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SARCOPENIA: BIOMARKERS AND IMAGING (INTERNATIONAL CONFERENCE ON SARCOPENIA RESEARCH)

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A first Task Force on Sarcopenia was organized in Castres, near Toulouse, in 2008, with a series of papers on Sarcopenia published in the JNHAin 2009 (1-10). Folowing this work a second international task force on Sarcopenia was organized in Rome in 2009 and the consensus paper was published in the JAMDA (11). Our third international Task Force was hosted in Albuquerque on Designing sarcopenia trials (12). We are happy to present now the abstract presented at our 4° International Task Force on Sarcopenia biomarkers just before the International Conference on Sacopenia research in 2011.

- Recommendations for imaging biomarkers in sarcopenia therapeutic trials; Defining Sarcopenia;
- Use of MRI and CT in Clinical Trials of Sarcopenia;
- Muscle quality and function: Implications for sarcopenia definitions and therapeutic targets;
- Modifiable risk factors for sarcopenia and mobility disability, which could be targeted in multicomponent interventions trials;
- Electrical Impedance Myography:A New Tool for the Assessment of Sarcopenia;
- Challenges in assessment of muscle strength and function - Lessons from als clinical trials;
- Novel Imaging Methods for Early Drug Development;
- Qualification of novel methodologies at the European Medicines Agency;
- Agrin-dependent sarcopenia.

The consensus paper from this symposium will be submitted for publication shortly.

IMAGING BIOMARKERS IN SARCOPENIA THERA-PEUTIC TRIALS, *M. Cesari, G. Abellan van Kan (France)*

Sarcopenia, defined as the age-related involuntary loss of muscle mass and strength, represents a major feature of the aging process[1], consequently representing an outstanding benchmark for gerontologists. Unfortunately, the study of this phenomenon has been (and still is) limited by controversies and uncertainties about its operative definition[2].

To date, numerous studies have suggested that sarcopenia is associated with major health-related events. Nevertheless, sarcopenia is still to be considered as a matter of research, not yet sufficiently characterized to also be clinically relevant. In other words, the design and implementation of therapeutic trials on sarcopenia imply that:

a. Sarcopenia can be defined and identified in a standardized fashion, and
b. It is a detrimental and common condition (thus, worth to be treated) and that specific therapeutic interventions may improve the individual's health status.

Taking into account these two propedeutical points, the following and consequent considerations should be made in the evaluation of the sarcopenia phenomenon:

a. The reduction of skeletal muscle (both in terms of quantity and quality) due to the aging process should be clearly distinguished from that occurring as consequence of concurring behavioral, clinical, biological, and environmental factors. The target population should allow the study of the aging phenomenon alone, reducing to the minimum potential biases due to confounders[3].
b. The bidimensional nature of sarcopenia implies that the operative definition should simultaneously capture both quantitative and qualitative declines occurring on the skeletal muscle with aging[4]. Muscle mass is different from muscle strength and/or physical performance[5,6]. At the same time, low muscle strength and poor physical performance differently capture unhealthy stati because exploring different domains.
c. Sarcopenia is a phenomenon systematically occuring to all the skeletal muscles of the organism (potentially with different extents and velocities). By proposing imaging biomarkers of sarcopenia, such differences might be important to be considered.

Table
Main imaging techniques for the evaluation of skeletal muscle (4)

Method	Strenghts	Weaknesses
Magnetic resonance imaging (MRI)	• Best resolution • Cross-sectional measurement of lean and fat areas in a specific part of the body • Evaluation of muscle quality parameters (i.e., intramuscular infiltrates of fat) • Technically difficult to perform	• Highly expensive • Images of a body part which may not be applicable to different body districts • Time-consuming • High space requirements
Computerized tomography (CT)	• Cross-sectional measurement of lean and fat areas in a specific part of the body • Evaluation of muscle quality parameters (i.e., intramuscular infiltrates of fat)	• Images of a body part which may not be applicable to different body districts • Exposure to radiation • Time-consuming • High space requirements • Technically difficult to perform
Peripheral quantitative computerized tomography (pQCT)	• Cross-sectional measurement of lean and fat areas in a specific part of the body • Evaluation of muscle quality parameters (i.e., intramuscular infiltrates of fat) • Portable • Does not require highly trained personnel	• Images of a body part which may not be applicable to different body districts • Limited accuracy compared to MRI or CT • Originally designed to evaluate bone parameters, it has lower application on muscle • Exposure to low dose radiation
Dual energy X-ray absorptiometry (DEXA)	• Sensitive and accurate method • Estimates of lean, fat, and bone tissues in the entire body or in specific parts of it • Usually present in clinic/research settings • Relatively cheap • Does not require highly trained personnel	• No information about muscle quality • Space requirements • Exposure to low dose radiation • Possible biased results due to limited differentiation between water and bone-free lean tissue
Bioelectrical impedance analysis (BIA)	• Relatively inexpensive • Minimal maintenance • Portable • Results immediately available • Does not require highly trained personnel	• Results based on body resistance • No measure of muscle quality • Lower accuracy compared to other methods (i.e., MRI, CT, DEXA)

d. The evaluation of sarcopenia should be specific of the skeletal muscle, reducing to the minimum the influence that other organs, systems, and apparati may exert on the assessment[7,8].
e. The methods designed to measure sarcopenia should be validated, standardized, repeatable, reliable, and accurate. All the available techniques are affected by different weaknesses, partly related to the instrument (e.g., accuracy, costs, availability…) and partly to the examined population (e.g., clinical conditions limiting the assessment and/or biasing the results). Consequently, all the efforts should be made to identify a unique "golden standard" to be systematically adopted.
f. The imaging biomarkers to be selected should present well-established and accepted thresholds distinguishing normal versus abnormal values. Only through the clear definition of such critical cut-points, a biological measure may reach a clinical relevance (thus allowing and justifying its study and treatment).
g. Since sarcopenia is a phenomenon of the advanced age, it is particularly important that techniques aimed at its assessment are suitable and acceptable for older persons. Moreover, since Phase II (but even more Phase III) trials will recruit a large number of participants, the operative definition should be based on biomarkers that are easy, quick, and unexpensive to be measures. The easiness in assessing the biomarkers of sarcopenia will simultaneously facilitate its future recognition as clinical condition.

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DEFINING SARCOPENIA, *J.E. Morley (USA)*

The term sarcopenia ("loss of flesh") was first introduced into the literature by Irving Rosenberg in 1995. It was defined operationally by Baumgartner as being 2 standard deviations below the level of the mean muscle mass of healthy young persons[1]. In recent years the utility of defining sarcopenia as purely a loss of muscle volume has been questioned[2-5]. Muscle strength, and therefore function, is not only dependent on muscle size but is also dependent on muscle function, fat and collagen infiltration aged associated decline in neuromuscular innervation blood flow to the muscle and alterations in the angle of pennation by which tendons insert into muscle. The desire to redefine sarcopenia has also been driven by the failure of regulatory agencies to accept that increasing muscle mass is a sufficient criteria to lead to approval of a new drug.

There is a logical gradation between loss of muscle mass and disability (Figure 1). Loss of muscle mass/function has been included as a clear component of the frailty syndrome[6-9]. The other components of frailty are loss of energy (fatigue) and loss

of weight. It is important to recognize that weight loss is not consistently associated with loss of muscle strength and muscle mass gain can occur without gain in strength[10].

The conundrum faced by those who wish to redefine sarcopenia is how far the definition should go. Frailty and disability clearly encompass a larger clinical syndrome than does pure alterations in muscle mass and function. Thus, definition creep needs to not blur the distinction between these two established geriatric conditions. It also needs to be questioned whether sarcopenia should be recognized as a geriatric syndrome or as a partial cause of geriatric syndromes such as disability (loss of activities of daily living) or frailty. There is at present no clear consensus of the magnitude of loss of muscle loss or force (strength) that will lead to deleterious clinical outcomes.

Figure 1
The Cascade from Loss of Muscle Mass to Disability

This does not necessarily inhibit the development of an operational definition. The definition of osteoporosis as two and a half standard deviations of bone mineral density below the mean of young persons (20 to 30 years) was purely arbitrary. This definition clearly ignored the fact that bone strength is a major factor in bone fracture, e.g., persons with Type II diabetes have large bone but an increase in fracture[11].

A second issue is no clear consensus on how to measure muscle mass. While dual-energy x-ray absorptiometry (DEXA) is relatively accurate and has the advantage that DEXA machines are readily available to measure bone mineral density, it is not universally accepted. Simple techniques such as measurement of midarm muscle circumference and calf circumference would be simple for clinicians to do (they are included as part of the MiniNutritional Assessment[12], they are generally not favored by clinical trialists. Magnetic resonance imaging and computed tomography are more expensive. Bioelectrical impedance is too inaccurate to be useful for individual measurements. Ultrasound clearly has a number of advantages but has had poor uptake in the field.

Endpoints that measure ability to generate power (dynapenia) therefore, may be an acceptable alternative. Walking speed/distance have been validated as useful endpoints to determine the utility of drug treatment in peripheral vascular disease and pulmonary hypertension. This raises the issue that factors other than muscle are responsible for these endpoints but they may be useful in the presence of loss of muscle mass and the exclusion of lung and heart disease and anemia.

Another problem with the definition of sarcopenia is to separate it from cachexia. One clear separation is that sarcopenia can be considered to be age-associated. Cachexia is directly related to severe underlying disease processes, and usually involves loss of adipose tissue as well as muscle mass[13-15]. Persons with sarcopenia appear to be at increased risk of developing cachexia ie, sarcopenia can be considered a risk factor for cachexia. In addition, many of the causes of sarcopenia (a multifactorial condition) overlap with those of cachexia[16-18].

A Working Definition: In December 2010, the Society of Sarcopenia, Cachexia and Wasting Disorders held a meeting in Washington, USA to help to resolve some of the conflicts and develop a working definition for sarcopenia to be utilized in clinical trials[19]. The term "sarcopenia with limited mobility" was chosen as a suitable target for therapeutic interventions.

Sarcopenia with limited disability is defined as "a person with muscle loss whose walking speed is equal to or less than 1 m/s or who walks less than 400m during a 6 minute walk. The person should have a lean appendicular mass corrected for height squared of more than 2 standard deviations below that of healthy persons 20 to 30 years of age of the same ethnic group. The limitations in mobility should not be attributable to the direct effect of a specific disease such as peripheral vascular disease with intermittent claudication, or central or peripheral nervous system disorders, or congenital or infectious muscle diseases, pain dementia or cachexia." It needs to be recognized that these cutoffs are arbitrary and in reality sarcopenia risk is a linear function. The committee also provided clinically significant improvements as an increase of 50 meters in the 6-minute walk or 0.1 m/s in gait speed. They accepted that sarcopenia is age-associated and suggested "myopenia" as a generalized term covering all muscle loss. It was felt that all persons 60 years and older should be screened for "sarcopenia with limited mobility."

Resistance exercise at present is the treatment modality of choice. High protein leucine enriched amino acid supplements should be considered as well as vitamin D for this population20. Sarcopenia is particularly prevalent in long term care residents and there is a need for drug trials in this population and in rehabilitation populations[21,22].

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USE OF MRI AND CT IN CLINICAL TRIALS OF SARCOPENIA, *R.A. Fielding (USA)*

It is estimated that in the next 20 years, the number of people greater than 65 years of age will rise from 40 to 70 million, and will account for 19% of the total population. Age-related decreases in muscle mass and function, known as sarcopenia, have been shown to be related to functional limitation, frailty and an increased risk of morbidity and mortality. Therefore, with an increasing elderly population, interventions that can improve muscle mass content and/or function are essential. However, analytical techniques used for measurement of muscle mass in young subjects may not be valid for use in the elderly. Therefore, the purpose of this review is to examine the applied specificity and accuracy of methods that are commonly used for measurement of muscle mass in aged subjects, and, to propose specific recommendations for the use of body composition measures in phase II clinical trials of function-promoting anabolic therapies.

The age-related decline in skeletal muscle mass and function are collectively referred to as sarcopenia[13]. Sarcopenia is a risk factor for functional limitations[3,11] and frailty[4], and added an excess cost to the US health care system of $18.5 billion, in the year 2000[12]. Elderly subjects within the age range 70-80 in the lowest quartile of muscle density were found to have a 51% higher risk of hospitalizations than those in the highest quartile[6], and may explain the sarcopenia-associated increased risk of morbidity and mortality[2]. Low levels of skeletal muscle cross-sectional area, density and mass have been shown to be directly related to the strength deficits[19] and mobility limitation[24] characteristic of sarcopenia. Therefore, use of analytic methods with adequate validity, precision and accuracy are necessary to identify high-risk groups for age-related muscle mass loss, and, to monitor potential intervention efficacy. However, the changes in body composition that occur during the aging process have been shown to not occur uniformly. For example, decreases in total body water, bone and muscle mass[23], and an increase in body adiposity[7] have each been found during aging.

Body composition measures in sarcopenia: Four main techniques are commonly used to measure skeletal muscle mass and/or quality: bioelectric impedance (BIA), dual energy X-ray absorptiometry (DXA), computed tomography (CT) and magnetic resonance imaging (MRI).

The principle of using DXA for measurements of body composition is based on the notion that when a beam of X-rays is passed through a complex material, the beam is attenuated in proportion to the composition and thickness of the material. The DXA scanner emits two X-ray beams comprised of photons at two differing energy levels (40 keV and 70 keV), and as a result of the interaction within the human body, the incident X-ray photon energy is exponentially attenuated. By knowing how many photons are transmitted with respect to the number detected, the amount of bone mineral and soft tissue (fat and fat-free mass) can be determined. Skeletal muscle and adipose tissue contain primarily water and organic compounds and each restrict the flux of X-rays less than bone[18]. Notable advantages of DXA include low cost, speed of measurement (whole-body scans require less than 20 min), exposure to low levels of radiation (<1 mrem), and the ability to perform fast serial-section measurements (Table 1). DXA has been reported to be the new gold standard for measurement of body composition.[21; for review see ref. 1]

BIA for the measurement of body composition is based on the notion that tissues rich in water and electrolytes are less resistant to the passage of an electrical current than lipid-rich adipose tissue. In theory, an individual with no adipose tissue would have minimum impedance, and impedance would increase to a maximum when all lean tissue was replaced by lipid-filled adipose tissue. The two main determinants of impedance, resistance and reactance, respond differently at any given frequency to intracellular and extracellular fluids. The reciprocal of the impedance is proportional to total body water for a current frequency ≥ 50 kHz or, to extracellular fluid, for frequencies below 5 kHz[17]. Impedance values are then converted into values specific for total body water or extracellular fluid and then, into fat-free mass by means of equations that are population specific. Once fat-free mass is known, total body fat is calculated as the difference between body weight and fat-free mass. However, BIA results have been shown to be confounded by fluid retention, as found in patients with COPD[20].

CT was first used to quantify arm skeletal muscle cross sectional area[9] and abdominal fat content[5] in 1979 and 1982, respectively. CT has been shown to be highly reliable in the evaluation of both adipose tissue[8] and fat-free mass[22]. One advantage of CT use is the ability to discriminate total fat content into subcutaneous and visceral components[22].

The principle underlying use of MRI involves a cylindrical magnet with an internal diameter large enough to enclose the human body, thereby allowing for the production of an external magnetic field. The presence of gradient coils creates a smaller identification field, known as a gradient field. The presence of

the external gravitational field in combination with the gradient field produces a net external magnetic field. The radio frequency coil generated by these magnetic fields provides the force necessary to rotate nuclear spin away from the direction of the external magnetic field. As the nuclear spins precess back toward the direction of the external magnetic field, they emit radio frequency signals (T1 and T2), which are combined to form an image. Variations in the radio frequency pulse sequence are then used to make determinations about adipose tissue or fat-free mass. For example, a short T1 and a long T2 proton relaxation time has been shown to be indicative of adipose tissue[14]. Furthermore, use of MRI has been shown to identify the relative percentage of Type I skeletal muscle fibers in human vastus lateralis[10,15,16].

Measurement of total body water (i.e., BIA) for the purpose of predicting skeletal muscle mass has shown conflicting results during aging. DXA has been shown to be a reliable method for measurement of fat-free mass during aging. Unfortunately, DXA is unable to measure fat-infiltration into skeletal muscle. CT and MRI have been well documented to detect within-skeletal muscle changes in fat content or connective tissue. However, the high cost of operation for both CT and MRI can be a limiting factor for their use. Based on the existing literature, we propose that DXA in conjunction with either CT or MRI is a valid approach for measuring changes in both muscle mass and quality in older populations, or to determine the efficacy of selected agents in phase II clinical trials of function-promoting anabolic therapies. *Acknowledgements:* This material is based upon work supported by the USDA, under agreement No. 58-1950-7-707 Any opinions, findings, conclusion, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture. Also supported by the Boston Claude D. Pepper Older Americans Independence Center (1P30AG031679). Dr. Fielding's contribution is partially supported by the U.S. Department of Agriculture, under agreement No. 58-1950-7-707. 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MUSCLE QUALITY AND FUNCTION: IMPLICATIONS FOR SARCOPENIA DEFINITIONS AND THERAPEUTIC TARGETS, *B.H. Goodpaster (USA)*

The loss of muscle mass with aging, known as sarcopenia, is associated with the loss of muscle strength and a decline in lower extremity function[1-3]. However, these associations are confounded by two key observations. First, older adults who are losing muscle mass are also becoming more obese[4,5]. Therefore, most older men and women are uniquely vulnerable to both the loss of muscle mass and gain in body fat. Second, despite actually having greater muscle mass compared to their normal weight counterparts, overweight and obese older adults are more likely to have poorer lower extremity function and low muscle quality (strength per unit muscle mass)[6]. Moreover, older men and women with type 2 diabetes have an accelerated loss of muscle mass[7]. These studies have more carefully scrutinized the combined roles of muscle and fat in aging relating to muscle function. In addition, fat accumulation within skeletal muscle tissue is associated with aging[8] and the loss of muscle function independent of muscle mass[9]. This has also helped to generate newer concepts of sarcopenic obesity to account for the increased overweight and obesity and loss of muscle mass in older adults. Moreover, this has underscored the need to examine intrinsic muscle properties as potential biomarkers of muscle quality.

The loss of muscle mass and strength with aging is associated with the loss of skeletal muscle proteins[10-14], and alterations in the balance between protein synthesis and degradation[13,15]. The molecular basis for these changes, including changes in the expression of several key genes and proteins implicated in growth, atrophy, autophagy, proteosome degradation and mitochondrial metabolism, is also well documented[12,14,16-19]. Recent evidence suggests that autophagy, a process of degrading damaged cellular components[20], may play

an important role in muscle wasting. Activation of the two major proteolytic systems in muscle, the ubiquitin proteasome and the autophagy lysosome, control protein degradation, which contribute to muscle loss and weakness[21].

A decline in mitochondria content and/or function often parallels the loss of muscle mass and function with age. Only until recently, however, have studies been conducted - in cell systems and animal models - to link mitochondria with muscle growth/atrophy signaling, autophagy and sarcopenia[16,19,22]. Although far from clear, these data from animal models have strongly suggested that there may be mechanistic links between mitochondria and muscle mass or function. For example, mice overexpressing PGC1- in skeletal muscle had reduced age-related muscle wasting and alterations in signaling proteins, e.g., mTOR and Akt, associated with this muscle loss[19]. In addition, increased PGC1- is associated with decreased FOXO3 expression, which attenuated the FOXO3-induced muscle atrophy gene expression[23]. It has also been reported that the activation of AMPK stimulates FOXO3 and subsequent muscle atrophy[24], and that induction of PGC1- reduces or prevents the influence of activated AMPK on FOXO3 and muscle atrophy[23]. Little is actually known about the potential functional consequences of these mitochondrial changes, i.e., whether they are related to the decline in muscle strength or function. Translational human clinical research is needed to determine whether these molecular and biochemical targets are implicated in age-related changes in muscle quality and in the preservation of muscle mass and function with aging.

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MODIFIABLE RISK FACTORS FOR SARCOPENIA AND MOBILITY DISABILITY, WHICH COULD BE TARGETED IN MULTI-COMPONENT INTERVENTIONS TRIALS,

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Age-related sarcopenia results from multiple concurrent factors, including comorbid, biological, behavioral and environmental factors. Therefore, the most effective intervention strategies to avert sarcopenia will target multiple modifiable risk factors. Several biological markers which could be targeted with specific interventions have shown strong associations with reduced skeletal muscle mass, strength and physical performance. Low levels of vitamin D and high parathyroid hormone levels have been shown to be significant determinants of loss of muscle strength and muscle mass in the Longitudinal Aging Study Amsterdam (LASA)[1]. High levels of CRP, TNF-alpha and IL-6 have been associated with lower muscle mass and strength in several studies[2-4]. In the InChianti study lower levels of dehydroepiandrosterone sulfate (DHEAS) were independently associated with lower strength and lower calf area among older men[5]. In the same study, anemia as assessed by lower levels of hemoglobin, was associated with disability, poorer physical performance, and lower muscle strength[6]. In the Women's Health and Aging Study (WHAS), protein carbonylation, an indicator of oxidative damage to proteins, was independently associated with low grip strength among older women living in the community[7]. In WHAS higher plasma concentrations of advanced glycation end products (AGEs) were independently associated with lower grip strength[8]. In the same study, higher carotenoid and alpha-tocopherol status were independently associated with higher strength measures, suggesting that oxidative stress is associated with sarcopenia in older adults[9]. In the Health Aging and Body Composition (Health ABC) Study, lower albumin concentrations, even above the clinical cutoff of 38 g/L, were associated with loss of in appendicular skeletal muscle mass in older persons, suggesting that low albumin concentration may

be a risk factor for sarcopenia[10]. In the MINOS study of older men low testosterone and decreased 25(OH)D concentrations were independently associated with lower relative appendicular skeletal muscle mass[11]. In the Framingham study higher IGF-1 predicted smaller loss of fat free mass in men than lower IGF-1 did[12]. The above mentioned biomarkers are specific for the etiology of sarcopenia, and are useful for designing mono- and multi-component intervention trials and for selecting target populations and interventions. Table 1 depicts potential biomarkers and risk factors for sarcopenia, and the corresponding interventions. However, such biomarkers are not specific for the skeletal muscle and are not sensitive to skeletal muscle changes in response to treatment.

Imaging techniques including Dual-energy X-ray absorptiometry (DEXA), magnetic resonance imaging (MRI) and X-ray computed tomography (CT) are used to directly estimate skeletal muscle mass. Particularly femoral muscle mass is associated with poorer lower extremity performance [13]. In a recent study comparing high and low functioning older adults in whom various skeletal muscle compartments were assessed by means of MRI, we found that femoral muscle mass is the major compartment associated with physical function in older adults. Isokinetic and isometric dynamometry are used to measure muscle strength. Both imaging and strength measures are highly specific to assess skeletal muscle function. Standardized physical performance measures are also useful indicators of sarcopenia. However, the imaging, strength and performance measures are not very sensitive, as it takes several months to detect changes in these measures in response to interventions aimed at averting sarcopenia. Furthermore, both strength and physical performance measures may be affected by other factors, such as cognition, mood, emotional stress, environment and learning effects.

To develop trials of new therapies for sarcopenia and to monitor their efficacy, biomarkers which have high specificity and sensitivity for skeletal muscle changes, are responsive to early changes, and are easy and practical to measure are needed. Skeletal muscle gene and protein expression profiling platforms have been used to identify several candidate early predictive biomarkers of muscle anabolism induced by short-term exposure to testosterone[14]. Collagen type III is produced in soft connective tissues, parenchymal organs, muscle and skin. Collagen III is produced from procollagen type III by means of cleavage of peptide fragments from the N- and C-terminal ends. Procollagen type III N-terminal peptide (P3NP) is released into the blood stream when collagen III is produced and it increases when skeletal muscle remodeling is activated. Chen et al. have found that plasma P3NP levels increased in a dose dependent manner one week after a single injection of testosterone[14]. While more validation studies are needed, P3NP is a likely promising early biomarker for monitoring skeletal muscle response to therapies for sarcopenia. A limitation is that P3NP can increase in several disease conditions such as acute respiratory distress syndrome, liver fibrosis, and myocardial infarction. Thus the presence of such conditions ought to be ruled out when monitoring interventions for sarcopenia. Biomarkers that target the neuromuscular junction and the synaptic serine protease neurotrypsin and agrin system are currently being investigated and appear to be promising[115,16].

Table 1
Target modifiable biological risk factors for sarcopenia and corresponding interventions to be tested in mono- or multi-component trials

Target modifiable biological risk factor	Possible intervention
Anemia of unknown origin (hemoglobin)	Erythropoietin
Inflammation (high IL-6, CRP, TNF-alpha)	NSAIDs
High advanced glycation end products (AGEs)	Low AGE diet
Low serum albumin	Nutrition, high protein diet
Low DHEA-S	DHEA
Low testosterone in men	Testosterone, SARM, nandrolone
Low vitamin D	High Dose Vitamin D vs. recommended dose
Low IGF-1	Growth hormone secretagogue
Protein carbonylation (oxidative stress)	Antioxidants (e.g. resveratrol)
Sarcopenia of any origin	Physical exercise, myostatin inhibitors

In conclusion, while several biomarkers related to the etiology of sarcopenia have been established, novel biomarkers that are highly specific and sensitive to skeletal muscle changes are needed to develop new therapies for sarcopenia and monitor their efficacy in mono- and multi-component intervention trials.

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ELECTRICAL IMPEDANCE MYOGRAPHY: A NEW TOOL FOR THE ASSESSMENT OF SARCOPENIA, *S.B. Rutkove (USA)*

Electrical impedance myography (EIM) is a painless and non-invasive methodology for the quantitative evaluation of muscle[1]. In EIM a high frequency, low-intensity electrical current is passed through a muscle and the consequent surface voltage patterns measured. Changes in muscle structure, integrity, composition, and mass are reflected in the electrical impedance data obtained and thus these data can serve as effective measures of disease status. Although EIM can be performed with commercial bioimpedance instruments, dedicated devices for rapid, reliable muscle measurement are also being developed[2]. To date, EIM has been applied to patients with a variety of neuromuscular conditions, including amyotrophic lateral sclerosis (ALS)[3], spinal muscular atrophy[4], and inflammatory myopathy[5]. In ALS, it has been found to be an especially sensitive marker of disease status and holds promise as an effective biomarker in early stage (Phase 2) clinical drug trials[3].

In addition to this work in primary nerve and muscle diseases, EIM has been applied to the study of muscle disuse. Ten subjects who sustained ankle fractures and were non-weightbearing for a period of 6 weeks were studied at the time their casts were removed and again several weeks to months later after they had returned to a normal level of function[6]. The EIM data mirrored the clinical course, showing substantial abnormalities initially that normalized when the patients reestablished their normal levels of activity.

Studies have also been performed assessing the change in EIM parameters with increasing age[7,8]. These studies have demonstrated marked age-dependent changes in one of the major EIM outcome parameters, the phase, both at a single frequency[7] and across a spectrum of applied frequencies (8). For example, there was a strong correlation between phase and age, with an r^2 value of 0.68 for men and 0.52 for women ($p < 0.001$ for both) for quadriceps for single frequency values. Moreover, a subgroup of individuals aged greater than 75 years were followed for up to 4 years; all demonstrated a 0.3 to 0.6 degree/year decline in EIM phase[7].

The mechanism underlying these alterations, both in neuromuscular disease and disuse atrophy and sarcopenia, is the subject of a separate series of investigations in animals. Surface EIM measurements are made on animals both at baseline and after the induction of a neuromuscular condition, such as nerve injury via sciatic nerve crush or muscle disuse via hind limb suspension[9]. In addition, genetic models of disease are studied including rat and mouse models of ALS and muscular dystrophy. After surface measurements are completed, the animals are sacrificed and the muscle is removed and placed into an impedance-measuring cell. Since the exact dimensions of the tissue being measured are known, when electrical current is passed through it in this way, the material or "dielectric" properties of the muscle can be accurately calculated. These properties include the muscle's ability to conduct electrical current (its conductivity) and its ability to store and release charge (its permittivity). The changes in these properties in disease are complex being both frequency-dependent and likely disease-specific. For example, after acute nerve injury, muscle conductivity increases substantially[9].

With these values in hand, the finite element method, a mathematical approach that allows one to determine how the muscle's inherent electrical properties impact the surface measured data, is employed. First, an approximate computer-based 3-dimensonal geometric representation of the body area being measured is created, including muscle, subcutaneous fat, bone, and fascial components. A mesh is then created within this structure, with millions of different elements. The measured material properties of the muscle and other tissues are input and the mesh-derived equations solved. The actual measured surface data are then compared with the predicted surface data obtained using the finite element method to determine the success of the model[10]. Ultimately, it may be possible to approximate a solution to the "inverse" problem—ie using surface measurements to approximate the intrinsic muscle qualities directly. These surface measurements could then provide a non-invasive approach to identifying muscle properties, including sarcopenia. Since different disorders result in different electrical properties of the muscle, EIM offers the possibility of providing direct diagnostic information about the muscle tissue.

In addition to our early investigations into sarcopenia[7], we are continuing our study of disuse in both rats and mice. We also plan to study senescent animals to determine how both the surface- and directly-measured properties of the tissue change with advancing age, such that we can more effectively utilize the technique in humans. Ultimately, with the dedicated devices now in development, we anticipate that EIM will provide a flexible tool for the non-invasive assessment of muscle that will be able to assist in the detection of sarcopenia and in its response to pharmacologic intervention.

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CHALLENGES IN ASSESSMENT OF MUSCLE STRENGTH AND FUNCTION –LESSONS FROM ALS CLINICAL TRIALS, *J.M. Cedarbaum (USA)*

The assessment of neuromuscular function presents several challenges. Individuals enrolling in clinical trials can vary tremendously in premorbid function (e.g., body size and mass, gender and age, level of physical fitness and customary activity). Likewise, disease-related rates of decline in performance also comprise a spectrum, and the course of decline is often not linear. Observed variability may also be a function of the choice of outcome measures that are chosen. The relevance of primary outcome measures to daily activities is also critical. For example, in assessment of neuromuscular disorders such as Motor Neuron Disease, Maximum Voluntary Muscle Isometric Muscle Contraction (MVIC)[2,6]; has often been used as an outcome measure, yet few activities of daily living require exertion of maximal forces. In setting up and conducting clinical trials, strict attention must be paid to control of measurement variability. Standardization for administration of assessment instruments and rater training are critical activities. Finally, data should be reviewed on an ongoing, blinded basis prior to final analysis to monitor for outlier values and for data trends that might represent rater "drift" or error. CK-2017357 is a novel activator of the fast skeletal muscle troponin complex, the first of a novel therapeutic class intended to improve skeletal muscle function. CK 2017357 slows the rate of calcium release from the regulatory troponin complex, thus sensitizing fast skeletal muscle fibers to calcium in vitro. In an in-situ nerve-muscle preparation CK-2017357 increased the force of muscle contraction at physiologically-relevant rates of nerve stimulation, increased the power of muscle contraction, and diminished the rate of development and degree of fatigue of both normal and ischemic muscle. CK-2017357 increased the force of contraction of the anterior tibialis muscle following stimulation of the peroneal nerve in healthy volunteers in a similar fashion to what was observed preclinically, confirming translation of mechanism into humans. In patients with ALS treated with single doses of CK-2017357 or placebo[1], we observed dose-related decreases in submaximal handgrip fatigue and an increase in maximal voluntary ventilation (MVV). Both patient and investigator global assessments also improved. However, improvements in maximal voluntary isometric muscle strength as measured by hand-held dynamometry were small and difficult to detect. Measurements of submaximal effort and/or repeated movements necessary for everyday living[8,9] may prove to be the best pharmacodynamic outcome measures for assessment of drugs for the treatment of neuromuscular and muscular disorders. However, measures of self-care activities and ability to function independently in Activities of Daily Living (ADL[1,3-5,7]; will likely be key outcome measures leading to drug approvals in the future.

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NOVEL IMAGING METHODS FOR EARLY DRUG DEVELOPMENT, *D. Laurent, D. Rooks, R. Roubenoff (USA, Switzerland)*

Sarcopenia, a growing public health problem, is defined as a muscle wasting condition that gradually develops during aging and results in a loss of strength, mobility and functionality. While by 50 years of age, the rate of muscle loss reaches 1-2%/year, the decrease in muscle strength may even be greater, up to 3% over 60 years[1]. Although the exact cause of sarcopenia is unknown, muscle disuse, loss of alpha motor neurons in the spinal cord, and lower hormone levels (e.g., IGF-1, testosterone, and growth hormone) are believed to be the three main factors involved in this progressive syndrome. Proposed therapeutic approaches aim at preserving and eventually restoring muscle mass and function as quickly as possible.

To better understand the underlying mechanism of age-related muscle wasting and develop well adapted treatments, comprehensive clinical trials are needed and must rely on efficient tools that also minimize the overall burden on the frail elderly subjects often participating in these studies. However, the field of biomarkers to assess muscle wasting is still in a rudimentary stage. Main efforts are directed towards biomarkers showing efficacy in early clinical trials, but are yet to be correlated with restoration of function. In this respect, non-invasive imaging may offer sensitive markers of change in muscle anatomy and physiology by quantifying alterations in muscle mass, tissue metabolism and contractile potential which

in turn would provide novel readouts for proof-of-concept/mechanism studies.

Magnetic resonance imaging (MRI) is uniquely suited for volumetric measurement of the skeletal muscle with a high level of accuracy anywhere in the body. The fact that MRI allows for comprehensive assessment of pathological conditions of the skeletal muscle that cause changes in muscle signal intensity (e.g., inflammation, fibrosis, fat infiltration etc) is a key advantage. Thus, MRI can also be used to select the appropriate site of biopsy. Presence of unsafe ferromagnetic metal implants, uncontrollable claustrophobia or the inability to fit into the machine (e.g. obesity, etc.) are the only contraindications for a subject to be scanned. In an effort to guarantee image quality, the subject should lie on a bed for 30 minutes prior to scanning to allow body water to re-equilibrate throughout the body and be comfortable on the table. Then, MRI scans can be acquired on any clinical systems (1.5 or 3 Tesla) using the inherent body coil, with the body part to be imaged (usually the lower/upper limb) being relaxed, parallel to the MRI table and free from compression.

A proton-density weighted MRI sequence encoding fat and water signals for a phase-sensitive three-point Dixon-type analysis is usually considered as an optimal approach. Images can be acquired throughout the limb in a relative short time without loss in image quality. Appropriate methods, preferentially based on interpolation and deformation of a parametric specific object may then be used for image analysis to reach a given level of precision. Muscle mass in kg, but also the subcutaneous (SC) fat and intermuscular adipose tissue (IMAT) mass visible in each MRI section are ultimately determined by multiplying volumes by respective density values. This overall approach allows detection of changes in muscle and fat mass in a specific body region as small as 2%. It also makes possible studies of rather short duration (~2 months follow up) with only 8-10 patients/group. By comparison, whole-body DXA scans are about half as sensitive in detecting similar changes. In any cases, use of a standardized scan acquisition protocol and appropriate analysis software are essential to achieve consistent results. Likewise, because of variability in interpretation of the scans, it is important to utilize centralized scan analysis by an experienced staff.

The age-related decrease in muscle mass and strength appears to be mainly caused by atrophy of the type IIa muscle fibers[2]. Not surprisingly then, specific muscles that are naturally rich in this fiber type, such as the vastus lateralis in the thigh, are most affected by age-related atrophy[3]. The determination of individual muscle volumes may help to better characterize specific therapeutic effects, while increasing statistical power of the clinical trial. However, the main difficulty resides in the the correct delineation of muscle boundaries, especially in areas where the muscle-muscle interface is blurred. Semi-automated software solutions[4] now exist to exactly address this challenge while allowing for a fast turnaround on the image reading. However, most of these tools still require thorough validation (against for instance data obtained from manual segmentation) before extending their use.

Given the multi-faceted nature of sarcopenia, other imaging applications may be considered beyond pure volumetric analyses. For instances, chronic subclinical inflammation observed in aged skeletal muscle has been linked to functional limitations in older persons, by negatively influencing skeletal muscle through lower protein synthesis rate[5], direct catabolic effects or indirect mechanisms[6]. As a specialized MR technique, diffusion-weighted imaging (DWI) provides molecular information regarding fluid motion in tissues. Recently, this technique was applied to inflamed muscles from myositis patients and showed clear differences in water diffusion properties compared to unaffected patient muscles[7]. Interestingly, using the same approach also allows differentiating edematous muscles from fat infiltrated muscles which have reduced water diffusion compared to control muscles. Finally, this technique may also inform on longitudinal changes in the fibrous muscle structure occurring during disease progression[7].

The loss of muscle strength as a consequence of aging is particularly obvious during isokinetic testing, but is more subtle under eccentric testing conditions. Although the mechanism is still unclear, the development of muscle stiffness in older adults seems partly related to the preservation of high velocity strength. During sarcopenia, there is a replacement of muscle fibres with fat and an increase in fibrosis, which contributes to tissue stiffening. Magnetic Resonance Elastography (MRE) is a non-invasive phase-contrast MRI technique that can directly measure propagating shear waves in tissue in response to a harmonic mechanical excitation. Recent data have shown that muscle stiffness can be estimated from the measured wave[8]. The assessment relies on a wave image formed from the pixel vibratory displacement. Such displacement is due to the spin-phase shifts in the received MR signal in response to the mechanical shear vibration imposed on the MRI gradient. Like any other MR application mentioned before, stiffness measurements using this technique can easily be combined to volumetric assessments in a single MRI scanning session. Thus far, no MRE data are available on patients with sarcopenia and it remains to be shown that such approach offers enough sensitivity to observe longitudinal changes. The same applies to muscle stiffness measurements using alternative imaging techniques such as the ultrasonography-derived Young's (or elastic) modulus approach[9]. The interest here lies in the fact that the stand-alone ultrasound device, which both induces and detects propagating shear waves in the muscle, can be used at the bedside while preserving comfort of the patient.

At a more intimate level, the decline in muscle cell contractility is another hallmark of sarcopenia. Newly developed technology using minimally invasive optical microendoscopy showed that high-speed data acquisition enables observation of sarcomere contractile dynamics with millisecond-scale resolution[10]. Data are typically acquired from a single fiber (or no more that 4 or 5 at a time) and clinical applications using a portable device are apparently considered

for the foreseeable future. However, given that most muscles are of a mixed fiber type composition, it will be critical to verify how reflective of the whole muscle function such contractility measurements are before rolling out this technology for a widespread use.

Finally, as pointed out earlier, alteration of muscle protein turnover could represent an early and sensitive marker of sarcopenia. Measurement of the nitrogen balance is the most commonly used method to assess protein homeostasis. However, it is also often recognized as not sensitive enough to determine the continuous exchange of amino acids between tissues, which depends on the metabolic status of the organism. The marginal-to-low protein intake compromises muscle size and function, despite a near zero nitrogen equilibrium. This is why the determination of the muscle protein fractional synthetic rate by continuous infusion of one or more labeled amino-acids (such as ^{13}C-Phe and ^{2}H-Tyr) is the current gold standard, even though it is an invasive, time-consuming and tedious approach. PET imaging using a specific radiolabeled amino acid may help circumvent these issues while providing information on individual muscle protein turnover. Of all the PET tracers tested so far, [^{11}C]MeAIB (methylaminoisobutyrate), an amino acid analogue already in the clinic, showed the most promise as a marker of muscle protein synthesis[11]. The main caveat is that the tracer, while being trapped in the cells, is not incorporated into muscle proteins. A preclinical validation experiment should help to verify how well the uptake rate of this tracer is a good measure of the protein synthesis rate.

To conclude, it is essential that new muscle imaging readouts be available soon as more precise markers of the therapeutic effects typically investigated in clinical trials. Such markers will also help to support trial duration. In order to meet this important need, issues around biological relevance, sensitivity, specificity, variability, safety (i.e., radiation exposure from PET tracers), implementation across multiple sites (i.e., harmonization) and cost will have to be resolved. It is very likely however that these imaging readouts will help fill the gap between the few clinical endpoints available (i.e., patient quality of life and physical function) and pathway-specific markers. Ultimately, the correlation of early changes in muscle size and function with long-term clinical endpoints should offer a unique application of imaging in Phase I and II trials in patients with musculoskeletal impairment.

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QUALIFICATION OF NOVEL METHODOLOGIES AT THE EUROPEAN MEDICINES AGENCY, *M. Isaac, S. Vamvakas (United Kingdom)*

The European Medicines Agency (EMA) in London is responsible for the Regulatory review of new medicinal products for Marketing Authorsiation, in which companies obtain 1 Marketing Authorisation valid throughout the EU.

The regulation (EC) No 726/2004 defines that medicinal products for neurodegenerative disorders must be evaluated centrally by the EMA. The evaluation and opinion is responsibility of the Committee for Medicinal Products for Human Use (CHMP), which has representatives from all Member States.

The Scientifc Advice Working Party (SAWP) is a standing Working Party of the CHMP (the only working party define in the regulation of Reg. 726/2004). The SAWP consists of experts in all therapeutic areas, including also non-clinical experts and experts in statistics among others. The SAWP has also a network of external experts and patient's representatives from the entire EU. In addition the SAWP has representatives from the Committee for Orphan Medicinal Products (COMP), 1 from The Committee for Advanced Therapies (CAT), 2 from the Paediatric Committee (PDCO). The SAWP is supported by a scientific and administrative secretariat.

The role of the SAWP is to give Scientific Advice (SA) to Applicants (mainly companies but also consortia, patients associations and other bodies) on studies for the development of medicinal products for registration.

Questions that the SAWP answers are very diverse including for example: a) CMC: starting materials, specifications, comparability, bridging; b) non-clinical: overall toxicology plan for registration, innovative models for assessing the pharmacological effect of the new product; c) clinical pharmacology: PK/PD, modelling & simulation, BE; d) clinical therapeutic areas: endpoints, population, comparator; e) methodology, statistics: size of the clinical trial, interim analysis, adaptive/seamless designs etc.

In contrast to the CHMP at the time of the Marketing Authorisation Application the SAWP does not undertake a formal assessment of submitted data.

Procedure for Qualification of Biomarkers and other Novel Methodologies: In 2008 the EMA established the new Qualification of Novel Methodologies procedure under the umbrella of the SAWP of the CHMP which has two very novel aspects: it can be independent of a specific medicinal product and it can include formal assessment of submitted data by the SAWP if it is related to this new procedure.

This new procedure foresees two possible outcomes:

- CHMP Qualification Advice on future protocols and methods for further development of the new method towards

qualification for regulatory use, based on the evaluation of the scientific rationale and on preliminary data submitted.

- CHMP Qualification Opinion on the acceptability of a specific use of the proposed method (e.g. use of a novel methodology or an imaging method) in a research and development (R&D) context (non-clinical or clinical studies), based on the assessment of submitted data.

The procedure is voluntary. Although sponsors are recommenced to contact the SAWP as early as possible to receive first Qualification Advice, it is entirely possible to approach the Authority with the results directly for a Qualification Opinion.

As the scientific knowledge and the intended use of a new method may change in line with the generation of additional data the EMEA qualification process may encompass an ongoing interaction between the CHMP and the Applicant. Prior to final adoption of a Qualification Opinion, the CHMP evaluation, being open to public consultation of the scientific community will ensure that CHMP shares information and is open to enlarged scientific scrutiny and discussion. The impact of the qualification on regulatory technical standard also requires that the international dimension of the scientific evaluations is accommodated for within the available confidentiality arrangements.

The EMEA envisages a truly broad scope of the new activity. The qualification process addresses innovative drug development methods and tools. It will focus on the use of novel methodologies developed by consortia, networks, public/private partnerships, learned societies and pharmaceutical industry for a specific intended use in pharmaceuticals R&D.

Among others a Qualification Opinion may define the purpose of the use of the novel biomarker for example as surrogate (supportive) endpoint or depending upon the outcomes of the studies performed as outlined in the Qualification Advice possibly at a certain point in time also as primary (main) endpoint. Alternatively a Qualification Opinion may justify the use of a biomarker as an inclusion criterion for a clinical study to enrich the study population with patients in early stages of the disease.

The existing Scientific Advice/Protocol Assistance procedure not affected by the new qualification procedure.

The CHMP via the SAWP gives a Qualification Opinion on the acceptability of a specific use of the proposed method (e.g. use of a certain biomarker) in a R&D context (non-clinical or clinical), based on the assessment of data.

The assessment is carried out by a Qualification team of several experts. After agreement with the Applicant Qualification Opinions are published for consultation before finalisation.

Earlier in the development the CHMP via the SAWP can also give CHMP Qualification Advice on future protocols and methods for further method development towards qualification. This early involvement of the SAWP and CHMP in the design of the strategy to qualify a new biomarker in combination with the the commitment to evaluate the data obtained from the agreed studies and to provide a Qualification Opinion aims to speed up drug development

So far the the CHMP via the SAWP has given Qualification Advice in developing biomarkers for cancer, dementia, preclinical kidney safety, diabetes, cardiovascular.

The CHMP has given non-clinical; Qualification Opinions and a clinical Qualification Opinion for biomarkers in the predementia stage of Alzheimer's disease: cerebro - spinal fluid related biomarkers for drugs affecting amyloid burden in which the methods used for qualification can be reviewed. *Disclaimer:* The views expressed in this article are the personal views of the authors and may not be understood or quoted as being made on behalf of or reflecting the position of the European Medicines Agency or one of its committees or working parties. 1. EMA guidance for companies requesting SA or PA http://www.emea.europa. eu/pdfs/human/sciadvice /426001en.pdf; 2. EMA Qualification of Novel Methodologies: http://www.emea. europa.eu/pdfs/ human/biomarkers/7289408en.pdf; 3. Qualification Opinion: http://www.ema.europa.eu/ema/pages/includes/document /open_document.jsp ?webContentId=WC500102018

AGRIN-DEPENDENT SARCOPENIA, *J.W. Vrijbloed, S. Hettwer, A. Shaheen, P. Dahinden, R.G. Fariello (Switzerland)*

Sarcopenia is characterized by reduced lean muscle mass associated with diminished functionality at old age leading to frailty, disability and increased mortality. Sarcopenia is imposing a heavy burden, not only on the affected individual but also on our rapidly aging society. Various causes for sarcopenia have been suggested, among which, on the basis of recent data in aged animals, a crucial role of the neuromuscular junction (NMJ), the sole link between motor neurons and muscle fibers, has emerged. As a consequence sarcopenia is commonly referred to as a syndrome of the NMJ. The extracellular matrix protein agrin is essential for the formation and stabilization of NMJs. Agrin forms a complex with LRP4, a low-density lipoprotein receptor (LDLR)-related protein and MuSK, a transmembrane tyrosine kinase. Agrin is cleaved by the pre-synaptic protease neurotrypsin at two sites thereby losing its NMJ stabilizing function. Agrin cleavage by neurotrypsin frees a soluble 22 kDa C-terminal Agrin Fragment (CAF) detectable in blood. Transgenic mice over-expressing neurotrypsin in motoneurons (SARCO mice) exhibit a sarcopenia-like phenotype. In these mice CAF is elevated in the serum. Conversely, in neurotrypsin knockout mice CAF is undetectable. SARCO mice exhibit reduced muscular mass, abnormal gait and diminished limb strength. Histopathology of SARCO mice muscles reproduces all the key features of the pathology found in muscles of sarcopenia patients.

Objectives: To test the hypothesis that over-activity of neurotrypsin as revealed by elevated levels of CAF in serum, may play a pathogenic role in the genesis of sarcopenia.

Material and Methods: Previous pilot studies demonstrated that in a healthy population of Swiss blood donors CAF is measurable in blood where it shows a narrow range of values that do not vary with aging.

Based on these observations, a pilot multi-center, non-randomized, open-label, vertical clinical study was designed. Briefly, 133 informed and consenting elderly (> 65 y.o.a) were recruited and assigned to a sarcopenia patients (SP) group defined according to up to date diagnostic criteria and an aged matched control (AMC). The SP group was characterized by a DXA scan value below -1. People with DXA values between -1 and 0 were assigned to the SP group if presenting weakness in their grip and knee strength and/or reported difficulties in daily living as to walking and lifting objects, with frequent falls. People with DXA values >0 were assigned to the AMC group. The SP group comprised 73 subjects (34 women and 39 men, aged 65 to 87), the AMC group comprised 60 subjects (28 women and 32 men, aged 65 to 88 years). Anthropometric data, DXA, blood levels of inflammatory and other markers (IL-1, hs-CRP, TNF-alpha, glucose, etc.) as well as grip and knee strength were assessed. As a further reference CAF values of a healthy population, serum from Swiss blood donors (BD; n = 169; 86 women and 83 men, age 19 to 74 years) were analyzed. The serum CAF values were determined using Western blot and assays were performed in blind.

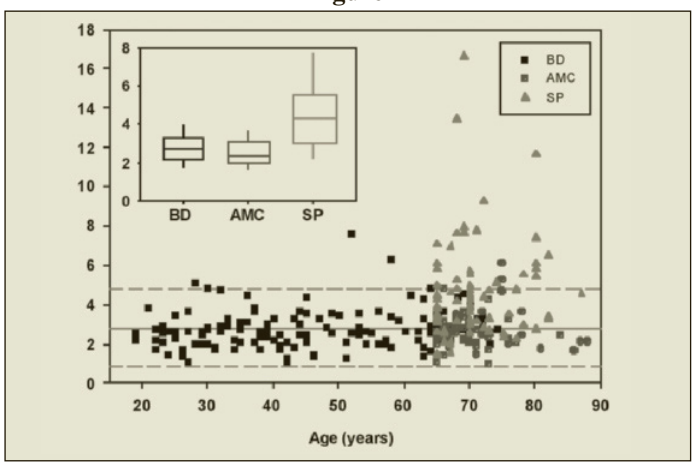
Results: The mean CAF level in the BD group was about 3 ng/ml without gender specific differences or age correlation and was indistinguishable from the AMC group. The SP group showed a 1.5-2 fold increased mean CAF level which was statistically highly significant (see Figure). Detailed analysis of the CAF levels showed that approximately 40% of the sarcopenia patients had non-overlapping values with the normal range. Interestingly, the CAF levels of the males in the SP group were significantly more elevated then the females indicating that males are probably more affected by agrin-dependent sarcopenia.

Discussion: The results of this study confirmed a role of the neurotrypsin/agrin axis in sarcopenia stressing the clinical relevance of our original animal data. CAF levels in the sarcopenic test group as a whole were significantly higher compared to both the age-matched controls and the healthy blood donors. None of the variables under observation that might have influenced the significance of the results (diabetes, renal function, vitamin D levels and inflammation markers) correlated to the CAF values. These results indicate that excessive agrin inactivation at the NMJ may be an important event in the development of sarcopenia. Thus, CAF detection in serum may be of diagnostic value. Measuring CAF blood levels in sarcopenia patients lead to the first causal classification of sarcopenia in an agrin-dependent form that is distinguishable from natural muscle aging. Further work needs to be performed in order to establish the selectivity and specificity of this test and to detect possible variations of CAF levels according to the evolution of sarcopenia. Some of these activities will be performed within the EU-funded project DISARCO during which an ELISA immunoassay will be developed for clinical use. Within this project Neurotune will provide the scientific background, Microcoat GmbH the ELISA technology and the Friedrich-Alexander University Erlangen-Nürnberg (Prof. Sieber), will perform a clinical trial.

Conclusions: Elevated agrin degradation occurs in a substantial subset of sarcopenia patients and can be used to identify those patients in whom a novel pathogenic target may be therapeutically exploited. Excessive degradation of agrin by neurotrypsin leading to fragmentation of the NMJs appears to be an important process in the pathogenesis of sarcopenia.

Disclosures: All the Authors are employees of NeurotuneAG, JWV and RGF hold stock options of the Company

Figure



Serum CAF levels of Sarcopenia patients (SP, N = 73), age matched controls (AMC, n = 60) and swiss blood donors (BD, N = 169) plotted against age. CAF concentrations were determined by Western blotting of serum and calibrated with recombinant human CAF. For swiss blood donors (black squares), a normal value of 2.76 ± 0.98 ng/ml was determined. The mean value is indicated by the solid line. Dashed lines indicate the upper and lower confidence interval (2 x standard deviation). Sarcopenic patients (red triangles) have an overall elevated CAF level of 4.71 ± 2.60 ng/ml compared to the age matched controls (blue circles) with 2.65 ± 0.96 which reproduce the value for the swiss blood donors. Inset: box plot of CAF levels displaying the mean values and 95 % range of the data set. *** $P < 0.0001$ in Student's T-test compared to controls.

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