	−2.02 kg	−3.6	−0.18
(Delmonico et al., 2009) HABC	Computed tomography	Thigh muscle CSA	M	73.6	5	813	−6.8 cm^2	−4.9	−0.98
			F	73.2	5	865	−3.2 cm^2	−3.2	−0.64
			F	70	5	49	−0.93 kg	−2.1	−0.16
(Koster et al., 2011) HABC	DXA	Lean leg mass	M	74.2	7	1129	−1.02 kg	−5.6	−0.8
			F	73.9	7	1178	−0.62 kg	−4.9	−0.07

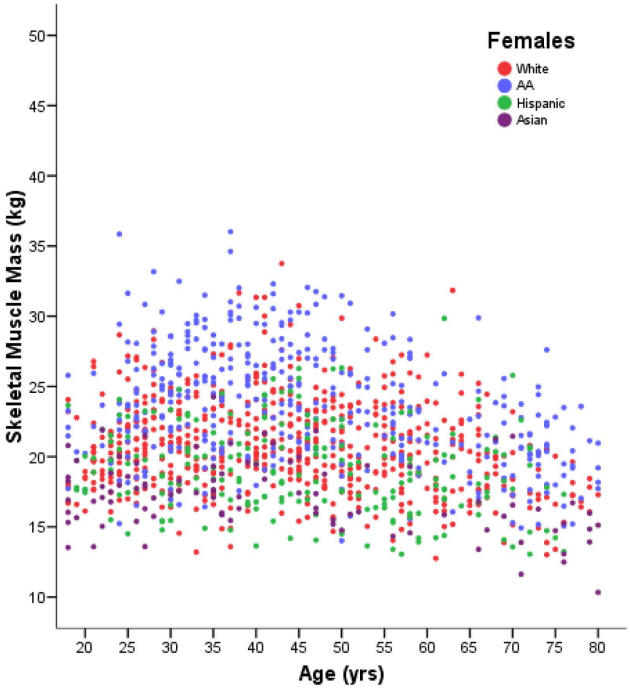

FIGURE 1 | Muscle mass in 1280 females aged 18–80 years, measured by DXA. With permission Z. M. Wang and A. M. Silva, adapted from Silva et al. (2009).

muscle (Janssen et al., 2000), supporting previous evidence from CT (Borkan et al., 1983) and DXA (Gallagher et al., 1997) based measurements.

Sex, race, and menopausal status may impact on age-related changes in muscle mass

At any given age males possess more muscle bulk than females, even after correction for height and weight. Men exhibit larger age-related decreases in muscle mass compared to women (Gallagher et al., 1997). However this difference all but disappears when loss is expressed as a proportion of peak muscle mass (Janssen et al., 2000; Silva et al., 2009).

It has been proposed that menopausal status has a significant impact upon the maintenance of muscle mass and that transition into the menopause has been associated with a reduction in muscle mass (Sirola and Kroger, 2011). However the evidence to support this claim has been equivocal. The first study proposing an accelerated loss of muscle at menopause used TBK to measure cell mass and demonstrated a negligible rate of loss in pre-menopause and a significant inverse correlation with age amongst post-menopausal women (Aloia et al., 1991). Another study used both the TBK technique and also DXA to measure appendicular muscle mass in both men and women and the data displayed very similar trends in both sexes, with different regression models being requires to describe the relationship between muscle mass and age before and after age 60 years (Kyle et al., 2001). Thus it may be argued that the increased loss of muscle seen after menopause is more age-related than menopause related. This is supported by another study that

shows leg lean mass to be inversely correlated with age but not menopausal status (Douchi et al., 2002).

Although men loose more muscle with aging, in absolute and relative terms, it seems that women suffer more from the consequences of lean tissue mass (Janssen et al., 2002), perhaps due to their lower starting mass and their greater longevity.

Studies have attempted to quantify separately the age-related changes in muscle bulk in different ethnic groups living in the same area. African American men and women have been shown to have higher peak muscle mass than Whites but experience greater absolute and relative age-related losses (Gallagher et al., 1997; Silva et al., 2009).

The rate of loss in high-level recreational athletes, who continue to train 4–5 times a week, was similar to subjects not selected according to athletic activity, albeit happening against a different baseline (Frontera et al., 1991; Wroblewski et al., 2011).

Limitations of cross-sectional observation

These cross-sectional studies provide a measure of muscle mass across ages at a moment in time. They cannot support the assumption that individuals follow trajectories of change calculated from arithmetic lines of best fit. Longitudinal studies, with all the associated difficulties in logistical aspects of execution, are the only way to assess trends in individuals.

Table 3 summarizes the few longitudinal studies that assess estimates of muscle mass over time. Given the huge size of the overlapping sample considered by both Koster et al. (2011), Delmonico et al. (2009) who used data from the health, aging and body composition (HABC) study, it should be recognized that their findings; an annual loss of 0.8–0.98 and 0.64–0.7% of leg lean mass per year throughout the eight decade, in men and women, respectively; are the most reliable to date for a population of initially well functioning community dwelling men and women. There is marked discrepancy with the results of the previous largest longitudinal study of Hughes et al. (2002), who considered total lean mass measured by underwater weighing and reported an annual loss of 0.2% in men and approximate stability in women throughout the seventh decade. Contributing to this discrepancy may be the older age of the HABC subjects; the method of recruitment, with HABC subjects being randomly selected Medicare beneficiaries whilst Hughes followed up patients responding to newspaper advertisements; and the asymmetrical loss with lower limb muscle atrophy exceeding upper limb. However these studies consistently report losses of less than 1% per year. Despite this a figure of 1–2% loss per year over 50 is widely quoted and misattributed to Hughes et al. (Thomas, 2007; Rolland et al., 2008; Peake et al., 2010; Sirola and Kroger, 2011).

Age-related loss of muscle is only a part of age-related change in body composition

Against a period of almost-constant muscle mass lasting up to four decades, most individuals gain total body weight due to an increase in fat mass (Kyle et al., 2001). Thus a reduction in relative muscle mass, expressed as a fraction of body weight, can be seen to drop from the third decade of life (Janssen et al., 2000). Most people continue to gain fat mass in the eighth decade when muscle mass is decreasing (Goodpaster et al., 2006).

Muscle mass does decrease with advancing age. On average, in every age group, men carry more muscle mass than women. Men loose more muscle both in absolute terms and a proportion of total. Historical methods of estimating changes in muscle mass have tended to overestimate loss. Throughout much of adult life, muscle mass remains fairly constant. Average rates of loss in those aged over 70 years are in the region of 0.5–1% per year. Most individuals aged over 70 years will possess about 80% of the muscle mass of those aged 20–30 years. There is great variability between individuals at any given age. Sarcopenia is a term that is widely used to describe the phenomenon of muscle loss with old age. There is still much debate surrounding its exact definition. The prevalence of sarcopenia varies with geography, ethnicity, and definition. It may affect half of octogenarians. Some studies show low muscle mass to be a risk factor for disability and death. The health impact of low muscle mass is not as consistent at the impact of low strength.

FUNCTION

Most skeletal muscles act through tendons to move joints against resistance. The force developed depends upon the number of sarcomeres acting in parallel and the force per sarcomere as well the mechanical advantage at which the muscle works. The maximum force that can be applied against an immovable object is the *isometric strength. Isokinetic strength* describes the peak torque that can be developed against a load that moves through an arc at a fixed angular velocity. *Power* describes the rate of energy transfer and is therefore the product of velocity and force of contraction. The velocity of shortening will depend upon the number of sarcomeres in series as well as the speed of shortening of each sarcomere. Concentric contractions occur within a shortening muscle that acts against a load which it overcomes; eccentric contractions occur when the muscle is actively lengthened by external forces. Thus intrinsic stiffness of a muscle will hinder force generation during concentric contractions but contribute to force generation during eccentric contractions. *Fatigability* describes the exercise-induced reduction in the ability to exert muscle force or power. As most muscles act across joints via tendons, changes in other connective tissue elements will indirectly affect the muscle's mechanical behavior (its length–force relation) and impact upon physical performance as will sensorimotor and cognitive processes such as balance, attention, and motivation (Narici and Maganaris, 2007). For these reasons assessing, interpreting, and describing changes in muscle function with age is much more challenging than changes in size of muscle mass, which can be expressed relatively easily as an absolute or relative change in mass or as a simple index thereof, such as muscle mass/height^2 or % body mass.

Hand grip strength and upper limb isometric strength

The use of simple hand grip strength near-isometric dynamometers has been established for more than seven decades (Fischer and Birren, 1946). These permitted an early quantification of changing muscle function with age and also raised awareness of the tendency for cross-sectional studies to underestimate each individual's rate of decline because of relatively fewer weak individuals surviving to be represented in the older samples (Clement, 1974). The NORA cohort of initially healthy, independent Scandinavian 75-year-old

men and women showed a drop in grip strength in 5 years of 20 and 15%, respectively. This was contrasted to a loss of fat free mass as measured by BIA of 3.6 and 2.1% (Dey et al., 2009) suggesting a dissociation between loss of muscle size and strength.

Hand grip dynamometers can be portable and inexpensive and therefore can easily be introduced into clinical practice. Low hand grip strength predicts disability, hospitalization, and mortality. Among a sample of circa 2500 independent living Mexican Americans over 65 years followed up for 7 years, incident disability was more common in the lowest quartile compared to the highest hand grip strength quartile even after adjustment for confounding factors (HR 1.9, 95% CI 1.14–3.17 in men and 2.28, 95% CI 1.59–3.27 in women; Al Snih et al., 2004). The Leiden 85-plus Study looked at Dutch individuals over 85 years and showed low handgrip strength to be a predictor of accelerated decline in activities of daily living (ADL)-ability and cognition (Taekema et al., 2010). Hospitalization was more common in those aged 70–80 years and in the lowest quartile of hand grip strength compared to the highest (OR 1.47, 95% CI 1.3–1.78; Cawthon et al., 2009). Among 463 Finnish people aged 75–84 years participating in the EVERGREEN project, the risk of death within 4 years was higher in those with grip strength below the sample mean (OR = 1.86, 95% CI 1.13–3.07; Laukkanen et al., 1995). Progressive increases in mortality amongst men aged >60 years have been observed between quartiles when ranked by hand grip, an effect that remained significant even after adjustment for total muscle mass as calculated by 24 h creatinine excretion (Metter et al., 2002). The HABC Study showed mortality to be associated with hand grip strength and this effect remained significant even after adjustment for arm lean tissue mass as measured by DXA (Newman et al., 2006). The Leiden 85-plus Study showed the increased mortality associated with low hand grip strength persists as far as the end of the ninth decade (Ling et al., 2010). These findings suggest that measurement of hand grip strength may have a role in clinical assessment and risk stratification in the elderly.

Knee extensor strength and lower limb isokinetic strength

Assessment of knee extensor isokinetic strength, or peak torque at a fixed angular velocity, allows a dynamic measure of lower limb function. Lower limb strength is lost more rapidly than upper limb strength (Frontera et al., 2000), in line with the loss of lean mass described above. However the loss in strength greatly exceeds the loss of muscle mass. Among 1678 older people followed for 5 years, men lost c. 16% of knee extensor strength but only 5% of thigh muscle mass whilst women lost 13% of strength but only 3% of mass. In those patients with stable body weight or who lost weight, the loss of strength exceeded the loss of muscle mass two- to five-fold. Individuals who gained body weight still lost strength despite an increase in muscle mass. Loss of muscle mass only accounted for a small fraction of the between subject variability in the loss of knee extensor strength (6 and 8% in men and women, respectively). This reinforces the notion of a clear dissociation between loss of muscle and loss of strength (Hughes et al., 2002; Delmonico et al., 2009).

Patients with low knee extension strength are at increased risk of disability and death. The HABC Study shows an increase in incident disability in the lowest compared to highest quartile by quadriceps strength (OR 2.02, 95% CI 1.39–2.94; Visser et al., 2005). The same study shows increased mortality by quadriceps strength per standard deviation of 48 Nm, with a crude hazard ration of 1.51 (95% CI 1.28–1.79) in men and 1.65 (95% CI 1.19–2.3) in women (Newman et al., 2006). The EVERGREEN project showed the risk of death within 4 years was higher in those with knee extension strength below the sample mean (OR = 2.52, 95% CI 1.50–4.42), a higher mortality risk than that associated with low hand grip strength in the same sample (Laukkanen et al., 1995).

The preservation of eccentric strength with aging

The evidence presented above shows compromise in strength of isometric contractions, when the muscle is held at a fixed fiber length during activity and in dynamic, concentric contractions when the muscle shortens during the generation of force. When peak torque is measure in eccentric contractions there is a relative preservation of strength in old age and other chronic disease states associated with muscle loss and weakness (Porter et al., 1997; Phillips et al., 1998; Klass et al., 2005). It has been proposed that the accumulation of non-contractile material within the muscle increasing passive stiffness and changes in the contractile properties of muscle fibers resulting in "active stiffness" both contribute to this phenomenon (Roig et al., 2010).

Loss of peak torque depends upon angular velocity

During concentric contractions the peak torque developed depends upon the angular velocity of the load. The loss of peak torque with aging is greater at higher angular velocities (Yu et al., 2007). In young men, the ratio of elbow flexion peak torque developed at 240 versus 60 s^{-1} is about 0.9. In elderly men this has been shown to drop to c. 0.5 (Pousson et al., 2001).

Loss of power

Power describes a muscle's ability to do work and is thus the rate of transfer energy. It is equal to the product of the force developed and the velocity of contraction. Power is lost faster than strength (Skelton et al., 1994; Izquierdo et al., 1999). Measuring and comparing power is much more difficult than measuring strength. The reasons for this are discussed in a review of the topic (Macaluso and De Vito, 2004).

Fatigability

Anecdotal evidence may suggest *fatigue* constitutes an element of normal aging. However the observation of increased rapidity of tiring upon repeated performance of the same task reflects the age-related decrease in strength, outlined above, more than fatigability of muscles. As maximum strength decreases, and body mass increases or stays relatively constant, the fraction of maximum force needed to perform the same physical task, e.g., stair-climb or rise from chair, will increase. Fatigue occurs more rapidly with increasing task intensity and the maximum endurance time decreases in an non-linearly fashion as task intensity increase (Frey Law and Avin, 2010).

However the physiological phenomenon of *muscle fatigue*, which has been defined as "an exercise-induced reduction in the ability to exert muscle force or power" (Bigland-Ritchie and Woods, 1984) would require that comparisons between age groups

be based upon performance at a constant task intensity (% of maximal force). A recent meta-analysis included data from 46 publications which reported fatigue tasks (voluntary activation) performed at relative intensity in both young (18–45 years) and old (>55 years). This work demonstrated significant fatigue resistance in the aged. Subgroup analysis by task showed significant fatigue resistance when tasks involved sustained or intermittent isometric tasks but this effect was lost when tasks were dynamic (Avin and Law, 2011). This is in keeping with studies that have showed an accelerated loss in Type II fibers (Larsson and Karlsson, 1978; Lexell et al., 1988).

Individual fiber observations

The *in vivo* human observations described above will reflect not only factors intrinsic to muscle fibers but will also be influenced by differences in intramuscular fiber orientation, differences in the mechanical leverage provided by the bony anatomy of joints, the elasticity of tendons, the pattern of motor unit recruitment, and the activation of antagonist muscles. Even multicellular muscle preparations, independent of these variables, will depend upon interpreting the contribution of different fiber types. The study of chemically skinned, single fibers allows the examination of myofilament behavior in a near physiological environment yet without the confounding effects of intercellular connective tissue.

It has thus been demonstrated that with aging, the maximum shortening velocity of type I and IIA fibers decreases by c. 20–46 and 10–30%, respectively, and these changes are seen in both males and females (Larsson et al., 1997; Yu et al., 2007). The small numbers of mixed type and type IIx fibers and the variability between fibers make assessment of these types difficult but a trend toward slowing is seen in all fiber types (D'Antona et al., 2003).

Consistent with a decrease in maximum shortening velocity of each fiber type is a decrease in the actin sliding velocity on purified myosin isoforms prepared from aged muscle determined by *in vitro* motility assays (Hook et al., 2001; D'Antona et al., 2003).

The specific tension, or force per unit CSA, has also been shown to decrease with age in both sexes; by c. 16–33% in type I, 14–25% in type IIA, and possibly up to 50% in type IIx (Larsson et al., 1997; D'Antona et al., 2003; Yu et al., 2007).

Within fibers, myosin concentration falls with age. Within each fiber type, the specific tension generated is almost proportional to the myosin concentration observed suggesting that the loss of specific tension is the result of dropping myosin concentrations (D'Antona et al., 2003).

Strength decreases with advancing age. Average rates of loss are 2–4% per year. This is 2–5 times faster than muscle mass is lost. Low strength, both assessed by hand grip and knee extension, predicts disability and death. Factors other than loss of mass account for much of the observed loss of strength. Age compromises the ability to generate torque more at high than low angular velocities. Power is lost more quickly than strength. Relative preservation of eccentric strength and fatigue resistance and is a feature of aged muscles. These phenomena are seen at a single fiber level. Underlying the observed loss of whole muscle function is a slowing of actin-myosin interaction and a reduction in myosin concentration.

QUALITATIVE CHANGES IN MUSCLE WITH ADVANCING AGE

Human skeletal muscle consists of fibers or myofibers, individual multinucleated terminally differentiated cells, usually 20–80 μm in diameter and up to many centimeters long. Fibers are sheathed in insulating endomesium. Nuclei are fixed in a non-random distribution throughout within each fiber, each nucleus regulating protein synthesis within a volume of cytoplasm, its myonuclear domain (MND). Muscle fibers may contain many thousand nuclei; for example in human vastus lateralis each fiber contains about 100 nuclei per millimeter length (Cristea et al., 2010). Arranged along the surface of each muscle fiber are satellite cells which function as a stem cell population; satellite cell mitosis can replenish myonuclear number and producing new satellite cells throughout life.

Running along the length of each fiber are myofibrils; the ultastructural elements responsible for force generation. Each myofibril is composed chiefly of interdigitating actin and myosin myofilaments. In a fiber from a large muscle like gastrocnemius, there may be 1000 myofibrils each composed of c. 1500 myosin and 3000 actin myofilament.

The alignment of interdigitating actin and myosin gives skeletal muscle its striated appearance on microscopy. A sarcomere describes an individual repeated unit of the myofilament and in humans it is about 2 μm long and so 5000 sarcomeres lie in series along each centimeter of muscle fiber. Groups of a few to a few hundred fibers are arranged into fascicles, each just visible to the naked eye and bound together with a connective tissue sheath, the perimesium.

The length of each fascicle will determine the number of sarcomeres in series and therefore the maximum velocity with which it contracts. The CSA of fibers will dictate the number of sarcomeres contracting in parallel and therefore the maximum force generated. Most muscles involved in locomotion are pennate; that is to say the long axis of each fascicle lies at an angle to the axis of traction. Pennation allows more fibers to act in parallel and with hypertrophy, the angle of pennation increases. Thus a physiological CSA of a muscle, perpendicular to the long axis of the fibers, can greatly exceed its anatomical CSA. The volume of a muscle will be the product of its physiological CSA and the fascicle length.

Alpha-motoneurons are the large lower motor neurons that form synapses on muscle fibers. Their cell bodies lie in the ventral horn of spinal gray matter. Each innervates a variable number of muscle fibers which, together with the nerve, constitute a motor unit. Average motor unit size ranges from less than three fibers in the case of the extraocular muscles, c. 180 in the case of soleus to >2000 fibers in the gastrocnemius. The total number of motor units making up some human muscles has also been quantified. This is highly variable between people. In one young sample the range in the number of motor units making up biceps brachi was 58–190 and in the median innervated thenar muscles was 102–421. As described below this changes with age. Due to the security of transmission at the neuromuscular junction between nerve and muscle it is normal for all muscle fibers in a motor unit to fire together making the motor unit the smallest increment of muscle recruitment.

Muscle fibers can be classified according to their predominant myosin heavy chain (MyHC) isoform expression. Type I fibers express MyHC I and demonstrate slow contractile velocity with numerous mitochondria and plentiful myoglobin and hence significant oxidative capacity. Type II fibers are subdivided type IIA and IIx (previously called IIB). Fibers expressing MyHC IIA are packed with more contractile elements each of which contracts faster and generates more force than type I but have fewer mitochondria, less myoglobin, and so less oxidative capacity and therefore less capacity for sustained force generation. Fibers expressing MyHC IIx contract more vigorously than type IIA fibers but with even lower aerobic capacity and are therefore more reliant upon glycolytic metabolism. Each motor unit is composed of fibers of the same type. Type I motor units consist of fewer fibers than Type IIx. Myosin isoform expression is dynamic and can change with training, immobility, or disease (Goldspink, 1985; Guyton, 1991; Purves et al., 2001; Narici et al., 2003; Davies Re and Gergely, 2012).

CHANGES IN MUSCLE ARCHITECTURE

The architecture of muscle describes the three-dimensional arrangement of its component fibers and is a major determinant of its force and excursion capability (Lieber and Friden, 2001). Muscle architecture is dynamic and changes with hypertrophy. Specific age-related change have also been described using ultrasound to measure fascicle length and pennation angle. For example, in the pennate plantar flexor gastrocnemius medialis, it has been shown that the decreases in volume comparing old to young men, measured at 24–31% by CT and MRI, were due not just to fewer, thinner fibers but also in part due to *shorter* fibers; fascicle length dropped by 10–20% (Narici et al., 2003; Thom et al., 2007). This will contribute to an age-related loss of shortening velocity as well as force generation. It also means physiological CSA will not drop as dramatically as anatomical CSA and actually suggests that the loss of specific torque in pinnate muscles, e.g., plantar flexors and knee extensors, is *greater* than that calculated using anatomical CSA as the divisor.

The decrease in fascicle length, and hence in number of sarcomeres in series, will reduce maximum shortening velocity. However, the observed impact on shortening velocity with aging exceeds that which could be explained by decreasing fascicle length. In one study a 38% decrease in calculated maximum shortening velocity of human gastrocnemius was reduced to a 16% decrease when adjusted for fascicle length, suggesting further age-related factors intrinsic to muscle play a role (Thom et al., 2007)

CHANGES IN FIBER MORPHOLOGY

Light microscopic investigation of whole-muscle slices of male human cadaveric vastus lateralis showed that the decrease in CSA with age was in part due to a loss in number of fibers; a 50% reduction in fiber numbers was seen between mean age 19 and 82 years in this sample which did show a greater than normal loss of muscle bulk. No particular fiber type was lost preferentially. However whilst type I fiber size remained constant with age, there was a significant decrease in type II fiber diameter. With advanced age there was also an accumulation of abnormal fibers; they tended to be small and angular and were found individually or in groups of similar such fibers. There were also some large hypertrophic fibers (Lexell et al., 1983, 1988). As described below these shrunken angular fibers are characteristic of denervated fibers.

There is further evidence supporting a selective decrease in size of type II fibers (Larsson, 1978; Coggan et al., 1992; Larsson et al., 1997; Cristea et al., 2010) though some also suggest a decrease in diameter of type I fibers (D'Antona et al., 2003). Another histological study suggested that whilst type II fiber size reduce by c. 57%, type I fibers shrunk by 25% between the third and ninth decades (Andersen, 2003). There is evidence that there may be sexual dimorphism in this phenomenon; it has been observed that type I fiber size remained constant with aging in both sexes with a significant decrease in type II fiber size observed only in males (Yu et al., 2007). Some small biopsy-based studies suggest a preferential loss of type II fibers and subsequent increase in proportion of type I but these should be interpreted with caution given the lack of fiber type homogeneity across muscle seen in old age (Larsson et al., 1997). Discrepancies between studies observing the impact of age on fiber size and type are discussed below.

CHANGES IN MYONUCLEI AND SATELLITE CELLS

A widely accepted paradigm in understanding myonuclear function proposes the constancy of the MND; with hypertrophy new myonuclei arise from satellite cell turnover and with hypoplasia myonuclei apoptose (Hall and Ralston, 1989). This has been called into question by observations of increased density of nuclei and hence reduced MND in disuse atrophy (Gundersen and Bruusgaard, 2008) and old age (Kadi et al., 2004) which has lead to the suggestion of decreased nuclear efficiency with age. However the most recent developments in assessing the spatial arrangement of myonuclei have demonstrated little or no change in average MND size with aging. Instead there is an apparent increase in the *variability* of size of MND and a clustering of myonuclei within grooves on the periphery of the fiber which may itself have implications in the efficiency of the myonuclear control of muscle protein synthesis (MPS; Cristea et al., 2010).

The number of satellite cells per muscle fiber has been shown to decrease by 24% in women and 37% in men when those aged 20–32 years were compared to those 70–83 years. Due to the increase in myonuclear density in this study there was an even larger decrease in the ratio of satellite cells to myonuclei. It is proposed this may lead to a loss of regenerative function (Kadi et al., 2004).

CHANGES IN FIBER BIOCHEMISTRY

In young humans most muscle fibers express a single MyHC isoform though different isoforms do coexist in some fibers, with type I/IIA and IIA/IIx being recognized and having contractile properties intermediate between classical fiber types (Larsson et al., 1997). In aged muscle there is a greater tendency for fibers to express a mixed pattern of MyHC isoforms. Within aged vastus lateralis, 20–28% of fibers were seen to express both type I and type IIA and 22–33% expressed both type IIA and type IIx. Some individual fibers expressing all three isoforms have been identified in biopsies of aged biceps brachii and vastus lateralis, a phenomenon not seen in young muscle (Klitgaard et al., 1990; Andersen, 2003). Other unusual hybrids seen in old age include type I/IIx and expression of neonatal myosin was unexpectedly observed (D'Antona et al., 2003).

A shift toward slow myosin isoform expression is seen in some studies (Gelfi et al., 2006) but not others (Marx et al., 2002; D'Antona et al., 2003). Some of the discrepancy between studies exploring the impact of age on fiber type and myosin isoform expression has been explained by D'Antona et al. They demonstrated dramatically different biochemical and morphological features when comparing muscle from elderly and elderly immobile patients. Immobility accelerated the observed reduction in fiber CSA, in myosin concentration and in specific tension. However, the elderly immobile had a shift toward *fast* isoform expression. MyHC 2X became the most plentiful isoform and fibers co-expressing IIA/X outnumbered those expressing type I. Accompanying this was the paradoxical *increase* in maximum shortening velocity of isolated skinned fibers from the old immobile even when compared to young controls. These data are consistent with observations made in "unloaded" muscle, e.g., during spaceflight or limb immobilization, of muscle wasting accompanied by a shift toward a fast twitch phenotype. Thus it is proposed that the actual expression pattern of myosin isoforms in the elderly is complex because it depends upon conflicting influences of aging and reduced activity tending to shift toward slow and fast isoforms, respectively (D'Antona et al., 2003).

Muscle architecture changes with age. Fibers become shorter as well as thinner and few in number. There is an accumulation of abnormal shrunken angular fibers both individually and in groups and there is clustering of fiber types. These changes suggest denervation and incomplete reinnervation. Type II fibers are more vulnerable to hypoplasia than type I. Fiber classification by MyHC isoform becomes less relevant as fiber increasingly expressed mixed characteristics. Aging and activity levels influence the pattern of MyHC isoform expression. Whist the average MND size changes little with age, its variability increases with age due to clustering of myonuclei.

UNDERLYING MECHANISMS OF SARCOPENIA

GENETIC DETERMINANTS OF SACROPENIA

Sarcopenia is determined by peak muscle mass and the subsequent rate of loss. The degree to which human skeletal muscle phenotypes are heritable has been widely explored. In young people, muscle size and strength are strongly heritable characteristics with h^2 of c. 90% in males in their 20s (Huygens et al., 2004) and in girls and boys aged 10–14 years (Loos et al., 1997). In older people a much smaller proportion of variance in muscle mass and strength is attributable to genetics, with h^2 for leg lean mass as low as 5% and hand grip strength 22%. Environmental factors explain most of the variability (Carmelli and Reed, 2000; Prior et al., 2007). It has been shown in a longitudinal study of male twins, baseline age 63 years, that the proportion of variance in hand grip strength due to genetics decreases from 35 to 22% over a 10 year period (Carmelli and Reed, 2000).

It is therefore unsurprising that the study the molecular genetic basis of sarcopenia remains an area of active research with results of studies identifying "sarcopenia genes" being at best tentative and occasionally inconsistent. The subject has recently been comprehensively reviewed (Garatachea and Lucia, 2011; Tan et al., 2012), with only five genes being identified as having contributed to variation in skeletal muscle mass or strength, in two or more studies. These are angiotensin 1 converting enzyme 1 (ACE), alpha actinin 3 (ACTN3), myostatin (MSTN), ciliary neurotrophic factor (CNTF), and vitamin D receptor (VDR). Linkage and association findings suggest insulin-like growth factor 1 (IGF-1), androgen receptor (AR), and interleukin 6 (IL-6) genes may also contribute to variation in muscle phenotypes.

It cannot be assumed that these genes will subsequently be shown to play a direct role in the pathogenesis of sarcopenia. MSTN attracted early attention as a sarcopenia gene. It's product MSTN was suggested to contribute to sarcopenia after it was shown that MSTN deficient mice did not experience the same age-related muscle atrophy as wild-type controls (Siriett et al., 2006). Anti-MSTN antibodies could even increase muscle mass and strength in an adult mouse model (Whittemore et al., 2003). Further, MSTN mRNA and protein expression levels appeared to increase in older men (Leger et al., 2008). However the hypothesis that increasing MSTN expression significantly contributes to sarcopenia has subsequently been refuted by a larger study comparing young and frail old men that showed no association between serum MSTN levels and knee extensor strength ($r^2 = 0.0001$, $p = 0.97$) or quadriceps CSA ($r^2 = 0.0121$, $p = 0.48$; Ratkevicius et al., 2011).

No single "unfavorable" genotype, associated with accelerated sarcopenia, is yet supported by solid data (Garatachea and Lucia, 2011). The strongest candidate to date is a Lys(K)153Arg(R) polymorphism in exon 2 of the MSTN gene. Studying large groups with this polymorphism has been difficult as the frequency of the mutant R allele is <5% in Caucasians and <20% in Africans. However there is evidence that the KR and RR phenotypes confer weakness on aged women (Seibert et al., 2001; Gonzalez-Freire et al., 2010).

ALTERATIONS IN PROTEIN SYNTHESIS

Protein balance

Muscles exist in a dynamic equilibrium with constant MPS and breakdown (MPB). To achieve proteostasis the two are closely matched, though diurnal fluctuations reflect normal intermittent feeding. In the fasted state and at rest, about 0.05% of the myofibrilar mass of leg muscles is synthesized each hour and this rate has not been shown to differ between young and old men. However in young men this rate more than doubles upon either feeding or exercise but in old men these responses are blunted (Cuthbertson et al., 2005; Kumar et al., 2009). As the age-related loss of muscle mass must reflect a period when average MPB exceeds MPS, a blunting of the anabolic response to these stimuli may be an important contributor to sarcopenia.

Further, MPB is inhibited in the postprandial period, mainly due to the availability of insulin. The ability of insulin to suppress leg muscle proteolysis has been shown to be diminished in older people. Thus there may a compromise in both the downregulation of MPB *and* the upregulation in MPS in response to feeding (Wilkes et al., 2009).

Changes to the proteome

Whilst loss of muscle mass requires protein breakdown to exceed synthesis, a loss of muscle quality of specific tension suggests a change in the profile of protein synthesis, i.e., the proteome. This has been explored using two-dimensional difference gel

electrophoresis as a quantitative differential analysis of protein expression to comparing young and aged human skeletal muscle. Numerous proteins show differential expression dependent upon age. Most are either components of the contractile system or enzymes involved in cellular metabolism (Gelfi et al., 2006).

Myosin heavy chain isoform expression changed dramatically with age. With aging there was an increase in the proportion of MyHC 1 from 48 to 68%; a decrease in the proportion of MyHC 2A from 39 to 31% and a near total loss of MyHC 2X, from 13 to 1%. Myosin light chain (MLC) expression patterns were also different, with a shift away from expression of a fast isoform Q14843 toward P10916, associated with slower twitch velocity. MLC phosphorylation was also lower in the elderly. *In vitro* studies suggest MLC phosphorylation increases calcium sensitivity (Sweeney et al., 1993). Further, troponin T and tropomyosis α-chain isoform, both known to confer calcium sensitivity, are downregulated with advancing age. Reduced calcium sensitivity and reduced calcium release due to dihydropyridine-ryanodine receptor uncoupling will result in excitation-contraction uncoupling (Delbono et al., 1995).

Most of those enzymes that are downregulated with aging participate in anaerobic metabolism. These include creatine kinase as well as several enzymes of the glycolysis pathway including glycogen phosphorylase, glyceraldehydes-3-phosphate dehydrogenase, triosephosphate isomerizes, and δ-enolase. Conversely several enzymes with roles in aerobic metabolism were upregulated in the elderly. These included ATP synthase δ-chain, dihydrolipoamide dehydrogenase, three isoforms of aconitase, malate dehydrogenase, and ubiquinol-cytochrome C reductase complex.

Together these proteomic observations back the *in vivo* observation of older muscles loosing strength and slowing in contraction velocity and are also consistent with the observed decreases in fatigability. The observation of decreased MyHC IIA and IIx expression has been described in some (Larsson et al., 1997) but not all studies (Marx et al., 2002; D'Antona et al., 2003) is discussed above.

CHANGES IN THE HORMONE AND CYTOKINE MILIEU

There are marked endocrine changes associated with advancing age (Lamberts et al., 1997). At menopause, there is an abrupt decrease in ovarian estrogen production. As described above, there is little evidence that this has a dramatic effect upon maintenance of muscle mass or strength.

A much more gradual but nonetheless significant decrease in testosterone and adrenal androgens is seen in males; this has been referred to as the *andropause*. Given the potent anabolic effects of testosterone (Urban et al., 1995), its decreased production may contribute to sarcopenia in males who do loose muscle mass and strength faster than women as described above. Bioavailable testosterone levels have been shown to positively correlate with peak knee extensor strength in older males (Haren et al., 2008).

Dehydoepiandrosterone (DHEA) is the most plentiful circulating steroid hormones and its levels also decrease with advancing age (Ganong, 2005). Most is of adrenal origin; as DHEA itself is weakly androgenic and its metabolites include active androgens, there has been much interest in the potential significance of its decline in later years and the role of therapeutic administration (Casson et al., 1998; Flynn et al., 1999; Percheron et al., 2003). As yet there is little evidence to supporting the hypothesis that DHEA decreases directly contribute to sarcopenia.

Activity in the pituitary growth hormone (GH)/hepatic IGF-1 axis also diminishes with age and this has been termed the *somatopause* (Lamberts et al., 1997). The ability of subcutaneous GH administration to increase lean mass and reduce fat mass in older men suggests that its decrease with aging may contribute to sarcopenia and the body composition changes seen in old age (Rudman et al., 1990).

High parathyroid hormone levels (≥4 pmol/L) approximately double the risk of sarcopenia (Visser et al., 2003).

Chronic inflammation and subsequent cytokine production has received attention as a potential contributor to sarcopenia. There is an age-related increase in circulating levels of the mildly catabolic IL-6 (Roubenoff et al., 1998; Pedersen et al., 2003). Il-6 levels have been shown to negatively correlate with quadriceps strength in healthy older people (Yende et al., 2006) and muscle function in elderly hospitalized patients (Bautmans et al., 2005). Some (Pedersen et al., 2003) but not all (Roubenoff et al., 1998) studies observed age-related increases in the more markedly catabolic, pro-apoptotic cytokine tumor necrosis factor (TNF)-α. Consistent with an age-related increase in chronic low grade inflammation within muscles and this playing a part in the development of sarcopenia is the observation of increasing expression of genes such as compliment C1QA, galectin-1, and FOXO3A which are involved in inflammatory signaling and apoptosis (Giresi et al., 2005).

IL-6 has been shown to downregulate IGF-1 levels both *in vivo* and *in vitro* (De Benedetti et al., 1997; Lelbach et al., 2001), suggesting a biological link between these factors. Further, the impact of elevated IL-6 and low IGF-1 (both individually and combined) on mobility, independence and survival among older women has been examined. Women in the highest quartile of IL-6 or the lowest quartile of IGF-1 had significantly compromised mobility when compared to those with neither risk factor and women with both risk factors had more disability and higher mortality (Cappola et al., 2003), suggesting a compounding impact of dysregulation in immunological and endocrine systems.

It is widely acknowledged that changes in the endocrine milieu, especially decreased androgens and GH/IGF-1 are contributing factor to sarcopenia (Doherty, 2003; Volpi et al., 2004; Narici and Maffulli, 2010). Increased catabolic cytokine signaling may also contribute though it is likely that this plays a more major role in those elderly patients with accelerated muscle loss associated with chronic inflammatory conditions such as chronic obstructive airway disease or rheumatoid arthritis.

LOSS OF INNERVATIONS

It has long been recognized that the pattern of histological changes seen in muscle, described above, suggested that denervation significantly contributed to wasting. The terms "disseminated neurogenic atrophy" and "grouped denervation atrophy" were used to describe the progressive accumulation and clustering of small, angular fibers with denervated appearance (Tomlinson et al., 1969). Alongside this there is a progressive loss of α-motoneurons. The total number of limb motoneurons in the human lumbosacral

region of the human spinal cord was found to average at 57–60,000 before 60 years dropping to 45,000 in octogenarians and 40,000 in non-agenarians (Tomlinson and Irving, 1977).

Electrophysiological studies have confirmed a decrease in the number of motor units with aging (Brown, 1972). Alongside a reduction in number of motor units is some increase in size of motor units (Doherty et al., 1993), suggesting some reinnervation effort. Studies suggest a compromised potential for terminal sprouting and reinnervation of muscle fibers, at least in aged rodentia (Einsiedel and Luff, 1992) Further evidence supporting rounds of denervation and reinnervation is based on the observation that the spatial distribution of fibers in each motor unit in rat muscle becomes more clustered with advancing age (Larsson, 1995). In young humans, fiber types appear randomly distributed across the muscle but they become increasingly grouped or clustered together in age. This may reflect a similar process of denervation and reinnervation (Nygaard and Sanchez, 1982; Lexell and Downham, 1991; Andersen, 2003).

Fewer, larger motor units have been observed in muscles in human upper and lower limbs, especially in smaller distal muscles though it may be a less pronounced phenomenon in more proximal muscles such as biceps brachii (Campbell et al., 1973; Galea, 1996).

It is therefore proposed that apoptosis of motoneurons with subsequent incomplete reinnervation of fibers by surviving neurons is a major contributor to the loss of strength and muscle mass with age (Luff, 1998).

NUTRITION AND ACTIVITY LEVELS

Despite the observed increases in body fat and obesity seen in old age, there is a decrease in food intake across the adult lifespan. The reasons for this anorexia of old age are complex and include visceral, hormonal, neurological, pharmacological, and psychological factors (Morley, 1997). In a survey of 60 years+ people in a developed country, over 50% had a usual intake of less than 1.0 g of high quality protein per kg body weight per day (Sayhoun, 1992). In another survey, 30% had less than 0.8 g/kg/day and 15% less than 0.6 g/kg/day (Roubenoff and Hughes, 2000). It has been suggested that the usual recommended daily amount of protein, 0.8 g/kg/day, is an underestimate and in old age 1.25 g could be considered an appropriate value (Evans and Cyr-Campbell, 1997).

An intake of 0.45 g/kg/day has shown to lead to dramatic and rapid loss of lean tissue and muscle function in older ladies (Castaneda et al., 1995). Dietary supplementation with oral amino acids, providing an additional 0.25 g/kg/day of protein has been shown to increase lean mass in sarcopenic patients (Solerte et al., 2008).

Caloric intake also decreases and this can contribute to sarcopenia as a negative energy balance will result in a negative nitrogen balance regardless of actual nitrogen intake (Roberts, 1995).

Further, vitamin D deficiency (serum 25-OHD $\leq$ 25 nmol/L) has been shown to more than double the risk of sarcopenia (Visser et al., 2003).

Since sarcopenia was first conceived as a concept there has been the realization that decreasing physical activity played a role in its development and held potential as an intervention (Rosenberg, 1989; Evans and Campbell, 1993; Evans and Cyr-Campbell, 1997). The role of exercise as an intervention is discussed below.

COMPROMISED VASCULARIZATION

Capillary density (/mm) has been seen to decrease with age in sedentary individuals (Coggan et al., 1992) but not in masters athletes (Coggan et al., 1990) but these findings are not consistent. Other studies show that a decrease in capillaries per fiber seen with aging is largely (Frontera et al., 1990) or entirely (Andersen, 2003) due to fibers being smaller and the resulting capillary density is unchanged. Despite the apparent preservation of capillary density there is a reduction in the increase in leg bulk flow on exercise (Proctor et al., 1998).

This may be due to compromised vasomotor responsiveness in age rather than reduced capillary numbers. There is evidence of a lower sensitivity of arteriolar tone to normal vasodilatory stimuli; endothelium dependent and nitric-oxide mediated; in arterioles from the mainly slow twitch, oxidative rat soleus. There may be heterogeneity between muscle types as this phenomenon was not observed in the fast twitch glycolytic gastrocnemius (Muller-Delp et al., 2002).

Compromised vascular responsiveness would expose aged muscles to potential hypoxia, free radical stress, and could compromise nutrient delivery.

INCREASED OXIDATIVE STRESS

With aging a reduction in the cellular anti-oxidant buffering mechanisms and an increase in the generation of free radicals due to dysfunction in the mitochondrial respiratory chain result in an increase in the oxidative stress to which the cell is exposed (Barreiro et al., 2006). This will result in damage to muscle components including DNA, myofibrilar, and mitochondrial proteins, the neuromuscular junction, and those elements of the sarcoplasmic reticulum which are responsible for the Ca^{2+} release that initiates contraction. It may contribute to α-motoneuron atrophy and the reduced number and function of satellite cells seen in old age. Levels of serum protein carbonyls, a measure of oxidative stress, are a strong predictor of weak hand grip strength in elderly women (Howard et al., 2007).

It has been proposed that damage to mitochondrial DNA may play a significant part in muscle aging. This tightly packed DNA resides close to the main generator of reactive oxygen species and is therefore at risk of accumulating genetic lesions. These genetic insults may lead to further mitochondrial dysfunction and a vicious-cycle of increasing oxidative stress (Hiona and Leeuwenburgh, 2008).

There is an important heritable element in aging skeletal muscle phenotypes but data published to date on individual genes tends to be tentative and controversial. These are likely polygenic traits and thus not reducible to specific polymorphisms. With increasing age the muscle phenotype is increasingly shaped by environment.

Older muscles demonstrate a blunted anabolic response to feeding and exercise. Enzymes involved in anaerobic metabolism are expressed less in old muscle whilst the aerobic pathways are preserved or enhanced. In healthy active aging, myosin isoform expression shifts away from fast type 2A and 2X and toward slower type I.

The production of anabolic hormones such as testosterone and IGF-1 drop in old age. Levels of the catabolic cytokines IL-6 increase.

Spinal α-motoneurons are lost with age resulting in fewer motor units. There is some increase in size of motor units but there is still histological evidence of denervation. Older people eat less protein and are less active than young people. Blood vessels are less able to respond to demand for changing blood flow. Oxidative stress increases in aged muscle and free radical damage ensues. The mechanisms that contribute to sarcopenia are complex, overlapping, and interdependent.

INTERVENTIONS IN SARCOPENIA

A wealth of literature supports the efficacy of resistive exercise for combating sarcopenia (Meltzer, 1994). For instance, 12 months resistive training in septuagenarian males was found to increase calf muscle volume by 12% and torque by 20% (Morse et al., 2005). Instead, older women (aged 80 years+) display a blunted anabolic response, as no increase in muscle size was found after 12 weeks of resistive training (Raue et al., 2009). Nevertheless, from a systematic review of 120 trials with 6700 older participants, it has been found that the gains in muscle mass and strength afforded by resistive training are associated with a small but significant improvement in physical performance (e.g., gait and ability to raise from a chair; Liu and Latham, 2009). It is noteworthy that lower intensity mechanical loading such as aerobic exercise, despite being considerably less effective for inducing muscle hypertrophy, has been found to promote protein synthesis and expression of growth-related genes and inhibit the expression of muscle breakdown-related genes (Harber et al., 2009). Also, exciting new research findings suggest that aerobic exercise (running) activity sustained for decades (as that performed by master athletes) affords protection against the age-related loss of motor unit number. These findings show that the number of motor units in the tibialis anterior muscle of master runners was similar to that of young runners and significantly higher than that of age-matched older inactive controls. These neuro-protective benefits of running seem restricted to the exercised muscles of the lower limbs since they were absent in the upper limbs (Power et al., 2012). Furthermore, since aerobic exercise training is a potent and effective intervention for the prevention and treatment of insulin resistance, it is likely also to provide protection against the blunting of insulin inhibition of proteolysis in old age, contributing therefore to prevent age-related sarcopenia (Wilkes et al., 2009).

The associated concepts of sarcopenia and dynapenia remain exciting fields of research. We need to refine prediction models for disability, institutionalization and mortality, and translate these into clinical practice. Through exercise based studies we must explore the degree of reversibility of the sarcopenic and dynapenic states. Successful interventions may allow us to infer causality between loss of muscle and strength and the associated health and survival impact. Evidence supporting the use of nutritional, pharmacological, hormonal, and anti-oxidant therapies may revolutionize the approach to treating sarcopenia.

CONCLUSION

With old age skeletal muscles get weaker and they get smaller but they get weaker much faster than they get smaller. The force generated per unit CSA decreases as muscle quality changes with age. Numerous underlying mechanisms contribute to sarcopenia. Evidence supports a role for resistive exercise training in combating sarcopenia. Nutritional and pharmacological interventions remain areas of active research.

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The genetic pleiotropy of musculoskeletal aging

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Musculoskeletal aging is detrimental to multiple bodily functions and starts early, probably in the fourth decade of an individual's life. Sarcopenia is a health problem that is expected to only increase as a greater portion of the population lives longer; prevalence of the related musculoskeletal diseases is similarly expected to increase. Unraveling the biological and biomechanical associations and molecular mechanisms underlying these diseases represents a formidable challenge. There are two major problems making disentangling the biological complexity of musculoskeletal aging difficult: (a) it is a systemic, rather than "compartmental," problem, which should be approached accordingly, and (b) the aging *per se* is neither well defined nor reliably measurable. A unique challenge of studying any age-related condition is a need of distinguishing between the "norm" and "pathology," which are interwoven throughout the aging organism. We argue that detecting genes with pleiotropic functions in musculoskeletal aging is needed to provide insights into the potential biological mechanisms underlying inter-individual differences insusceptibility to the musculoskeletal diseases. However, exploring pleiotropic relationships among the system's components is challenging both methodologically and conceptually. We aimed to focus on genetic aspects of the cross-talk between muscle and its "neighboring" tissues and organs (tendon, bone, and cartilage), and to explore the role of genetics to find the new molecular links between skeletal muscle and other parts of the "musculoskeleton." Identification of significant genetic variants underlying the musculoskeletal system's aging is now possible more than ever due to the currently available advanced genomic technologies. In summary, a "holistic" genetic approach is needed to study the systems's normal functioning and the disease predisposition in order to improve musculoskeletal health.

Keywords: aging, sarcopenia, tendon, bone, cartilage, musculoskeleton, pleiotropic genes, genome-wide studies

Aging of the musculoskeletal system starts early and is detrimental to multiple functions of the whole organism, since it leads to disability and degenerative diseases. The age-related musculoskeletal changes are important in medical risk assessment and care because they influence the responses to treatment and outcomes of therapy. Challenges posed by this growing problem of health care and social medicine are well recognized and are a focus of efforts by many health professionals, as well as biomedical scientists.

There are two major problems that one faces while trying to disentangle the biological complexity of the musculoskeletal aging: (a) it is a systemic, rather than "compartmental," problem, which should be dealt with accordingly, (b) the aging *per se* is neither well defined nor reliably measurable. A unique challenge of studying any age-related process is a need of distinguishing between the "norm" and "pathology," which are interwoven in the aging. When another dimension is added, namely genetics underlying the system's functioning, even less is known about this aspect, and attempts to decipher genetic relationships between the system's components are few. By definition, genetic study of a complex system should explore pleiotropic relationships among the system's parts; however, this is challenging both methodologically and conceptually.

To disentangle the aging-related pathology from the homeostasis particular for aging steady-state, is a challenging task. Despite the multiple definitions of the aging process were proposed, there is no single agreed upon and reliable measurement (Karasik, 2011), therefore underlying molecular mechanism of aging is still not fully understood (de Magalhaes et al., 2009). The definition of aging is complicated by the occurrence of various diseases that modify body functions and tissue structures; these disease-related changes that are common in older persons are often hard to delineate from the aging process *per se* (Nair, 2005). Disease processes and environmental factors also need to be taken into consideration since they affect the rate of aging. Therefore, for our purpose, the "aging" will be considered as a changing environment in which the musculoskeletal system's homeostasis takes place. Aging-related factors provide an additional challenge for the study of genetic effects, since parameters such as co-expression of genes and penetrance of genetic variants can be "masked" by aging. It is necessary to detect genes with pleiotropic functions which are involved in the aging process, since this can provide insights into the potential biological mechanisms underlying inter-individual differences in susceptibility to the aging (Karasik, 2011). In turn, the disentangling effects of these genes in some of the facets of the musculoskeletal system

might contribute to the knowledge related to the aging process of the entire organism.

Identification of genetic variants associated with traits related to the musculoskeletal system's physiology is now possible, due to the rapid development of various novel sequencing technologies, each aspiring to reduce costs to the point at which the genomes of individual humans could be sequenced as part of routine health care (Shendure et al., 2004), as well as the possibility to replicate associations in large human cohorts. Although many recent reviews have been dedicated to aging changes in the musculoskeletal system, most focused on the contribution of advanced age to one of the components of the system and/or on development of one of the musculoskeletal diseases—e.g., osteoporosis (Khosla et al., 2011), osteoarthritis (Loeser, 2010), sarcopenia (Rolland et al., 2008; Fielding et al., 2011), tendinitis (Rechardt et al., 2010) and similar. In turn, this perspective will focus mostly on the muscular part; although we do not attempt to assign the muscle a central role in the "musculoskeletal" system, it seems that the biological importance of the muscle is frequently overlooked while studying other components, and undeservedly so.

PARTS OF THE MUSCULOSKELETON IN ADULTHOOD AND AGING

The term "musculoskeletal system" is most frequently used to describe the biomechanical relationships between muscle, tendon, fascia, bone, and cartilage, which are most evident in locomotion or mastication. However, these organs are interconnected at many other levels, including biochemically and microstructurally. Musculoskeleton includes muscle, tendon, fascia, bone, and cartilage. Skeletal (striated) muscle is a tissue on its own, while other components of the musculoskeleton belong to the connective tissue and have different embryonic origin. Tendon, ligament, bone, and cartilage are connective tissues of mesenchymal origin. Morpho-functional components of the system also include fascia and aponeuroses, which are beyond the scope of the present review. It is important to note that many components of the skeletal muscle are also built by connective tissue. Muscle extracellular matrix (ECM) is often subdivided into epimysium (around the whole muscle), perimysium (around groups of muscle cells), and endomysium (around the muscle cell), and the basement membrane which is considered to be distinct from the endomysium. However, this simplified presentation does not fully explain the transmission of force from the myofiber to the tendon. Detailed studies of the transition zones between endomysium, perimysium, epimysium, and tendon are lacking.(Gillies and Lieber, 2011).

Muscle fibers are multinucleated, post-mitotic, highly differentiated cell agglomerates. Muscles of different functions are probably physiologically distinct; some are chronically active, perform antigravity functions, operate at long lengths, or are involved in rapid movements (Gillies and Lieber, 2011). Based on contractile and metabolic properties, muscle fibers are commonly classified as slow-twitch and fast-twitch, which are further subdivided to more aerobic (Type I) and the more anaerobic glycolytic (Type II), fibers. Adult fast/type-II fibers express one or more of the six type II sarcomeric myosin heavy chain (MHC) genes, while slow/type I fibers express β-MHC isoform (Resnicow et al., 2010). There are different types of collagen expressed during skeletal muscle development, although fibrillar types I and III predominate in adult endo-, peri-, and epimysium (Gillies and Lieber, 2011).

Under normal conditions, skeletal muscle is relatively quiescent, however, it harbors considerable regenerative capacity due to the presence of tissue-resident muscle stem cells known as the satellite cells (Sacco et al., 2010). However, with aging, cellular changes occur that reflect a reduced regenerative capacity of muscle (Collier et al., 2011). Common conditions of muscle aging include sarcopenia (muscle wasting), myosteatosis (gradual infiltration of muscle with fatty tissue), and fibrosis (accumulation of excess ECM). Morphologically, with advanced age, there are alterations in muscle morphology: the muscle tissue is progressively replaced by adipose and fibrotic tissue. Muscle mass is well maintained through the fourth decade of life (Nair, 2005) and shows a moderate decline between 50 and 60 years of age, followed by a more accelerated rate of loss beyond 60 years of age (Deschenes, 2004). Sarcopenia is manifested by decreases in muscle strength and muscle mass with age. The age-specific response to damage seen in muscles of older people includes higher protein expression for nuclear factor kappa B (Nf-κB) and heat shock protein 70 (Mann et al., 2011). Senescence is associated with a decrement in the number of muscle fibers along with a reduction in the size of type II, but not type I fibers (Deschenes, 2004); the relative percentages of different fiber types appear to be unaffected by age (Deschenes, 2004).

Sarcopenia seems to occur by mechanisms that partly are unique to it and partly are common to other forms of atrophy (Mann et al., 2011). There are several potential causes of sarcopenia, among which the most prominent are age-related changes in the hormonal status, low levels of physical activity, reduced protein intake, and increased oxidative stress (Poehlman et al., 1995). Physiologically, after acute injury, such as after sports injuries, damaged or dead fibers are first removed by inflammatory cells, and they are then repaired or replaced by satellite cells (Cosgrove et al., 2009; Mann et al., 2011), which play a major role in post-natal muscle growth and repair. Successful regeneration of healthy muscle thus requires an inflammatory response with the migration and proliferation of fibroblasts, in order to produce new temporary ECM components, such as collagen types I and III, fibronectin, and others. The primary mononuclear cell in normal muscle ECM is the fibroblast, which is responsible for producing the majority of ECM components, including collagen, fibronectin, and proteoglycan (Gillies and Lieber, 2011). Fibroblasts are able to convert mechanical signals into altered gene expression [see review by Gillies and Lieber (2011)]. When tissue is damaged, fibroblasts migrate into the damaged area and begin to produce and remodel the ECM in response to profibrotic cytokines such as transforming growth factor-β (Mann et al., 2011). They serve to stabilize the tissue and act as a scaffold to which the new fibers can migrate, which is necessary for effective repair of damaged tissues.

However, with advanced aging, the chronic inflammatory response drives unrestrained wound healing and tissue fibrosis, characterized by excessive and persistent fibrin/fibrinogen

deposition (Mann et al., 2011). Alternatively-activated macrophages have been shown to promote fibrosis in several different pathogenic conditions (Wynn, 2008). Satellite cells can also differentiate into fibroblasts (Brack et al., 2007).

Fibrin(ogen) can also directly stimulate the expression of collagen; in turn, collagen type I can markedly suppress differentiation of C2C12 cells (the cell line established to become muscle), thus muscle fibrosis is seen as a self-perpetuating mechanism of collagen overproduction (Mann et al., 2011). One might hypothesize that, similar to sarcopenia, advanced muscle fibrosis will have a detrimental effect also on bones, both due to diminished muscle strain and a lack of the myokines due to insufficient population of the myocytes.

With "normal" aging, there is a marked increase in the formation of advanced glycation end-products (AGEs). AGEs are produced by the spontaneous non-enzymatic glycation of proteins (Grillo and Colombatto, 2008) and crosslinking. These collagen modifications increase the stiffness of muscle connective tissue, thereby contributing to impaired muscle function in the older person (Mann et al., 2011). AGEs do not only affect muscles: the accumulation of AGEs in articular cartilage with aging (independent of diabetes) may be attributed in large part to the very slow turnover of cartilage matrix components (Lotz and Loeser, 2012). It has been suggested that in bones, AGEs enhance osteoclast-induced bone resorption, and AGE modification of bone proteins disturbs bone remodeling (Hein et al., 2006). Interestingly, it was shown that AGE-specific binding sites are present in cultured osteoblast-like cells, thus AGEs can regulate proliferation and differentiation of osteoblastic lineage (Hein et al., 2006).

In an alternative path to muscular degeneration with age, intermuscular adipose tissue content and intramyocellular lipid deposits increase, and adipose tissue appears to replace muscle tissue (Song et al., 2004). Muscle lipid content can be reliably measured as muscle attenuation with computed tomography (CT) (Miljkovic et al., 2009). Intermuscular adipose tissue may accumulate in skeletal muscle of elderly people as a result of satellite cells differentiating into adipogenic cells. In aged muscle, there is also an accumulation of the intramuscular, extracellular fat: these fatty deposits are generally localized to the perimysium (Gillies and Lieber, 2011) (more details on the intermuscular adipose tissue are provided below).

TENDONS

Tendons are dense, fibrous connective tissue bands that connect muscle to bone, transmitting the muscular forces to perform movement. Fibroblasts aid in tendon development and maintenance (Gillies and Lieber, 2011). Tendons are built mostly of collagen; however, expression of fibrillar collagens varies along the bone insertion: tendon fibroblasts expressed type I collagen, fibrochondrocytes in the transitional tissue between tendon and bone expressed type II and X collagen, and osteoblasts in bone again expressed type I collagen (Thomopoulos, 2011). The proteoglycans found in tendons, including decorin, fibromodulin, biglycan, and lumican, act to lubricate and organize collagen fiber bundles (Ito et al., 2010). Tendon and perimysium both contain primarily type I collagen, and the primary proteoglycan for both structures is decorin. Perimysium seems to be continuous with tendon (Gillies and Lieber, 2011): collagen in tendon becomes much more organized than collagen in perimysium; high tensile loads on the tendon may organize collagen fibers to align with the muscle axis (Gillies and Lieber, 2011). With aging, enzymatic and nonenzymatic cross-linking is observed. Although cross-linking might signify an aberrant collagen, and thus mechanically less stable tendon, some proposed this might be a mechanism to maintain the mechanical properties of the tendon into advanced age (Couppe et al., 2009). Generally, tendon damage from overuse or degeneration is a common clinical problem in geriatrics and orthopedics, because damaged tendon heals very slowly and rarely recovers completely (Ito et al., 2010).

BONE

Bones develop during embryonic period by either intramembranous ossification (flat bones) or endochondral ossification. The majority of the bones develop endochondrally; those include the long, short, and irregular bones, thus most of the bones are developmentally related to cartilage. Bone cells include osteoclasts, osteoblasts, and osteocytes, whose simplified roles can be described as bone resorption, bone formation, and sensoring/mechanosensitivity, respectively. Bones are built by two compartments, cortical and cancellous (trabecular). Cortical bone consists of a repeating unit, named osteon; the unit structure of cancellous bone is a trabeculum (with a built-in "hemi-osteon"). Muscle and tendon interconnect to transmit force to bone, and the tendon inserts to the bone frequently via fibrocartilage (enthesis) (Gillies and Lieber, 2011; Thomopoulos, 2011). The fibrocartilaginous enthesis forms partly through endochondral ossification (Thomopoulos, 2011). The decrease in collagen fiber orientation in enthesis along with a functionally graded insertion of tendon to bone are major determinants of ensuing stiffness. The linear increase in mineral accumulation on collagen fibers provides significant stiffening of the fibers, but only for concentrations of mineral above a "percolation threshold" corresponding to formation of a mechanically continuous mineral network (Thomopoulos, 2011).

Osteoporosis is among the most common skeletal disorders; it is characterized by low bone mineral density (BMD) and an increased risk of fragility fractures. BMD predictably declines with advanced age; it is the best clinical predictor of future osteoporotic fracture risk. However, BMD measures are relatively ineffective in predicting the skeleton's ability to withstand the forces that produce fractures (Christiansen and Bouxsein, 2010). BMD is also a complex trait controlled by multiple environmental and genetic determinants with individually modest effects, which makes it a difficult phenotype to use in biological exploration. Degenerative bone diseases have been described in detail in a number of recent reviews (Michaelsson et al., 2007; Shanmugarajan et al., 2007) and are therefore not discussed further here.

JOINTS

Joints can be classified into three groups based upon how the bones within the joint are connected: fibrous, cartilaginous, and synovial (Benjamin et al., 1995). Joint tissues include articular

cartilage, ligaments, and menisci, which are frequently surrounded by synovial membrane and fluid. Articular cartilage contains only post-mitotic cells of mesenchymal lineage; with a very low rate of replication during adulthood, which makes them vulnerable to trauma and most affected by the aging process (Lotz and Loeser, 2012). The major collagens of the cartilage are Type II (characteristic of chondrocytes) and Type X (characteristic of hypertrophic chondrocytes) (Thomopoulos, 2011). Cells of cartilage, chondrocytes, and osteoblasts are well interrelated: notably, the chondrocytes frequently transdifferentiate into osteoblasts. Cartilage thus has an innate ability to ossify. Fibrous suture joints in the skull are eventually replaced by bone in childhood. Cartilaginous joints are found in growth regions of the long bones (Wu et al., 2011), where they are also gradually replaced by bone during childhood. A highly prevalent change in aging cartilage is cartilage calcification—by deposition of calcium-containing crystals, mainly calcium pyrophosphate and basic calcium phosphate due to increased pyrophosphate production by chondrocytes from aged cartilage (Lotz and Loeser, 2012).

Notably, similar to cartilage, there is an increased incidence of *heterotopic ossification*, a condition where bone forms within muscles, with aging; it can cause muscle necrosis. The new bone seems to actually form in connective tissue between muscle planes and not in the muscle itself. Heterotopic bone formation occurs also in ankylosing spondylitis, hypertrophic osteoarthrosis, and diffuse idiopathic skeletal hyperostosis. A useful review on inherited human diseases of heterotopic bone formation was recently published (Shore and Kaplan, 2010). Degeneration of other joint tissues, such as ligaments and menisci has been examined at some level (Pauli et al., 2011), however at a lesser extent than the aging of cartilage.

ETIOLOGY OF COMMON MUSCULOSKELETAL AGE-RELATED CONDITIONS

The musculoskeletal system can be regulated by multiple biological mechanisms, including anatomical (anabolic), physiological (anaerobic threshold, hormones), or even behavioral (desire to exercise, pain tolerance) pathways. To get a better understanding of the system's molecular organization and higher-level function, novel pathways with pleiotropic effects of the musculoskeletal system's components should be discovered. Pleiotropy is usually implied when a single gene seems to control multiple phenotypic traits. Strictly speaking, this would be a "direct" pleiotropy; usually, there might be a mediation of a gene's effect via one trait onto the other, or even a confounding by some third factor.

Each compartment of the musculoskeletal system—muscle, tendon, bone, and cartilage—undergoes aging. Despite years of intense studies, we are still relatively ignorant of the molecular basis of the majority of genetically-influenced musculoskeletal phenotypes. In terms of genetics, each degenerative condition is a common and complex disease; even some "rare" syndromes, such as Duchenne Muscular Dystrophy (DMD) or rheumatoid arthritis (RA), are also complexly-regulated traits. It can be anticipated that with the broad use of next generation sequencing (NGS), most causative genes for rare musculoskeletal diseases will be identified in the next few years (Laing, 2012). "Rare" diseases are thus expected to provide some insight into the genetics of common diseases of the musculoskeletal aging, since they share etiology with the latter [see, for example, *LRP5* gene, originally discovered in the rare diseases such as high-bone-mass trait and osteoporosis-pseudoglioma (Boyden et al., 2002; Little et al., 2002) but further being associated with BMD and fracture risk in the general population (Ferrari et al., 2005; Kiel et al., 2007; Estrada et al., 2012)].

Degenerative musculoskeletal process (Lotz and Loeser, 2012) seems to be a reflection of a general aging, which can be seen as disorganization of the cellular homeostasis mechanisms. Survival and normal function of post-mitotic cells like muscle fibers and mature articular chondrocytes depends on their ability to cope with persistent wear and tear. Conceptually, age-related pathologies originate from limitations in the maintenance and repair mechanisms of DNA, by e.g., anomalies in the antioxidant mechanisms that contribute to the detoxification of reactive oxygen species (ROS) (Lotz and Loeser, 2012).

TELOMERES AND MITOCHONDRIA IN MUSCULOSKELETAL AGING

Shortening of the telomeres and mitochondrial somatic mutations were suggested to have an impact on aging skeletal muscle [see recent studies by Dillon et al. (2012) and Payne et al. (2011)]. The shortening of the telomeres at the ends of chromosomes is considered to be a measure of cell senescence; it has been associated with age-related disease and mortality (Wheeler and Kim, 2011). It was also shown that centenarians and their offspring maintain longer telomeres compared with controls and that longer telomeres are associated with protection from age-related diseases, better cognitive function, and lipid profiles of healthy aging (Atzmon et al., 2010; Wheeler and Kim, 2011). A recent study identified a common haplotype of *hTERT*, coding for the human telomerase reverse transcriptase gene, that is enriched in centenarians and associated with longer telomere length (Atzmon et al., 2010). Aging of mitotically active human tissues with high turnover, including blood, liver, skin, testis, and kidneys, is accompanied by telomere shortening (Aikata et al., 2000). In contrast, analysis of telomeres in skeletal muscle during aging reveals only a mild shortening (Decary et al., 1997; Renault et al., 2002), presumably reflecting the low rate of proliferation of myogenic progenitors and muscle tissue turnover during normal aging (Sacco et al., 2010). Telomere length was shorter in women with osteoporosis. In bones, telomere shortening and telomerase activity have been linked to *in vitro* osteoblast senescence and to increased secretion of pro-inflammatory cytokines (Valdes et al., 2007). Telomere shortening can also be promoted by extrinsic or "stress-induced" factors such as the chronic effects of oxidative damage and inflammation (Loeser, 2010; Lotz and Loeser, 2012).

Mitochondrial dysfunction and excessive ROS production contribute to oxidative stress and chronic inflammation of the musculoskeleton. For example, ROS-mediated adverse effects of diabetes are seen on bone formation and maintenance, as ROS greatly influence the generation and survival of osteoclasts, osteoblasts, and osteocytes (Manolagas, 2010). Excessive levels of ROS can contribute to aging in many tissues (Ahima, 2009): in muscles, ROS may trigger different signaling pathways leading to

diverging responses, including either autophagy or apoptosis in the tissues (Barbieri and Sestili, 2012). In joints, there is increased apoptosis in OA chondrocytes following exposure to oxygen radicals; also, oxidized low-density lipoprotein (LDL) could form when LDL present in synovial fluid reacts with ROS (Lotz and Loeser, 2012).

MOLECULAR CASCADES INVOLVED IN THE INFLAMMATION

A direct relationship exists between aging and increasing incidence of chronic diseases, which is in part due to the underlying chronic inflammatory state. However, the molecular mechanisms underlying this chronic inflammatory condition, such as local infiltration by inflammatory cells, are presently unclear, as well as whether aging-related inflammation can be triggered by cellular senescence.

The circulating concentrations of cytokines and C-reactive protein are often elevated in people with age-related diseases, including obesity and Type 2 diabetes, osteoporosis, RA, and Alzheimer disease (Tan et al., 2007; Peake et al., 2010). Some form of inflammatory response is necessary to repair damaged tissues effectively; however, chronic inflammatory responses drive unrestrained wound healing and fibrosis especially in aging (Mann et al., 2011). Intermuscular adipose tissue may accumulate in skeletal muscle of elderly people as a result of satellite cells differentiating into adipogenic cells. Satellite cells can also differentiate into fibroblasts in "old" muscles (Le Grand and Rudnicki, 2007). Both adipocytes and fibroblasts, which replace myoblasts, produce proinflammatory cytokines, which in turn may contribute to the inflammatory state of skeletal muscle in the elderly.

Local inflammation in adipose and synovial tissues is a causative factor in some of the degenerative changes in these and neighboring tissues. Anti-inflammatory cytokines (e.g., IL-4, IL-10, IL-13) play central role in resolving local inflammation and repairing muscle tissue following injury (Peake et al., 2010). It is unclear whether "overflow" of inflammatory cytokines and reactive oxygen/nitrogen species from these tissues and their "spill-over" into the circulation can cause inflammation in neighboring tissues, such as bone. Systemic inflammation in elderly people has been hypothesized for a long time to produce a background milieu (Finch and Ruvkun, 2001; Peake et al., 2010), which affects even distant organs.

Paradoxical relationships between exercise, which triggers the skeletal muscle inflammation, and a positive effect of the exercise on the bones, are of a particular interest. For example, inflammatory cytokines IL-6 and IL-7 are released as a result of physical activity and ensuing muscle contraction; they are, however, activating bone resorption and turnover. On the contrary, an over-secretion of interleukin-15 occurs due to resistance exercise, and its beneficial effects involve a decrease in adiposity and increase in bone mass (Quinn et al., 2009).

ROLE OF MUSCLES IN ENERGY METABOLISM AND INSULIN SENSITIVITY

The adipose tissue is an endocrine organ producing a variety of factors which regulate energy metabolism and insulin sensitivity (Sell et al., 2006). There are distict types of the adipose: white, brown; subcutaneous, visceral, and inter/intramuscular tissue. Adipose tissue protects other cell types from "lipotoxicity" by providing a safe haven for surplus energy. On the other hand, increased adipose tissue mass is associated with insulin resistance, systemic dyslipidemia, hyperglycemia, hypertension, and components of the metabolic syndrome (Dandona et al., 2005; Despres, 2006). Lately, Schafer et al. (2010) found that fat infiltration of muscle was higher in adults with diabetes or impaired glucose metabolism than in those with normal glucose levels ($P < 0.001$). Furthermore, fat infiltration of muscle was independently associated with a 19% increased risk of incident clinical fracture (hazard ratio = 1.19; 95% confidence interval = 1.04–1.36); this association did not differ across glucose metabolism groups. Skeletal muscle is a major depot responsible for glycogen synthesis; therefore, there is an ongoing interest in the metabolic interplay between adipocytes and skeletal myocytes. There are suggestions of a negative crosstalk between body fat and skeletal muscle, which results in disturbances of insulin signaling in skeletal muscle as well as insulin resistance (Sell et al., 2006). Direct adipocyte–myocyte interaction was first explored by Dietze et al. (2002), who demonstrated a direct cross-talk between human adipocytes and myocytes in a co-culture model in which both cell types shared the same medium. Co-culture with adipocytes resulted in insulin resistance in skeletal muscle cells (Dietze et al., 2002; Dietze-Schroeder et al., 2005). There is an effect of local fat also on bone: using normal human osteoblasts co-cultured with differentiating pre-adipocytes *in vitro*, Elbaz et al. showed that inhibition of fatty acid biosynthesis prevents adipocyte lipotoxicity on osteoblasts (Elbaz et al., 2010). After 3 weeks in co-culture, osteoblasts showed significantly lower levels of differentiation and function, with lower mineralization and expression of alkaline phosphatase, osterix, osteocalcin, and Runt-related transcription factor 2 (Runx2).

Evidence has been presented that adipose tissue communicates with the rest of the body not only via free fatty acids (FFAs) but also through adipose-derived cytokines (adipokines) (Sell et al., 2006), which have been implicated in the impairment of insulin sensitivity. On the contrary, adipokines leptin and adiponectin have each been shown to increase fatty acid oxidation and decrease in triglyceride storage in muscle, which may explain, in part, the insulin-sensitizing effect of these cytokines (Dyck, 2009). Recently, Tenta et al. (2012) explored the relationship of adiponectin with bone mass indices and bone metabolic markers in middle-aged post-menopausal women without diabetes, and showed significant associations with osteoprotegerin and IGF1 levels, suggesting an anabolic role of adiponectin. Together with the co-culture experiments, these observations may contribute in the understanding of the interplay between adipose tissue derived hormones and bone metabolism (Tenta et al., 2012).

Koonen et al. (2010) provided insight into the mechanisms by which aging becomes a risk factor for the development of insulin resistance in middle-aged mice, demonstrating that limiting skeletal muscle fatty acid transport is an effective approach for delaying the development of age-associated insulin resistance and metabolic disease during exposure to a high-fat diet. As opposed to the high-fat diet, caloric restriction is frequently used to improve function in aged animals. Thus, Selman et al. (2009)

established that *S6K1*$^{-/-}$ mice live longer and show improved health in later life than control mice. In these mice, the gene S6 Kinase 1 (*S6K1*) was inactivated. *S6K1* is a ribosomal S6 kinase, a component of the nutrient-responsive mammalian target of rapamycin (mTOR) signaling pathway. Microarray data analysis helped to characterize the gene-regulatory changes accompanying the long-lived animals compared with wild-type mice. For example, increased expression of genes associated with pathways known to be associated with aging and with caloric restriction (*Ppargc1a*, *Ppara*, *Foxo1*, *Foxo3a*, *Pdk4*, *Glut1*, *Sirt1,* and *Ucp3*), was observed in the muscle of *S6K1*$^{-/-}$ mice. Down-regulation of ribosomal S6 protein kinase signaling was reported to regulate mammalian lifespan supposedly by increasing activity of AMP activated protein kinase (AMPK) (Selman et al., 2009), a master regulator of cellular energy homeostasis (Hardie, 2007). AMPK inhibits the mTOR pathway, an important regulator of growth control and metabolism. Skeletal muscle mitochondrial uncoupling activates AMPK (Gates et al., 2007; Neschen et al., 2008), and in turn, AMPK has been shown to regulate energy metabolism by modulating the activity of the histone/protein deacetylase SIRT1 (Canto et al., 2009), a molecule supposed to be involved in gene expression changes that mediate the increase in longevity induced by caloric restriction (Ruderman et al., 2010).

PARACRINE AND ENDOCRINE CROSSTALK BETWEEN MUSCLE AND ITS "NEIGHBORS"

There are several pathways with a plausible pleiotropic effect on musculoskeletal system's homeostasis, which seem to be among major players in the aging of musculoskeleton.

GROWTH FACTORS

The contribution of growth factors to the muscle and other parts of the system at different stages of development and aging, is well documented. Growth hormone (GH) is an important regulator of different physiological processes necessary for somatic growth and development, starting with the differentiation of muscle and bone cells, as well as metabolism of lipids and carbohydrates (Perrini et al., 2010). GH regulation and signaling can occur directly, through activation of specific GH receptors (Giustina et al., 2008), or indirectly, through insulin-like growth factor 1 (IGF-1) (Laviola et al., 2007). IGF-1 is produced primarily by the liver as an endocrine hormone in response to GH stimulation (Ohlsson et al., 2009) or in target tissues in a paracrine or autocrine fashion under the control of systemic hormones (Laviola et al., 2007; Kapoor et al., 2008). It was shown that the changes in GH and IGF-1 secretion that occur with aging are paralleled by a progressive loss of muscle mass and BMD (Bohannon, 1997). The contribution of IGF-1 to the maintenance of parts of the musculoskeleton is well documented. Mechanical stimulation of bone cells may induce elevated levels of IGF-1, which signals the differentiation of osteoblasts into osteocytes (Schmid et al., 1980); which maintain bone mass in response to normal load (Bonewald and Johnson, 2008). IGF-1 levels are positively correlated with muscle protein synthesis rates, specifically myofibrillar protein (actin and myosin filaments) and MHC synthesis (Waters et al., 2000). Overexpression of insulin-like growth factor and the simultaneous loss of myostatin in muscle *in vivo* have been found to have synergistic effects on myofiber growth and lessened fibrosis (Mann et al., 2011). The anabolic response to IGF-1, measured as proteoglycan or collagen synthesis, declines in human articular chondrocytes from older donors with increasing age (Lotz and Loeser, 2012).

Other growth factors regulating muscle mass include mechano-growth factor, myostatin, vascular endothelial growth factor (VEGF), or hepatocyte growth factor (HGF). In skeletal muscle, at least two different IGF-1 isoforms are expressed due to alternative splicing of the primary IGF-1 transcript (Goldspink, 2004). For the overview of growth factors and cytokines secreted by muscle, as well as their potential effects on bone metabolism, the reader is referred to a recent review by Hamrick (2011).

Mechano-growth factor (MGF) is a member of the IGF-1 superfamily that is induced in response to physical activity (Wu et al., 2011). It has a marked ability to induce satellite cell fusion for muscle repair and maintenance. Recent findings of up-regulation of MGF in prostatic cancer cells might highlighted its role also in cancer biology (Armakolas et al., 2010); this might suggest that caution should be exercised for its therapeutic use in older men (Thomis and Aerssens, 2012). Recently, Juffer et al. (2012) investigated whether mechanical loading by pulsating fluid flow modulates the messenger RNA (mRNA) and/or protein levels of muscle anabolic and metabolic factors in MLO-Y4 osteocytes. They showed that loaded MLO-Y4 osteocytes differentially express anabolic factors involved in the adaptive response of muscle to mechanical loading (i.e., IGF-1, MGF, VEGF, and HGF). Similarly to muscle fibers, mechanical loading enhanced expression levels of these growth factors in primary bone cells. The authors concluded that osteocytes respond to mechanical loading by producing more VEGF and HGF, which suggests that these proteins previously played unrealized roles in bone remodeling mediated by loading.

Fibroblast growth factor 2 (FGF2) is another polypeptide growth factor that stimulates satellite cells (a.k.a. muscle precursor cells, MPCs). MPCs isolated from sarcopenic animals exhibit a decreased proliferative response to FGF2 (Jump et al., 2009). FGF2 is also a well-known osteogenic factor; it has positive effects on bone formation in estrogen-deficient rodents (Hamrick, 2012).

TRANSFORMING GROWTH FACTOR-β (TGFβ) SUPERFAMILY, MYOSTATIN, AND ACTIVIN RECEPTORS

TGFβ is a multifunctional protein that controls proliferation, differentiation, and other functions in many cell types. In the muscle, TGFβ seems to play an important role in aging-associated fibrosis and muscle impairment. Overexpression of active Notch2 in C2C12 cells (the cell line established to become muscle) prevents TGFβ from inducing the expression of collagen I, whereas transient knockdown of Notch2 by siRNA in cultured myoblasts results in the differentiation of C2C12 myoblasts into myofibroblastic cells that express fibrotic markers (Mann et al., 2011). For the recent review on role of TGFβ in osteoblast differentiation and bone formation, see Chen et al. (2012). The review also highlights the different modes of cross-talk between TGF-β/BMP and the signaling pathways of MAPK, Wnt, Hedgehog, Notch, and FGFs in osteoblast differentiation and bone formation

(Chen et al., 2012). The role of TGFβ in muscle fibrosis of aging has been the subject of many studies [see review Dequeker et al. (2003)]. Activated TGFβ induces fibroblasts to produce type I collagen, fibronectin, and connective tissue growth factor (cTGF) and suppresses matrix metalloproteinases (MMPs). Notably, MMP levels in uninjured muscle are generally low, but secreted MMPs can degrade type IV collagen, fibronectin, proteoglycans, and laminin (Gillies and Lieber, 2011). When repeated muscle injury occurs, elevated TGFβ continues to stimulate ECM production and eventually leads to a fibrotic response (Gillies and Lieber, 2011). Logically, TGFβ inhibitors can be used to reduce aging-associated fibrosis in skeletal muscle (Mann et al., 2011).

A lot of research in the last decade was focused on myostatin (growth differentiation factor 8, GDF8), a secreted TGFβ protein family member that inhibits muscle differentiation and growth. Myostatin is produced primarily in skeletal muscle cells, circulates in the blood and acts on muscle tissue, by binding a cell-bound receptor called the activin type II receptor [for the recent review, see Hamrick (2012)]. Thus, elevated levels of myostatin expression are evident in disuse atrophy and cachexia (of cancer and AIDS) (Hamrick, 2011). Contrarily, inactivating mutations of the myostatin *(MSTN*, a.k.a. *GDF8)* gene induce a hypermuscular phenotype in mammals, with pronounced effect on bones (Hamrick et al., 2003). Importantly, myostatin is released from muscle during traumatic and catabolic conditions that may inhibit and suppress bone repair. Lee and McPherron (2001) previously showed that inhibition of myostatin by genetic elimination using knockout mice or by increasing the amount of the propeptide follistatin, resulted in greatly increased muscle mass. Follistatin (also known as activin-binding protein) is an autocrine glycoprotein that is expressed in nearly all tissues of higher animals (Tortoriello et al., 2001). Follistatin is known to regulate myostatin activity and muscle growth (Lee and McPherron, 2001). Kota et al. (2009) showed that regulation of follistatin via gene therapy also resulted in muscle growth and increases in strength in non-human primates. The authors agreed with the findings in mice detected by Lee and McPherron (2001), so the findings of both studies point out that gene therapy may improve muscle mass and function in patients with certain degenerative muscle disorders.

Myostatin not only regulates the growth of muscle cells, but also fibroblast activation and hence the progression of fibrosis in the muscle. In the absence of myostatin, the improved regeneration and decreased fibrosis took place (Mann et al., 2011). It appears that myostatin also regulates the structure and function of tendon tissues, as the stiffness of tendons is 14 times higher in myostatin-deficient mice than in the wild-type controls (Mendias et al., 2008). Importantly, myostatin expression is increased by glucocorticoids; this suggests that myostatin is required for the catabolic effects of glucocorticoids, leading to muscle atrophy (Gilson et al., 2007). It is well known that glucocorticoid-induced enhanced proteolysis also corresponds to increase in bone loss (Kanis et al., 2007).

Myostatin binds to the soluble activin type IIB receptor (ACVR2B) and to a lesser extent to activin type IIA receptor (ACVR2A), which act as myostatin antogonists and thus exert differential anabolic activity in bone and muscle (Lee et al., 2005). Digirolamo et al. presented data on muscles and bones in three groups of mice given either one of two types of ACVR2 or placebo (Digirolamo et al., 2011). Their study findings suggest that both ACVR2 and ACVR2B produce anabolic effects on muscle and bone; however, ACVR2's effect was greater on bone than muscle, whereas ACVR2B's effect was greater on muscle than bone. The activin receptor types and their related function in the musculoskeletal system are summarized in **Table 1**. *In vitro* and *in vivo* studies have demonstrated that both activin A and its antagonist follistatin play opposite roles in bone formation (Eijken et al., 2007): follistatin increases bone formation in mice, by inhibiting activin A *in vivo* using a decoy soluble activin A receptor (Vallet et al., 2010). Activin A treatment can inhibit mineralization in cultured osteoblasts. Loss-of-function of activin type I receptor (ACVR1) in osteoblasts increases bone mass and activates canonical Wnt signaling through suppression of Wnt inhibitors sclerostin (SOST) and DKK1 (Kamiya et al., 2011).

Table 1 | Activin receptor types and their function in the musculoskeletal system.

Activin receptors/sub-units	Function in bone/muscle	References
ACTIVIN TYPE I RECEPTOR		
ACVRA1	Essential for skeletal development. Involved in ossification of muscles and joints in fibrodysplasia ossificans progressiva (FOP) disease, through mutations in ACVR1 and noggin gene. A mutation causes endothelial cells to transform to mesenchymal stem cells and then to bone	Chen et al., 2004; Lucotte et al., 2009; van Dinther et al., 2010
ACVR1B	Involved in muscle strengthening	Windelinckx et al., 2011
ACVR1C	Is used as an antagonist of myostatin, which inhibits muscle cells proliferation	Digirolamo et al., 2011
ACTIVIN TYPE II RECEPTOR		
AVCR2A	Regulates muscle growth and bone formation	Lee et al., 2005; Lotinun et al., 2010
AVCR2B	Involved in the signaling pathway essential for initiating osteoblast differentiation	Liu et al., 2012

A point mutation in ACVR1 can cause fibrodysplasia ossificans progressiva (FOP) in which ectopic ossification occurs in skeletal muscles and deep connective tissues (Lucotte et al., 2009; van Dinther et al., 2010).

VITAMINS

The connection between muscle and bone can be regulated by vitamins, most prominently, by vitamin D. Vitamin D, a key regulator of bone metabolism, is also known to be significantly associated with muscle strength: a lack of vitamin D, which is associated with aging, can cause myopathy. In the elderly, vitamin D deficiency is linked to muscle weakness, increased body sway, and increased susceptibility to falls and fractures. The mechanisms underlying the effect of vitamin D on muscle strength are not fully understood; thus, vitamin D action requires activation of the vitamin D receptor, which is widely distributed in various tissues including skeletal muscle (Endo et al., 2003; Grundberg et al., 2004; Windelinckx et al., 2007). Gilsanz et al. demonstrated that serum 25OHD was inversely related to the percent fat of skeletal muscle, a relation that was independent of body mass or subcutaneous and visceral fat depots. Compared with women with normal serum 25OHD concentration, vitamin D-insufficient women had approximately 24% greater muscle fat infiltration (but no significant differences in the cross-sectional area of their thigh muscles) (Gilsanz et al., 2010). Non-straightforward seems to be the relationship between the serum levels of vitamin D and osteoarthritis. Vitamin D has been shown to stimulate synthesis of proteoglycan by mature articular cartilage *in vitro*, and this suggests that vitamin D may directly affect articular cartilage metabolism, but its relationships with the clinical OA seem to be confounded by other aging-related processes.

Other vitamins are also have roles in the musculoskeleton regulation, however, less is known. Vitamin K deficiency has been linked to a variety of age-associated conditions, including loss of BMD or increased fracture risk, arterial calcification, and inflammation (Shea et al., 2009). Bone-related conditions and arterial calcification have been most widely studied in relation to vitamin K availability (McCann and Ames, 2009), while there are virtually no data on the vitamin K effect on skeletal muscle. On the contrary, a role of Vitamin E in the muscle physiology seems to be well known. Vitamin E could reduce muscle inflammation (Tiidus and Houston, 1995). Since a large majority of vitamin E is found in adipose tissue, this might reflect anti-oxidant properties of vitamin E and indicate its supplementation can help reduce muscle damage caused by free radicals. Interestingly, mice with a genetically-induced vitamin E deficiency, have high bone mass as a result of a decrease in bone resorption. Vitamin E thus decreases bone mass by stimulating osteoclast fusion according to Fujita et al. (2012).

MENOPAUSE AND SARCOPENIA: IS THERE EVIDENCE FOR A CAUSAL RELATIONSHIP?

Menopause is associated with a decline in estrogen levels, which is translated into an increase in fat mass, as well as a decrease in bone density, muscle mass, and muscle strength. Decline in muscle mass (sarcopenia) is frequently observed in post-menopausal women (Messier et al., 2011). During the menopausal transition, there is a sharp fall in estrogen levels, therefore it has been suggested that changes in female estrogen levels may play a role in the development of sarcopenia during menopause. Estradiol (E2), together with estriol (E3) and estrone (E1), belong to estrogens. So far, there is limited evidence whether the loss of estradiol negatively affects muscle mass and physical function. Ratiani et al. (2012) were exploring lately the influence of estrogen on the intensity of oxidative metabolism in women of reproductive and menopausal age. For this study, two groups of women—less than 45 years old (reproductive age) and older than 45 (menopausal age) were tested. It was found that the physiological reduction in estrogen levels during menopause by itself contributes to impairment of oxidative metabolism and intensification of inflammation, oxidative stress, hypoxia, and related conditions.

Aging is associated with a loss of sex hormone not only in women but also in men; this phenomenon is called the "andropause" or the "male menopause." In men, reduction in the levels of the androgen testosterone can trigger declines in muscle mass, bone mass, and overall physical function (Horstman et al., 2012). It was previously shown that administration of supplemental testosterone orally to older relatively hypogonadal men resulted in an increase in muscle mass and a decrease in body fat (Wittert et al., 2003). Lately, the Toledo Study for Healthy Aging (Carcaillon et al., 2012) found that there are sex differences in the association between serum levels of free testosterone and frailty (a syndrome of aging-related loss and dysfunction of skeletal muscle and bone) in an elderly population. The authors suggested that although the age-associated decline in testosterone occurs in both men and women, this decline does not arise to the same extent in both sexes, suggesting a possible differential impact on frailty according to sex.

Adverse effects of menopause on bones are well known (Kanis, 1996); less obvious are effects of "andropause" on bones (Ferrari et al., 2005). The uncertainties of the roles of major sex hormones in muscle and in bones are illustrated in **Figure 1**. Recently, a Finnish study investigated a link between the estrogen replacement therapy (ERT) and skeletal muscle transcriptome (Ronkainen et al., 2010). Long-term use of ERT was associated with subtle differences in muscle transcript profiles and a better muscle fiber composition, although no differences were observed in mitochondrial DNA copy number or oxidative capacity per muscle cross section. Similar to the menopausal etiology of osteoporosis, the role of estrogen has been a long-standing theme in osteoarthritis research, since OA is more common in women than in men (a ratio of ∼3:1). OA often begins around the time of menopause, and incidence rises faster in menopausal women than in men of the same age. Joint pain is a common complaint at the time of menopause or ERT withdrawal.

In addition to changes in androgen and estrogen levels, another important player in the development of sarcopenia in elderly population might be myostatin, a secreted TGFβ family member that inhibits muscle differentiation and growth (as detailed above). The relationship between testosterone and myostatin in the humans is not fully understood, although testosterone is known to reduce myostatin release in the muscle (Kovacheva et al., 2010). Studies indicated that myostatin release in the muscle is not necessary related to sarcopenia.

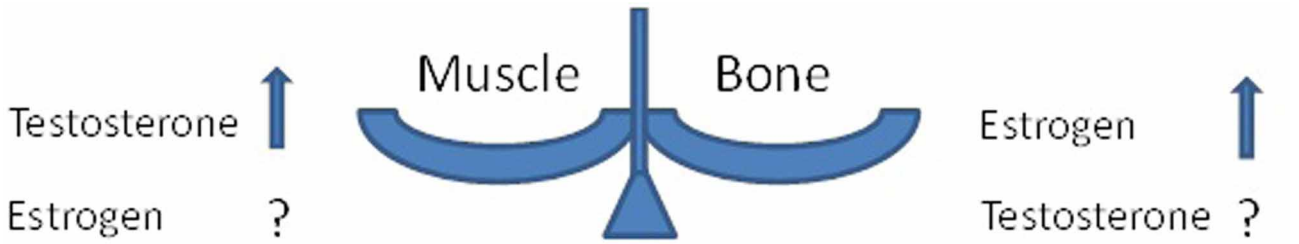

FIGURE 1 | Possible changes in muscle and bone in response to sex hormone levels.

Ratkevicius et al. (2011) showed that serum concentrations of myostatin and myostatin-interacting proteins do not differ between young and sarcopenic elderly men. The authors suggested that altered serum concentrations of myostatin-interacting proteins might not contribute to sarcopenia with the possible exception of follistatin-related gene (FLRG). FLRG encodes a secreted glycoprotein that is highly homologous to follistatin and binds activins and bone morphogenetic proteins (Wang et al., 2003). In a different study, in young and old men treated with graded doses of testosterone, myostatin levels were significantly higher on day 56 than on baseline in both young and older men. Notably, changes in myostatin levels were significantly correlated with changes in total and free testosterone only in young men, probably suggesting that response to testosterone is affected by age (Lakshman et al., 2009).

GENETICS OF SARCOPENIA AND OTHER COMMON MUSCULOSKELETAL AGE-RELATED CONDITIONS: EVIDENCE OF PLEIOTROPY?

RELATIONSHIP BETWEEN MUSCLE AND BONE MASS IN ADULTS

Skeletal muscle has a close functional relationship with bone, starting in embryonic period. Developmentally, osteoblasts and muscle cells derive from a common mesenchymal precursor, the pluripotent mesenchymal stem cells. For example, the C2C12 cell line is established to become muscle but can be induced to differentiate into osteoblasts in the presence of BMP-2 (Darcy et al., 2012).

Studies of both humans (adults and children) and laboratory animals have documented a strong, positive correlation between muscle strength and bone mass (Gilsanz et al., 2006). Developmentally, this process is a reflection of allometry (usually defined as the covariance between the form, shape, and size of body part and size of the whole organism) (Karasik and Kiel, 2010). Further in the life, muscle atrophy is concomitant with the observed bone loss (Judex et al., 2004). There is a "biomechanical" explanation for this phenomenon. Mechanical loads activate new bone formation on cortical and trabecular surfaces; strain can activate some bone cells, which then respond with gene activation, increased metabolism, growth factor production, and building the matrix (Forwood, 2001; Frost, 2003). For example, in their recent study, Zhang et al. (2012) found that osteogenic response of bone mesenchymal stem cells to continuous mechanical strain is dependent on the signaling of extracellular regulated protein kinase (ERK) 1/2 and Runx2. Runx2 is a key transcription factor known to regulate the differentiation and/or function of osteoblasts. It is also an important mediator of the ability of metastatic breast cancer cells to directly modulate both osteoclast and osteoblast function. Sears et al. (2007) showed there is an evolutionary association between Runx2 repeats (changes in glutamine-alanine tandem-repeat ratio) and facial skull length in carnivores suggesting Runx2 tandem repeats providing a flexible genetic mechanism to rapidly changing the timing of ossification. Facial length is co-regulated with mastication, which is a product of muscle forces. Changes in bone length thus probably reflect a co-evolution of muscles and bones and suggest a pleiotropic role for Runx2 in mammalian evolution (Sears et al., 2007).

Furthermore, muscle strains are needed for fracture repairs. According to Hao et al. (2012), bony union was affected in rats whose quadriceps were treated with botulinum toxin-A (BXTA); saline was injected into the contralateral quadriceps. At different time points up to 8 weeks post-fracture, a gap was still visible on X-ray images of that side and no mature osseous calluses or woven bone were found by histology on the BXTA-treated side. Finally, biomechanical testing indicated that the femora of the BXTA-treated side exhibited inferior mechanical properties compared with the control side.

Beyond an obvious biomechanical importance of muscle strains for fracture repair, locally-released factors seem playing a role: covering bone fractures with muscle hastened healing and resulted in an increase in union strength. Thus, muscle-derived stromal cells exposed to an adjacent fracture differentiate into osteoblasts *in vitro*; the osteogenic potential of these cells exceeds that of adipose and skin-derived stromal cells and is equivalent to bone marrow stromal cells. The recruitment and differentiation of muscle-derived stromal cells in response to tumor necrosis factor-α (TNF-α) was shown to promote an accelerated healing of the fracture in an *in vivo* murine model (Glass et al., 2011). A local injection of recombinant human TNF-α on the first two days after fracture-inducing surgery accelerated healing of the fracture. By day 28, TNF-α treated animals showed significantly higher callus mineralization compared to controls. Importantly, TNF-α is one member of a large family of inflammatory cytokines that share common signal pathways, including activation of the transcription factor Nf-κB. NF-κB is a key regulator of inflammation in skeletal muscle (Peake et al., 2010), thus plays a critical role in muscle atrophy. As a skeletal catabolic agent, TNF-α stimulates osteoclastogenesis while simultaneously inhibiting osteoblast function (Nanes, 2003). Paradoxical role of

TNF-α in fracture repair is interesting, since it probably points out at a necessity of resorption of bone edges and the regulated apoptosis in the callus for its ossification, osteon integrity, and thus an efficient fracture healing.

Both bone and muscle show major changes during aging and in the same direction, with sarcopenia and osteoporosis contributing to frailty (Matthews et al., 2011). Similarly to bone, muscle tissue deteriorates with the age. Sarcopenia term has been coined which is based on lower muscle quality, just as osteoporosis is typified by both decreased bone mass and structural integrity (Matthews et al., 2011). In addition, based on the mechanostat theory, the muscle-bone unit has been defined as a functional system whose components are under the common control of several hormones (Zofkova, 2008). Sex steroids modulate the function of the muscle-bone unit in adulthood and aging. Therefore, based on theoretical principles of allometry and the ample empirical data on co-regulation (provided above), the existence of genes determining characteristics of both traits is plausible (Karasik and Kiel, 2010).

GENETIC CORRELATION BETWEEN THE MUSCLES AND BONES AND QUANTITATIVE TRAIT LOCI AFFECTING BOTH

Evidence for genetic correlations was described in the literature between geometric parameters of femoral bone neck and total body lean mass in men and women (Sun et al., 2006). It is not surprising, since lean mass was positively associated with section modulus and cross-sectional area in both sexes ($r = 0.36$–0.55, $p < 0.05$) (Moseley et al., 2011). Deng et al. (2007) performed a bivariate genome linkage analysis which produced two chromosomal regions, 5q35 and 10q24, with pleiotropic effects on these phenotypes. Results in the Framingham Osteoporosis Study demonstrated similar bivariate genetic correlations between leg lean mass and cross-sectional femoral geometry; bivariate linkage analysis identified significant quantitative trait loci (QTL) of leg lean mass shared with shaft CSA on chromosome 12p12–12p13 and with neck shaft angle, on 14q21–22 (Karasik et al., 2009). One cannot exclude that a third trait (such as fat mass) might be involved in pleiotropic relationships between bone and muscle, probably even as a mediator of their relationship.

There is an importance studying complex diseases and aging related processes through large animal models and translate animal research findings to future biomedical sciences. As of now, mostly small organisms are being used for this purpose, for example, zebra fish are being explored as a model for investigating the effect of aging on male reproduction (Kanuga et al., 2011). In livestock, we only find genetic evidence for skeletal muscle mutations which affect muscle growth and development but the effect on aging is not yet being explored. In sheep for example, the *callipyge (CLPG)* mutation causes post-natal muscle hypertrophy, which is localized in the pelvic limbs and loin (Cockett et al., 2005). Muscles from *CLPG*-expressing lambs enlarge to different degree and not all muscles are affected (Cockett et al., 2005). The *CLPG* trait in sheep exhibits a novel mode of inheritance termed "polar over-dominance" while the only animals that express the *CLPG* phenotypes are those heterozygotes who inherited the *CLPG* mutation from their sire (Georges et al., 2003). Enhanced skeletal muscle growth is also observed in animals with the *Carewell* (or *rib-eye muscling)* mutation, and a double-muscling phenotype has been documented for animals of the Texel sheep breed (Cockett et al., 2005). QTL were identified for traits related to bony carcass and meat quality in sheep while genome-wide significant QTLs were mapped for muscle (Karamichou et al., 2006) and bone (Campbell et al., 2003) densities. Since clinical studies are not easy to be implemented, large animals can be used as models to explore aging-related complex diseases and their effect on the musculoskeletal system. Future identification of the actual genes or mutations responsible for these bone and muscle related QTLs in livestock and especially their role in aging, will increase the understanding of vertebrate musculoskeletal biology.

PLEOTROPIC RELATIONSHIPS BETWEEN BONE AND MUSCLE: IS THIS A "TRUE" PLEIOTROPY OR A MEDIATION?

There are several potential mechanisms underlying associations between genetic variants and the parts of the musculoskeletal apparatus, including the mediation of environmental influences by genetic factors (Karasik, 2011). It was previously postulated that the spectrum of pleiotropic effects of a gene on a morphological trait may include direct or indirect effect with a possible continuum in-between (Kelly et al., 2006). We previously mentioned two possible scenarios of gene actions. The first one includes pleiotropic effects of gene polymorphisms on two traits, Trait1 and Trait2 (say, muscle and bone), and the second includes a conditional model (mediation), in which a gene is associated with one trait, and that trait in turn influences or affects another trait. (see scheme in **Figure 2**, scenarios **A** and **B**). Additional possible scenario can be suggested where a gene is affecting a third—unknown—trait (Trait3), which in turn affects directly both muscle and bone (confounding). In any case, products of the same gene(s) can be used as biomarkers for two traits simultaneously.

An example for cumbersome relationships when pleitropy is suspected, comes from a naturally occurring recessive mutation, named "mini muscle" (MM), that causes some 50% reduction in hind limb muscle mass of mice (Kelly et al., 2006). Observed reduction of both muscle mass and bone structure could represent direct pleiotropic effects of the MM allele if the gene is expressed in the stem cell populations early in development. Alternatively, the gene might act intrinsically only on the muscle cells and not the bone cells, and the MM bone phenotype could arise via weaker mechanical input from the reduced hind limb musculature (Wallace et al., 2012).

Pleiotropy in humans is ubiquitous (Sivakumaran et al., 2011); there are multiple examples in the literature, starting with a textbook example of phenylketonuria (PKU), caused by a deficiency of the enzyme phenylalanine hydroxylase, which is necessary to convert the essential amino acid phenylalanine to tyrosine. A defect in the single gene that codes for this enzyme therefore results in the multiple phenotypes associated with PKU, including mental retardation, eczema, and pigment defects that make affected individuals lighter skinned (Paul, 2000). In the most recent study, a single nucleotide polymorphism (SNP) rs11655470 in the region where the *CRHR1-MAPT* genes are located was related to infant head circumference (Taal et al., 2012). Variants in or near *CRHR1* have been previously associated

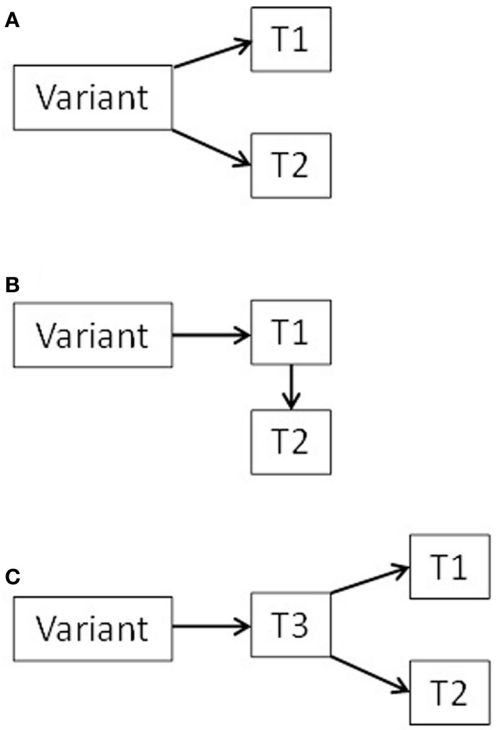

FIGURE 2 | Diagram of three possible scenarios (A, B, and C) for pleiotropic bone-muscle interactions (*T* = trait).

with brain development and BMD (Rivadeneira et al., 2009). In a parallel paper, Ikram et al. (2012) showed that a correlated SNP in the same region (rs9303525; linkage disequilibrium, LD, $r^2 = 0.22$ with rs11655470) is associated with adult intracranial volume. Since LD between the variants found to be low, it is possible that they represent separate, independent signals. After a series of statistical models with adjustments of one trait for the other, the investigators concurred that these signals reflect a third marker influencing both phenotypes, the head circumference and intracranial volume. In the above example of the MM allele, the authors similarly rise a possibility that there exists a circulating hormone or growth factor that regulates both muscle and bone development (Wallace et al., 2012). As occurs when the confounded scenario is a correct one, frequently there is a third SNP which seems to be associated with both traits, whose relationship with a combination of traits is stronger than with either trait. The biological causality underlying the potentially-pleiotropic associations is largely unknown, and cannot be resolved with statistical means; the definite answer should come from the "bench"—functional studies.

USING GENOMIC TOOLS IN ORDER TO DETECT GENETIC VARIANTS INVOLVED IN PLEIOTROPIC MECHANISMS

Recently, in parallel with the upsurge of the whole-genomic genotyping and sequencing techniques, large-scale analyses and data mining methods have been developed to identify associations at a genomic level. These high-throughput techniques have enabled genome-wide association studies (GWAS), in which about 100,000—1,000,000 SNPs (representative of millions of SNPs in human genome) are tested. Linkage studies, GWAS and re-sequencing as well as new bioinformatic tools pointed out some interesting musculoskeletal genes, whose, in turn, contribute to identifying new pathways of possible pleiotropic value. We summarized the candidate genes with plausible biological pleiotropic effects on muscles and bones, as well as other tissues, in **Table 2**. For example, a possible candidate for pleotropic effect between bone and muscle is α-actinin 3 (*ACTN3*) gene which is highly expressed in fast skeletal muscle fibers (Yang et al., 2011). ACTN3 is one of the two isoforms of α actinin which are found in Z-discs of skeletal muscle (Chan et al., 2008; Yang et al., 2011). Genetic studies previously suggested that the absence of ACTN3 is detrimental to sprint and power performances in elite athletes and in the general population (Chan et al., 2008; MacArthur et al., 2008). Yang et al. (2011) recently showed that ACTN3 deficiency is associated with reduced bone mass in humans and mice.

As follows from the Table, along with the biological candidates, some genetic studies produce "serendipitous" pleiotropic findings. Similarly, genetic association studies for rare diseases have their merit by uncovering some facets of the complex musculoskeletal biology. Often methodologically different, both belong to the domain of human genetic study. Here are examples of recent discoveries of genes with a potentially-pleiotropic role in the musculoskeleton.

In a large GWAS performed by the GEFOS consortium, a locus at 5q14—near myocyte enhancer factor 2C (*MEF2C*)—was found to be significantly associated with BMD related traits (Rivadeneira et al., 2009). Besides being involved in myogenesis (Cesana et al., 2011) like the other myogenic basic helix-loop-helix proteins, members of MEF2 family of regulatory proteins are participating in bone-relevant pathways including endochondral ossification (Kramer et al., 2012). Notably, by virtue of being widely pleiotropic, *MEF2C* was associated with different traits, such as platelet count, retinal vascular caliber, tonometry, and adult height. Future research should be performed to decipher a "real" musculoskeletal pleiotropy of this molecule, as opposed to a mediation effect via some primary basic mechanism active early in development.

Another segue, now into the pathobiology of tendons, was provided by a recent GWAS of Dupuytren contractures (Dolmans et al., 2011). Dupuytren's disease is a fibromatosis of the flexors, whose prevalence increases with age and leads to flexion contractures affecting fingers. Six of the 9 loci identified by the study included genes known to be involved in the Wnt-signaling pathway, including *WNT4*, *WNT2*, *WNT7B*, *SFRP4*, *SULF1*, and *RSPO2*. The latter gene encodes R-spondin, a member of the family interacting with frizzled receptors and LRP5/6 to induce β-catenin signaling; *Rspo2* expression is required for Wnt11-mediated osteoblast maturation. Wnt-signaling pathway is among the leading regulators of bone mass, as is shown time and again (Rivadeneira et al., 2009; Zhang et al., 2010; Estrada et al., 2012). In muscle, a conversion of myogenic into fibrogenic lineages could be abrogated experimentally by treating mice with Wnt inhibitors (Mann et al., 2011). Similarly, injection of the Wnt antagonist DKK1 into the skeletal muscles of *mdx* mice (see below) significantly reduced fibrosis. Indeed, there

Table 2 | Candidate genes and pathways with pleiotropic action on the musculoskeletal system [modified from (Karasik and Kiel, 2010) with permission].

Gene (abbrev.)	Gene title (alias)	Action	Reference
SEX HORMONES			
AR	Androgen receptor	Decreased AR activity results in a loss of bone mass	Bhasin and Buckwalter, 2001
		CAGn repeats associated with fat-free mass in men	Walsh et al., 2005
ESR1	Estrogen receptor 1	In a meta-analysis, *XbaI* polymorphism associated with BMD and fracture risk in women	Ioannidis et al., 2002
		Esr1 knock-out mice unable to respond to physical exercise with a periosteal bone expansion compared to wildtype mice	Lee et al., 2003
		PvuII polymorphism may modulate the effect of exercise on BMD	Suuriniemi et al., 2004
		Note: no relationship between *TA*-repeat polymorphism and muscle mass and strength in young adult women	Grundberg et al., 2005
COMT	Catechol-O-methyltransferase	Val158Met polymorphism was associated with peak BMD in young men and an interaction of *COMT* with physical activity on BMD was found in the same young men	Lorentzon et al., 2004, 2007
		Girls with $COMT^{LL}$ compared to $COMT^{HH}$ genotype had more lean mass as measured by DXA, and an increased muscle area in the tibia as measured with pQCT	Eriksson et al., 2005
		mCSA differed by Val158Met genotypes (significantly larger in LL than HL individuals) in *older Finnish women*	Ronkainen et al., 2008
GROWTH HORMONE/INSULIN-LIKE GROWTH FACTORS			
IGF1	Insulin-like growth factor I	CA-repeat promoter polymorphism has effects on femoral bone geometric parameters	Rivadeneira et al., 2004
		CA-repeat polymorphism was associated with increased bone strength and muscle volume and strength	Kostek et al., 2005
		Alternative splicing was involved in the mechanotransduction of bone cells	Goldspink and Yang, 2004; Tang et al., 2004
TRANSFORMING GROWTH FACTOR-β SUPERFAMILY			
*TGFB1**	Transforming growth factor-β1	Association of 29C > T polymorphism in the transforming growth factor-β1 gene with lean body mass in community-dwelling Japanese population	Fuku et al., 2012
		SNPs in *TGFB1* found to be associated with peak bone mass in young healthy Caucasian females	Tzakas et al., 2005
*MSTN**	Myostatin	Myostatin-null mice had significantly greater cortical bone mineral content and larger entheses than normal mice	Hamrick et al., 2003; Hamrick, 2003
		mRNA levels were reduced in response to heavy-resistance strength training in older adults	Roth et al., 2003
		SNPs (rs2293284 and rs7570532) were associated with hip peak BMD variation in Chinese women	Zhang et al., 2008
VITAMIN D SIGNALING			
VDR	Vitamin D receptor	In a meta-analysis, *Cdx-2* polymorphism was associated with risk for vertebral fractures in women	Uitterlinden et al., 2006
		BsmI polymorphism was associated with decreased vertebral area and femoral narrow neck width	Fang et al., 2007
		BsmI polymorphism was associated with muscle strength	Windelinckx et al., 2007; Grundberg et al., 2004

(Continued)

Table 2 | Continued

Gene (abbrev.)	Gene title (alias)	Action	Reference
		FokI polymorphism was associated with fat-free mass and sarcopenia in older men	Roth et al., 2004
		Interactions between leisure physical activity and VDR *BsmI* genotype on the lumbar spine BMD in active post-menopausal women	Blanchet et al., 2002
		Association between *VDR* polymorphisms and falls, balance and muscle strength	Barr et al., 2009
INFLAMMATORY CYTOKINES			
IL6	Interleukin 6	*–174 GC* polymorphism was associated with increased risk of wrist fracture in post-menopausal women and with hip BMD in post-menopausal women	Nordstrom et al., 2004; Ferrari et al., 2004
		–174 GC was associated with fat-free mass in men but not women	Roth et al., 2003
		Exercise increases IL-6 receptor production in human skeletal muscle	Keller et al., 2005
IL15	Interleukin 15	Transgenic mice (overexpressing IL-15 in skeletal muscle, with elevated circulating levels) show increased bone mass	Quinn et al., 2009
TNF	Tumor necrosis factor (a.k.a. TNF-α)	*TNF* promoter polymorphisms associated with muscle phenotypes in humans	Liu et al., 2008
		TNF negatively regulates bone formation in rats	Zhou et al., 2006
OTHER PATHWAYS			
BMP2	Bone morphogenetic protein-2	Young males with the *rs15705* C/C genotype were associated with an increased gain in skeletal muscle volume ($P = 0.0060$) following resistance training	Devaney et al., 2009
		BMP2 was linked and associated with BMD at different skeletal sites	Xiong et al., 2006; Styrkarsdottir et al., 2003
*PPARG**	Peroxisome proliferator-activated receptor γ	Polymorphisms in the PPARγ were associated with aBMD in both mice and humans	Ackert-Bicknell et al., 2008
		Mutations in PPARγ result in increased fatty acid flux to the skeletal muscle	Savage et al., 2003
MEF2C	Myocyte enhancer factor 2C	Responsible for controlling bone development, by activating chondrocyte hypertrophy.	Arnold et al., 2007
*NR3C1**	Nuclear receptor subfamily 3 group C member 1 (a.k.a. Glucocorticoid receptor)	Contributed both to bone and lean mass in older persons, muscle strength in younger males	van Rossum et al., 2003, 2004
		ER22/23EK polymorphism was associated with lower trochanteric BMD in elderly women	van Schoor et al., 2007
PTN	Pleiotrophin	Over-expression affects mouse long bone development, fracture healing and bone repair	Li et al., 2005
		Potential mediator of mechanotransduction signaling in regulating periosteal bone formation and resorption in mouse	Xing et al., 2005
		Expression levels lowered in response to spaceflight	Nikawa et al., 2004
NOTCH1	Notch homolog 1, translocation-associated	NOTCH1 inhibits bone resorption, both directly on osteoclast precursors and indirectly via osteoblast lineage cells	Bai et al., 2008
		Significantly lower expression found in muscle biopsies from older men compared to muscle from younger men	Carey et al., 2007
NOTCH2	Notch homolog 2, neurogenic locus notch homolog protein 2	Mutations in NOTCH2 cause Hajdu-Cheney syndrome, a disorder of severe and progressive bone loss	Simpson et al., 2011

(Continued)

Table 2 | Continued

Gene (abbrev.)	Gene title (alias)	Action	Reference
RETN	Resistin	Serum levels showed a significant negative correlation with lumbar spine BMD in middle-aged men	Oh et al., 2005
		Polymorphisms associated with muscle and bone phenotypes in men and women	Pistilli et al., 2007
SOX17	Transcription factor SRY (sex determining region Y)-box 17	Involved in endochondral bone growth	Agoston et al., 2007
		Downregulated in older men (as a part of the "sarcopenia signature")	Giresi et al., 2005
SOX6	(sex determining region Y)-box 6	Associated with BMD in GWAS studies	Liu et al., 2009; Rivadeneira et al., 2009
		Expressed in a wide variety of tissues, most abundantly in skeletal muscle	Smits et al., 2001
NFKB1	Nuclear factor of kappa B (a.k.a. *NF-kB*)	NF-kB proteins implicated in muscle wasting (short-term hindlimb unloading in rodents)	Kandarian, 2008
		Activation of NF-kB can induce muscle atrophy in transgenic mice	Cai et al., 2004
LMNA	Lamins A/C	Mutations cause primary laminopathies, including skeletal muscular dystrophies	Jacob and Garg, 2006
		Lamin A/C knockdown had a negative impact on osteoblastogenesis and bone formation *in vitro*	Akter et al., 2009
FOXO1	Forkhead box protein O1	Skeletal muscle transgenic mice (FKHR) have less skeletal muscle mass, down-regulated type I fiber genes and impaired glycemic control	Kamei et al., 2004
		Positive regulator of bone formation, required for osteoblast proliferation	Rached et al., 2010
VCP	Valosin-containing protein	Mutations cause inclusion body myopathy with early-onset Paget's disease and frontotemporal dementia (IBMPFD) syndrome	Watts et al., 2004
		Transgenic mice expressing mutant forms VCP/p97 recapitulate the full spectrum of IBMPFD syndrome including degeneration in muscle, brain and bone.	Custer et al., 2010
ACTN3	α-Actinin-3	Muscle deficiency is detrimental to sprint and power performance in humans	Chan et al., 2008; MacArthur et al., 2008
		Deficiency is associated with reduced bone mass in human and mouse	Yang et al., 2011

**According to GeneCards (http://www.genecards.org)—Last accessed on April 29th 2012.*

was reduced fibrosis and enhanced muscle regeneration in aged muscle injected with DKK1, whereas no change was observed in young similarly treated muscle (Brack et al., 2007). A mechanism of Wnt-induced cell-fate changes from myogenic to non-myogenic cells in resting satellite cells awaits further validation (Mann et al., 2011). Notably, a role of another important Wnt antagonist, SOST in skeletal muscle has not been explored yet.

Other evidence for the importance of muscle-to-bone cross-talk in bone health and disease comes from the rare diseases of muscles. Thus, DMD is an X-linked disease where the gene encoding the protein dystrophin is affected by various mutations. Fractures are a significant problem in patients with DMD (young boys). Two recent studies investigated bones affected by the deficiency of dystrophin, which is mutated in DMD. Both used dystrophin-deficient *mdx* mice, a model of human DMD. Thus, one study (Novotny et al., 2011) showed that tibiae of *mdx* mice had up to 50% lower strength and stiffness compared to wild-type mice; they had reductions in cortical cross-sectional moment of inertia, cross-sectional area, and in trabecular bone volume. Importantly, this compromised bone strength was already obvious in very young mice (Novotny et al., 2011), which corresponds to poor bone health in DMD boys. The second group (Nakagaki et al., 2011) investigated the changes that occur in the femur of young *mdx* mice, at 21 days of age. They also demonstrated a lower strength, stiffness and energy absorption capacity in *mdx* femora, which were shorter, had a smaller cortical area and thickness, and manifested changes in the ECM and collagen organization. Interestingly, at 3 weeks of age the muscle damage in mice was still not significant, thus it is a lack of DMD expression, even in the absence of significant muscle fiber degeneration, which seems to be affecting bones (Nakagaki et al., 2011). The underlying molecular mechanisms for the effects on bone, whether direct or indirect, have not yet been elucidated in detail, which makes this exploration tantalizing. The above—semi-serendipitous—findings of pleiotropy, call out for a systematic study dedicated to identifying genetic variants underlying muscle and other related traits, for shared genetic mechanisms underlying parts of the musculoskeleton. In the next sub-section we discuss a framework that may be useful in analyses of the "-omics" data.

POST-GWAS AND GENE EXPRESSION STUDIES

Our knowledge of the genetic architecture of common musculoskeletal diseases remains rudimentary, in part due to the complex relationship between the phenotype and genotype (and the environmental effects). Only recently has it been possible to address the need for high throughput gene expression studies (RNA-seq, transcriptome) focusing on multiple relevant tissues, such as differential expression of skeletal and muscular genes with aging. Transcriptional profiles of many human tissues, including muscle (Welle et al., 2004; Zahn et al., 2006; Wallace et al., 2012), skin (Lener et al., 2006), tendons (Jones et al., 2006), and cartilage (Swingler et al., 2009); have been generated; however, for human bones, there are fewer available resources (Grundberg et al., 2008; Reppe et al., 2010).

Mantila Roosa et al. (2011) study used the rat forelimb loading model to evaluate the extent of alternative splicing in bone under mechanical loading. Animals were subjected to loading sessions every day, and ulnae were sampled at 11 time points, from 4 h to 32 days since loading started. They identified multiple alternatively spliced genes encoding cytokines, ion channels, solute carriers, and notably, muscle-related genes. Previously, Paic et al. (2009) isolated osteocyte and osteoblast cell populations for microarray analysis. Multiple muscle-related genes were downregulated in osteoblasts with respect to osteocytes, including many of the same genes downregulated by loading in the study of Mantila Roosa et al. (2011). Although the involvement of muscle-related genes and proteins in bone biology is not well understood, it is clear that they are highly regulated in bone cells. One can speculate that finding of the muscle-related genes being downregulated both during bone formation and in osteoblasts compared to osteocytes is linked to mechanosensitivity, which is characteristic of osteocytes.

Transcription factors that play a role in ossification during development are expected to participate in post-natal fracture repair since the endochondral bone formation that occurs in embryos is recapitulated during fracture repair (Reumann et al., 2011). The knowledge of their interplay during the bone formation, as well as functioning as parts of muscle-bone unit, should provide the strategic basis for interventions aimed at improving tissue repair after fracture, by mechanical stimuli or some humoral factors, or by a combination of both.

Several studies have used gene profiling to identify gene clusters and individual genes in muscle that change with age (Roth et al., 2002; Giresi et al., 2005; Dennis et al., 2008, 2009; Thalacker-Mercer et al., 2010). Furthermore, expression profiling was used to identify genes expressed in adult rat and human tendon tissue (Jelinsky et al., 2010). Using this technique, approximately 1,600 transcripts appeared to be selectively expressed in rat tendon tissue and approximately 300 transcripts appeared to be selectively expressed in human tendon tissue, with ∼20 genes overlapping between both human and rat tendon tissue. Of these common tendon-selective genes, thrombospondin-4 and tenomodulin were found to have the highest tendon-selective expression compared to other tissues examined. Interestingly, expression of these tendon-selective genes, which are present in primary tendon fibroblasts, is lost when these cells are placed in two-dimensional culture systems (possibly suggesting that mechanoception is a factor for expression of these genes). Identification of tendon-selective genes provides potential molecular tools to facilitate a better understanding of tendon development and repair.

Drummond et al. (2011) profiled microRNA (miRNA) expression patterns in aging human skeletal muscle followed by in-depth functional and network analysis. In a classical fashion of such experiments, muscle biopsy samples from 19 younger men (age 31 ± 2) were compared with the older (73 ± 3 yrs old; $n = 17$). Eighteen miRNAs were differentially expressed, including Let-7 miRNA family members, Let-7b and Let-7e (Drummond et al., 2011). A higher expression of Let-7 family members that may down-regulate genes related to cellular proliferation fits well into our understanding that older human muscle is characterized by reduced muscle cell renewal and regeneration.

Expression QTLs (eQTLs) can be used as a tool assessing genetic regulation of the expression levels of mRNA in desired tissues. These eQTLs can be mapped to a variant in a local

gene (*cis* eQTLs) or to variant on a different chromosome (*trans* QTLs). In porcine, identification of eQTLs of genes expressed in longissimus dorsi found to be associated with meat quality traits (Ponsuksili et al., 2010). As an example for the adipose tissue, Small et al. (2011) and the MuTHER consortium demonstrated that the Type 2 diabetes and HDL-cholesterol associated *cis*-acting eQTL of the maternally-expressed transcription factor *KLF14* acts as a master *trans*-regulator of adipose gene expression. Expression levels of genes regulated by this *trans*-eQTL are highly-correlated with concurrently-measured metabolic traits, and a subset of the *trans*-genes harbor variants directly associated with metabolic phenotypes. The authors suggested that by leveraging "-omics" data from multiple sources they are able to discover new biological and functional insights. A note of caution should be sounded while interpreting these experiments, since the cell culture and animal models may not reflect the complex dynamic interactions between genes and the environment that form the basis of human phenotype (Franceschi et al., 2007).

CONCLUSION

The pathobiology of many of the musculoskeletal diseases remains obscure, as do factors affecting disease severity (Laing, 2012). Thus, for example, the establishment of new therapies, such as regenerative medicines, for injured tendons has been delayed by a limited understanding of tendon biology (Ito et al., 2010). Several markers have been shown to correlate with the burden of musculoskeletal diseases; additionally new techniques must be developed to identify and quantify the biomarkers in order to support both genetic diagnostics and a genetic study, which is a powerful tool in biological discovery. We need to focus on genetic aspects of the cross-talk between muscle and its "neighboring" tissues, to find previously unknown pathways of inter-compartmental communication. Multivariate methods for large-scale data analysis and mining of results were proposed and validated (Shriner, 2012). Ideally, a multivariate GWAS, identifying genetic variants underlying both bone and muscle, should be replicated in large human cohorts. Similarly, tantalizing findings of pleiotropy between the genes regulating muscle and energy metabolism, insulin resistance, fat depots, and reproductive aging, should be explored further.

Several studies have been proposed in this Perspective that could address the areas of immediate interest, and the key questions can be summarized as follows. Focus should be on a possibility that growth factors (inflammatory cytokines, myokines, etc.) expressed in muscle could affect signaling in bone cells. New methods are needed to accurately measure the *in vivo* mechanical responses and in particular, how aging may affect the ability of mechanical loading to stimulate muscles by activating satellite cells (Thomis and Aerssens, 2012) and further trigger anabolic responses in bones (Wu et al., 2011). Therapeutic applications of the biological knowledge of muscle-bone-cartilage interface can further be extended to the fractures: risk prevention by soft-tissue cushion, fracture repair by adjacent muscles, or improving tendon-bone attachment in orthopedics. This knowledge would help institute treatment interventions aimed at improving bone tissue repair and successful regeneration of healthy muscle, thus reducing adverse outcomes in vulnerable populations, such as aged people and others (e.g., professional athletes). Combined strategies will be crucial to ameliorate muscle loss with the ensuing inflammation, fatty infiltration, and fibrosis, including systemic delivery of anti-inflammatory or anti-aging agents and gene-corrected cells, adapted for the local milieu (Mann et al., 2011). More fundamental study is desired to learn how aging contributes to musculoskeletal diseases. Answers to these questions will vastly improve our understanding of normal function of the musculoskeleton as a whole. Finally, identifying mechanisms by which etiologies of several diseases are interconnected, will provide potential targets for systemic therapeutic interventions. From a general gerontological perspective, a rapidly aging system such as the musculoskeletal provides challenges for studying its normal decline and trying to prevent it but also forthcoming rewards if our efforts succeed.

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Physiology and pathophysiology of musculoskeletal aging: current research trends and future priorities

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"Physiology and Pathophysiology of Musculoskeletal Aging" is a Frontiers Research Topic aimed at increasing our understanding of healthy musculoskeletal aging. By gaining a better understanding of healthy musculoskeletal aging we can provide better care and new therapies for common musculoskeletal problems.

Sarcopenia is a term utilized to define the loss of muscle mass and strength and the consequent functional impairment that occurs with aging (Baumgartner et al., 1999; Waters et al., 2000; Morley et al., 2001). It can begin as early as the third decade of life. However, in most cases it affects individuals in the fourth decade and beyond. The gradual loss of muscle tissue (atrophy) reduces total body mass. Decreased physical activity with aging appears to be the key factor involved in producing sarcopenia (Morley et al., 2001). The age-related alterations in muscle involve age-related muscle fiber transitions and metabolic shifts in aging muscle. These alterations also affect posture and gait and become particularly evident in the seventh, eighth, and ninth decades of life. Mitchell and colleagues highlight these issues by reviewing the current knowledge of the decline in human muscle mass and strength with advancing age and the associated risk to health and survival (Mitchell et al., 2012). They also review the underlying changes in muscle characteristics and the etiology of sarcopenia. The authors draw on evidence from cross-sectional studies that have compared young and old muscle to show that in people aged 75 years, muscle mass is lost at a rate of 0.64–0.70% per year in women and 0.80–0.98% per year in men with more rapid concomitant losses in muscle strength; loss of muscle strength is thought to be 2–5 times greater than loss of mass. The loss of muscle strength is a more consistent risk factor for the development of disability and death than loss of muscle mass (Mitchell et al., 2012).

In their review article David Karasik and Miri Cohen-Zinder from Bar-Ilan University argue the need for identification of genes with pleiotropic functions in musculoskeletal aging (Karasik and Cohen-Zinder, 2012). The authors point out that musculoskeletal aging is detrimental to multiple bodily functions. This is a challenging area given the fact that the process of musculoskeletal aging becomes apparent in the fourth decade of an individual's life. Exploring pleiotropic relationships is difficult both methodologically and conceptually. However, in order to identify the biological mechanisms underlying these changes we need a "holistic" genetic approach to investigate the cross-talk between muscle and closely related tissues (i.e., tendon, bone, and cartilage). This strategy may allow us to find the links between skeletal muscle and other parts of the "musculoskeleton" (Karasik and Cohen-Zinder, 2012).

Kay Ohlendieck takes a molecular approach to musculoskeletal aging by reviewing the applications of proteomic profiling to aging muscle and the "fast-to-slow" muscle transitions that are thought to occur during aging (Ohlendieck, 2011a). The author has recently published a review article that discusses current proteomic approaches for studying skeletal muscle and associated technical challenges and emerging techniques (Ohlendieck, 2011b). His review article in this Research Topic discusses proteomic profiling approaches that have helped to establish an age-related shift to slower protein isoforms of myosin heavy chain, myosin light chain, actin and tropomyosin, as well as subunits of troponin (Ohlendieck, 2011a). These studies have confirmed previous assertions concerning the disproportionate age-related atrophy of type IIa muscle fibers and an age-related decrease in the synthesis rate of myosin heavy chain (Morley et al., 2001). Ohlendieck's mini-review also discusses the "glycolytic-to-oxidative" shift that occurs in slower-twitching senescent muscles and the newly identified proteins that are altered in aging muscle using proteomic profiling (Ohlendieck, 2011a). Proteomic profiling has also revealed an increase in mitochondrial enzymes and a concomitant decrease in glycolytic enzymes during the fast-to-slow transformation process in aging skeletal muscle. This timely mini-review which has previously been accompanied by a dedicated Editorial (Mobasheri, 2011b) also discusses alterations in metabolic and contractile elements that can be used to define a "sarcopenia-specific" biomarker signature.

Aging is also a major contributor to the development and progression of osteoarthritis (OA). OA is a long and slow continuum of joint changes and symptoms that has no clear-cut onset (Englund, 2010). Therefore, the use of "omics" technologies offers great promise for understanding factors that contribute to disease initiation and progression and how they are affected by aging.

Gharbi and co-workers (Gharbi et al., 2011) review the applications of proteomic techniques in OA research. One of the main objectives of applying proteomics to the study of cartilage

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aging and OA is to discover disease-specific biomarkers, which may reveal new therapeutic targets and facilitate targeted drug development. Gharbi et al., summarize proteomic techniques and their applications to OA research and discuss technical limitations and practical problems associated with sample preparation and strategies to overcome them. Proteomic studies of cartilage and other joint tissues will be particularly relevant to diagnostic and therapeutic research in OA (Mobasheri, 2011a).

Fernández-Moreno and colleagues from Blanco's group assess the link between a number of biomarkers and the mitochondrion-related phenotype in patients with OA (Fernandez-Moreno et al., 2012). They measured the serum levels of the OA-related biomarkers including matrix metalloproteinase-1 (MMP-1), matrix metalloproteinase-3 (MMP-3), matrix metalloproteinase-13 (MMP-13), myeloperoxidase (MPO), hyaluronic acid (HA), human cartilage glycoprotein 39 (YKL-40), cartilage oligomeric matrix protein (COMP), cathepsin K, and several peptides derived from type II collagen including Coll2-1, Coll2-1NO2, C2C, and CPII. The biomarkers were analyzed in 48 OA patients and 52 healthy controls carrying the mitochondrial haplogroups H and J. MMP-13 was the only biomarker that significantly increased in OA patients compared to healthy controls in both mitochondrial haplogroups H and J. The collagen type II biomarkers, Coll2-1, Coll2-1NO2, the Coll2-1NO2/Coll2-1 ratio, C2C, CPII, and the C2C:CPII ratio were significantly increased in OA patients carrying haplogroup H compared to OA carriers of the haplogroup J. Therefore, mitochondrial DNA haplogroups are potentially useful biomarkers of OA and may be used in conjunction with traditional protein biomarkers (Fernandez-Moreno et al., 2012).

Adrian Hunnisett and Christina Cunliffe put forward a positioning paper on the role of chiropractic treatment as a primary care intervention for better musculoskeletal health in the aging population in the United Kingdom (Hunnisett and Cunliffe, 2012). The elderly are encouraged to take more exercise to ensure, and maintain, musculoskeletal health. The authors propose chiropractic treatment as an Intervention in musculoskeletal aging and advocate patient-focused care for the management of musculoskeletal disorders. Chiropractic intervention and maintenance programs can promote health and ensure continued musculoskeletal functioning of the elderly population and encourage the mechanistic and evidence-based integration of chiropractic into the National Health Service of the United Kingdom (Hunnisett and Cunliffe, 2012).

Musculoskeletal aging is a complex process. It involves tissue atrophy and loss of function in muscle, bone, tendon, ligament, intervertebral disk, and articular cartilage. Musculoskeletal aging is also accompanied by reduced neuromuscular integrity. The progressive loss of musculoskeletal mass and function highlights the importance of physical activity and exercise in elderly people. Increased adiposity (sarcopenic obesity) is another mechanism that contributes to age-related musculoskeletal deterioration. Increasing exercise capacity and physical fitness are likely to results in regeneration and remodeling within musculoskeletal tissue compartments. However, the molecular mechanisms that underscore these changes are poorly understood. Understanding age-related musculoskeletal deterioration requires co-ordinated basic, clinical, and translational studies that can generate novel and clinically testable approaches to healthier musculoskeletal aging. The elegant and original articles in this Research Topic highlight some of the major issues facing researchers in the area of musculoskeletal health and aging. We hope that this Research Topic will serve as a platform for encouraging further research into musculoskeletal aging.

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Disparities in the consequences of sarcopenia: implications for African American Veterans

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INTRODUCTION

The phenomenon of age-related muscle wasting has been long recognized by geriatricians, rehabilitation specialists, and public health practitioners. However, Federal agencies are slow to recognize sarcopenia as a diagnostic term despite the ubiquitous observation of muscle loss as a signature event of aging. The formal adoption of sarcopenia as a clinical diagnosis has been complicated by its evolving operational definition and the varied approaches to the assessment of lean body mass (LBM). Currently, formal assessments of LBM rarely occur within the context of standard geriatric care. The failure to adequately assess muscle tissue within the larger context of geriatric medical care has significant public health implications given the importance of adequate muscle function for "successful aging" and the maintenance of independent living (Visser and Schaap, 2011).

The prevalence of sarcopenia is approximately 25% of the U.S. population over 70 years of age (Baumgartner et al., 1998; Lorenzo, 2009). Additionally, U.S. health care expenditures ascribed to sarcopenia, based on the Third National Health and Nutritional Examination Survey (NHANES III) data, have been conservatively estimated at 11.8 billion dollars (Janssen et al., 2004a). Given the national trends in the Veteran population with respect to age, reported disability levels, and increased representation of African American soldiers, the potential impact of sarcopenia on the Veterans Health Administration (VHA) healthcare system merits consideration.

AFRICAN AMERICANS AND THE VHA HEALTHCARE SYSTEM

The total Veteran population was estimated to be over 22 million in 2012 which also includes approximately 2.2 million women. There was significant minority representation among Veterans during this same period with African Americans members totaling 2.6 million men and 430,000 women. The 2011 Veteran Population Projection Model (VA Office of the Actuary, 2011) projects a nearly 35% decrease in the total Veteran population between 2013 and 2040. Nonetheless, this population trend will be countered by an anticipated concomitant increase in the percentage of minority Veterans from 21 to 34% during this same time period. African Americans will constitute the largest proportion of this projected demographic shift in the Veteran population (National Center for Veterans Analysis and Statistics, 2011). These changes reflect the advent of the All-Voluntary Force and recent military conflicts such as the Gulf War I and II which were notable for the high proportion of participating minority service members with nearly one in three soldiers being African American (Census Bureau, 2010). It is important to note that the Veteran population is older than the general population with a median age of 62 in comparison with a non-Veteran median age of 43. The oldest Veterans reflect a larger proportion of Caucasians (Census Bureau, 2010; National Center for Veterans Analysis and Statistics, 2011) as their median age is 64 in comparison to minority Veterans who have a median age of 54–57. However, the relatively younger subset of minority Veterans coupled with the projected demographic changes suggest that the need to effectively manage geriatric syndromes in African Americans within the VHA healthcare system will continue to increase over time.

SARCOPENIA: DIAGNOSTIC CONSIDERATIONS FOR AFRICAN AMERICANS

Sarcopenia is an age-related syndrome characterized by a decrease in muscle mass and associated with a loss of strength and power, diminished functional performance, and increased disability (Newman et al., 2003; Morley et al., 2011). The observation that basic mobility and independent living status may be more dependent on lower extremity muscle performance rather than LBM in older adults (Visser et al., 2000; Kamel, 2003) was the impetus for some investigators to ascribe specific terms (e.g., dynapenia and kratopenia), to characterize the age-related loss of muscle force and power (Morley et al., 2011). Moreover, the Foundation for the National

Institutes of Health Sarcopenia Project has proposed to qualify sarcopenia as a form of *skeletal muscle function deficit* to reflect the varied etiology of mobility impairments (Correa-de-Araujo and Hadley, 2014). The recognition of total body mass or body fat as independent factors that influence functional status gave rise to sarcopenic index measures that combine these variables with estimates of LBM (Newman et al., 2003). Consensus groups such as the European Working Group on Sarcopenia in Older People (EWGSOP) (Cruz-Jentoft et al., 2010) have proposed a staged diagnostic criteria for sarcopenia that include elements of LBM, strength, and functional performance in response to these contemporary research findings (**Table 1**). These consensus groups have recommended customary walking speed as a key component of the sarcopenia screening criteria, and the Society for Sarcopenia, Cachexia and Wasting Disorders (SSCWD) has recommended that only older adults with mobility deficits and/or a history of falls should be considered for sarcopenia screening (Morley et al., 2011). This screening model has potential shortcomings that may be cause for concern. Just as bone fracture is the signal event of osteoporosis, an observed functional limitation may be deemed a signal event of sarcopenia. Moreover, it remains an open question if these aforementioned conditions for sarcopenia screening, derived largely from population-based studies involving relatively healthy community-dwelling adults (Dam et al., 2014), are appropriate for usage among a Veteran population with a high prevalence of comorbid conditions.

RACIAL/ETHNIC CONSIDERATIONS FOR BODY COMPOSITION ASSESSMENT

Despite the recent evolution of the diagnostic criteria for sarcopenia, the assessment of LBM remains vital to our understanding and clinical management of age-related muscle dysfunction. A critical issue to consider in addressing possible sarcopenia disparities in African Americans is the influence of racial/ethnic characteristics on common methods of body composition assessment. African Americans have approximately 5–8% higher levels of muscle mass in comparison to Caucasians based on total body potassium assessment (TBK), and this difference applies to both men and women and persists from childhood to old age (Ortiz et al., 1992). African Americans may also have 7–10% greater levels of bone mineral density (BMD) as measured with dual-energy X-ray absorptiometry (DXA), even when controlling for LBM and stature in men (Ettinger et al., 1997). However, the *rate* of age-related LBM and BMD loss may be similar between the two races (Cohn et al., 1977). In addition, the distribution of body fat differs between these groups as Caucasians have greater skinfold thickness in their chest, abdomen,

Table 1 | Categories of the sarcopenia syndrome based on causative factors, body composition, and stages of severity.

Causative factors		Body composition		Staging	
Primary Sarcopenia	Age-related only	**Sarcopenia**	LBM loss criterion only[b]	**Pre-sarcopenia**	LBM loss criterion only[b]
Secondary Sarcopenia Contributing comorbid factors and behavioral conditions	• Activity-related • Disease-related • Nutrition-related	**Sarcopenia class: aLM/h^2** • Class I (−1 to −2 SD) • Class II (↓ −2 SD)	A range of criteria based on LBM (relative to stature)[c]	**Sarcopenia**	LBM loss criterion, and strength loss or functional status criteria met[f]
Myopenia All-cause designation, independent of age	LBM loss, affecting functional status or mortality	**Sarcopenia class: SMI** • Class I (−1 to −2 SD) • Class II (↓ −2 SD)	A range of criteria based on ratio of LBM to body mass (relative to body mass)[d]	**Severe Sarcopenia**	All criteria are met
Skeletal Muscle Function Deficit All-cause designation, independent of age	Diminished muscle performance[a], affecting functional status	**Sarcopenic obesity**	LBM maintenance or loss, and body fat criterion met[e]		

[a] *Muscle performance as measured by a given measure or estimate of strength, power, or capacity (e.g., endurance/fatigue; Correa-de-Araujo and Hadley, 2014).*

[b] *Appendicular lean mass (aLM/h^2; men and women, respectively): 7.26 and 5.45 kg/m^2 (Baumgartner et al., 1998); 6.12 and 5.29 kg/m^2 (African American cohort; Kelly et al., 2009); aLM scaled to body mass index (BMI): 0.789 and 0.512 (aLM$_{BMI}$; men and women, respectively; Dam et al., 2014); use of a single criterion for sarcopenia involving lean body mass (LBM) may include adjustments for height, body fat, and/or body mass by some investigators (Cruz-Jentoft and Morley, 2012).*

[c] *Class I: 8.51–10.75 kg/m^2 in men, and 5.76–6.75 kg/m^2 in women; Class II: ≤8.50 kg/m^2 in men, and ≤5.75 kg/m^2 in women (Janssen et al., 2004a,b).*

[d] *Class I: 31–37% in men, and 22–28% in women; Class II: ≤30% in men, and ≤21% in women [SMI, skeletal muscle mass index; (muscle mass/body mass) * 100; Janssen et al., 2002].*

[e] *Body fat percentage (BF%): 27% in men and 38% in women (Baumgartner, 2000); BMI: ≥30 kg/m^2 (Schrager et al., 2007); cohort specific criteria: 2 highest quintiles of BF% and 2 lowest quintiles of lean body mass (Gomez-Cabello et al., 2011).*

[f] *Grip strength: <30 kg in men, and <20 kg in women (Murphy et al., 2013); <26 kg in men, and <16 kg in women (Dam et al., 2014); gait speed: <0.8 m/s (Cruz-Jentoft et al., 2010); gait speed or distance: <1.0 m/s or <400 m during the 6-min walk test (Morley et al., 2011).*

and thighs, whereas African Americans have greater subscapular skinfold thickness (Zillikens and Conway, 1990; Nindl et al., 1998). These phenotypic patterns may confound body composition assessment methods that do not account for these factors. Consequently, an alternative LBM density value of 1.13 g/cm^3 has been proposed for African Americans instead of the 1.10 g/cm^3 value typically used in body composition models (Schutte et al., 1984).

The most common whole body tissue composition methods used in sarcopenia research include anthropometry, bioelectrical impedance (BIA), and DXA. Anthropometry is often used in community-health settings and constitutes a broad assessment category that features extremity circumferential measurements, body mass index (BMI), and skin fold measurements. While circumferential measurements have been shown to be associated with diminished activities of daily living (ADL) (Baumgartner et al., 1998), their use as proxy body composition measures systematically underestimate LBM in African Americans based on comparative differences in LBM density, body fat distribution, and even relative body proportions (Wagner and Heyward, 2000). BMI has served as a valuable predictor of critical health outcomes (Kane et al., 2011), but also does not account for racial/ethnic differences in LBM density or body fat distribution and may overestimate the obesity rates in African Americans (Kleerekoper et al., 1994). Skin fold measurements have limitations similar to the aforementioned anthropometric methods, and have also been shown to underestimate LBM density in African Americans (Schutte et al., 1984). In addition, BIA has been used extensively to characterize body composition in population-based studies (Janssen et al., 2002, 2004b; Chien et al., 2008). The interpretation of body composition data involving African Americans and BIA measures may be limited due to assumptions concerning body fat distribution and relative body proportions. However, Wagner et al. (1997) have identified a validated predictive model appropriate for use in this population. Their use of the Segal adiposity-specific equations, with expanded categories per the Stolarczyk modification, yields fairly accurate body composition measures for African Americans, underestimating FFM by 1.8 kg. While lacking the portability of anthropometry and BIA, DXA estimates of muscle mass have a high degree of association with TBK and the imaging method serves as a direct measure of BMD (Wagner and Heyward, 2000; Cruz-Jentoft and Morley, 2012). Therefore, DXA may not be unduly influenced by racial/ethnic differences in LBM density and may serve as an ideal method to assess sarcopenia in studies with multiracial participants and for clinical practice in hospital settings (sarcopenic LBM criterion values, **Table 1**).

THE PREVALENCE AND CONSEQUENCES OF SARCOPENIA IN AFRICAN AMERICANS

Despite the substantial clinical and financial burden attributed to sarcopenia, this geriatric syndrome remains an underdiagnosed condition and is rarely subject to a systematic screening process as a part of customary medical care for older adults (Fielding et al., 2011). The sarcopenia syndrome diminishes functional independence and results in a 3–4 times increased likelihood of developing a disability. In addition, sarcopenia may exacerbate hip fracture incidence due to the loss of requisite strength to maintain dynamic balance (Kamel, 2003). However, while some investigators have shown a significant relationship among diminished LBM, impaired lower extremity strength, functional performance, and disability (Malmstrom et al., 2013), others have reported that a negligible association exists between LBM and disability in community dwelling older adults (Visser et al., 2000). Walking speed, fall risk, negotiating stairs, basic mobility, and independent living status may be more dependent on lower extremity strength and power rather than LBM in older adults (Visser et al., 2000; Kamel, 2003).

The discordance between the age-related loss of muscle mass and diminished function has been observed across ethnic groups. However, this non-intuitive clinical observation is most evident in African Americans. Few studies have reported epidemiological data by ethnic or racial group for sarcopenia. Investigators using data from the Health Aging and Body Composition Study found that 14.3 and 18.4% of African American men and women, respectively, were classified as sarcopenic when adjusting for height and body fat (Newman et al., 2003). Others have noted a prevalence in Caucasians above 25% for men and women across all ages, whereas these values were reported as low as 6–12% in African Americans (Kan, 2009). Nevertheless, studies involving older, community-dwelling, African Americans consistently demonstrate that they have diminished functional performance, and higher levels of dependence with ADLs and risk of disabling conditions, in comparison to other racial/ethnic groups (Miller et al., 2005). What accounts for older African Americans having a higher prevalence of functional limitations and ADL difficulty despite their lower prevalence of sarcopenia?

Both Janssen and Visser have recognized high body fat levels as a confounder in the investigations into the association of LBM with functional limitations and disability (Janssen et al., 2002; Visser et al., 2002). The consideration of body fat and body mass adds an important dimension to the sarcopenia syndrome given that unchanging muscle mass coupled with increased body fat may portend significant functional and metabolic decline (Cruz-Jentoft and Morley, 2012). Moreover, myosteatosis (an increased proportion of intramuscular adipose tissue) has been observed in older adults of African ancestry, and this deleterious adaptation to aging may occur to a greater degree in comparison to Caucasians even when subcutaneous and visceral fat levels are similar among the groups (Song et al., 2004; Miljkovic-Gacic et al., 2008). While the higher risk of disabling conditions observed in African Americans likely involves an array of socioeconomic factors (Fuller-Thomson et al., 2009), high levels of myosteatosis are strongly associated with impaired lower extremity function and may serve as a possible explanation for the departure in linearity between muscle wasting and physical performance (Goodpaster et al., 2001).

Non-contractile features of muscle also affect health outcomes as age-related changes in skeletal muscle may be a critical progenitor of metabolic abnormalities (Albu et al., 2005). While preliminary

findings suggest that relative muscle mass may be more strongly associated with insulin resistance than strength (Bijlsma et al., 2013), there are divergent views on this point (Kamel, 2003; Sayer et al., 2005). Data from the NHANES III cohort reveal that sarcopenia is strongly associated with the homeostasis model assessment of insulin resistance and higher HbA1C levels in both non-obese and obese individuals under 60 years of age (Srikanthan et al., 2010). However, the relationship between sarcopenia and a higher prevalence of type 2 diabetes was only observed in obese individuals. Miljkovic et al. (2009) determined that Afro-Caribbean men had higher myosteatosis levels and a lower body fat percentage in comparison to Caucasian men. Myosteatosis was associated with type 2 diabetes in both groups, but 44% of the Afro-Caribbean men were diagnosed with the metabolic disorder in comparison to 13% of the Caucasian men. While the negative impact of low muscle mass on glucose homeostasis cannot be discounted (Bijlsma et al., 2013), excessive intramuscular adipose tissue and intramyocellular lipid content may disrupt insulin receptor activity, limit glucose transport, and potentially result in adipokine release from local concentrations of adipose tissue (Miljkovic et al., 2009). The case could be made to incorporate age-related changes in muscle quality into the broad view of the sarcopenia diagnosis. However, more prospective study will be needed to better understand the role of myosteatosis in the pathogenesis of diminished insulin sensitivity and diabetes. Given that African Americans are 1.6–1.7 times as likely to have diabetes in comparison to Caucasians within the VA and civilian population, it may be a compelling interest of the Federal health agencies, professional and scientific organizations, and other stakeholders to study the impact of age-related muscle changes and how they interact with racial/ethnic group factors in health disparities (National Center for Veterans Analysis and Statistics, 2011).

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