universität wien

DISSERTATION / DOCTORAL THESIS

Titel der Dissertation / Title of the Doctoral Thesis

„Age-related loss of muscle mass and function in Kosovan older men and women and its association with the ACTN3 genotype“

verfasst von / submitted by

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angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Doctor of Philosophy (PhD)

Wien, 2022 / Vienna, 2022

Studienkennzahl lt. Studienblat / degree programme code as it appears on the student record sheet:	UA 794 680 481
Dissertationsgebiet lt. Studienblatt / field of study as it appears on the student record sheet:	Sportwissenschaft (Lebenswissenschaften)
Betreut von / Supervisor:	Assoz. Prof. Dipl.-Ing. Dr. Barbara Wessner, Privatdoz.

„For centuries I have been selling blood
and raised I’ve been with my own sold blood,
for centuries I have been eating with myself
and never knew how to laugh with myself excessively...“

Ali Podrimja (1942 – 2012)

CONTENTS

Abstract

Ageing is one of the most interesting and intriguing processes of human being, which raised wonders throughout their existence. From the scientific point of view, age-related changes in body composition, together with the progressive decline of body functioning have been subject of numerous investigations on population-wide, physiological, cellular and even molecular levels. Meanwhile, it became evident that the level of exposure to various environmental and lifestyle factors together with the genetic make-up of the individual are responsible for the observed differences during the ageing process. One particular topic gaining an ever-growing interest, especially in countries with high income levels and predictions of high life expectancy, is sarcopenia, the progressive decline of muscle mass and function (including strength and physical performance). There is no doubt that the situation from the perspective of Kosovo, a developing European country with the lowest life expectancy within the continent, has to be deeper explored as reliable data on the ageing population of Kosovo is scarce. Therefore, the general aim of the thesis has been to investigate a broad range of underlying factors of sarcopenia including basic but also socioeconomic and genetic ones in older Kosovan adults.

Establishing consistency within the assessment techniques (muscle mass and other body composition components, isometric muscle strength, physical performance and isokinetic dynamometry) over a wide age range has been the main goal of study one. The test-retest reliability found in almost all parameters in both younger (n = 57, mean age (SD) = 22.6 (3.7)) and older participants (n = 61, mean age (SD) = 70.7 (6.1)) was acceptable (ICCs > 0.7). Only average power of knee flexion at 60^0/s and 120^0/s, as well as peak torque flexion at 120^0/s amongst older participants were somewhat lower with ICCs being 0.63, 0.67 and 0.69, respectively. Besides confirming the usability of the assessment methods (in the following cross-sectional study of the thesis, but also in future longitudinal studies), the data obtained from the young adults have been used to determine population-specific diagnostic cut-off points for sarcopenia and its related conceptual stages as suggested previously.

Although sarcopenia has been acknowledged as a disease, its diagnosis remains subject for discussion as several guidelines have been developed using different measures, algorithms and cut-off points. The aim of study two was not only to cross-sectionally describe the prevalence of sarcopenia in a convenient sample of Kosovan adults aged 60 years and above (n = 240, 47.1% female), but also to compare different algorithms and the influence of using Kosovan-derived cut-off points instead of generally published ones from the European Working Group of Sarcopenia in Older People (EWGSOP). Sarcopenia was observed to be low in all cases, but higher percentages of pre-/probable sarcopenia was evidenced with population-specific cut-off points (22.9% vs. 2.1% and 5.4% following EWGSOP and EWGSOP2, respectively). Sarcopenia was observed more frequently in males and related to higher age and risk for malnourishment, but lower body mass index, skeletal muscle mass, physical performance score, educational level and nutritional status. Other factors such as living environment, marital status, financial status and self-perceived health condition were not related to sarcopenia stages.

Three hundred and eight participants (40 - 91 years old, 51.9% females) from studies one and two provided saliva DNA samples to be used for determining the impact of a specific genetic polymorphism that has been associated with muscle mass and strength in previous studies, especially in athletic populations. Whether this genetic variant of the ACTN3 gene (R577X-rs1815739) is associated with sarcopenia-determining factors in a general population is less clear and was the main aim of genetic study. Genotype allocation was not associated to any of the measured muscle traits in the overall population. Interestingly, a sex-specific impact on knee extensor peak torque have been detected with female XX carriers exerting a higher isokinetic knee extension peak torque than the RX+RR group.

In conclusion, the conducted studies provided evidence that sarcopenia can be observed in Kosovan older adults, whereby its prevalence can vary up to ten-fold based on the diagnostic criteria to be applied. This is an important point to be considered when comparing prevalence rates to those from other countries. In any case internal characteristics, together with socioeconomic factors and lifestyle habits should be taken in consideration for their modifying potential and carefully evaluated before taking general population-wide countermeasures. Although genetic variation in the ACTN3 gene only seems to provide a marginal impact, genetic diversity may play a role in the individual susceptibility to loss of muscle mass and function during ageing. Whether genetic variants in the ACTN3 or other genes can be used as “biomarker” to identify people at risk may be elucidated. Taken together, data from this PhD thesis should be seen as a starting point for future studies to understand especially country-specific determinants of sarcopenia to be used for scientists, health-authorities, policy-makers and last but not least the general population.

Zusammenfassung (German abstract)

Der Alterungsprozess stellt einen der der interessantesten und faszinierendsten Vorgängen dar, der im Laufe des Lebens immer wieder für Verwunderung sorgt. Aus wissenschaftlicher Sicht sind die altersbedingten Veränderungen der Körperzusammensetzung sowie die fortschreitende Verschlechterung der Körperfunktionen Gegenstand zahlreicher Untersuchungen auf bevölkerungsweiter, physiologischer, zellulärer und sogar molekularer Ebene. Inzwischen hat sich gezeigt, dass das Ausmaß der Exposition gegenüber verschiedenen Umwelt- und Lebensstilfaktoren zusammen mit der genetischen Ausstattung des/der Einzelnen für die beobachteten Unterschiede während des Alterungsprozesses verantwortlich sind. Ein besonderes Thema, das vor allem in Ländern mit hohem Einkommensniveau und prognostizierter hoher Lebenserwartung immer mehr an Bedeutung gewinnt, ist die Sarkopenie, der fortschreitende Rückgang der Muskelmasse und -funktion (einschließlich Kraft und körperlicher Leistungsfähigkeit). Es besteht kein Zweifel daran, dass die Situation im Kosovo, einem europäischen Entwicklungsland mit der niedrigsten Lebenserwartung auf dem Kontinent, eingehender untersucht werden muss, da nur wenige zuverlässige Daten über die alternde, kosovarische Bevölkerung vorliegen. Daher bestand das übergeordnete Ziel dieser Arbeit darin, ein breites Spektrum von Faktoren zu untersuchen, die der Sarkopenie bei älteren kosovarischen Erwachsenen zugrunde liegen, einschließlich grundlegender bestimmender als auch sozioökonomischer und genetischer Faktoren.

Das Hauptziel der ersten Studie bestand darin, die Konsistenz der Bewertungsmethoden (Muskelmasse und andere Komponenten der Körperzusammensetzung, isometrische Muskelkraft, körperliche Leistungsfähigkeit und isokinetische Dynamometrie) über eine breite Altersspanne hinweg festzustellen. Die Test-Retest-Reliabilität war bei fast allen Parametern sowohl bei jüngeren (n = 57, Durchschnittsalter (SD) = 22.6 (3.7)) als auch bei älteren Teilnehmern (n = 61, Durchschnittsalter (SD) = 70.7 (6.1)) akzeptabel (ICCs > 0,7). Lediglich die durchschnittliche Leistung der Kniestrecker bei 60°/s und 120°/s sowie das Spitzendrehmoment bei 120°/s waren bei den älteren Teilnehmenden mit ICCs von 0,63, 0,67 bzw. 0,69 etwas niedriger. Neben der Bestätigung der Anwendbarkeit der Bewertungsmethoden (in der folgenden Querschnittsstudie der Dissertation, aber auch in zukünftigen Längsschnittstudien) wurden die von den jungen Erwachsenen gewonnenen Daten verwendet, um wie in vorangegangenen Studien vorgeschlagen bevölkerungsspezifische diagnostische Grenzwerte für Sarkopenie und die damit verbundenen konzeptionellen Stadien festzulegen.

Obwohl Sarkopenie als Krankheit anerkannt ist, bleibt ihre Diagnose Gegenstand von Diskussionen, da mehrere Leitlinien entwickelt wurden, die unterschiedliche Messgrößen, Algorithmen und Cut-off-Punkte verwenden. Ziel der zweiten Studie war es nicht nur, die Prävalenz der Sarkopenie in einer geeigneten Stichprobe kosovarischer Erwachsener im Alter von über 60 Jahren (n = 240, 47.1% Frauen) im Querschnitt zu beschreiben, sondern auch verschiedene Algorithmen und den Einfluss der Verwendung von im Kosovo entwickelten Grenzwerten anstelle der

allgemein veröffentlichten Grenzwerte der European Working Group of Sarcopenia in Older People (EWGSOP) zu vergleichen. Die Sarkopenie wurde in allen Fällen als gering eingestuft, aber bei Verwendung bevölkerungsspezifischer Cut-off-Punkte wurde ein höherer Prozentsatz an prä- sowie wahrscheinlicher Sarkopenie festgestellt (22,9 % gegenüber 2,1 % und 5,4 % nach EWGSOP bzw. EWGSOP2). Sarkopenie trat häufiger bei Männern auf und hing mit einem höheren Alter und einem höheren Risiko für Unterernährung, aber einem niedrigeren Body-Mass-Index, einer geringeren Skelettmuskelmasse, einem niedrigeren Wert für die körperliche Leistungsfähigkeit, einem niedrigeren Bildungsniveau und einem niedrigeren Ernährungsstatus zusammen. Andere Faktoren wie das Lebensumfeld, der Familienstand, der finanzielle Status und der selbst wahrgenommene Gesundheitszustand standen in keinem Zusammenhang mit dem Sarkopenie-Stadium.

308 Teilnehmende (40 – 91 Jahre alt, 51,9% Frauen) aus Studie eins und zwei stellten Speichel-DNA-Proben zur Verfügung, um die Auswirkungen eines bestimmten genetischen Polymorphismus zu bestimmen, der in früheren Studien, insbesondere bei Sportlern, mit Muskelmasse und -kraft in Verbindung gebracht wurde. Ob diese genetische Variante des ACTN3-Gens (R577X-rs1815739) mit Sarkopenie-bestimmenden Faktoren in der Allgemeinbevölkerung in Verbindung steht, ist weniger klar und war das Hauptziel der Genetische Studie. Die Verteilung des Genotyps war mit keinem der gemessenen Muskelmerkmale in der Gesamtbevölkerung assoziiert. Interessanterweise wurde ein geschlechtsspezifischer Einfluss auf das Spitzendrehmoment der Kniestrecker festgestellt, wobei weibliche XX-Träger ein höheres isokinetisches Spitzendrehmoment der Kniestrecker aufwiesen als die RX+RR-Gruppe.

Zusammenfassend lässt sich sagen, dass die durchgeführten Studien den Nachweis erbrachten, dass Sarkopenie bei älteren Erwachsenen im Kosovo beobachtet werden kann, wobei die Prävalenz je nach den anzuwendenden diagnostischen Kriterien bis zum Zehnfachen variieren kann. Dies ist ein wichtiger Punkt, der beim Vergleich der Prävalenzraten mit denen anderer Länder berücksichtigt werden muss. In jedem Fall sollten interne Merkmale zusammen mit sozioökonomischen Faktoren und Lebensgewohnheiten auf ihr Veränderungspotenzial hin untersucht und sorgfältig bewertet werden, bevor allgemeine bevölkerungsweite Gegenmaßnahmen ergriffen werden. Obwohl die genetische Variation im ACTN3-Gen nur einen marginalen Einfluss zu haben scheint, könnte die genetische Vielfalt eine Rolle bei der individuellen Anfälligkeit für den Verlust von Muskelmasse und -funktion während des Alterns spielen. Es wird sich zeigen, ob genetische Varianten im ACTN3-Gen oder in anderen Genen als "Biomarker" verwendet werden können, um Risikopersonen zu identifizieren. Insgesamt sollten die Daten dieser Doktorarbeit als Ausgangspunkt für künftige Studien zum Verständnis insbesondere der länderspezifischen Determinanten der Sarkopenie gesehen werden, die Wissenschaftler/innen, Gesundheitsbehörden, politischen Entscheidungsträger/innen und nicht zuletzt der allgemeinen Bevölkerung zugutekommen sollten.

List of publications

This thesis is organized, developed and elaborated based on the following published articles (see appendix):

Publication 1:

Boshnjaku A, Bahtiri A, Feka K, Krasniqi E, Tschan H, Wessner B (2021). Test-retest reliability data of functional performance, strength, peak torque and body composition assessments in two different age groups of Kosovan adults. **Published:** Data in Brief, 36:106988. https://doi.org/10.1016/j.dib.2021.106988

Dataset (from publication 1):

Boshnjaku A, Bahtiri A, Feka K, Krasniqi E, Tschan H, Wessner B. Raw data for test-retest reliability of physical performance in young and old adults.
Published: Mendeley data. V1. https://doi.org/10.17632/ntsrj6hf87.1

Publication 2:

Boshnjaku A, Bahtiri A, Feka K, Krasniqi E, Tschan H, Wessner B (2022). Impact of Using Population-Specific Cut-Points, Self-Reported Health, and Socio-Economic Parameters to Predict Sarcopenia: A Cross-Sectional Study in Community-Dwelling Kosovans Aged 60 Years and Older.
Published: Journal of Clinical Medicine, 11(19), 5579; https://doi.org/10.3390/jcm11195579

Publication 3:

Boshnjaku A, Krasniqi E, Tschan H, Wesner B (2021). ACTN3 Genotypes and Their Relationship with Muscle Mass and Function of Kosovan Adults.
Published: International Journal of Environmental Research and Public Health, 18(17), 9135; https://doi.org/10.3390/ijerph18179135

1 Introduction

1.1 Ageing

Ageing is a multidisciplinary concept, both a process in the physiological point of view, just as much as a psychological and social phenomenon [1, 2]. This multidimensionality comes from a complex of interactions that occur in between different factors involved on it, being them either internal of biological or genetic aspect, or external from different lifestyle, environmental or socioeconomic covariates. The global phenomenon of population ageing is today widely accepted as a human success story, as a reflection of the advancement of public health, medicine, economic and social development, together with their contribution disease control, injury prevention and premature death risk reduction [3]. In fact, population ageing is one of the key components of current global demographics as described by the United Nations (UN), which together with the other "mega-trends" such as population growth, urbanization and international migration are expected to continue having substantial and lasting impacts on future sustainable development [3]. This trend is resulting in the increase of population aged 65 or older from 6% in 1990, to 9% in 2019, and is projected to rise further to 16 % by 2050[3], thus allowing an increase of expected life expectancy to come along simultaneously with the ageing of population. The life expectancy at birth has currently reached 72.3 years (74.7 years in women and 69.9 years in men) [3], with an estimated increase of 2 years per decade since 1840 starting at the developed countries (initially) [4].

With the increasing of human lifespan, there is an ever-growing need to further understand the mechanisms behind the ageing process as a whole, to explore deeply into the molecular levels, as well as to better understand the impact and interaction of internal factors on organisms' behavior within different circumstances. At the biological level, ageing results from the impact of continual accumulation of a range of molecular and cellular damage over the time, leading towards a gradual decrease in both physical and mental capacity, a growing risk of disease, and ultimately death [5]. Ageing is also considered as the single highest risk factor for a range of diseases [6], thus particularly imposing the necessity to understand major problems associated with senescence, starting from the gradual decline in the systems and organs that determine physical fitness, notably skeletal muscle tissue [7]. It is important to mention that the nowadays human is well aware of his progress through time, which was significantly increased in the modern era coming with the industrialization and globalization in particular. This includes the acknowledgement that achievements and benefits from this general development come to a certain cost that needs to continuously be investigated and better understood.

1.1.1 Physiological mechanisms of ageing

As a complex process characterized by the progressive loss of physiological functioning over the time, while correlating with an increased vulnerability to different conditions and diseases within all systems of organs [8], aging presents a serious issue to be dealt with. Humans have always been trying to understand in depth the mechanisms of ageing, with an ever-increasing interest having attracted the eyes of the researchers in the last decades. A landmark

article by López-Otín C and colleagues [9] has categorized and described remarkably the "hallmarks of ageing", as follows:

- Genomic instability – a phenomenon characterized by the accumulation of genetic damage over time from either exogenous or endogenous factors, this way overshadowing the organisms complex mechanisms for DNA repair [10] and resulting in highly variable genetic lesions in either nuclear or mitochondrial DNA, or even in nuclear architecture;
- Telomere attrition – an age-related process that is characterized by the progressive shortening of telomeres (the highly specialized nucleotide sequences in the terminal chromosomal end, who serve as protector from deterioration) upon the cellular division [8], thus leading towards the cell senescence and Hayflick limit (which represents the extent to which normal somatic cells divide before ceasing division) [11];
- Epigenetic alterations – different epigenetic modifications that are acquired during lifetime can alter cellular functions and gene expressions, including here the histone modifications, DNA methylation, chromatin remodeling, transcriptional alterations through non-coding RNA (ncRNA) and the reversion of epigenetic changes [8, 9];
- Loss / collapse of proteostasis – an impaired cell regulation process on the level of protein homeostasis, proper chaperone-mediated protein folding, as well as the proteolytic systems for degradation of misfolded proteins (namely autophagy-lysosomal and ubiquitin-proteasome systems) [8];
- Deregulated nutrient sensing – an important perspective that is supported by the current state of art is the potential of decreased nutrient signaling or caloric intake (dietary restriction) to extend longevity, as well as the anabolic signaling itself to accelerate the ageing process [12], while considering the prospects of decreased caloric intakes on extending longevity through preventing the age-related decline in autophagy [13];
- Mitochondrial dysfunction – a tendency of age-related diminishing of the respiratory chain efficacy, with mitochondria becoming progressively inefficient to work properly as well as potentially toxic [14]; the increased production of reactive oxygen species as a consequence to this mitochondrial dysfunction leads towards further internal deterioration and systemic damage[8];
- Cellular senescence – a process through which cells do terminate their replication together with their progress throughout the cell cycles, resulting as a consequence a set of different factors [8];
- Stem cell exhaustion – an undoubtedly trademark of the ageing process in general is considered the progressive decline of organisms regenerative potential, which comes as a consequence mainly to the decrease of stem cells reserves across different tissues throughout the body, starting from skin, hematopoietic, immune, musculoskeletal to nervous systems [15];
- Altered intercellular communication and inflammation – a process characterized by changes within the level of intercellular communication, though beyond the emphasized cell-autonomous alterations, notably with inflammation highlighted amongst these alterations [9]; this particular age-related, low grade, chronic pro-inflammatory state has been emphasized and named "inflammaging"[16].

In a recent review, Grote and colleagues [8] proposed another important hallmark of ageing which they entitled "abnormal crosslinking", while referring to the accumulation of different abnormal links in between the molecules. Furthermore, it was also suggested that it is either intra- or inter- molecular bonding that will lead towards altered translation, transcription and tissue properties, with the manifestations being seen in from the skin elasticity loss, stiffening of blood vessels, changes within the eye lenses and delaying in the wound healing process [17, 18].

1.1.2 Genetic determinants of ageing

An encompassing and very comprehensive review on the genetics of ageing [19] described ageing of human being as a process driven in the balance between damage and repair, which is simultaneously influenced by environmental exposures and genetic variation (Figure 1). Obviously, different environmental factors assert influence consistently, proportionally to the exposure, whereas the importance of genetic factors seem to be emphasized in certain cases or upon the exposure to a certain stimuli. The impact and interaction of environmental exposures on ageing and longevity have been studied more in comparison to the genetic influences, which could probably be due to the wider scope of the environmental context. Regardless, potential impact of genetic component in longevity has been reported from 20 – 30% in twin studies, increasing to 48% in males and 33% in females in families with a centenarian [20]. However, two recent massive family tree analysis studies reported a heritability of not more than 16% [21] and 10% [22]. Conversely, it should be noted that notwithstanding the influence that any of the factors might have in particular cases, when it comes to successful ageing and longevity, every detail accounts and could make the difference.

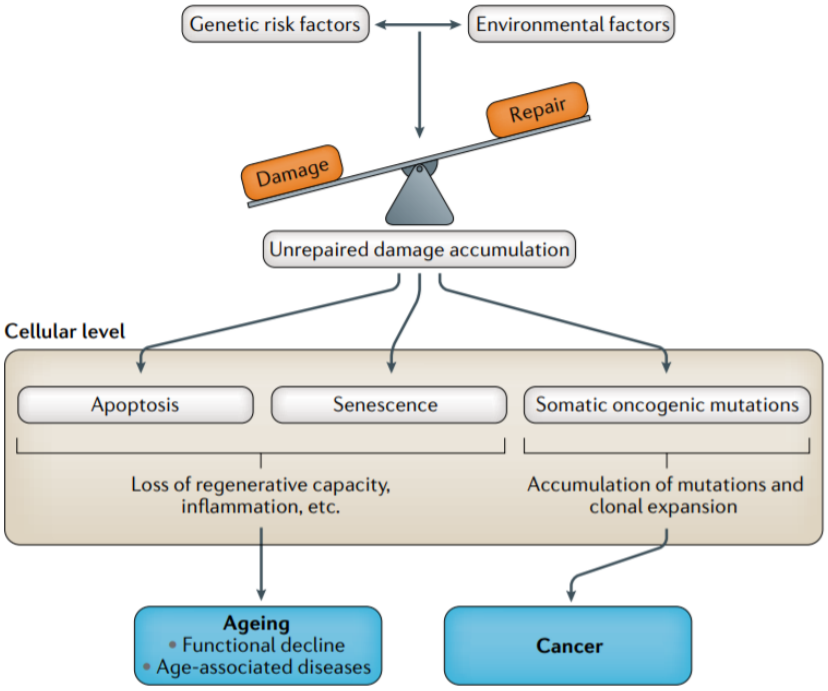

Figure 1: The major impacts and mechanisms of human ageing. With permission from Melzer D et al. [19] and Springer Nature

As described previously in the hallmarks of ageing, senescence is a process occurring in both molecular and cell levels, which makes it rather difficult to measure directly in genetic association studies with larger samples. This leads towards the need for alternative research forms, namely the development of so-called "proxy phenotypes of ageing" [19] such as:

- Lifespan – the individual's timeframe from birth to death;
- Disease and physiological markers – the clinical diagnosis of age-related disorders, diseases and cancers;
- Challenges in studying ageing – obstacles such as terminology, environmental exposure, quantification and standards etc.

Additionally, the parental lifespan, genetic associations with longevity and gene-environmental interaction of certain variants present other pathways towards the better understanding the ageing process [19]. In this context, extensive studies conducted around the world have tried to better understand and analyze the different candidate genetic variants that might be related to ageing and age-related specificities. Most of these studies in genotype-phenotype associations within large genetic samples are the Genome Wide Association Studies (GWAS) with stringent statistical significance threshold for avoiding the confounding false positive findings, whereas the more variants studied, the better chances would be for finding an association [19, 23]. To date, different GWAS studies have been describing the hallmark mechanisms of ageing, such as several multi trait loci and variants related to inflammation and obesity [19]. Notwithstanding the fact that there are still many incomprehensible aspects of ageing, just the idea of the existing potential to better understand and perhaps even affect and influence upon, looks as the beginning of what seems to be a bright prospect for our future of mankind.

1.1.3 Skeletal muscle and ageing

Skeletal muscle presents an essential component for the vital human functions such as movement, breathing, postural support, thermogenesis [24], maintaining systemic glucose homeostasis and providing the site for glycogen synthesis and fatty acid metabolism to occur [25, 26]. Further on, together with extracellular matrix of skeletal tendon-bone, are integral for maintaining the tissue structure in one hand and enabling the processes of the contraction of muscle and the transmission of force, in the other [4]. Skeletal muscles themselves are made from bundles of muscle fibers, each of which consisting of small functional units that are responsible for contraction and relaxation, called sarcomeres. Importantly, skeletal muscles are distinguished for their plasticity, the process described as the ability to adapt towards the pressure imposed on it [26]. Still, with ageing and the whole body's homeostatic misbalance, this ability progressively declines and diminishes as well.

Muscles, together with bone, cartilage and tendon are the primary tissues within the musculoskeletal system to be affected by age [4]. The skeletal muscle mass (SMM) begins declining after the middle of the third decade of life (Figure 2), with an estimated 10% of muscle that can be lost by 50 years of age [27], a median annual decline of about 0.37% in females and 0.47% in males (increased to 0.64 - 0.70% in females and 0.80 - 0.98% in males aged 75 years and above) [28], always decreasing the protective resources against neurodegenerative and cardiovascular diseases, diabetes mellitus type II and age related cancer forms such as colon and breast cancers [29, 30]. The age-related muscle

mass decline is observable in the muscle fiber size as well, but only specific to type II muscle fibers (10 - 40% smaller in older comparing to younger adults) [31, 32]. This is particularly worrisome since the type II muscle fibers, also known as fast twitch fibers, are generally responsible for higher intensity and highly fatiguing activities and therefore their decline may also decrease muscle strength and be reflected on performing the daily life activities [33]. In contrary, type I muscle fibers, also known as slow twitch (muscle fibers), are known for a sustainable recruitment since they're normally recruited firstly due to their highly aerobic capacity and capability for endurance activities they possess. An interesting accompanying phenomenon observed with ageing and the decline of type II muscle fiber size, was the reduction of type II muscle fibers stem cell (also named as satellite cell) content and function, which presents an issue of serious concern, especially having in mind their importance in skeletal muscle fiber growth, repair and regeneration throughout human life [33-35]. For a better understanding, these satellite cells normally reside beneath the skeletal muscle fibers in a quiescent state, waiting to be activated, and start proliferating (if activated) and either differentiate to form new mononuklei or return to quiescence upon the appropriate stimuli [35].

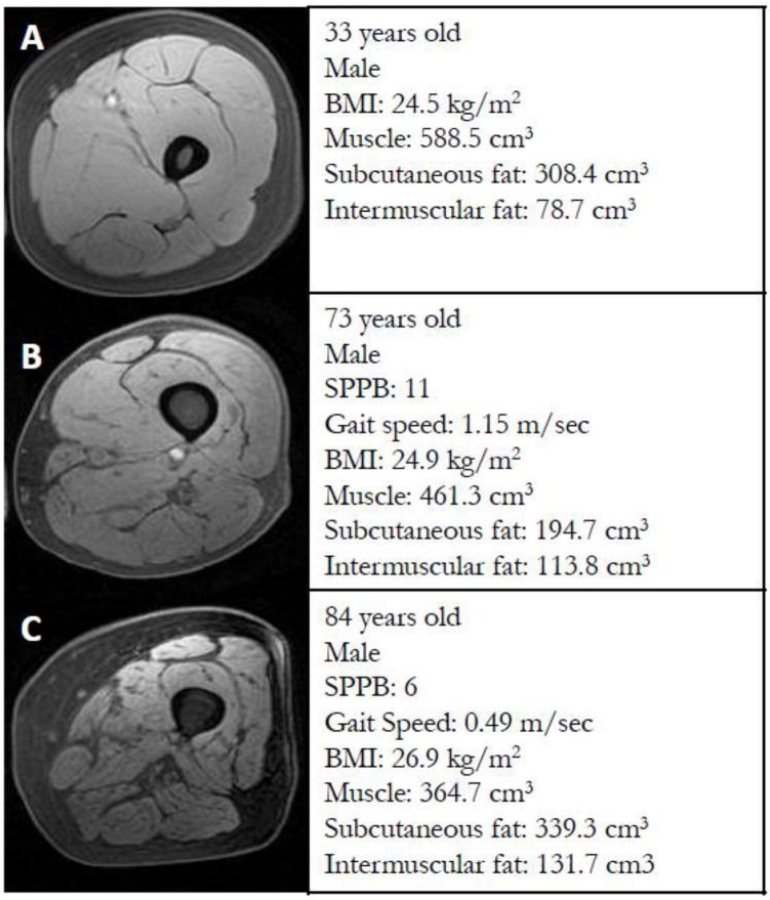

Figure 2: Male femoral region's Magnetic Resonance Imaging (MRI) in: A) young, B) high-functioning older adult, 1and C) low-functioning older adult. With permission from Buford TW et al. [36] and Elsevier

One other concerning characteristic observed with skeletal muscle ageing in terms of the architectural changes, besides the changes in the elastic fiber system, is the increase in the infiltration of adipose tissues within the skeletal muscles [37]. The age-related increase of inter-muscular fat was shown to be associated with declines in mobility and physical performance amongst older adults [37, 38], while serving as an important risk alarm for optimal individual capabilities and independence with ageing. It should be mentioned that the skeletal muscles architectural changes also include the increased level of muscle fibrosis (the excessive formation of scar tissues fibrous bands in between the muscle fibers), but to this day there is no evidence supporting this in humans, which is mainly due to the difficulties in assessing fibrosis in human population studies in comparing to findings in rats [33, 39].

In conclusion, age-related deterioration of skeletal muscle (in particular) has undoubtedly severe health consequences, and is a field requiring and also attracting an ever-growing attention. Nevertheless, it should be noted that even though inevitable to all humans, this process is typically slow while the functional altering differs significantly amongst individuals [40].

1.1.4 Ageing and muscle strength, power, mass and function

Up to the 5th decade of life, the number and size of muscle fibers remain relatively stable, whereas afterwards, a decline in size, number of fibers and motor units proceeds [41, 42]. The progressive age-related decline in skeletal muscle function is presented as a consequence of qualitative and quantitative changes within muscle structure and function [40]. It has been described that muscle strength declines between 1 - 1.5% per year in older adults [43], reaching a decline of approximately 20 - 40% below that of a typical young adult by the seventh and eighth decades, with the potential to decline up to 50% amongst the very old [44]. Additionally, the muscle fibers contractile velocity has been shown to decrease in older adults as a result of an approximately 18 – 25% of speed reduction within the actin sliding on myosin [45, 46]. With respect to the muscle power, Alcazar and colleagues [47] explored and described its decline after the fourth decade of life, mainly due to the age- and sex-specific changes [47]. Otherwise, age-related decline of muscle strength has been shown to be independently related to all-cause death and cancer in men aged 20 – 82 years [48], to be the most reliable measure of muscle function to date, as well as better than mass in the prediction of adverse outcomes [49], and a strong predictor of longer hospitalization [50], increased mobility impairments [51, 52] and disability [28]. Therefore, keeping in mind that muscle strength is undoubtedly a fundamental component of the locomotor system and organism itself, its progressive age-related decline should be seen as a sign for serious concern and precursor to several functional limitations that might just as well lead towards disability, morbidity and mortality (Figure 3).

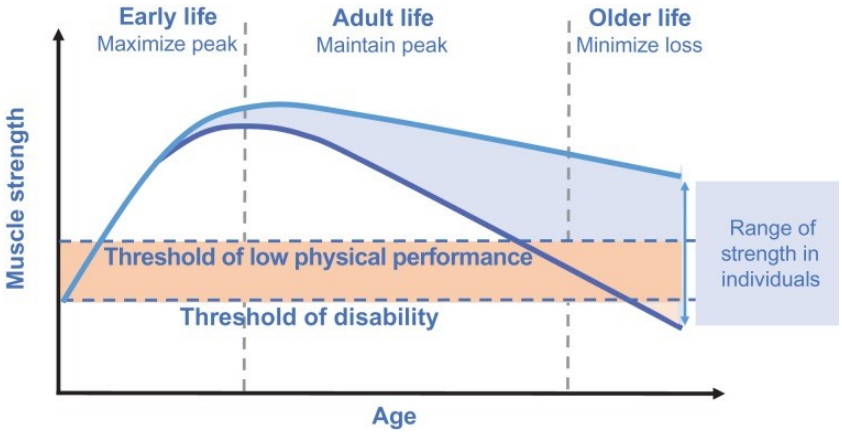

Figure 3: Muscle strength through life course. With permission from Cruz-Jentoft AJ et al. [49] and Oxford University Press

One important component of skeletal muscle is muscle quantity or muscle mass, which as described in the previous chapter (see 1.1.3.), starts declining by the second part of the third decade, reaching an estimated decline around 0.37% in females and 0.47% in males per year [28]. Muscle quantity is normally reported either as SMM, appendicular skeletal muscle mass (ASMM) or cross sectional area (CSA) of a particular muscle group or certain body location [49], when measured by any of the established techniques such as MRI, computerized tomography (CT), dual energy X-ray absorptiometry (DXA) or bioelectrical impedance analysis (BIA). Muscle quality is another concept that was proposed as a surrogate parameter to narrate the generation of force per capacity per unit of muscle CSA [28], or to explain the micro- and macroscopic features within the muscle composition and architecture [49], highlighted mainly due to muscle strengths relation to adverse outcomes no matter if muscle mass is preserved [41]. Therefore, muscle quality is destined to follow declining of other muscle components and features, notably muscle strength.

It has been suggested lately that both muscle quantity and quality remain impediment as primary parameters for defining sarcopenia [52-54] whereas the revised consensus on definition and diagnosis of sarcopenia by European Working Group in Sarcopenia for Older People (EWGSOP2) went in favor of muscle strength overtaking muscle mass as the principal determinant [49] (see 1.2.). However, it should be mentioned that there are other studies that have been describing a stronger relation of power components (30 sec chair stand test) with physical performance (gait speed) in comparison to muscle strength [55, 56], thus endorsing muscle power as a potentially better path to follow with a greater clinical relevance towards sarcopenia detection. Losa-Reyna and colleagues identified particularly the association of muscle power with lower gait speed (at usual pace), frailty and quality of life [56]. Nevertheless, using muscle power assessment in older people have not been particularly encouraged in clinical practice [56] due to several technical and economic barriers [57], as well as the necessity for feasible and standardized protocols [58].

Finally, physical performance presents another key feature of skeletal muscle and locomotor system, which is a multidimensional concept defined as an objectively measured locomotion-related whole-body function, integrating muscles function with central and peripheral nervous systems, as well as balance [41, 49, 58]. Physical functioning,

like muscle strength and muscle mass, is another component that declines with ageing. This characteristic decline is directly observed when performing activities of daily life, and is measured through a wide range of existing physical performance tests based on the recommended diagnostic guideline criteria. Amongst these testing procedures the most commonly used are Short Physical Performance Battery (SPPB) with the recommended set of tests, usual gait speed and timed up-and-go test (see 5.2.).

1.1.5 "Successful ageing" and impairments

Successful ageing is a term unveiled with the increasing of expected life expectancy with birth as well as the increased longevity. Its definition has shifted from the initially rather biomedical approach (what is now often regarded as "healthy ageing"), towards more subjective aspects, as a necessity for a more informative approach instead of focusing on single health outcomes [59]. Nowadays the concept of "successful ageing" has become an important tool for describing the quality of ageing itself, with it being widely regarded as a combination of both subjective and objective aspects, the biomedical approach and the psychosocial factors [60]. Investigations trying to better understand and distinguish in between the predisposing factors and other co-variants on longevity and successful ageing are continuously conducted. Recently it was suggested that superior bone health [61] and bone/muscle interaction [62] should promote a longer lifespan and quality of life [63]. This bone/muscle interaction origins from the muscle-bone unit [64] and the interrelated co-functioning in between these two integral body components. In this line, the importance of initially having and further maintaining a good musculoskeletal health with age is presented as an undoubtedly beneficiary component within the human beings ageing process.

Notwithstanding the above mentioned facts, the continual increase of longevity and the percentage of older population comes with other burdens, notably the gradual increase on the incidence of limitations on the physical performance. In fact, it has been described that as much as 42% of older population in western societies have shown difficulties on performing daily life activities (e.g. standing from a chair or simple walking speed), 15 - 30% reported the inability to carry 4.5kg and more than 30% are directly confronted with physical disabilities [65], with these physical limitations directly increasing the risk of co-morbidity, falls, institutionalization and premature death [33]. The causes of these age-related physical limitations are multifactorial, from biomedical (physiological and genetic [8] towards psychosocial (fear of falling [66], loneliness [67] of even self-efficacy [68]). A recent review also suggested for the possibility of stress and various other similar insults as contributors to senescence in the in-vivo cells [69].

1.1.6 Ageing in a developing society

With the recent achievements of modern human, especially in terms of lower mortality, higher longevity, better life quality and education in general, much of the attribute is given to the societal organization and countries general development. Yet, disparities between countries based on the level of development could be visible, as something that has long been an interest of scientists [70, 71]. For better understanding, the World Bank classifies countries based on their per capita gross national income level in three major groups, low-income, middle-income and high-income countries [72], whereas the first two groups (lower- and middle-income countries (LMIC's)) are often integrated

within the same subgroup versus higher-income countries (HIC's). Ageing in particular, is a global issue that affects all populations, notwithstanding the region or the level of development. As a direct function of the economic and social development, for a century, ageing has been mainly associated with the populations of industrialized countries of Europe, North America and Japan in Asia [73]. Lately, with the fast growing new economies, improvement of education and the increase of the quality of life in the less developed countries, the expected life expectancy at birth is rapidly increasing. Still, differences are seen in this indicator as well in between the established developed and developing countries.

In this aspect, remarkable results were found by Paprotny D [74] while analyzing the differences within the context of economy, health, education and environment during a whole century (starting from 1920 and ending up to 2020), finding out that the majority of developing countries and the developing populations as a whole, have reduced their lag in most indicators during this time, whereas the life expectancy together with education were the most successful components in these advancements. Furthermore, a recent UN report on the world population ageing [3] has estimated the fact that more than two-thirds of older adults' population will be expected to live within LMIC's by 2050. This undoubtedly presents a point of concern, since the increase of expected life expectancy at birth together with the increase of the percentages of this population within the developing world means that certain measures have to be taken, whereas the public interest has to start being diverted towards other less traditional sectors as well (notably free health care approach, better health care quality and social insurance for people aged 65 and above within an ever growing social group).

Within Europe, Western Balkan countries like Kosovo, Albania, Bosnia and Herzegovina, Montenegro, North Macedonia and Serbia are officially classified by the Organization for Economic Cooperation and Development (OECD) as Upper Middle Income Countries [75]. The case of Kosovo is interesting for its lower percentage of people aged 65 years and older (6.7% out of 1.739.825 inhabitants; 7.2% amongst females and 6.2% amongst males [76]) in comparison to Europe (20.8% in total, 23.2% in females and 18.3% in males) and world (9.3% in total, 10.4% in females and 8.3% in males), as well as a lower expected life expectancy at birth (72.5 years in total, 74.8 years in females and 70.3 years in males) in comparison to European average (81.1 years in total, 83.8 years in females and 78.4 years in males) and the world average (72.7 years in total, 75.0 years in females and 70.6 years in males [77]). However, the emerging of the new pandemics from the infection by novel SARS COVID-19 virus and particularly its affinity to affect severely the more vulnerable groups of society including the older adults [78], is expected to potentially change the current situation with respect to the worldwide numbers of older population in general. This might be reflected in developing countries as well, taking in consideration their often fragile healthcare systems and response towards public health emergencies. Nonetheless, the outcomes are yet to be seen.

1.2. Sarcopenia (diagnostic algorithms and cut-off points)

As stated in the previous chapters, human organism is characterized (amongst others) with age-related progressive decline in muscle mass, strength and performance. However, other factors might affect these traits in certain forms, as seen in Figure 4. Different systematic diseases together with their direct or indirect influence on skeletal muscle are

amongst the distinctive causes of sarcopenia (the so-called "secondary sarcopenia"). Lack of appropriate levels of physical activity and malnutrition represent additional potential influencers on sarcopenia occurrence.

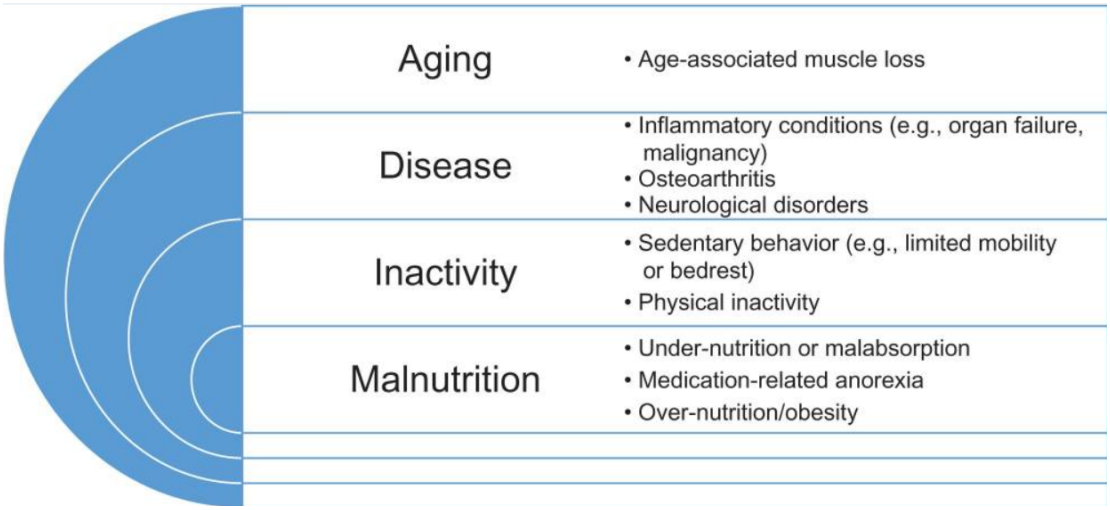

Figure 4: Factors affecting the reducing of muscle mass, strength and performance. With permission from Cruz-Jentoft AJ et al. [49] and Oxford University Press

"Sarcopenia" is a term initially introduced by Rosenberg [79] as a phrase formed by the Greek words "sarx" meaning "flesh" and "penia" meaning "loss" or "deficiency", in order to describe the age-related decrease of muscle mass in older people. What started as a research hypothesis for a novel issue at the time, immediately began raising eyebrows and attracting attention within the scientific community. New ideas on this skeletal muscle phenomenon started coming up, allowing it to grow progressively and evolve. The issue itself was brought to attention amongst the clinicians and others working in clinical settings, steadily involving them in the process as well. The quickly absorbed new concept of sarcopenia, from the initial key component (muscle mass), embraced two other important and integral components on its journey: muscle function and muscle strength. Different working groups started working worldwide, aiming to establish a consensus definition and diagnostic criteria for sarcopenia [49, 80-85]. Table 1 provides a comprehensive description of different international working groups on sarcopenia and their suggested diagnostic algorithms and cut-off points.

Table 1: Comparison of the diagnostic recommendations from the major working groups in sarcopenia

Working group	**Recommendations**		
	Muscle function		**Muscle mass (quality)**
	Strength	**Physical performance**	
The New Mexico Elder Health Survey [86]	n / a	n / a	**DXA –** ASM Predictive equation including weight, height, hip circumference, grip strength, sex and standard error of estimation DXA - ASMI (ASM/Ht^2) 2SD below mean of young reference group
European Working Group on Sarcopenia for Older People (EWGSOP) [80]	Grip strength [29] ♂ < 30 kg ♀ < 20 kg	Gait speed (6 m course) [88] < 1 m/s	**DXA –** ASMI 2SD below mean of young reference group [86] ♂ < 7.26 kg/m²

	BMI based [87]	(other cut-off values for different course lengths [29, 87]) SPPB [89] ≤ 8 points	♀ < 5.5 kg/m² sex specific lowest 20% [90] ♂ < 7.25 kg/m² ♀ < 5.67 kg/m² sex specific lowest 20% [91] ♂ < 7.23 kg/m² ♀ < 5.6 kg/m² linear regression on ALM adjusted for fat mass and height [91] ♂ - 2.29 ♀ - 1.73 **BIA –** ASMI [92] ♂ < 8.87 kg/m² ♀ < 6.42 kg/m² NHANES III [93, 94] Severe sarcopenia ♂ ≤ 8.50 kg/m² ♀ ≤ 5.75 kg/m² Moderate sarcopenia ♂ 8.51 – 10.75 kg/m² ♀ 5.76 – 6.75 kg/m² Normal muscle ♂ ≥ 10.76 kg/m² ♀ ≥ 6.76 kg/m²
International working group on sarcopenia (IWGS) [82]	n / a	Gait speed < 1 m/s (4 m course)	DXA - appendicular fat lean mass (aLM) to height squared (aLMI/Ht²) ♂ ≤ 7.23 kg/m² ♀ ≤ 5.67 kg/m² Cut-off less than 20%tile of values for healthy young adults
The Foundation for the National Institutes of Health (FNIH) [83]	Handgrip strength ♂ < 26 kg ♀ < 16 kg BMI adjusted grip ♂ < 1.0 ♀ < 0.56	n / a	ALM (BMI adjusted) ♂ < 0.789 ♀ ≤ 0.512 ALM ♂ < 19.75 kg ♀ < 15.02 kg
Asian Working Group on Sarcopenia (AWGS) [84]	Handgrip strength ♂ < 26 kg ♀ < 18 kg	Gait speed: < 0.8 m/s	2SD below mean of young reference group [86] The lower quintile as the cutoff value determination [90, 91] ASMI - DXA ♂ ≤ 7.0 kg/m² ♀ ≤ 5.4 kg/m² ASMI - BIA ♂ ≤ 7.0 kg/m² ♀ ≤ 5.7 kg/m²
Revised Asian Working Group for Sarcopenia (AWGS2) - 2019 Consensus Update [85]	Handgrip strength ♂ < 28 kg ♀ < 18 kg	Gait speed (6 m course) < 1.0 m/s SPPB score ≤ 9 points 5-time chair stand test ≥12 seconds	ASMI - DXA ♂ ≤ 7.0 kg/m² ♀ ≤ 5.4 kg/m² ASMI - BIA ♂ ≤ 7.0 kg/m² ♀ ≤ 5.7 kg/m²
Revised European Working Group on Sarcopenia for Older People (EWGSOP2) – 2019 Consensus Update [49]	Handgrip strength [95] ♂ < 27 kg ♀ < 16 kg Chair stand [88] < 15 s for 5 rises	Gait speed ≤ 0.8 m/s SPPB [96, 97] ≤ 8 point score TUG [98]: ≥20 s 400 m walk test [99] Non-completion or ≥6 min for completion	DXA and BIA ASMM [83] ♂ < 20 kg ♀ < 15 kg ASMI [100] ♂ < 7.0 kg/m² ♀ < 5.5 kg/m²

Baumgartner and colleagues [86] presented a significant work in the field by being the first to propose an operational definition of sarcopenia and describing that the prevalence of sarcopenia and its severity significantly increases with age. The suggested operational definition encompassed the measuring of lean soft tissue by DXA, out of which a predictive equation was used for estimating the ASMM per height squared (Ht^2) (involving components such as weight, height, hip circumference, grip strength, sex and standard error of estimation), and finally calculating sarcopenia as 2 standard deviations (SD) of ASMM per height squared (m^2) below a young reference group's mean [86]. Later on, Janssen and colleagues [93] presented the association of relative skeletal mass with functional impairments and disability in older adults in general and female sex in particular, as well as raising concerns for sarcopenia being a potentially reversible cause of morbidity and mortality on older people. This time Janssen et al [93] were using the BIA, while following the operational definition from Baumgartner and colleagues [86]. In 2010, the EWGSOP came with a consensus on definition and diagnosis of sarcopenia, by suggesting to recognize sarcopenia as a geriatric syndrome characterized by the presence of low muscle mass and low muscle function (strength, performance, or both) [80]. The EWGSOP recommendations were embraced immediately amongst the field experts, while remaining the most cited recommended consensus in the field of sarcopenia to date. Another characteristic of EWGSOP was the lack of defined specific cut-off values, which was complemented by the suggestion of a range of previously used diagnostic alternatives, tools and cut-off points, as a measure and also tendency to involve as much diagnostic approaches as possible. EWGSOP also recommended three conceptual stages of sarcopenia: "Presarcopenia" (low muscle mass alone), "Sarcopenia" (low muscle mass and either low muscle strength or low physical performance) and "Severe Sarcopenia" (low muscle mass, physical performance and muscle strength)[80]. In the next year, an international working group of geriatricians and scientists gathered to form the International Working Group on Sarcopenia (IWGS) [82], presenting a consensus definition (of sarcopenia) as age-associated loss of both skeletal muscle mass and function, as well as suggesting that the presence of lower physical performance (gait speed) should be considered for quantitative measurement of body composition by DXA. IWGS additionally provided defined diagnostic cut-off values. Asian experts on sarcopenia came with their consensus report on 2014 (AWGS), suggesting a similar diagnostic algorithm to the European reference (EWGSOP) ("decline of skeletal muscle plus low muscle strength and / or physical performance") [84], yet with a considerably narrower range of diagnostic alternatives and specific recommended cut-off values to be used for diagnostic purposes in comparison to EWGSOP. In the same year (2014), the Foundations for the National Institutes of Health (FNIH) in their "Sarcopenia Project" [83] provided a recommended definition for sarcopenia including muscle mass and muscle strength, together with the respective recommended cut-off values to be used for diagnostic purposes.

Finally in 2016, sarcopenia was eventually recognized as a muscle disease upon the receiving of an ICD-10-MC code (International Classification of Diseases and Related Health Problems), M62.84 in particular [101]. This and other events led towards the need for revision and update of recommendations consistently with the current state of art. The first was the revised European consensus on definition and diagnosis of sarcopenia (EWGSOP2), which came with an interesting shift of attention from low muscle mass towards low muscle strength as the key characteristic of sarcopenia, suggested to be followed by the detection of low muscle quantity and quality to confirm diagnosis, and this way

emphasizing the poor physical performance as an indication for severe sarcopenia [49]. EWGSOP2 recommended sarcopenia as a muscle disease (muscle failure) that is rooted in adverse muscle changes that develop during the lifetime, while being associated with increased possibility for adverse outcomes such as falls, fractures, physical disability and mortality [49]. "Probable sarcopenia" was presented as a novel term instead of the previous "presarcopenia", and was recommended to be used in the cases of lower muscle strength alone, as an alternative conceptual stage towards the (already established) sarcopenia and severe sarcopenia. One very positive aspect of this consensus was the offering of clear cut-off points that are specific for sarcopenia and its conceptual stages. Upon the publication of EWGSOP2 recommendations, AWGS anticipated as well with their updated consensus criteria for definition and diagnosis of sarcopenia (AWGS2). This revised version [85] was characterized by three important aspects: 1) muscle mass remained the key characteristic whereas in addition with low muscle strength, and / or low physical performance fulfills diagnostic criteria of sarcopenia, or altogether (low muscle mass, strength and function) being classified as "severe sarcopenia", 2) the term "possible sarcopenia" was introduced as the reduced muscle strength and / or physical performance, 3) diagnostic cut-off points remained mostly intact from the initial suggested consensus report (AWGS).

All these consensus groups together with their outcomes are important in the process of making and establishing sarcopenia as an age-related phenomenon, geriatric syndrome and / or muscle disease. However, the differences between each other remain to date, further complicating things particularly for the population comparison data and worldwide prevalence. To this day, the initial EWGSOP [80] and the revised EWGSOP2 [49] consensus definition on sarcopenia, endorsed by both the initial AWGS [84] and the revised AWGS2 [85] present the most highlighted and referred definition and diagnostic guidelines worldwide. Nevertheless, population specific cut-off values as described by Baumgartner and colleagues [86] are suggested to be used as well, as a mean of ensuring outcomes as appropriate and precise as possible in accordance to the specific characteristic of the population.

1.2.1 Types of sarcopenia

With the development of sarcopenia from the initial concept towards what is now known, the need to further divide it in smaller categories was imposed. There are different factors affecting the muscle quantity and quality, and consequently the cause of sarcopenia, allowing sarcopenia to be classified in two main categories [80]:

- Primary – age-related sarcopenia with no other specific cause identified;
- Secondary – sarcopenia caused by other factors than (or in addition to) ageing, such as systematic disease (e.g. those invoking malignancy or organ failure), physical inactivity or malnourishment.

This suggested classification from the initial EWGSOP was made for operational and diagnostic purposes, but it should be kept in mind that mixed sarcopenic etiology is a phenomenon that is often encountered in practice. To address this issue and facilitate the further diagnostic steps of this process, the EWGSOP2 [49] provides a novel division of sarcopenia into two subcategories:

- Acute sarcopenia – the cases lasting for less than six months and that are usually related to any acute illness, injury or condition;

- Chronic sarcopenia – the cases lasting for six months and more, characteristic for their potential association to different chronic and progressive conditions, as well as presenting a higher risk of mortality.

Sarcopenic obesity is another distinct condition, characterized with the developing of sarcopenia simultaneously with an increase in adiposity [102]. This proposed concept and its differences to others (concepts) as well as its particular consequences are still being investigated while generating increasing attention within the scientific and clinical communities.

1.2.2 Genetic impact in Sarcopenia and its components

Variations in human muscle phenotypes may be attributed to different environmental or genetic factors, and / or the mutual interactions between environment and genes [103-105]. To date, studies have evidenced a substantial genetic influence on muscle strength with an estimated heritability of 30 – 65% [106-108], lean body mass 52 – 80% [109, 110], muscle size (measuring CSA) 70 – 90% [111] and fat free mass 45 – 65% [112]. Nonetheless, evidence with respect to the particular genes and variations behind the heritability of skeletal muscle traits are currently inconclusive and to some extent even contradictory, whereas further investigation is needed to address this topic [104].

Muscle strength as one of the integral components of sarcopenia and the key characteristic as per the latest EWGSOP2 revised consensus [49], features a progressive decline with ageing. Handgrip strength together with chair stand test are the two suggested measurement techniques by EWGSOP2 [49] for estimating muscle strength, with handgrip strength also proposed as a biomarker for identifying older adults at risk for poor health status and a predictor for several morbidities and all-cause mortality [113]. A recent large-scale GWAS identified a range of variants associated with handgrip strength, out of which, genes involved in both structure and function of skeletal muscle fibers (actin gamma 1 (ACTG1)), neuronal maintenance and signal transduction (peroxisomal biogenesis factor 14 (PEX14), transforming growth factor alpha (TGFA), synaptotagmin 1 (SYT1)), or monogenic syndromes with involvement of psychomotor impairment (PEX14, leucine rich pentatricopeptide repeat containing (LRPPRC) and lysine acetyltransferase 8 (KAT8) regulatory NSL complex subunit 1 (KANSL1)) [114]. Total lean body mass is another component of sarcopenia whose variations due to several genetic influences have been analyzed [115, 116], with a notable GWAS identifying eight novel single nucleotide polymorphisms (SNPs) in/near hydroxysteroid 17-beta dehydrogenase 11 (HSD17B11), versican (VCAN), ADAMTS like 3 (ADAMTSL3), insulin receptor substrate 1 (IRS1), and FTO alpha-ketoglutarate dependent dioxygenase (FTO) for total lean body mass [117]. Another interesting study identifying the heritability of lean soft tissue mass and muscle CSA, suggested the potential of genetic influence to differ as a function of sex and age [103].

A comprehensive systematic review conducted by Pratt and colleagues [104] identified the key genetic associations that are believed to be related with ageing muscle, by emphasizing the particular supporting evidence for alpha actinin 3 (ACTN3), angiotensin I converting enzyme (ACE) and vitamin D receptor (VDR) genes, followed by other genes such as insulin like growth factor 1/ insulin like growth factor binding protein 3 (IGF1/IGFBP3), tumour necrosis factor alpha-like (TNFα), apolipoprotein E (APOE), ciliary neurotrophic factor/receptor (CNTF/R) and uncoupling protein 2/3 (UCP2/3) with significant association demonstrated in two or more studies. Nonetheless, further ongoing

studies are trying to identify other candidate targets that could be potentially associated to the muscle phenotypes and sarcopenia related components [118].

1.2.3 ACTN3

The sarcomere, which encompasses the region flanked by two Z-lines and containing a M brand in its center, presents the contractile apparatus of skeletal muscles. It is formed by the bipolar myosin filaments that are interpolated to the polar actin filaments, and shorten the sarcomere by sliding of the later and pulling the myosin filaments [119]. ACTN3 represents the responsible gene for encoding of the sarcomeric protein α-actinin-3 as an integral component that is localized within the contractile apparatus at the Z-line (Figure 5), which is particularly characterized for its expression mainly in a subset of type II muscle fibers [120, 121].

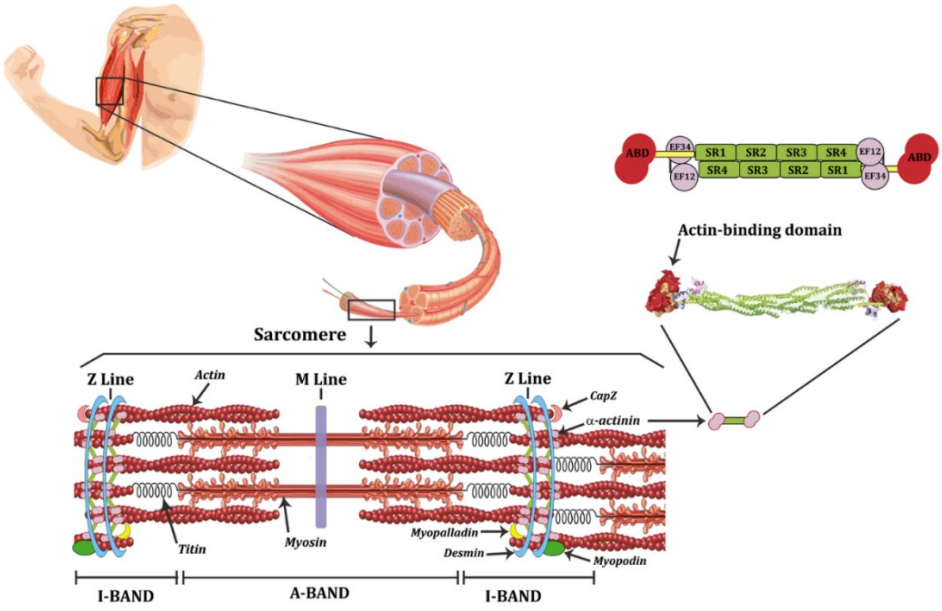

Figure 5: α-actinin localization in skeletal muscle. With permissions from Ribeiro EA et al. [122] and Elsevier, as well as the adapted version by Del Coso J et al. [123] and Springer Nature

It has been shown that α-actinin-3 is absent in certain groups of individuals within the human populations, whereas the homozygosity for a premature stop codon (named R577X - rs1815739) accounts for most cases of α-actinin-3 deficiency in type II muscle fibers [124]. This particular X-allele (characterized by α-actinin-3 deficiency) has been suggested to have been gone under a positive selection in various human populations, due to its potential effects on skeletal muscle metabolism, notably association with muscle strength, muscle mass and fast-twitch muscle fiber diameter [125]. The importance of this potential association lays upon the possibility it provides on the early identification of components from age-related diseases or geriatric conditions such as sarcopenia. Thus, studies have been investigating these suggestions and evidenced potential influence that ACTN3 genotypes might have in muscle strength [126-130], mass [126, 131-133], power [126, 134] or functional performance [135-137] amongst older subjects. In this context, a set of two interesting studies from the team led by Zempo and colleagues [131, 138]

evidenced a significantly lower thigh CSA amongst XX homozygotes, a phenomenon that was observed only within an older group in comparison to a younger group. Furthermore, it was also suggested that older physically active people with ACTN3 RR genotype might have a bigger incident disability protection [139], whereas other studies evidenced that those with ACTN3 XX genotype might be associated with age and / or sex-specific declines in physical performance [135] and a 33% (10-61%) elevated risk of falling [140]. Moreover, ACTN3 genotype has been shown to potentially influence gait speed as well, as a response to high-speed power training in particular [141]. Eventually, Cho and colleagues [133] found out a significant association of sarcopenia prevalence with XX genotype. Notwithstanding, there were several other studies not proving these claims [132, 142-144], adding further contradiction within and imposing the need for further investigations.

The ACTN3 577X allele frequency distribution varies between different regions worldwide, with the lowest in Africa (0.093), followed by Middle East (0.392), Europe (0.443), Central and South Asia (0.502), East Asia (0.477), and the highest in Oceania (0.495) and Americas (0.764) [145]. This difference is observed in specific populations as well, from as low as 0.03, 0.08, 0.11 in Africa (Mbuti Pygmies, Yoruba and Bantu in South Africa, respectively), towards 0.35, 0.39, 0.49 in Europe (Finnish, Spanish and British, respectively), 0.41, 0.49, 0.5 in Central and South Asia (Han Chinese, Japanese and Mongolia, respectively), to as high as 0.6, 0.76, 0.89 in the native American populations (Cabecar, Tepehuano and Aymara, respectively) [145]. Likewise, another study by Friedlander and colleagues [146] emphasized the differences in ACTN3 XX genotype distribution throughout the world, ranging from as low as 0% in Nigeria (Yoruba), 1% in Kenya and Bantu, towards 16.9% in China, 18% in Israel, 18.3% in Greece, 19.9% in Spain, 21.6% in Italy and 25% in Sweden. Friedlander et al. [146] even suggested that ACTN3 577XX genotype has evolved in association to the environmental conditions that are related to the global latitudinal gradient.

2 Research gaps, aims and hypothesis

To outline the previous chapters and the current state of the art within the complex relationship of ageing and skeletal muscle, it is important to firstly understand the multi-dimensional context of ageing and the multi-directional consequences ageing exerts on it. Age-related loss of muscle strength, mass and performance (known as sarcopenia) is observed as a worldwide emerging concern affecting all populations, though attracting higher attention within the developed countries with high-incomes and predictions of high life expectancy [147]. In contrary, many LMIC's often prioritize other direct life-threatening and emergency health issues within their healthcare systems [147], paving the path for a scarcity of data on ageing within their respective populations. Kosovo as a developing European country with the lowest life expectancy within the continent represents a typical such example. In this context, many international working groups have been trying to find the better and more applicable diagnostic cut-off criteria for sarcopenia ant its conceptual stages. EWGSOP and AWGS even revised their initial consensus definitions and suggested diagnostic criteria (EWGSOP2 [49] and AWGS2 [85], respectively) in pursue of a more versatile diagnostic pathway. The additional usage of population-specific diagnostic criteria as a potentially more accurate instrument has also been suggested [49, 95, 148, 149]. Nevertheless, a general worldwide consensus is still missing. In addition to that, the multi-factorial impact on age-related decline of muscle traits, including the level of exposure to various environmental and lifestyle factors presents a burden for defining shares within the final outcomes. The involvement of genetic make-up of the individual, the multifactorial genetic cause and the combinations of numerous genetic variants with external influencing covariates complicate things even further. To date, studies have been investigating the potential impact of different genetic variations on muscle phenotypes. Some promising directions were observed [114, 129, 138], though outcomes remain inconclusive. ACTN3 emerged as distinguished and promising gene in this aspect, suggested for a positive selection in humans [125]. However, the population differences in genetic background with the ACTN3 577X allele frequency distribution varying in between different world regions [145] makes it difficult to clearly understand and draw conclusions regarding the exact influence it might exert. This issue gets further obscured with the general lack of studies performed in older adults particularly, or comprehensive studies including altogether all the phenotypes related to sarcopenia (muscle strength, power, mass and physical function) and analyzing their potential association with different ACTN3 genotypes (RR, RX or XX) or dominant (RR + RX) and recessive (RX + XX) models with the matched genotypes (XX and RR, respectively).

Upon these foundations, the contribution of this thesis lays on the potential to establish consistency within the assessment techniques of muscle mass, isometric muscle strength, physical performance and isokinetic dynamometry, as well as providing population specific cut-off points for the diagnosis of sarcopenia and its conceptual stages. Furthermore, it intends to not only cross-sectionally describe the prevalence of sarcopenia in a convenient sample of Kosovan adults aged 60 years and above based on the different recommended algorithms and diagnostic cut-off points (initial EWGSOP [80], revised EWGSOP2 [49] and Kosovo-specific), but also to compare the different algorithms and try to differ the more versatile ones to follow. Additionally, investigating the influence of environmental and genetic covariates on the outcomes intends to bring light on this complex situation. As emphasized previously, this

study is performed in community-dwelling adults of a developing upper middle-income European country such as Kosovo. Even though the major diagnostic consensus criteria (notably the initial EWGSOP and the revised EWGSOP2) are ideated, designed and suggested to be implemented for European populations (including the Western Balkan region) which are predominantly based on Caucasian populations [150] (similar to Kosovo's case), it is also important to better understand what could be the impact of using the population specific diagnostic cut points on the final outcomes. Furthermore, the consequences of shifting focus from lower muscle mass (EWGSOP) to lower muscle strength / function (EWGSOP2) either through using the EWGSOP(2) recommended cut off points or through the population specific ones will shed light on the matter and help detect the more accurate diagnostic pathways to follow. In line with the above stated research gaps, this set of studies aims to:

1) analyze the test-retest reliability of muscle strength, body composition parameters, functional performance tests and peak torque assessments in younger (aged 18 - 35 years) and older (above 60 years) community dwelling adults, as well as provide sex-specific diagnostic cut-off points for the studied population;
2) determine sarcopenia and its conceptual stages (pre - / probable -, severe -) as defined by the initial EWGSOP and the revised EWGSOP2 consensus criteria, as well as the population specific cut-off points (using EWGSOP1 and EWGSOP2 algorithms) in the ageing Kosovo adults (aged 60 years and above);
    a. investigate if sarcopenia presents an issue of concern within the developing world, from the perspective of Kosovo;
    b. analyze potential differences between the different algorithms and diagnostic cut-off points;
    c. determine the more robust and versatile diagnostic approach to follow;
    d. analyze the influence of various covariates (including socio-demographic characteristics - age, biological sex, living environments, education, marital status, financial status and self-perceived poverty level, smoking status, alcohol consumption, self-perceived health condition, self-reported comorbidities, polypharmacy and nutritional state) on sarcopenia status;
3) determine the ACTN3 genotype distributions (RR, RX and XX) amongst Kosovo mature adults (above 40 years) in total and in sex specifically, as well as analyze the potential association between these genetic variants (of ACTN3 gene) with muscle strength, isokinetic peak torque, mass and function.

Based on the these emerging study aims, the following hypotheses were evinced:

- the measured parameters of muscle mass and function provide acceptable test-retest reliability with interclass correlation coefficient (ICC) > 0.70, in field and laboratory tests, in both young and older adults;
- sex specific cut-off points (valid for the Kosovan population) for functional performance, muscle strength and peak torque can be derived from the young population (2 SD below the respective mean of a young population [86]); these outcomes can be used as future reference data in both research and clinical settings;
- sarcopenia (and its conceptual stages) is higher in older ages as well and it increases with aging;

- the occurrence of sarcopenia (and its conceptual stages) in Kosovo population might differ due to the lower expected life expectancy at birth and lower percentages of older populations within this country (those that are more prone towards the development of sarcopenia);
- differences between diagnostic cut-off points and the recommended algorithms are observable;
- secondary endpoints such as sex, education level, marital status, environment of living, financial status and self-perceived poverty level, self-perceived health condition, co-morbidities, smoking status, alcohol consumption, nutritional status and medication usage might affect the occurrence of sarcopenia and its conceptual stages;
- differences in muscle strength, isokinetic peak torque, mass and physical performance between the three groups of ACTN3 genetic variants (RR, RX and XX) in Kosovo mature adults and sexes specifically are detectable.

3 Studies included in this theses

The organization of studies together with subjects' participation process is described in Figure 6. Initially, a total 57 young (aged 18-35 years) and 61 older (60 years of age and above) participants were included, which underwent the exact same assessments in two different time periods. Once establishing the reliability of assessment techniques, 308 other participants aged 40 years and above underwent the assessments. Amongst, those aged 60 years and above (n = 240) were included in the sarcopenia study, out of whom those willing to provide DNA saliva samples in association with others aged between 40 – 60 years that were willing to provide saliva samples were included in the genetic study (n = 299).

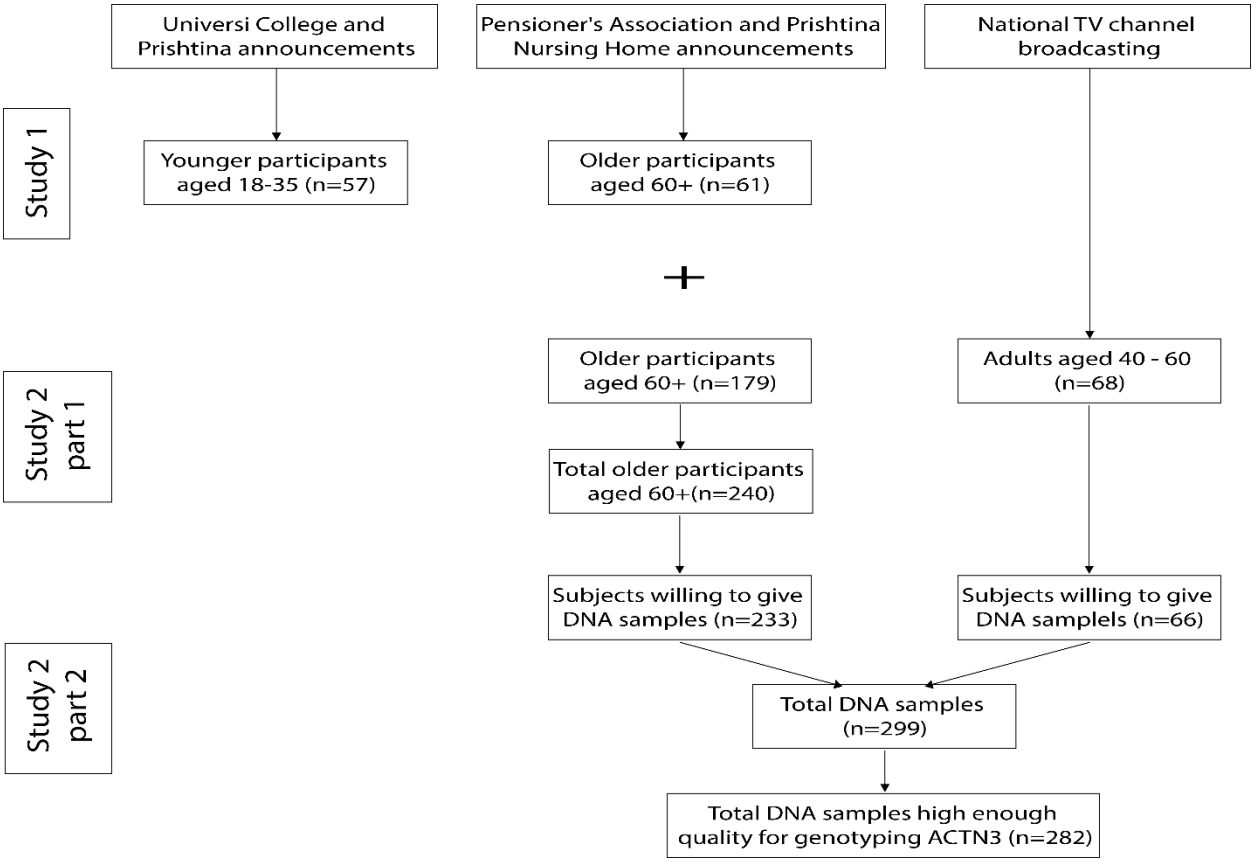

Figure 6: Flow chart of study work

Study 1 (test-retest reliability study) as described in details within the published paper [151] was organized as a cross-sectional study, involving a group of younger and another group of older community dwelling adults, participating in two separate identical test sessions (7 - 97 days in between; median (25^{th} - 75^{th} percentile): 14 (13 – 21) days), following uniformly organized order of tests by the 3 members of research team, each of whom doing most of the same tests. Assessments were performed from August 2016 to January 2017 in the Sports Medicine Laboratory and the sports hall at the "Universi College" in Prishtina (Kosovo), with the inclusion criteria assessed at the entrance by 2 health

professionals. The data collection process began in the morning after an overnight fast, starting with personal information (including self-perceived health status, co-morbidities and socio-economic status [152]), continuing with anthropometric assessments (following the International Standards for Anthropometric Assessment [153]), body composition analysis (using BIA) and concluding with isometric and isokinetic measurements, together with the functional performance assessments.

SMM, skeletal muscle index (SMI), ASMM and appendicular skeletal muscle index (ASMI) were obtained to determine the muscle quantity / mass, whereas isometric handgrip strength, isokinetic peak torque and average power knee extension and flexion (at 60^0/s and 120^0/s) were used to estimate the reliability of muscle strength and power. Arm curl, chair-stand, 6-minute walk and chair sit and reach tests from the senior fitness test manual [154, 155], together with timed-up and go and gait speed (both normal and fast pace) were obtained to estimate the reliability of functional performance. The values obtained in younger subjects were used for calculating the cut-off points in sarcopenia-related components for older subjects following the Baumgartner and colleagues [86] guidelines of 2 SD below the respective mean of a young population (See 4.2.1). In total, 57 healthy younger participants (aged 18 - 35 years; 26 females and 31 males) as informed via advertisements throughout the city, and 61 older community dwelling participants (aged 60 years and above; 39 females and 22 males) from the Kosovo Pensioners' Association participated. Previous studies have already shown that 50 persons are sufficient for attesting the quality criteria within such assessments [156-158]. As mentioned above, the 2 healthcare professionals assessed the inclusion criteria at the entrance through defining if participant belonged to the age group(s), sex, were an inhabitant within the region of Prishtina and had a Kosovo nationality, had a lack of any underlying health condition with potential to significantly impair the outcomes by preventing or limiting the conduction of assessments (e.g. cardiac implantable electronic devices) as screened by the physical activity readiness questionnaire (PAR-Q) [159].

Study participation was on voluntary basis, whereas written informed consent for participation and publication of data was obtained by everyone prior to the inclusion in the study. Each participant was well informed with the participation conditions while being aware that they could drop out of the study at any time if seen appropriate by them, even though being strongly recommended against. Study itself was approved by the Ethical-Professional Committee of the University Clinical Center of Kosovo (no 1246/15.09.2016), and was performed in accordance with the ethical standards of the revised version of the Declaration of Helsinki [160].

The 1st part of study 2 (sarcopenia study) included only community dwelling older adults aged 60 years and above that were living in the biggest of seven regions in Kosovo, region of Prishtina (two neighboring municipalities within the region, Prishtina and Fushë Kosova). Measurements for this study, together with the overall organization of assessment process, were performed uniformly to the 1st study, at the same laboratory, using the same facilities, following the exact same inclusion criteria (for the older participants), testing order and research team (Figure 6). Older participants' data that were collected from the initial testing session from the 1st study, were also included (total 61 subjects, excluding the retesting phase).

The calculated cut-off points for sarcopenia-related components that derived from the 2 SD below the respective mean of a young population [86], were used for diagnostic purposes besides the initial EWGSOP [80] and revised EWGSOP2 recommended diagnostic approaches [49] within this study and consequently paper 2[161].

The third part of the thesis (genetic study) included a total of 308 subjects (160 females and 148 males) aged 40 years and above, including the data from 240 participants (113 females and 127 males) aged 60 years and above from the sarcopenia and its conceptual stages assessment study. This number represented an estimated 0.8% of the total eligible sample from the selected age group [76]. If required, subjects could bring their partners in the study, mainly as an additional precaution for ensuring heterogeneity from the genetic point of view, as well as avoiding potential inbreed amongst the participants (since the same participants were recruited upon their permission in the genetic study as well). Data collected from the rest of the subjects from the initial 308 subjects (the age group in between 40 – 60 years) were preserved for being calculated and analyzed in the genetic study [162]. The recruiting process encompassed the same steps and procedures as mentioned previously in the 1st study for the older subjects [151], including the added promotion in national TV channels broadcasting and advertisements in the Prishtina nursing home, while the inclusion and exclusion criteria was also followed as set at the beginning of the 1st study.

Figure 7: Promoting the study in national TV broadcast [163]

Genetic study [162] was organized in two separate integral parts (Figure 6). The first part encompassed the previously performed measurements and collected data (from test-retest and sarcopenia studies, including the 68 adults aged 40 - 60 years that were recruited and measured, but not included in the paper 2) on personal information, anthropometric assessments, body composition analysis, isometric and isokinetic measurements, and the functional performance assessments. The second part involved the collection of saliva samples from participants willing to give it in order for isolating the DNA. This process was conducted by trained reviewers in the Laboratory (if subjects were willing to come personally for a second visit), in the Prishtina and Fushë Kosova branches of the Kosovo Pensioners' Association (in the nursing room) or even at home if a subject was willing, but for an understandable reason (i.e. acute sickness or other understandable personal issue) could not participate.

DNA material for the genetic study [162] was obtained from 299 participants out of the total 308 people aged 40 and above with reported data on self-perceived health state, socio-economic status, behavioural characteristics, anthropometrics and muscle traits. For inclusion in this study, subjects were required to have been already enrolled in the previous phases (test-retest reliability and sarcopenia study, excluding younger participants aged 18 - 35 from study 1 as a necessity to have comparable data within the studied genetic variants), meaning they wold have had fulfilled the previously set inclusion criteria prior to their initial inclusion, and to be willing to provide their personal DNA for the study. Upon the collection of all samples, they were then sent to the Laboratory for Molecular Exercise Physiology at the Centre for Sport Science and University Sports, University of Vienna. Further analyses were performed in the collected samples, starting with the extraction of genomic DNA following standardised procedures [164], and completing with the genotyping process (as described further in the published paper [162]).

4 Methods

The three parts encompassing this thesis involved very similar assessment techniques, starting with the collection of personal information from the two healthcare professionals (as described previously in 4.1.1. and 4.1.2.) through the WHO STEPS instrument (assessing the self-perceived health status, co-morbidities and socio-economic status) [152], mini nutritional assessment questionnaire (MNA) - long form (assessing the self-perceived nutritional status) [165], brief medication questionnaire 1 (BMQ1, assessing the medication usage) [166], after successfully fulfilling the set inclusion criteria (as described specifically for each study). Upon the completion of data collection, the assessment continued with anthropometrics (starting with height and weight), body composition analysis (SMM, SMI, ASMM, ASMI, whole body fat mass and percentage), a short 30 minutes break with a standardized light meal, followed with isometric handgrip strength, isokinetic measurements (isokinetic peak torque and average power of knee extension and flexion at 60^0/s and 120^0/s) and concluded with the functional performance assessments (physical performance tests from as described in 4.1.1.).

4.1 Anthropometric assessment and body composition analysis

As described previously in section 4.1.1. and in the published manuscripts [151, 162], anthropometric assessments were performed following standardized international procedures [153], with height measured by a portable stadiometer with a precision of 5 mm using the stretch stature method, while the participants were barefoot and wearing light indoor clothing, (DT05L, Kinlee, Zhongshan Jinli Electronic Weighing Equipment Co. Ltd, Guangdong, China).

Segmental multifrequency BIA (operating frequencies ranging from 1, 5, 50, 250 to 500 kilo Hertz (kHz) and 1 mega Hertz (mHz)) while following the manufacturers calculation algorithm [167, 168] (Inbody 770 device, Biospace Co., Ltd., Seoul, Korea) was used to assess the whole body mass and body composition, as a recommended technique to be used in research [49] that is described to present a high correlation degree with DXA regarding the muscle mass assessments [169, 170]. The process itself was plain and simple, with participants standing vertically with the barefoot soles above the tactile foot electrodes, while holding each of the hand electrodes with the respective hand in a relaxed and non-tensed condition.

4.2 Isometric and isokinetic dynamometry

Isometric dynamometry was assessed by a portable, hand-size adaptable and hydraulic dynamometer (JAMAR, Patterson Medical, Warrenville, IL, USA) on the self-reported dominant hand, as suggested previously [171, 172]. Participants were sat in a chair with an unsupported elbow, bent at the 90^0. After a size adaptation and brief demonstration of the process by the test administrator, the sat participant with an unsupported elbow that was bent at the 90^0 were instructed to perform two trials of squeezing the dynamometer for maximal isometric contraction within 4 - 5 seconds (s), with a one minute (min) rest in between trials (the better results recorded for further analyses).

For assessing the isokinetic peak torque and average power of knee extension (quadriceps femoris) and flexion (hamstrings muscle: semitendinosus, semimembranosus and biceps femoris) at 60^0/s and 120^0/s, isokinetic dynamometry was used [173] (Biodex System 4 Pro, Biodex Medical Systems, Inc., Shirley, NY, USA). The assessment was made as suggested previously [172, 174] unilaterally on the self-reported dominant side, with participants holding the sideway handles while being fixed in a sitting position on the device's chair, with hips flexed at 90^0, strapped torso, pelvic, thigh and calve for fixation and for avoiding any other potential movement. After a general warming up, and a tryout for familiarization with the method, absolute and relative peak torque at 60^0/s and 120^0/s were measured. Administrator encouraged participants for achieving the maximum effort during the whole assessment process.

4.3 Functional performance tests

All testing procedures and their specificities of physical functioning tests (as described in the previous sectors and the publications as well [151, 162]) were taught and explained carefully to each participant by the test administrators before the beginning of each test. Participants were instructed to perform a repetition practice in order of familiarizing with the technique before the registered testing. Noteworthy, (as described in 4.2.2.) upon the completion of inform consent every subject was well aware that participation in the tests was on voluntary bases and dropping out without the request of the test administrator is possible (for any reasonable explanation or if just found appropriate by the participant), though not recommended.

4.3.1 Arm curl test

Arm curl test performance presents the assessment for estimating the upper body strength (endurance), based on the fully repeated elbow flexion and extension within 30 seconds of time, as described previously by Jones and Rikli [154, 155]. The test was performed on the self-reported dominant hand of the seated participant's supinated arm while lifting a dumbbell (5 pounds for woman and 8 pounds for men). As a rule in this and all similar tests, the test administrator stood next to the participant signaling the beginning and completion of time, counting the successful repetitions on a stop watch, and deciding positively in the last attempt if the subject mastered more than 50% of the motion in time [154, 155].

4.3.2 30 second chair stand test

The 30 seconds (s) chair stand performance test is conducted to assess the lower body strength (endurance) based on the fully repeated stand up and sit down cycles within 30 seconds of time, from an armless chair of 46 centimeter (cm) high that was placed against a wall . The test administrator instructed participants to keep the arms crossed over the chest, while standing next to him / her holding a stop watch, signaling the beginning and ending of the test, as well as counting the successfully completed repetitions. The positive decision on the last attempt was made if the subject managed to master more than 50% of the range of movement prior to timeline [151, 154, 155, 162, 172].

4.3.3 6 minute walking test

The 6 minute walking test (6MWT) presents a measurement for assessing aerobic endurance of the tested individual, by assessing the total distance that can be covered by a person within 6 min of time [154]. Thus, participants were instructed to walk alone as fast as possible within a 30 m shuttle truck (signaled with cones) during the time limit, with the test administrator on his / her side holding and controlling a stopwatch, signaling the initiation time, the time remained after each minute, the last 15 s and the finish sign. Even though encouraged to be faster, participants were allowed to accommodate the speed to their capacities (accelerate, decelerate or even rest if seen appropriate). Upon the completion of 6 min, the subject stop and allowed the test administrator to register the covered distance in m as a test outcome [172, 175].

4.3.4 Chair sit and reach test

Chair sit and reach presents another assessment method from the senior fitness test manual [154, 155]. This assessment technique was used to evaluate the flexibility of lower body of the participant's siting on a chairs front, with an extended self-reported dominant leg and hands above each other reaching towards toes of that same leg. The test administrator measures the distance in cm between the tips of the fingers and toes during a 2 s fixed position required by the participant.

4.3.5 Timed up-and-go (TUG) test

TUG was used as suggested by Podsiadlo and colleagues [176] in order to assess agility / dynamic balance of participants, based on the time needed to successfully complete the task of standing up from an armless chair, walking fast forward though a straight line for 3 m, turn back and return to the seated position as fast as possible without running. Administrator (as in other cases) stood next signaling the initiation and carefully followed the executing process. However, participants were instructed to perform a repetition practice in order of familiarizing with the technique before the registered testing [176]

4.3.6 Gait speed (slow and fast pace)

Gait speed presents one of the key components of performance in the diagnostic process of sarcopenia. For performing the test, participants were instructed to walk without stopping a constant normal pace of their usual gait speed in a 10 m course, with time registered only for the 6 m in between, whereas the first 2 m were used for acceleration and the last 2 m for deceleration [171, 175]. As explained in previous sections and likewise to other tests, the administrator stood next to participants signaling the test initiation, following the performance while recording manually on a stop watch the time needed for completion the task (expressed in m/s). Two trials were performed with a short resting pause in between, and the better results were used. This same procedure was followed for gait speed fast mode, with participants instructed to walk with their fast pace but without running [171, 175].

4.4 Muscle mass (quality)

BIA was used to assess body composition as described previously (5.1). The outcome variables included whole body SMM and fat mass, calculated while using the manufacturer's algorithm [167, 168]. ASMM was calculated as the total amount of arms and legs lean mass altogether.

In the sarcopenia study, a previously suggested multivariate regression model was used as a mean to predict the DXA estimation of muscle mass measured by BIA method [169, 177]. Body weight, SMM and ASMM divided by height squared (expressed as kg/m2) were used to determine body mass index (BMI), SMI, and ASMI [94].

4.5 Genetic analysis

Genetic analyses were performed to the participants that had previously participated in the sarcopenia study and for whom data on self-perceived health status, co-morbidities, socio-economic status, anthropometrics, body composition, isometric, isokinetic and the functional performance were already collected. Within these subjects, those willing to participate in the genetic study and thus provide their saliva samples were included. As described in 4.3.1. the collected tubes, which contained 2 milliliters (ml) of saliva mixed with 2 ml of stabilization buffer, were stored at -20^0 C environment and in less than 3 months' time were sent to the Laboratory of Molecular Exercise Physiology at the Centre for Sport Sciences and University Sports in Vienna, Austria.

GeneFiX Saliva-Prep DNA Kit (Isohelix, Kent, United Kingdom) was used for genomic DNA extraction, NanoDrop spectrophotometer (Peqlab, Erlangen, Germany) assessed the quality and quantity of DNA, whereas Real time PCR (QuantStudio 7 Flex real-time PCR System, ThermoFisher Scientific, Vienna, Austria) and a commercially available TaqMan™ SNP genotyping assay enabled the detection of ACTN3 R577X polymorphism (rs1815739, C_590093_1, ThermoFisher Scientific, Vienna, Austria).

The particular steps, protocols and procedures followed during this study are extensively described in the genetic paper [162].

4.6 Statistical analyses

Statistical analysis were performed using IBM SPSS statistical package version 26 for Windows (SPSS Inc, Chicago, IL). The specific statistical approaches and methods are extensively detailed in each manuscript.

5 Publications

Published papers from this cumulative dissertation thesis are presented within this chapter.

5.1 Summary of results

The major endpoint of the 1st study was to establish the test-retest reliability of laboratory and field based functional performance tests, strength and peak torque assessments, as well as body composition in younger and older adults in a Kosovo. These findings are published in the paper 1. The other important milestone achieved in this study was the calculation of population specific diagnostic cut-off points for sarcopenia related components, derived from the young reference population.

The core of 2nd paper (sarcopenia study) lays upon the exploring of the occurrence of sarcopenia as a generalized and progressive skeletal muscle disorder [49] in a developing upper-middle income European country like Kosovo. Furthermore, sarcopenia and its conceptual stages were evaluated using different suggested algorithms (the initial EWGSOP and the revised EWGSOP2), as well as different diagnostic cut-off points (as recommended by EWGSOP1 and EWGSOP2, including the population specific ones as defined in the study 1). Finally, the impact of health and socio-economic parameters to predict sarcopenia and its conceptual stages were assessed, allowing to detect the potential effects of not just different approaches and diagnostic criteria, but also health and lifestyle related covariates in determining the presence of age-related decline in muscle strength, mass and performance in Kosovo.

The final endpoint of this dissertation was arraigned in the genetic study, where analysis of genetic influence on muscle phenotypes and its relation with age-related decline and sarcopenia related components were investigated. ACTN3 gene and its three different genotypes (RR, RX and XX) have already been emphasized as a suggested target for potential association [104], whereas this study brought further light on this matter.

Because of the specificities that the published article with the data from the 1st study provides [151] ("Data in Brief" is a journal providing articles only directed towards data description, while avoiding their interpretation), summary from the main results in addition to the personal contribution (which is described in each study) on that particular article are detailed in the following sections. Dataset from the 1st study was published online [178] and is available at the Mendeley data.

5.1.1 Publication 1: Test-retest reliability data of functional performance, strength, peak torque and body composition assessments in two different age groups of Kosovan adults

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Status:

Published by *Data in Brief* on March 23, 2021;36:106988. https://doi.org/10.1016/j.dib.2021.106988

Personal contribution

- Conceptualization
- Methodology
- Investigation
- Data curation
- Writing – Original draft preparation

Data in Brief 36 (2021) 106988

Contents lists available at ScienceDirect

Data in Brief

journal homepage: www.elsevier.com/locate/dib

Data Article

Test-retest reliability data of functional performance, strength, peak torque and body composition assessments in two different age groups of Kosovan adults

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ARTICLE INFO

Article history:
Received 17 January 2021
Revised 8 March 2021
Accepted 17 March 2021
Available online 23 March 2021

Keywords:
Test-retest reliability
Physical fitness
Body composition
Age-related muscle loss

ABSTRACT

This article reports test-retest reliability data of laboratory- and field-based performance tests as well as body composition analyses of younger and older Kosovan adults. In total, 57 healthy young (18–35 years) and 61 older (>60 years) participants took part in two identical test sessions, with a median [25th – 75th percentile] of 14 [13–21] days in between. Functional performance tests included 30-s chair stand test (CST), 30-s arm curl test (ACT), six-minutes walking test (6MWT), sit and reach test, timed up and go test (TUG), as well as the assessment of gait speed (GS) at normal and fast pace. Isometric handgrip strength (HGS) was used to estimate strength of the dominant hand. Isokinetic peak torque (PT) and average power (AvgP) for knee extension and flexion were determined at velocities of 60°/s and 120°/s. Body composition assessments included body fat

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https://doi.org/10.1016/j.dib.2021.106988

percentage, skeletal muscle mass (SMM) and index (SMI) as well as appendicular skeletal muscle mass (ASMM) and index. Secondary endpoints included self-perceived health status and potential co-morbidities. All performance test outcomes as well as body fat percentage, SMM, ASMM, and self-perceived health were significantly better in young as compared to older participants ($p < 0.001$). Improvements from test to retest were observed for CST ($p < 0.001$), $PT_{flexion}$ (60°/s: $p = 0.001$, 120°/s: $p = 0.041$), $AvgP_{flexion}$ (60°/s: $p < 0.001$, 120°/s: $p < 0.001$), $AvgP_{extension}$ (120°/s: $p = 0.050$), but also for SMM ($p = 0.021$) and SMI ($p = 0.021$). Only for CST and HGS a time x age group interaction was detected ($p < 0.05$). Acceptable reliability (ICC > 0.7) was observed for all parameters in both age groups, except for some of the measures from the isokinetic dynamometry, where ICCs were generally lower in older participants, but fell below 0.7 for $AvgP_{flexion}$ at 60°/s (ICC = 0.6) and at 120°/s (ICC = 0.67) as well as for $PT_{flexion}$ at 120°/s (ICC = 0.69). These data's importance lay upon their potential use in epidemiological studies observing muscle strength, peak torque, power, physical performance and body composition over various age groups, either in the same or similar populations, or for comparison to other populations.

Specifications Table

Subject	Sport Sciences, Therapy and Medicine
Specific subject area	Assessment of functional performance, muscle strength, muscle mass, body composition
Type of data	Table
How data were acquired	Stadiometer for height (DT05L, Kinlee, Zhongshan Jinli Electronic Weighing Equipment Co. Ltd, China); Segmental multifrequency bioelectrical impedance analyser for body mass and composition (Inbody 720, Biospace Co., Seoul, Korea); Biodex dynamometer for isokinetic peak torque knee extension and flexion (Biodex System 4 Pro-Isokinetic Dynamometer, Shirley, New York, USA); Handgrip dynamometry for isometric handgrip strength (JAMAR, Patterson Medical, USA); Track (10 m) and stop watch for gait speed (sports hall); Track (3 m), armless chair (46 cm seat height) and stop watch for timed up and go test (sports hall); Armless chair (46 cm seat height) and stop watch for 30-s chair stand test (sport hall); Dumbbell (5 pounds for woman and 8 pounds for men), armless chair (46 cm seat height) and stopwatch for 30-s arm curl test (sports hall); 30-meters shuttle track and stop watch (sports hall) for six-minutes walking test (sports hall); Measuring ruler and armless chair (46 cm seat height) for sit and reach test (sports hall).
Data format	Raw data
Parameters for data collection	Human test-retest study on physical performance and body composition parameters of young (18–35 years) and older (above 60 years) subjects living in the region of Prishtina, Kosovo

(*continued on next page*)

Description of data collection	Body composition data were collected in the morning after an overnight fast by the same research team at the Sports Medicine Laboratory at the "Universi College" in Bardhosh, Prishtina. After a break of 30 min and provision of a light standardized meal, isometric, isokinetic and functional performance tests were assessed in the sport hall of the same institution. Retest was performed in the same manner after a period of 19 ± 16 days.
Data source location	Institution: Universi College Kosovo City/Town/Region: Bardhosh, Prishtina, 10,000 Country: Kosovo Latitude and longitude for collected samples/data: 42.7228° and 21.1476°
Data accessibility	Mendeley Data Repository [1]

Value of the Data

- The data of this article are important for cross-sectional and longitudinal studies observing functional performance, strength, power and body composition assessments throughout the life span, where there is a need to choose tests with similar reliability in younger and older adults.
- Reliability data of laboratory and field tests for functional performance, strength and power as well as body composition allow researchers, clinicians, sport scientists, physiotherapists or other health experts to compare these methods with respect to their measurement accuracy.
- Population-specific data of physical performance in two different age groups and for both sexes are given. Thus, the data can be reused for calculation of population-specific reference ranges needed i.e. for sarcopenia diagnostics in the Kosovan/Balkans area.

1. Data Description

The present data focus on test-retest reliability of functional performance tests together with strength, power and body composition assessments in young and older Kosovan adults. The data set comprises various parameters relevant to assess age-related changes in physical performance. Those parameters have been suggested by the European Working Group in Sarcopenia for Older People (EWGSOP) in its initial and revised consensus statements to be used for the diagnosis and treatment of sarcopenia [2,3].

Age group and sex-specific baseline characteristics of the participants including anthropometric data as well as health status are provided Table 1. Test-retest reliability of various functional performance tests included in the senior fitness test manual [4], such as the 30-s chair stand test, 30-s arm curl test, the six-minutes walking test (6MWT), the sit and reach test, the timed up and go test as well as gait speed data are shown in Table 2. In Table 3, test-retest reliability of laboratory-based assessments of isokinetic peak torque as well as average power at two different velocities (60°/s and 120°/s) is shown for knee extension and knee flexion. Finally, Table 4 summarizes test-retest reliability of parameters derived from bioimpedance analyses such as body fat percentage, skeletal muscle mass (SMM), skeletal muscle index (SMI), appendicular skeletal muscle mass (ASMM) and appendicular skeletal muscle index (ASMI).

2. Experimental Design, Materials and Methods

2.1. Experimental design and subjects

Young participants were recruited via advertisements in the city of Prishtina (Kosovo). Older subjects were approached via the Kosovo Pensioners' Association. In total, 57 healthy younger

Table 1
Baseline characteristics of participants.

	Young Participants	Young Females	Young Males	Old Participants	Old Females	Old Males	*p-value*
Participants [number (%)]	**57**	26 (45.6%)	31 (54.4%)	**61**	39 (63.9%)	22 (36.1%)	
Age [years]	**22.6 ± 3.7**	23.6 ± 4.3	21.8 ± 2.9	**70.7 ± 6.1**	69.6 ± 5.7	72.7 ± 6.5	*<0.001*
Height [m]	**1.75 ± 0.10**	1.68 ± 0.08	1.81 ± 0.07	**1.64 ± 7.69**	1.60 ± 5.42	1.70 ± 7.60	*<0.001*
Body mass [kg]	**70.3 ± 14.0**	62.0 ± 11.4	77.2 ± 12.2	**80.8 ± 14.7**	79.6 ± 14.0	83.0 ± 15.9	*<0.001*
BMI [kg/m²]	**22.8 ± 3.3**	22.0 ± 3.4	23.5 ± 3.0	**30.1 ± 4.9**	30.8 ± 4.6	28.8 ± 5.1	*<0.001*
Body fat [%]	**19.5 ± 8.6**	23.8 ± 8.5	15.8 ± 6.9	**39.4 ± 8.2**	43.1 ± 5.3	32.5 ± 8.4	*<0.001*
SMM [kg]	**34.6 ± 7.8**	25.6 ± 5.8	36.7 ± 5.2	**26.5 ± 4.9**	24.2 ± 3.4	30.6 ± 4.5	*<0.001*
SMI [kg/m²]	**10.2 ± 1.6**	9.0 ± 1.4	11.2 ± 1.0	**9.8 ± 1.1**	9.4 ± 0.9	10.6 ± 1.0	*0.075*
ASMM [kg]	**23.9 ± 5.9**	19.3 ± 4.3	27.7 ± 4.1	**20.3 ± 3.9**	18.6 ± 2.9	23.5 ± 3.6	*<0.001*
ASMI [kg/m²]	**7.7 ± 1.2**	6.8 ± 1.0	8.4 ± 0.7	**7.5 ± 0.9**	7.2 ± 0.9	8.1 ± 0.8	*0.398*
Self-perception of health [good/not good], n (%)	**47/10 (82.5/17.5)**	23/3 (88.5/11.5)	24/7 (77.4/22.6)	**27/34 (44.3/55.7)**	19/20 (48.7/51.3)	8/14 (36.4/63.6)	*<0.001*
Chronic diseases, n (%)	**0 (0)**	0 (0)	0 (0)	**46 (75.4)**	29 (74.4)	17 (77.3)	*<0.001*
Cardiovascular disease, n (%)	**0 (0)**	0 (0)	0 (0)	**28 (45.9)**	19 (48.7)	9 (40.9)	*<0.001*
Diabetes, n (%)	**0 (0)**	0 (0)	0 (0)	**11 (18.0)**	9 (23.1)	2 (9.1)	*0.001*
Osteoporosis, n (%)	**0 (0)**	0 (0)	0 (0)	**7 (11.5)**	5 (12.8)	2 (9.1)	*0.008*
Degenerative rheumatic problems, n (%)	**0 (0)**	0 (0)	0 (0)	**11 (18.0)**	6 (15.4)	5 (22.7)	*0.003*

Abbreviations: BMI (Body Mass Index); SMM (Skeletal Muscle Mass); SMI (Skeletal Muscle Index); ASMM (Appendicular Skeletal Muscle Mass); ASMI (Appendicular Skeletal Muscle Index). Values are shown as mean ± standard deviation; p-values refer to differences between young and old participants (independent student's *t*-test for continuous variables and Chi-square test for categorical variables).

Table 2
Test-retest reliability of functional performance tests and isometric handgrip strength.

Functional performance test	Age group (years)	Test (Mean ± SD)	Retest (Mean ± SD)	Difference (Mean ± SD)	95% CI of Difference	Typical error (95% CI)	ICC (95% CI)	Time	Age group	Sex	Time × Age group	Time × Sex
30-s chair stand [rep]	18–35	23.8 ± 5.9	24.2 ± 5.7	0.5 ± 1.9	−0.05 → 0.96	1.34 (1.13–1.65)	0.95 (0.91–0.97)	**<0.001**	**<0.001**	**<0.001**	**0.007**	0.838
	60 +	11.7 ± 3.3	13.1 ± 3.0	**1.4 ± 1.6*** **	0.95 → 1.77	1.13 (0.96–1.37)	0.80 (0.37–0.92)					
30-s arm curl [rep]	18–35	27.7 ± 6.6	27.8 ± 6.5	0.1 ± 2.3	−0.53 → 0.67	1.60 (1.35–1.96)	0.94 (0.90–0.97)	0.065	**<0.001**	**<0.001**	0.157	0.628
	60 +	14.7 ± 2.7	15.3 ± 3.4	**0.6 ± 2.1****	0.02 → 1.10	1.49 (1.27–1.82)	0.76 (0.62–0.85)					
6MWT [m]	18–35	748.3 ± 102.7	750.3 ± 105.8	2.0 ± 27.1	−5.16 → 7.20	19.19 (16.20–23.54)	0.97 (0.94–0.98)	0.355	**<0.001**	**<0.001**	0.654	0.813
	60 +	425.1 ± 143.8	431.5 ± 142.1	6.4 ± 43.2	−4.66 → 17.44	30.51 (25.90–37.15)	0.95 (0.93–0.97)					
Sit and reach [cm]	18–35	5.5 ± 7.1	5.6 ± 6.8	0.1 ± 1.3	−0.23 → 0.44	0.89 (0.75–1.09)	0.98 (0.97–0.99)	0.267	**<0.001**	0.107	0.581	0.879
	60 +	−1.3 ± 9.4	−1.0 ± 8.8	0.3 ± 2.7	−0.40 → 0.97	1.90 (1.61–2.31)	0.96 (0.93–0.97)					
Timed up and go [s]	18–35	4.1 ± 0.6	4.0 ± 0.6	−0.1 ± 0.3	−0.14 → 0.04	0.24 (0.20–0.29)	0.84 (0.74–0.90)	0.806	**<0.001**	**<0.001**	0.520	0.070
	60 +	7.2 ± 2.1	7.3 ± 2.4	0.1 ± 0.8	−0.12 → 0.30	0.58 (0.49–0.70)	0.94 (0.89–0.96)					
Gait speed [m/s]	18–35	1.4 ± 0.2	1.4 ± 0.2	0.0 ± 0.1	−0.01 → 0.03	0.05 (0.05–0.07)	0.89 (0.82–0.93)	0.595	**<0.001**	**0.045**	0.347	0.869
	60 +	1.1 ± 0.2	1.1 ± 0.2	0.0 ± 0.1	−0.03 → 0.02	0.07 (0.06–0.08)	0.91 (0.85–0.94)					
Gait speed - fast [m/s]	18–35	2.1 ± 0.3	2.1 ± 0.3	0.0 ± 0.2	−0.07 → 0.06	0.18 (0.15–0.21)	0.84 (0.74–0.90)	0.255	**<0.001**	**<0.001**	0.383	0.854
	60 +	1.5 ± 0.3	1.5 ± 0.3	0.0 ± 0.1	−0.07 → 0.00	0.10 (0.09–0.12)	0.91 (0.86–0.95)					
Handgrip strength [kg]	18–35	41.5 ± 10.7	42.3 ± 11.0	**0.8 ± 2.7***	0.10 → 1.52	1.88 (1.59–2.31)	0.97 (0.94–0.98)	0.339	**<0.001**	**<0.001**	**0.034**	0.229
	60 +	29.2 ± 9.2	28.8 ± 9.3	−0.4 ± 2.7	−1.07 → 0.31	1.90 (1.61–2.31)	0.96 (0.93–0.97)					

Abbreviations: 6MWT (Six-minutes walking test); rep (repetitions); SD (standard deviation); CI (confidence interval); ICC (interclass correlation coefficient); *** $p<0.001$, ** $p<0.01$, * $p<0.05$ between test and retest as analysed per paired *t*-test; main effects of time, age group and sex as well as their interaction with time were determined by three-way mixed ANOVA, exact p-values are shown;; significant differences are marked in bold; young participants ($n = 57$), older participants ($n = 61$).

Table 3
Test-retest reliability of peak torque and average power assessments for knee extension and flexion.

	Age group (years)	Test (Mean ± SD)	Retest (Mean ± SD)	Difference (Mean ± SD)	95% CI of Difference	Typical Error (95% CI)	ICC (95% CI)	Time	Age group	Sex	Time × Age group	Time × Sex
Peak torque extension, 60°/s [Nm]	18–35	153.7 ± 60.2	154.8 ± 52.2	1.1 ± 30.1	−6.86 → 9.10	21.26 (17.95–26.08)	0.86 (0.77–0.92)	0.296	**<0.001**	**<0.001**	0.633	0.691
	60+	68.5 ± 36.7	70.9 ± 42.8	2.5 ± 25.1	−4.12 → 9.06	17.71 (14.98–21.69)	0.80 (0.69–0.88)					
Peak torque flexion, 60°/s [Nm]	18–35	94.9 ± 43.2	102.8 ± 44.4	**7.8 ± 19.9****	2.53 → 13.11	14.09 (11.89–17.28)	0.88 (0.79–0.93)	**0.001**	**<0.001**	**<0.001**	0.451	0.284
	60+	44.2 ± 21.6	48.9 ± 24.6	**4.7 ± 17.6***	0.03 → 9.27	12.41 (10.49–15.20)	0.70 (0.54–0.81)					
Avg power extension, 60°/s [W]	18–35	83.2 ± 36.1	84.3 ± 32.9	1.1 ± 19.9	−4.18 → 6.35	14.03 (11.85–17.22)	0.84 (0.74–0.90)	0.151	**<0.001**	**<0.001**	0.470	0.438
	60+	30.7 ± 16.6	33.4 ± 21.4	2.7 ± 14.3	−1.06 → 6.45	10.10 (8.54–12.37)	0.72 (0.57–0.82)					
Avg power flexion, 60°/s [W]	18–35	56.7 ± 29.2	63.2 ± 31.9	**6.5 ± 14.3****	2.69 → 10.30	10.14 (8.56–12.44)	0.87 (0.76–0.93)	**<0.001**	**<0.001**	**<0.001**	0.255	**0.007**
	60+	21.9 ± 11.3	24.8 ± 13.7	**2.9 ± 10.6***	0.11 → 5.69	7.52 (6.35–9.20)	0.63 (0.44–0.62)					
Peak torque extension, 120°/s [Nm]	18–35	115.9 ± 48.5	116.5 ± 44.5	0.6 ± 22.3	−5.31 → 6.55	15.80 (13.34–19.38)	0.89 (0.81–0.93)	0.202	**<0.001**	**<0.001**	0.379	0.825
	60+	46.4 ± 27.6	50.0 ± 30.8	3.5 ± 17.4	−1.03 → 8.10	12.28 (10.38–15.03)	0.82 (0.71–0.89)					
Peak torque flexion, 120°/s [Nm]	18–35	80.8 ± 42.5	85.0 ± 40.8	4.2 ± 29.2	−3.53 → 11.96	20.64 (17.42–25.32)	0.75 (0.62–0.85)	**0.041**	**<0.001**	**<0.001**	0.889	0.942
	60+	33.6 ± 18.3	38.5 ± 18.8	**4.9 ± 14.1***	1.18 → 8.61	9.99 (8.45–12.23)	0.69 (0.51–0.81)					
Avg power extension 120°/s [W]	18–35	115.4 ± 56.5	118.3 ± 55.5	2.9 ± 31.0	−5.34 → 11.13	21.94 (18.53–26.92)	0.85 (0.75–0.91)	**0.050**	**<0.001**	**<0.001**	0.449	0.553
	60+	36.3 ± 24.9	42.0 ± 31.4	**5.7 ± 19.4***	0.63 → 10.83	13.72 (11.60–16.79)	0.75 (0.61–0.85)					
Avg power flexion 120°/s [W]	18–35	84.5 ± 48.8	94.8 ± 52.4	**10.3 ± 24.0****	3.96 → 16.67	16.93 (14.30–20.77)	0.87 (0.76–0.93)	**<0.001**	**<0.001**	**<0.001**	0.327	0.116
	60+	27.9 ± 18.9	33.7 ± 21.7	**5.7 ± 16.0****	1.52 → 9.93	11.31 (9.56–13.84)	0.67 (0.48–0.79)					

Abbreviations: Avg (average); SD (standard deviation); CI (confidence interval); ICC (interclass correlation coefficient); ** $p<0.01$, * $p<0.05$ between test and retest as analysed per paired *t*-test; significant differences are marked in bold; main effects of time, age group and sex as well as their interactions were determined by three-way mixed ANOVA and exact p-values are shown; young participants ($n = 57$), older participants ($n = 58$).

Table 4
Test-retest reliability of body composition parameters.

	Age group (years)	Test (Mean ± SD)	Retest (Mean ± SD)	Difference (Mean ± SD)	95% CI of Difference	Typical Error (95% CI)	ICC (95% CI)	Time	Age group	Sex	Time x Age group	Time x sex
Body fat (%)	18–35	19.5 ± 8.6	19.5 ± 8.3	0.0 ± 1.4	−0.38 → 0.37	1.00 (0.84–1.32)	0.99 (0.98–0.99)	0.595	**<0.001**	**<0.001**	0.584	0.148
	60 +	39.4 ± 8.2	39.1 ± 8.1	−0.3 ± 1.6	−0.67 → 0.15	1.10 (0.93–1.35)	0.98 (0.97–0.99)					
Skeletal muscle mass (kg)	18–35	31.8 ± 7.6	32.0 ± 7.6	0.2 ± 0.7	−0.03 → 0.36	0.52 (0.44–0.64)	1.00 (0.99–1.00)	**0.021**	**<0.001**	**<0.001**	0.851	0.411
	60 +	26.5 ± 4.9	26.7 ± 4.7	**0.2 ± 0.8***	0.02 → 0.45	0.59 (0.50–0.72)	0.98 (0.97–0.99)					
Skeletal muscle index (kg/m^2)	18–35	10.2 ± 1.5	10.3 ± 1.5	0.1 ± 0.2	−0.01 → 0.11	0.17 (0.14–0.20)	0.99 (0.98–0.99)	**0.013**	0.420	**<0.001**	0.579	0.327
	60 +	9.8 ± 1.1	9.9 ± 1.1	**0.1 ± 0.3***	0.02 → 0.18	0.23 (0.19–0.28)	0.95 (0.92–0.97)					
Appendicular skeletal muscle mass (kg)	18–35	23.9 ± 5.9	23.9 ± 5.9	0.0 ± 0.5	−0.10 → 0.17	0.36 (0.30–0.44)	1.00 (0.99–1.00)	0.802	**0.001**	**<0.001**	0.960	0.816
	60 +	20.3 ± 3.9	20.3 ± 3.9	0.0 ± 1.5	−0.35 → 0.43	1.07 (0.91–1.31)	0.93 (0.88–0.96)					
Appendicular skeletal muscle index (kg/m^2)	Young	7.7 ± 1.2	7.7 ± 1.2	0.0 ± 0.2	−0.03 → 0.06	0.12 (0.10–0.14)	0.99 (0.98–0.99)	0.813	0.782	**<0.001**	0.968	0.865
	60 +	7.5 ± 0.9	7.5 ± 0.9	0.0 ± 0.6	−0.14 → 0.17	0.42 (0.35–0.51)	0.80 (0.69–0.88)					

Abbreviations: SD (standard deviation); CI (confidence interval); ICC (interclass correlation coefficient); * $p<0.05$ between test and retest as analysed per paired *t*-test; main effects of time, age group and sex as well as their interaction with time were determined by three-way ANOVA, exact p-values are shown, significant differences are marked in bold; young participants ($n = 57$), older participants ($n = 60$).

(18–35 years) and 61 older (>60 years) participants took part in the study. Two health professionals evaluated self-perceived health status and co-morbidities by adhering to the WHO-STEPS instrument [5]. Inclusion criteria involved both sexes, an age between 18 and 35 or above 60 years, living in the region of Prishtina, Kosovo and lack of any underlying health condition that would prevent the conduction of the physical performance tests.

Measurements took place from August 2016 until January 2017, in the Sports Medicine Laboratory and the sports hall at the "Universi College" in Prishtina, Kosovo. For assessing test-retest reliability of physical performance, strength and power parameters, participants were asked to take part in two identical test sessions. Study participants were instructed to repeat the test within 1 to 16 weeks, with a strong encouragement to finalize the second test within one to four weeks. This was achieved by 110 (93.2%) persons finally leading to a period of 7–97 days in between the two tests (median [25th–75th percentile]: 14 [13–21] days).

All data were collected in the morning after an overnight fast, uniformly by the same research team consisting of three qualified persons, with the same person being responsible for a specific test. Data collection started at the laboratory with reporting the individual's personal information, anthropometric data and body composition. After a break of 30 min during which a light standardized meal was provided, isokinetic peak torque, handgrip strength and functional performance were assessed in the laboratory or the sport hall of the same institution.

2.2. Anthropometric and body composition

Anthropometric data collection followed standardized procedures by the International Standards for Anthropometric Assessment [6] with participants wearing light indoor clothing and being barefoot. Height was measured by means of a portable stadiometer with a precision of 5 mm using the stretch stature method (DT05L, Kinlee, Zhongshan Jinli Electronic Weighing Equipment Co. Ltd, China). Body mass and composition were measured on a segmental multifrequency bioelectrical impedance analysis device with operating frequencies of 1, 5, 50, 250, 500 kHz and 1 mHz (Inbody 770 device, Biospace Co., Ltd., Seoul, Korea). Participants were instructed to stand upright with the soles of both feet over the tactile foot electrodes while holding the hand electrodes. Data output (as determined using the manufacturer's algorithm) included body fat and skeletal muscle mass (SMM) and appendicular skeletal muscle mass (ASMM) being the sum of the lean mass of both arms and legs. Body mass index (BMI), skeletal muscle index (SMI) and appendicular skeletal muscle index (ASMI) were determined as body mass, SMM or ASMM divided by height squared (expressed as kg/m^2) [7].

2.3. Muscle strength and power assessments

Isometric handgrip strength of the self-reported dominant hand was measured by portable hydraulic handgrip dynamometry (JAMAR, Patterson Medical, USA). Upon a brief demonstration and adjustment for hand-size, participants were asked to squeeze the dynamometer for a maximal isometric contraction time of 4–5 s. This was performed on two different trials with one minute rest in between and the better results being considered for analyses [8].

Isokinetic concentric peak torque and average power of knee extensors and flexors were measured in a sitting position at 60°/s and 120°/s on the self-reported dominant leg (Biodex System 4 Pro-Isokinetic Dynamometer, Shirley, New York, USA). The testing range of motion was set from 10–90° of knee flexion. Subjects were instructed to use the support handles at the side of the machine while shin, thigh, pelvic, and upper crossing torso stabilization straps were used for fixation [9].

2.4. Functional performance measurements

To assess lower body strength (endurance), the 30-s chair-stand test was used [4]. Participants were instructed to stand up and sit down with arms crossed over the chest as often as possible within 30 s from a 46 cm high armless chair that was placed against a wall. The test administrator stood next to the participant, measured the time by using a stop watch, while counting the number of full stands completed in 30 s between the start and the stop sign that he signalled. The very last attempt was counted, if the subject mastered more than 50% of the range of motion before the timeline.

Upper body strength (endurance) was assessed by the 30-s arm curl test [4], with the participant lifting a dumbbell (five pounds for woman and eight pounds for men) on the self-reported dominant side, while sitting on an armless chair. Similarly to the 30-seconds chair-stand test, the tester stood next, holding and controlling time with a stop watch and counting the number of biceps curls (elbow flexion/extension with supination) completed in 30 s. Again, the very last attempt was counted, if the subject mastered more than 50% of the range of motion before the timeline.

The six minutes walking test (6MWT) was used to assess aerobic endurance [4,10], with participants being instructed to walk as fast as possible on a 30-m shuttle track for six minutes. While recording the time, tester signalled the starting point, remaining time after every minute (5, 4, 3, 2, 1 min to go), the last 15 s reminder and the stopping sign. Participants were allowed to reduce the speed or even to rest, if the speed was too high to be sustained. The covered distance in meters upon the completion of 6MWT was registered as the test result.

To assess lower body flexibility, the chair sit and reach test was used [4]. Participants were sitting on the front edge of a 46 cm high chair placed against a wall. The dominant leg was extended to the front, with the heel on the floor and the foot dorsiflexed at about 90°. The other foot was bent and stabilised on the floor with the sole of the foot. Participants were asked to slowly bend forward at the hip joint while keeping the spine and head as straight as possible, reaching down to the toes above the extended leg with palms down on top of each other and the middle fingers tips even. This position was held for a brief 2-s static position, whereas the test administrator registered the reached distance using a ruler parallel to the lower leg. Reaches short of the toes were registered as minus scores, whereas those beyond the toes were registered as plus scores.

Timed up and go (TUG) test served to assess mobility [4,11], with participants being instructed to stand up from an armless chair, walk a distance of three meters, turn around a mark, walk back to the chair and sit down. The test administrator signalled the start of the test and registered the time using a stopwatch upon the completion of the task.

Gait speed was assessed at normal and fast speed [10]. Participants were instructed to walk at a "normal, comfortable speed" (gait speed) and "as fast as you can safely walk" (gait speed fast) in two consecutive trials on a marked 10-m path, with the first two meters used for acceleration and the two last meters for deceleration. Upon the starting signal, time for completion of the middle six meters was measured by using a stopwatch. The better result of the two trials was used and expressed in m/s [8]. Two older subjects (3.3%) had to use a cane to perform the tasks that involve walking (6MWT, TUG and gait speed).

2.5. Statistical analyses

Statistical evaluations were performed using IBM SPSS Statistics for Windows Version 27.0 (IBM Corp, Armonk, NY). Descriptive statistics including mean and standard deviation for continuous variables and relative frequencies for categorical variables are provided. Differences between age groups were calculated using independent *t*-test for continual variables and $\chi 2$ test for categorical variables. Test-retest reliability was determined by using respective spread sheets as provided by Hopkins [12]. Differences between test and retest data were described by mean

differences including standard deviation and 95% confidence interval (95% CI). Furthermore, the typical error (95%CI) was assessed.

Intraclass correlation coefficients (ICC) and their 95% CI were calculated IBM Statistics version 27 based on a single-rating, absolute agreement, two-way mixed effects model [13,14]. Based on Koo and Li [14], an ICC between 0.50 and 0.90 would be considered as 'moderate' or 'good'. With a sample size of $n = 61$ per group we would be able to ensure that for an ICC of 0.80 the lower limit of a 95% one-sided confidence limit is not less than 0.65 with an 80% assurance probability [15].

Finally, a three-way mixed ANOVA was performed providing data for main effects of time, age group and sex as well as time $\times$ sex and time $\times$ age group interactions to assess whether general test-retest results would be affected by age group or sex.

Ethics Statement

The work described has been carried out in accordance with The Code of Ethics of the World Medical Association [16] for experiments involving humans, and was approved by the Ethical-Professional Committee of the University Clinical Centre of Kosovo (no 1246/15.09.2016). Participation was voluntary, with written informed consent obtained from all participants.

CRediT Author Statement

Arben Boshnjaku: Conceptualization, Methdodology, Investigation, Data curation, Writing - Original draft preparation; **Abedin Bahtiri**: Investigation, Reviewing final manuscript; **Kaltrina Feka**: Investigation, Reviewing final manuscript; **Ermira Krasniqi**: Investigation, Reviewing final manuscript; **Harald Tschan:** Methodology, Writing- Reviewing and Editing; **Barbara Wessner:** Conceptualization, Supervision, Data curation, Writing – Reviewing and Editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships, which have or could be perceived to have influenced the work reported in this article.

Funding

This work was supported by the Universi College Kosovo, Bardhosh, 10000 Prishtina, Kosovo [grant number 2802] and the Research Platform Active Ageing (University of Vienna, Austria).

Acknowledgments

Open access funding was provided by the University of Vienna, Austria.

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5.1.2 Dataset (from publication 1): Raw data for test-retest reliability of physical performance in young and old adults

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Status:

Published by *Mendeley data* on March 8, 2021. V1. https://doi.org/10.17632/ntsrj6hf87.1

Personal contribution

- Conceptualization
- Methodology
- Investigation
- Data curation
- Writing – Original draft preparation
- Formal analysis;
- Validation;
- Visualization.

Raw data for test-retest reliability of physical performance in young and old adults

Published: 8 March 2021 | Version 1 | DOI: 10.17632/ntsrj6hf87.1

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Description

The present data focus on test-retest reliability of functional performance tests together with strength, power and body composition assessments in young and older Kosovan adults. The data set comprises various parameters relevant to assess age-related changes in physical performance. Those parameters have been suggested by the European Working Group in Sarcopenia for Older People (EWGSOP) in its initial and revised consensus statements to be used for the diagnosis and treatment of sarcopenia. In total, 57 healthy young (18-35 years) and 61 older (>60 years) participants took part in two identical test sessions, with a median [25th – 75th percentile] of 14 [13 – 21] days in between. Functional performance tests included 30-s chair stand test (CST), 30-s arm curl test (ACT), six-minutes walking test (6MWT), sit and reach test, timed up and go test (TUG), as well as the assessment of gait speed (GS) at normal and fast pace. Isometric handgrip strength (HGS) was

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Boshnjaku, Arben; Bahtiri, Abedin; Feka, Kaltrina; Krasniqi, Ermira; Tschan, Harald; Wessner, Barbara (2021), "Raw data for test-retest reliability of physical performance in young and old adults", Mendeley Data, V1, doi: 10.17632/ntsrj6hf87.1

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used to estimate strength of the dominant hand. Isokinetic peak torque (PT) and average power (avgP) for knee extension and flexion were determined at velocities of 600/s and 1200/s. Body composition assessments included body fat percentage, skeletal muscle mass (SMM) and index (SMI) as well as appendicular skeletal muscle mass (ASMM) and index. Secondary endpoints included self-perceived health status and potential co-morbidities.

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5.1.3 Publication 2: Impact of using population-specific cut-points, self-reported health, and socio-economic parameters to predict sarcopenia: a cross-sectional study in community-dwelling Kosovans aged 60 years and older

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Status:

Published by *Journal of Clinical Medicine* on September 22, 2022. 11(19):5579. Special Issue: "Risk Factors, Prevention and Management of Frailty in Elderly". https://doi.org/10.3390/jcm11195579

Personal contribution:

- Conceptualization;
- Data curation;
- Investigation;
- Methodology;
- Resources;
- Writing – Original draft;
- Writing – review and editing;

Journal of
Clinical Medicine

MDPI

Article

Impact of Using Population-Specific Cut-Points, Self-Reported Health, and Socio-Economic Parameters to Predict Sarcopenia: A Cross-Sectional Study in Community-Dwelling Kosovans Aged 60 Years and Older

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Citation: Boshnjaku, A.; Bahtiri, A.; Feka, K.; Krasniqi, E.; Tschan, H.; Wessner, B. Impact of Using Population-Specific Cut-Points, Self-Reported Health, and Socio-Economic Parameters to Predict Sarcopenia: A Cross-Sectional Study in Community-Dwelling Kosovans Aged 60 Years and Older. *J. Clin. Med.* **2022**, *11*, 5579. https://doi.org/10.3390/jcm11195579

Academic Editor: Wing Hoi Cheung

Received: 9 August 2022
Accepted: 20 September 2022
Published: 22 September 2022

Abstract: The age-related decline of muscle strength, mass, and physical performance (sarcopenia) has been raising concerns among the scientific and healthcare communities. This decline may differ between populations, age groups, and sexes. Therefore, we aimed to explore sarcopenia together with the impact of health and socio-economic parameters in mature Kosovans. A cross-sectional study was conducted on community-dwelling adults aged ≥ 60 years (n = 240, 47.1% female) from the Prishtina region. Sarcopenia was identified using the following criteria: (i) the European Working Group in Sarcopenia for Older People (EWGSOP1), (ii) the revised EWGSOP2 algorithms, and (iii) sex-specific cut-points derived from the Kosovan population. In males, pre-sarcopenia/probable sarcopenia was detected from the EWGSOP1, EWGSOP2 and Kosovan-specific criteria at values of 3.1%, 5.5%, and 28.3%; sarcopenia was detected at 1.6%, 5.5%, and 0.0%, and severe sarcopenia was detected at 4.7%, 2.4%, and 4.7%, respectively. Pre-sarcopenia was lower in females (0.9%, 5.3%, 16.8%), with no cases of sarcopenia or severe sarcopenia detected by either algorithm. Sarcopenic males were older, had a lower weight, BMI, skeletal muscle mass, performance score, nutritional status ($p < 0.001$), educational level ($p = 0.035$), and higher malnourishment risk ($p = 0.005$). It is notable that high overweight and obesity levels were also detected (93.8% of females, 77.1% of males). This study highlights the importance of using population-specific cut-points when diagnosing sarcopenia, as otherwise its occurrence may be underestimated, especially in obese persons. Age, body composition, physical performance, health, and socio-economic conditions can influence the occurrence of sarcopenia.

Keywords: sarcopenia diagnostics; developing country; older adults; EWGSOP; population-specific cut points

1. Introduction

Sarcopenia presents an issue that is rapidly gaining interest among the scientific community, and it is clinically used to describe the age-related changes in the skeletomuscular system and their impact on the health of older people [1]. In 2010, a practical clinical definition and consensus diagnostic criteria for age-related sarcopenia was developed by

the European Working Group on Sarcopenia in Older People (EWGSOP1) [2]; it focused on lower muscle mass as the key characteristic accompanied by either lower muscle strength or worse physical performance (PP), or both (sarcopenia and severe sarcopenia, respectively). The revised 2019 version (EWGSOP2) [3] defined sarcopenia as a muscle disease (muscle failure) rooted in adverse muscle changes that occur across a lifetime, which was also associated with an increased likelihood of adverse outcomes, including falls, fractures, physical disability, and mortality. With the EWGSOP2 criteria, the attention was shifted towards the recommendation of using muscle strength as the primary parameter for sarcopenia diagnosis (probable sarcopenia). Low muscle quantity or quality would confirm the condition (confirmed sarcopenia), and the combined presence of low muscle strength, low muscle quantity/quality, and low physical performance characterizes the state of severe sarcopenia [3]. In addition to modifying the diagnostic algorithm, population-specific cut points were also proposed for use, namely to assess two standard deviations below the mean of a young comparison population [4].

Global estimates of sarcopenia prevalence vary from 10% to 27% depending on the definition used, ethnicity, age, and sex [5]. Sex emerges as a particularly interesting sarcopenia influence factor because of the age-related hormonal effects on muscle phenotypes, notably through estrogens and estradiol in particular [6,7]. However, ageing is a process that, aside from the progressive decline of muscle quality and quantity, is often characterized with a sex-specific visceral fat increase as well. Visceral fat itself has been shown to be related and even induce inflammation [8], which may subsequently contribute to the development of sarcopenia [9]. Additionally, it has been shown that a population's general health status as well as their educational and socio-economic status might affect the prevalence levels [10,11]. To date, the majority of studies have been predominantly based on Caucasians from high-income countries [12], but data from low- and middle-income countries involving a Caucasian population within Europe are scarce [13,14]. Kosovo declared independence in 2008 and has been recognized by more than 100 United Nations members as well as 23 out of 28 members of the European Union (EU). Despite its development to an upper-middle-income country and experiencing a solid economic growth over the last decade, life expectancy is still in the lower range compared with the EU (70.3 years for males and 74.8 years for females) [15]. Sarcopenia has been associated with an increased risk of all-cause mortality [16], but no study so far has determined neither its occurrence nor the more robust diagnostic criteria and cut-points for the Kosovan population. Therefore, this study aimed to determine sarcopenia levels as defined by different diagnostic criteria (EWGSOP1, EWGSOP2, and population-specific cut points) in older Kosovan adults and evaluated the impact of socio-economic, health-related, and lifestyle factors on sarcopenia status.

2. Materials and Methods

2.1. Study Design and Sample

This cross-sectional study examined data from community-dwelling males and females aged over 60 years living in the region of Prishtina, the largest region in Kosovo. This region is home to 477,312 people (27.4% of the total population of Kosovo), and 6.7% of the total Kosovan population are over 60 years old [17]. An a priori sample size estimation has been performed by using the following equation: $n = (z^2) \times P \times (1 - P)/d^2$, where n = sample size, z = z statistic for the level of confidence (1.96), P = expected prevalence, and d = allowable error. Hence, for an expected prevalence between 10% and 90% (P = 0.1 to 0.9), a d of $\pm$5% has been suggested as a reasonable choice [18]. Therefore, a sample size of n = 144 was considered necessary to detect a 10% prevalence. However, we enabled participation for all interested persons that fulfilled the inclusion criteria. In total, 240 adults (113 females and 127 males) aged $\geq$60 years participated, representing about 0.8% of the eligible sample. Participants were recruited through announcements (written and verbal) in the Kosovo Pensioners' Association branches in Prishtina and Fushë Kosova (neighboring municipality within the same region), Prishtina Nursing Homes,

and national TV broadcasting. This recruitment strategy intended to notify every eligible participant for the possibility to take part in the study and thus avoid a potential healthy subject bias through the most approachable means: associations (of which aside from the formal offices also includes a restaurant, coffee shop, and two board game rooms), one of the most frequented places during the daytime; nursing homes, the places where some of the target community lives; and TV programs, which could explain the study and approach towards the interested participants within their homes. Inclusion criteria were set in accordance with the study's specifications and measurement techniques and participants were assessed at the entrance by two healthcare experts; the assessment involved determining the sex, age, living region, and lack of any underlying condition or disease that could prohibit the participant from performing measurements (e.g., any cardiac implantable electronic devices).

2.2. Data Collection and Anthropometric Measurements

Data collection was organized at the Sports Medicine Laboratory at Universi College in Prishtina. All measurements were uniformly performed with respect to the testing order by the same research team during the entire data collection period. Anthropometric measurements were performed in accordance with the International Standards for Anthropometric Assessment in the morning after an overnight fast, starting with height, weight, and body composition [19]; subjects wore light indoor clothing and were barefoot. Heights were measured with a precision of 5 mm using the stretch stature method with a portable stadiometer (DT05L, Kinlee, Zhongshan Jinli Electronic Weighing Equipment Co. Ltd., Zhongshan, China). Body compositions were measured using segmental multi-frequency bioelectrical impedance analyses (BIA), with operating frequencies of 1, 5, 50, 250, 500 kHz and 1 mHz using a device that also allowed for the assessment of body mass (Inbody 720, Biospace Co., Seoul, Korea). Body composition variables included the whole body skeletal muscle mass (SMM) and fat mass. Appendicular skeletal muscle mass (ASMM) was assessed as the sum of the lean mass of both arms and legs. In order to predict the dual-energy X-ray absorptiometry (DXA) estimation of muscle mass when measuring by the BIA method, a multivariate regression model was used, as previously evaluated [20,21]. Body mass index (BMI), skeletal muscle index (SMI), and appendicular skeletal muscle index (ASMI) were determined as the body mass, SMM, or ASMM divided by height squared (all expressed as kg/m^2) [22].

2.3. Physical Performance and Strength Measurements

After the anthropometric assessments, a light standardized meal was provided 30 min before starting the strength and physical performance measurements. The isometric handgrip strength was measured by using handgrip dynamometry (JAMAR, Patterson Medical, Saint Paul, MN, USA). Each participant was asked to perform two trials with 1 min of rest in between the trials. The adaptable dynamometer was squeezed for a maximal isometric contraction time of 4–5 s using the self-reported dominant hand. The better result of the two trials was considered for the analyses [23].

For assessing gait speed, participants were asked to walk in their usual gait speed on a path six meters long with an additional two meters for acceleration and deceleration (a total course length of 10 m). The time for the completion of the six meters was manually recorded by the tester using a stopwatch; the gait speed was expressed in m/s. The better result of the two trials was used [23].

For the timed up and go test (TUG), subjects were required to stand up from a chair without armrests upon the starting signal, walk three meters on a linear course, turn around, and return to the starting position, where they had to sit down again. The time taken to complete this task was measured in seconds using a stopwatch [24].

Lower body strength endurance was tested by the 30-seconds (30-s) chair stand test as previously described using an armless chair with a height of 46 cm and placed against a wall. Participants were instructed to stand up and sit down as often as possible within 30 s

while having their arms crossed over their chest. Fully completed repetitions (including the last attempt if completing more than 50% of the task before the time expired) were counted by the tester who stayed next to the participant, holding a stopwatch and signaling the beginning and end of the time. Similarly, the 30-s arm curl test estimated the upper body strength endurance of the participants by counting the number of repetitions of elbow flexion with supination. The participants lifted a dumbbell (5 pounds for women and 8 pounds for men) while sitting upright on a chair upon receiving the starting and ending signal by the tester, who followed a similar protocol as the previous test (standing next to the participant and counting the repetitions, including the last repetition if the participant completed more than 50% of the task before the time expired). The task was performed for the self-reported dominant side [25].

For determining aerobic endurance, the 6-min walking test (6MWT) was used. Subjects were requested upon being signaled to walk as fast as possible for 6 min on a 30-meter shuttle track while being alone on the track. Testers strictly followed the process, counted the time, and signaled the ending. If necessary, the speed could be reduced or the person was allowed to rest if the selected speed was too high to be sustained, and the covered distance was reported in meters as the test outcome [26]. In four cases (1.7%), the use of a cane was necessary to perform the tasks that involved walking (gait speed, TUG, and 6MWT).

2.4. Assessment of Sarcopenia

Sarcopenia was diagnosed according to the EWGSOP1 and EWGSOP2 algorithms using the SMI/ASMI, handgrip strength, and gait speed tests, as indicated in the recommendations [2,3]. In order to compare the different modalities, we used either the suggested cut-points or population- and sex-specific cut-points for the Kosovan population. The latter were derived as suggested by Baumgartner et al. [4] through the subtraction of two standard deviations from the respective mean of a given parameter; we derived the cut-points from a young Kosovan sample [27]. Sex-specific thresholds were set to 5.74 kg/m^2 (male) and 4.77 kg/m^2 (female) for ASMI, 32.83 kg (male) and 19.64 kg (female) for handgrip strength, and 1.14 m/s (male) and 1.03 m/s (female) for gait speed.

2.5. Secondary Endpoints

The self-perceived health status and comorbidities were collected by two healthcare professionals. The nutritional status was assessed by the Mini Nutritional Assessment (MNA) questionnaire (long form), an 18 item tool comprising anthropometric measurements (BMI, weight loss, mid-arm, and calf circumferences) dietary intake (feeding autonomy, amount of food and fluids intake, number of meals consumed), general lifestyle assessment (living environment, medication consumption, mobility, pressure ulcers, presence of stress, depression, or dementia), and subjective self-assessment (self-perception of own health and nutritional level) [28]. Medication usage was assessed by the Brief Medication Questionnaire 1 [29]. The environment of living, education level, marital status, financial status and self-perceived poverty level, self-perceived health condition, comorbidities, smoking status, and alcohol consumption were collected and analyzed using the WHO STEPS instrument [30].

A physical performance score including the five performance tests (gait speed, TUG, 30-s arm curl test, 30-seconds chair stand test and 6MWT) was created using the weighted-sum method as has been previously described. Briefly, the test results of the individual tests were scaled to Z-scores and subjected to a principal component analysis in order to obtain the loading value of each test. The performance scores for each subject were calculated by multiplying the Z-scores with their associated loading value before summation [31].

2.6. Statistical Analysis

The means, standard deviations (continuous variables), and frequencies (categorical variables) were calculated to describe the general, anthropometric, and physical fitness

variables. A chi-square test was used to compare the frequencies between groups. Differences between the continuous variables were defined by an independent *t*-test and one-way ANOVA test, the latter followed by Tukey post hoc analyses, which also suggested homogenous subgroups. The homogeneity of variances were checked by a Levene test. Effect sizes were calculated to estimate the magnitude of the effect. Cohen's d was used when comparing males and females, whereas ω^2 was applied together with a one-factorial ANOVA test. A multinomial logistic regression was used to ascertain the impact of various covariates on the sarcopenia status. "No sarcopenia" was set as the reference group, and financial condition and health condition were included as factors; age, BMI, body fat mass, SMM, PP, and MNA scores were included as covariates. As "no sarcopenia" is present in more than 10% of the cases, the odds ratio (OR) derived from the logistic regression might overestimate the risk ratio (when it is more than 1) or underestimates the risk ratio (when it is less than 1). Therefore, the relative risk (RR) was estimated using the formula provided by Zhang and Yu [32]: RR = OR/[(1 − P0) + (P0 × OR), where P0 indicates the incidence of no sarcopenia. The statistical significance was set at $p < 0.05$, and all data were analyzed using the statistical package SPSS 26 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Participants' Characteristics

Figure 1 shows the flowchart for study participants. From the total of 477,312 people aged 60 years and older within the region of Prishtina, 20,175 and 3202 subjects from the municipalities of Prishtina and Fushë Kosova, respectively, were eligible to participate. From those, 261 expressed their interest to participate, out of whom 240 persons fulfilled the inclusion criteria and took part in the study. Data from the first 61 of these participants were used to assess the reliability of the assessment parameters and calculate diagnostic cut-off points for sarcopenia [27].

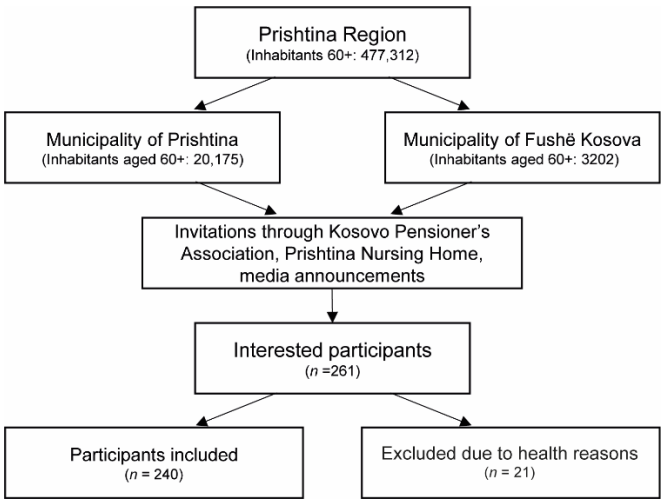

Figure 1. Selection of study participants.

The characteristics of the 240 subjects included in the study are summarized in Table 1. Males were observed to be older ($t_{238} = 5.187$, $p < 0.001$, d = 0.672) and taller ($t_{238} = 15.438$, $p < 0.001$, d = 2.001), with a higher SMM ($t_{218.4} = 12.801$, $p < 0.001$, d = 1.732) and SMI ($t_{223.9} = 6.816$, $p < 0.001$, d = 0.911) but a lower ASMI ($t_{238} = -3.055$, $p < 0.001$, d = −0.396). Females had a higher BMI ($t_{238} = 7.733$, $p < 0.001$, d = 1.003), whole body fat mass ($t_{238} = 8.688$, $p < 0.001$, d = 1.126), and fat percentage ($t_{232.2} = 14.741$, $p < 0.001$, d = 1.935). No differences were found for body mass between the sexes ($t_{238} = -0.670$, $p = 0.504$, d = −0.087). Additionally, the females presented a lower nutritional score (from the MNA long form, $t_{238} = -3.911$, $p < 0.001$, d = −0.507) and consequently a higher prevalence of malnourishment and risk for malnourishment ($p < 0.001$) in comparison to

the males. The female participants also reported a lower frequency of smoking ($p = 0.001$), lower level of education ($p < 0.001$), and worse financial condition ($p = 0.022$), but reported a higher medication intake compared with the males ($p < 0.001$). When observing the sex differences in physical performance, a significantly lower physical performance score was seen in females ($t_{238} = -4.428$, $p < 0.001$, d = −0.574), which was also the case for the individual performance tests (all $p < 0.001$, with Cohen's d ranging from −1.794 to 0.391); the one exception was the 30-s arm curl test ($t_{238} = -1.650$, $p = 0.100$, d = −0.214).

Table 1. Anthropometric, physical performance, lifestyle, and socio-demographic characteristics.

	Total (n = 240)	Female (n = 113)	Male (n = 127)	p Value
Sex (%)	100	47.1	52.9	
Age (years)	70.3 ± 5.8	68.4 ± 5.3	72.1 ± 5.7	<0.001
Height (m)	1.64 ± 0.09	1.57 ± 0.06	1.70 ± 0.07	<0.001
Body mass (kg)	79.9 ± 12.7	79.3 ± 11.7	80.4 ± 13.6	0.504
BMI (kg/m^2)	29.7 ± 4.7	32.0 ± 4.3	27.7 ± 4.2	<0.001
Whole body fat mass (kg)	29.6 ± 11.1	35.3 ± 8.9	24.4 ± 10.3	<0.001
Whole body fat percentage (%)	36.4 ± 10.6	44.1 ± 6.5	29.6 ± 8.6	<0.001
Skeletal muscle mass (kg)	26.9 ± 5.4	23.3 ± 3.2	30.2 ± 5.0	<0.001
SMI (kg/m^2)	9.9 ± 1.2	9.4 ± 0.9	10.3 ± 1.3	<0.001
Appendicular skeletal muscle mass (kg)	19.4 ± 2.9	18.2 ± 2.2	20.5 ± 3.1	<0.001
ASMI (kg/m^2)	7.2 ± 0.8	7.3 ± 0.7	7.0 ± 0.8	0.003
Hand grip strength (kg)	30.1 ± 8.8	24.1 ± 5.1	35.4 ± 8.0	<0.001
Gait speed (m/s)	1.08 ± 0.21	1.01 ± 0.19	1.14 ± 0.22	<0.001
Timed up and go test (s)	7.12 ± 1.98	7.50 ± 2.20	6.78 ± 1.70	0.005
30-s arm curl test (repetitions)	14 ± 3	14 ± 3	15 ± 3	0.100
30-s chair stand test (repetitions)	11 ± 3	11 ± 3	12 ± 3	0.004
6-min walking test (m)	420 ± 139	381 ± 127	455 ± 140	<0.001
Physical performance Score (-)	−1.26 ± 1.86	−1.78 ± 1.81	0.8 ± 1.79	<0.001
Mini nutritional status (-)	25 ± 3	24 ± 3	25 ± 2	<0.001
Malnourished (yes/risk/no, n (%))	4/64/172 (1.7/26.6/71.7)	4/41/68 (3.5/36.3/60.2)	0/23/104 (0.0/18.1/81.9)	<0.001
BMI categories (underweight/normal weight/overweight/obese, n (%))	2/34/104/100 (0.8/14.2/43.3/41.7)	0/7/35/71 (0.0/6.2/31.0/62.8)	2/27/69/29 (1.6/21.3/54.3/22.8)	<0.001
Smoking status (smoker/quit smoking/non-smoker, n (%))	53/24/163 (22.1/10/67.9)	20/4/89 (17.7/3.5/78.8)	33/20/74 (26/15.7/58.3)	0.001
Self-perceived health condition (good/not good, n (%))	103/137 (42.9/57.1)	50/63 (44.2/55.8)	53/74 (41.7/58.3)	0.694
Self-declared chronic disease (yes/no, n (%))	183/57 (76.2/23.8)	90/23 (79.6/20.4)	93/34 (73.2/26.8)	0.244
Intake of medication (yes/no, n (%))	186/54 (77.5/22.5)	95/18 (84.1/15.9)	91/36 (71.6/28.4)	0.021
Number of medications (n (%))	2.3 ± 1.9	2.8 ± 1.9	1.9 ± 1.7	<0.001
Education (no formal/1–8 years/>8 years, n (%))	10/95/135 (4.2/39.6/56.2)	7/62/44 (6.2/54.9/38.9)	3/33/91 (2.4/26/71.6)	<0.001
Marital status (single/partnership or married/widowed, n (%))	7/166/67 (2.9/69.2/27.9)	7/66/40 (6.2/58.4/35.4)	0/100/27 (0/78.7/21.3)	0.003
Financial condition (enough to cover the month/not enough, n (%))	166/74 (69.2/30.8)	70/43 (61.9/38.1)	96/31 (75.6/24.4)	0.022

Data are expressed as the means ± standard deviation or as absolute numbers; Abbreviations: BMI, body mass index; SMI, skeletal muscle index; ASMI, appendicular skeletal muscle index; m, meters; kg, kilograms; s, seconds. An independent t-test was used to determine the differences between females and males in the continuous variables and a chi-square test was used in the categorical variables; $p < 0.05$ was considered to be statistically significant.

3.2. Sarcopenia and Conceptual Stages in the Study Population

The case finding process that followed the two recommended algorithms from the EWGSOP1 and EWGSOP2 is described in Figure 2. Pre-sarcopenia was detected in 2.1% (3.1% of males and 0.9% of females) of the participants when following the EWGSOP1-suggested cut-points. Sarcopenia was observed in 0.8% (1.6% of males and 0.0% of females),

and 2.5% (4.7% of males and 0.0% of females) were identified to have severe sarcopenia. Using the EWGSOP2-suggested cut-points revealed probable sarcopenia in 5.4% (5.5% of males, 5.3% of females), sarcopenia in 2.9% (5.5% of males, 0.0% of females), and severe sarcopenia in 1.3% (2.4% of males, 0.0% of females) of participants, indicating slightly higher rates in all categories in comparison to the EWGSOP1. The Kosovo-specific cut-points (following the EWGSOP2 algorithm) revealed higher percentages of probable sarcopenia [22.9% (28.3% of males, 16.8% of females)], no cases with sarcopenia [0.0% (0.0% of males, 0.0% of females)], and severe sarcopenia [2.5% (4.7% of males, 0.0% of females)], compared with the EWGSOP2. Taken together, all algorithms confirmed the higher sarcopenic states among the males as compared with the females (EWGSOP1: $Chi^2 = 9.054$, $p = 0.029$; EWGSOP2: $Chi^2 = 9.333$, $p = 0.025$; EWGSOP2 (Kosovo-specific): $Chi^2 = 10.928$, $p = 0.004$).

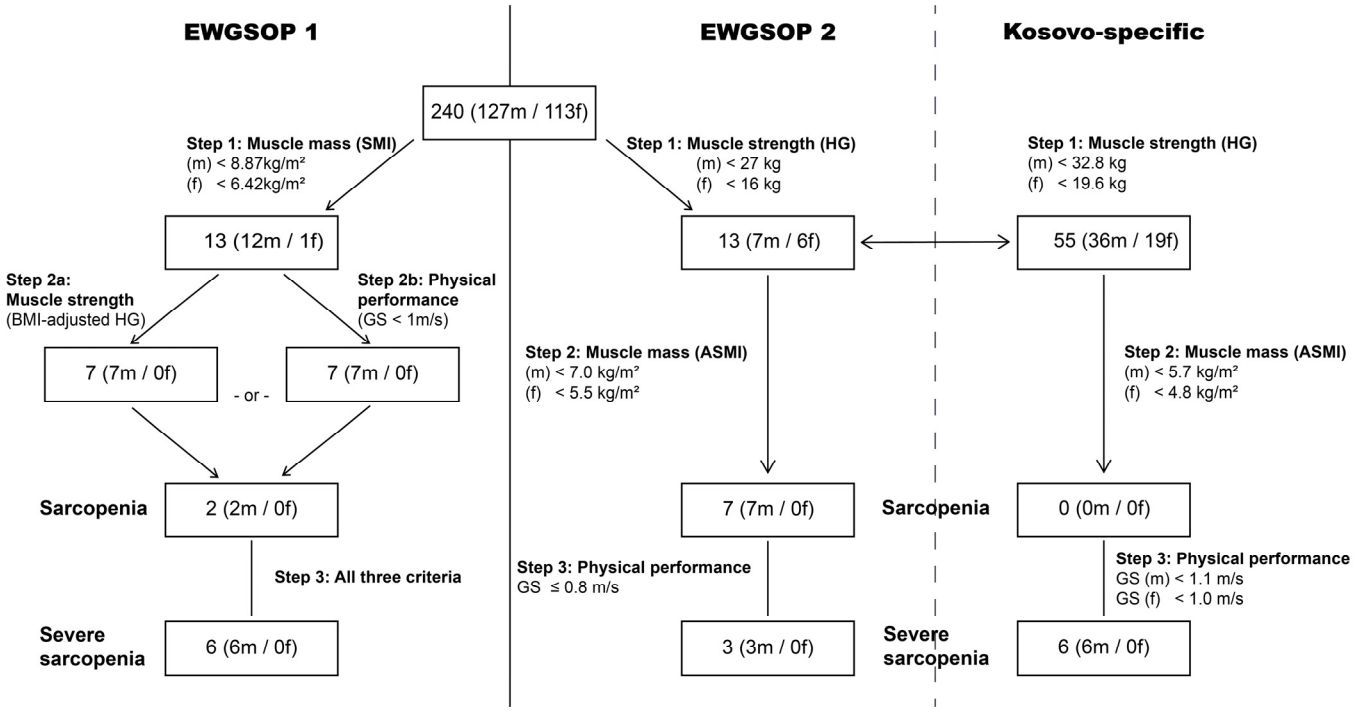

Figure 2. Sarcopenia case finding according to the EWGSOP1, EWGSOP2 and population-specific cut-points. EWGSOP1: pre-sarcopenia, low SMI; sarcopenia, low SMI and low HG or PP; severe sarcopenia, low SMI, low HG, and low PP. EWGSOP2 and Kosovo-specific: probable sarcopenia, low HG; sarcopenia, low HG and low ASMI; severe sarcopenia, low HG, low ASMI, and low PP. ASMI, appendicular skeletal muscle index; BMI, body mass index; GS, gait speed; HG, handgrip strength; SMI, skeletal muscle index; PP physical performance. Specific cut-points are given in the Figure. For the BMI-adjusted HG (EWGSOP1), the following values were used: males (m): ≤29 kg for BMI ≤ 24 kg/m^2, ≤30 kg for BMI 24.1–28 kg/m^2, ≤32 kg for BMI > 28 kg/m^2; females (f): ≤17 kg for BMI ≤ 23 kg/m^2, ≤17.3 kg for BMI 23.1–26 kg/m^2, ≤18 kg for BMI 26.1–29 kg/m^2, ≤21 kg for BMI > 29 kg/m^2.

3.3. Impact of EWGSOP2-Derived Sarcopenia States on Health-Related and Socio-Economic Factors

To generate comparable data to already published literature, the EWGSOP2 algorithm and suggested cut-points were used for first analyses. Due to the low number of cases, females were excluded, whereas the comparisons were made between the four groups in males of not sarcopenic ($n = 110$), probable sarcopenic ($n = 7$), sarcopenic ($n = 7$), and severe sarcopenic ($n = 3$). The groups differ in age ($F_{3,123} = 5.121$, $p = 0.002$, $\omega^2 = 0.089$), height ($F_{3,123} = 8.366$, $p < 0.001$, $\omega^2 = 0.164$), SMM ($F_{3,123} = 6.435$, $p < 0.001$, $\omega^2 = 0.134$), ASMM ($F_{3,123} = 6.823$, $p < 0.001$, $\omega^2 = 0.086$), and ASMI ($F_{3,123} = 5.521$, $p = 0.001$, $\omega^2 = 0.031$). With

respect to the physical performance tests, differences were found for the total performance score ($F_{3,123}$ = 8.554, $p < 0.001$, ω^2 = 0.115), handgrip strength ($F_{3,123}$ = 43.721, $p < 0.001$, ω^2 = 0.513), relative handgrip strength ($F_{3,123}$ = 27.795, $p < 0.001$, ω^2 = 0.482), gait speed ($F_{3,123}$ = 5.926, p = 0.001, ω^2 = 0.061), TUG ($F_{3,123}$ = 8.870, $p < 0.001$, ω^2 = 0.056), 30-s arm curl test ($F_{3,123}$ = 4.199, p = 0.007, ω^2 = 0.063), 30-s chair stand test ($F_{3,123}$ = 3.020, p = 0.032, ω^2 = 0.041), and 6MWT ($F_{3,123}$ = 4.083, p = 0.008, ω^2 = 0.059).

Post hoc analyses revealed that the sarcopenic and severe sarcopenic individuals particularly differ from non-sarcopenic ones in most of the variables; the interpretation might be affected by low case numbers. However, handgrip strength (both in absolute and relative measurements) was already lower in males with any of the three sarcopenia conceptual stages compared with the participants without sarcopenia being directly related to the diagnostic algorithm. Most interestingly, the PP score was the only parameter that was able to discriminate between sarcopenia and severe sarcopenia ($p < 0.05$). The number of participants with their marital status described as single (without partner) was lower in the sarcopenic and severe sarcopenic groups (p = 0.002), whereas no other lifestyle or socio-economic factor was associated with sarcopenic status (Table 2).

3.4. Impact of Kosovan-Specific Cut-Points on Health-Related and Socio-Economic Factors

When comparing the suggested EWGSOP2 cut-points to the Kosovo-derived ones (both using the EWGSOP2 algorithm), it was observed that 179 participants (74.6% of the total population) were classified as non-sarcopenic by both calculation methods, a further 13 subjects (5.4% of total population) with probable sarcopenia, and 2 subjects (0.8% of the total population) with sarcopenia or severe sarcopenia. Furthermore, 46 participants (19.2%) differed in the sarcopenia diagnosis (confirmed by Chi^2 test: χ^2 (4) = 84.822, $p < 0.001$). The most striking difference when using the Kosovo-derived cut-points might be the higher number of cases of probable sarcopenia (36 males (28.3%), 19 females (16.8%)). In the next step, we assessed (again only in males) whether the diagnosis was associated with differences in general, anthropometric, physical performance, lifestyle, socio-economic, and health parameters (Table 3).

Sarcopenia was associated with a higher age ($F_{2,124}$ = 8.682, p = 0.001, ω^2 = 0.108) but lower height ($F_{2,124}$ = 15.223, $p < 0.001$, ω^2 = 0.183), body mass ($F_{2,124}$ = 11.299, $p < 0.001$, ω^2 = 0.140), BMI ($F_{2,124}$ = 11.693, $p < 0.001$, ω^2 = 0.144), whole body fat mass ($F_{2,124}$ = 3.960, p = 0.022, ω^2 = 0.045), whole body fat percentage ($F_{2,124}$ = 3.293, p = 0.040, ω^2 = 0.035), skeletal muscle mass ($F_{2,124}$ = 21.463, $p < 0.001$, ω^2 = 0.244), MNA ($F_{2,124}$ = 8.917, $p < 0.001$, ω^2 = 0.111), ASMM ($F_{2,124}$ = 14.101, $p < 0.001$, ω^2 = 0.171), and ASMI ($F_{2,124}$ = 15.823, $p < 0.001$, ω^2 = 0.189). Regarding the physical performance tests, sarcopenic subjects showed a lower total performance score ($F_{2,124}$ = 24.484, $p < 0.001$, ω^2 = 0.270), handgrip strength ($F_{2,124}$ = 114.468, $p < 0.001$, ω^2 = 0.641), gait speed ($F_{2,124}$ = 17.672, $p < 0.001$, ω^2 = 0.208), TUG ($F_{2,124}$ = 10.420, $p < 0.001$, ω^2 = 0.129), 30-s arm curl test ($F_{2,124}$ = 14.790, $p < 0.001$, ω^2 = 0.178), 30-s chair stand test ($F_{2,124}$ = 9.871, $p < 0.001$, ω^2 = 0.123), and 6MWT ($F_{2,124}$ = 8.917, $p < 0.001$, ω^2 = 0.111). Post hoc analyses revealed that handgrip strength was already lower in the probable sarcopenic males, but in contrast to the use of the EWGSOP2 general cut-points, differences were detected between probable and sarcopenic/severe sarcopenic males. Only the skeletal muscle mass was markedly different between all three groups.

Similar to the regular EWGSOP2 analyses, the educational level differed between groups (p = 0.035). In addition, participants from the sarcopenic/severe sarcopenic group showed a lower nutritional status ($F_{2,124}$ = 8.917, $p < 0.001$, ω^2 = 0.111) together with a higher percentage of risk for malnourishment (p = 0.005) and lower percentages of overweight and obese cases ($p < 0.001$). Interestingly, the self-perceived health or medication intake and socio-economic variables did not differ between the groups (Table 3).

Table 2. Differences between the non-sarcopenic, probable sarcopenic, sarcopenic, and severe sarcopenic male subjects using the EWGSOP2 criteria.

	No Sarcopenia (n = 110)	Probable Sarcopenia (n = 7)	Sarcopenia (n = 7)	Severe Sarcopenia (n = 3)	p Value
Age(years)	71.4 ± 5.2 [a]	74.5 ± 8.1 [a,b]	79.2 ± 6.7 [b]	73.9 ± 0.8 [a,b]	0.002
Height (m)	1.71 ± 0.06 [a]	1.65 ± 0.07 [b]	1.61 ± 0.10 [b]	1.66 ± 0.08 [a,b]	<0.001
Body mass (kg)	81.2 ± 14.2	78.3 ± 5.3	74.5 ± 7.6	69.5 ± 3.3	0.280
BMI (kg/m^2)	27.6 ± 4.3	28.9 ± 1.7	28.8 ± 4.5	25.4 ± 2.7	0.579
Whole body fat mass (kg)	24.7 ± 10.7	22.5 ± 4.7	23.5 ± 11.2	22.7 ± 4.0	0.933
Whole body fat percentage (%)	29.4 ± 8.4	29.0 ± 6.7	31.7 ± 14.6	32.6 ± 4.1	0.830
Skeletal muscle mass (kg)	30.9 ± 4.5 [a]	26.9 ± 6.0 [a,b]	24.5 ± 7.0 [b]	25.3 ± 0.7 [a,b]	<0.001
SMI (kg/m^2)	10.5 ± 1.2 [a]	9.9 ± 1.7 [a]	9.3 ± 2.1 [a]	9.2 ± 0.9 [a]	0.037
Appendicular skeletal muscle mass (kg)	23.7 ± 3.5 [a]	23.2 ± 3.1 [a]	18.3 ± 3.4 [b]	19.6 ± 0.7 [a,b]	<0.001
ASMI (kg/m^2)	8.0 ± 0.9 [a]	8.6 ± 0.9 [a]	7.0 ± 0.6 [b]	7.2 ± 0.5 [a,b]	0.001
PP score (-)	1.06 ± 2.56 [a]	−0.31 ± 2.08 [a]	−0.86 ± 1.87 [a]	−5.65 ± 0.66 [b]	<0.001
Handgrip strength (kg)	37.7 ± 5.8 [a]	22.6 ± 2.7 [b]	19.8 ± 4.3 [b]	19.8 ± 7.8 [b]	<0.001
Relative handgrip strength (kg/kg)	0.47 ± 0.08 [a]	0.29 ± 0.03 [b]	0.27 ± 0.06 [b]	0.28 ± 0.10 [b]	<0.001
Gait speed (m/s)	1.16 ± 0.22 [a]	1.08 ± 0.14 [a]	1.04 ± 0.14 [a,b]	0.68 ± 0.14 [b]	0.001
Timed up and go test (s)	6.64 ± 1.52 [a]	6.63 ± 0.91 [a]	7.13 ± 2.37 [a]	11.30 ± 2.01 [b]	<0.001
30-s arm curl test (repetitions)	15 ± 3 [a]	13 ± 3 [a,b]	13 ± 3 [a,b]	10 ± 2 [b]	0.007
30-s chair stand test (repetitions)	12 ± 3 [a]	12 ± 3 [a]	10 ± 2 [a,b]	8 ± 2 [b]	0.032
6-min walking test (m)	470 ± 139 [a]	369 ± 82 [a,b]	384 ± 121 [a,b]	261 ± 92 [b]	0.008
Mini nutritional status (-)	25 ± 2	25 ± 3	26 ± 2	22 ± 3	0.059
Malnourished (yes/risk/no, n (%))	19/91 (17.3/82.7)	1/6 (14.3/85.7)	0/1/6 (0/14.3/85.7)	0/2/1 (0/66.7/33.3)	0.175
BMI categories (underweight/normal weight/overweight/obese, n (%))	2/24/60/24 (1.8/21.8/54.5/21.8)	0/0/5/2 (0.0/0.0/71.4/28.6)	0/2/2/3 (0/28.6/28.6/42.9)	0/1/2/0 (0/33.3/66.7/0)	0.781
Smoking status (smoker/quit smoking/non-smoker, n (%))	31/19/60 (28.2/17.4/54.5)	0/0/7 (0.0/0.0/100.0)	2/0/5 (28.6/0/71.4)	0/1/2 (0/33.3/66.7)	0.212
Self-perceived health condition (good/not good, n (%))	48/62 (43.6/56.4)	3/4 (42.9/57.1)	2/5 (28.6/71.4)	0/3 (0/100)	0.421
Self-declared chronic disease (yes/no, n (%))	80/30 (72.7/27.3)	4/3 (57.1/42.9)	6/1 (85.7/14.3)	3/0 (100/0)	0.459
Intake of medication (yes/no, n (%))	19/91 (17.3/82.7)	3/4 (42.9/57.1)	1/6 (14.3/85.7)	2/1 (66.7/33.3)	0.069
Number of medications (-)	1.9 ± 1.7	1.9 ± 1.8	1.9 ± 1.8	3.0 ± 1.7	0.741
Education (no formal/1–8 years/>8 years, n (%))	2/25/83 (1.8/22.7/75.5)	0/2/5 (0.0/28.6/71.4)	1/4/2 (14.3/57.1/28.6)	0/2/1 (0/66.7/33.3)	0.057
Marital status (single/partnership or married/widowed, n (%))	0/88/22 (0/80.0/20.0)	0/6/1 (0/85.7/14.3)	0/5/2 (0/71.4/28.6)	0/1/2 (0/33.3/66.7)	0.002
Financial condition (enough to cover the month/not enough, n (%))	85/25 (77.3/22.7)	4/3 (57.1/42.9)	6/1 (85.7/14.3)	1/2 (33.3/66.7)	0.191

Data shown as the means ± standard deviations or the absolute and relative frequencies. Differences were analyzed by a one-factorial ANOVA test followed by Tukey HSD post hoc tests. Different superscript letters ("[a]" and "[b]") indicate the statistically significant differences. Differences between the categorical data were analyzed by chi-square tests. $p < 0.05$ was considered to be statistically significant. Abbreviations: ASMI (appendicular skeletal muscle index), BMI (body mass index), PP (physical performance), and SMI (skeletal muscle index).

Table 3. Differences between the non-sarcopenic, probable sarcopenic, and sarcopenic male subjects using the Kosovo-specific cut-points following the EWGSOP2 algorithm.

	No Sarcopenia (n = 85)	Probable Sarcopenia (n = 36)	Severe Sarcopenia (n = 6)	p Value
Age (years)	70.7 ± 4.5 [a]	75.0 ± 6.4 [a]	74.2 ± 10.0 [a]	<0.001
Height (m)	1.72 ± 5.51 [a]	1.66 ± 7.51 [a,b]	1.70 ± 4.15 [b]	<0.001
Body mass (kg)	83.0 ± 14.0 [a]	78.0 ± 9.3 [a]	58.7 ± 8.0 [b]	<0.001
BMI (kg/m^2)	27.9 ± 4.1 [a]	28.5 ± 3.6 [a]	20.3 ± 2.1 [b]	<0.001
Whole body fat mass (kg)	25.2 ± 10.6 [a]	24.5 ± 9.1 [a]	13.2 ± 7.5 [b]	0.022
Whole body fat percentage (%)	29.5 ± 7.8 [a]	31.1 ± 9.6 [a]	21.5 ± 10.7 [b]	0.040
Skeletal muscle mass (kg)	31.7 ± 3.9 [a]	27.7 ± 5.2 [b]	22.3 ± 4.6 [c]	<0.001
SMI (kg/m^2)	10.7 ± 1.0 [a]	10.0 ± 1.4 [a]	7.7 ± 1.4 [b]	<0.001
Appendicular skeletal muscle mass (kg)	21.2 ± 2.9 [a]	19.5 ± 2.6 [a]	15.6 ± 1.3 [b]	<0.001
ASMI (kg/m^2)	8.2 ± 0.8 [a]	7.9 ± 0.9 [a]	6.3 ± 0.5 [b]	<0.001
PP score (-)	1.72 ± 2.29 [a]	−1.15 ± 2.27 [b]	−2.19 ± 2.78 [b]	<0.001
Handgrip strength (kg)	39.9 ± 4.5 [a]	26.7 ± 4.7 [b]	24.0 ± 8.7 [b]	<0.001
Relative handgrip strength (kg/kg)	0.49 ± 0.07 [a]	0.35 ± 0.07 [a]	0.43 ± 0.20 [b]	<0.001
Gait speed(m/s)	1.21 ± 0.20 [a]	1.00 ± 0.19 [b]	0.95 ± 0.13 [b]	<0.001
Timed up and go test (s)	6.37 ± 1.39 [a]	7.40 ± 1.90 [a,b]	8.77 ± 2.10 [b]	<0.001
30-s arm curl test (repetitions)	16 ± 3 [a]	12 ± 3 [b]	12 ± 3 [b]	<0.001
30-s chair stand test (repetitions)	12 ± 3 [a]	10 ± 2 [a,b]	10 ± 3 [b]	<0.001
6-min walking test (m)	489 ± 138 [a]	390 ± 113 [a,b]	355 ± 149 [b]	<0.001
Mini nutritional status (-)	26 ± 2 [a]	25 ± 2 [a]	22 ± 3 [b]	<0.001
Malnourished (yes/risk/no, n (%))	12/73 (14.1/85.9)	7/29 (19.4/80.6)	4/2 (66.7/33.3)	0.005
BMI categories (underweight/normal/overweight/obese, n (%))	0/17/50/18 (0.0/20.0/58.8/21.2)	0/6/19/11 (0.0/16.7/52.8/30.6)	2/4/0/0 (33.3/66.7/0.0/0.0)	<0.001
Smoking status (smoker/quit smoking/non-smoker, n (%))	24/14/47 (28.2/16.5/55.3)	5/5/26 (13.9/13.9/72.2)	4/1/1 (66.7/16.7/16.7)	0.055
Self-perceived health condition [good/not good, n (%)]	38/47 (44.7/55.3)	11/25 (30.6/69.4)	4/2 (66.7/33.3)	0.158
Self-declared chronic disease (yes/no, n (%))	62/23 (72.9/27.1)	27/9 (75/25)	4/2 (66.7/33.3)	0.908
Intake of medication (yes/no, n (%))	63/22 (74.1/25.9)	24/12 (66.7/33.3)	4/2 (66.7/33.3)	0.681
Number of medications (-)	1.9 ± 1.7	2.0 ± 1.7	1.0 ± 1.7	0.406
Education (no formal/1–8 years/>8 years, n (%))	0/20/65 (0.0/23.5/76.5)	2/11/23 (5.6/30.6/63.9)	1/2/3 (16.7/33.3/50.0)	0.035
Marital status (single/partnership or married/widowed, n (%))	0/70/15 (0/82.4/17.6)	0/25/11 (0/69.4/30.6)	0/5/1 (0/83.3/16.7)	0.273
Financial condition (enough to cover the month/not enough, n (%))	65/20 (76.5/23.5)	28/8 (77.8/22.2)	3/3 (50.0/50.0)	0.323

Data shown as the means ± standard deviations or the absolute and relative frequencies. Differences were analyzed by a one-factorial ANOVA test followed by Tukey HSD post hoc tests. Different superscript letters ("[a]", "[b]" and "[c]") indicate the statistically significant differences between groups. Differences between the categorical data were analyzed by chi-square tests. $p < 0.05$ was considered to be statistically significant. Abbreviations: ASMI (appendicular skeletal muscle index), BMI (body mass index), PP (physical performance), and SMI (skeletal muscle index).

3.5. Determinants of Sarcopenic States in Male Kosovan Older Adults (Kosovo-Derived Cut-Points)

To model the relationship between the observed sarcopenia categories (no sarcopenia/probable sarcopenia/severe sarcopenia) and several potential predictors (age, BMI, body fat mass, SMM, PP score, MNA score, and self-perceived financial and health conditions), a multinomial logistic regression was performed using only the data from the male

participants. The addition of the predictors to a model that only contained the intercept significantly improved the fit between the model and data [χ^2 (16) = 51.550, Nagelkerke R^2 = 0.538, $p < 0.001$]. Age [χ^2 (2) = 8.604, $p = 0.014$], BMI [χ^2 (2) = 9.557, $p = 0.008$], body fat mass [χ^2 (2) = 9.971, $p = 0.007$], and SMM [χ^2 (2) = 16.043, $p < 0.001$] significantly contributed to the model, whereas PP score [χ^2 (2) = 4.905, $p = 0.086$], MNA score [χ^2 (2) = 0.584, $p = 0.747$], financial condition [χ^2 (2) = 5.431, $p = 0.066$], and health condition [χ^2 (2) = 5.690, $p = 0.058$] did not.

Table 4 presents the results of the unadjusted and adjusted multinomial logistic regression. Being older and having a lower PP score was associated with a higher risk for being severely sarcopenic in both models (age, adjusted model: RR = 1.03, 95%, CI: 1.01–1.04, $p = 0.008$; PP score, adjusted model: RR = 0.94, 95%, CI: 0.86–1.00, $p = 0.039$). A higher BMI (adjusted model: RR = 1.07, 95%, CI: 1.02–1.10, $p = 0.009$) but lower body fat mass (adjusted model: RR = 0.96, 95% CI: 0.93–0.99, $p = 0.007$) were associated with probable sarcopenia, but not with severe sarcopenia. A lower SMM predicted probable (adjusted model: RR = 0.96, 95% CI: 0.92–0.99, $p = 0.023$) as well as severe sarcopenia (adjusted model: RR = 0.94, 95% CI: 0.90–0.98, $p = 0.003$). A lower reported health condition was associated with severe sarcopenia (adjusted model: RR = 0.34, 95% CI: 0.03–0.99, $p = 0.045$). MNA and financial condition did not contribute to sarcopenia status.

Table 4. Multinomial logistic regression predicting sarcopenia status.

	Unadjusted Model		**Adjusted Model**			
	Probable Sarcopenia	**Severe Sarcopenia**	**Probable Sarcopenia**	**Severe Sarcopenia**	**Probable Sarcopenia**	**Severe Sarcopenia**
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**RR (95% CI)**	**RR (95% CI)**
Age (years)	1.099 (0.971–1.244)	1.172 (1.058–1.299) **	1.034 (0.890–1.200)	1.227 (1.054–1.427) **	1.004 (0.984–1.023)	1.025 (1.007–1.042) **
BMI (kg/m^2)	1.067 (0.908–1.254)	1.012 (0.868–1.179)	1.928 (1.182–3.147) **	1.351 (0.896–2.039)	1.069 (1.021–1.100) **	1.036 (0.985–1.073)
Body fat mass (kg)	0.978 (0.900–1.062)	0.986 (0.922–1.054)	0.784 (0.658–0.935) **	0.864 (0.725–1.029)	0.965 (0.935–0.991) **	0.979 (0.952–1.004)
SMM (kg)	0.836 (0.711–0.984) *	0.775 (0.667–0.900) ***	0.770 (0.615–0.965) *	0.693 (0.546–0.880) **	0.962 (0.923–0.995) *	0.944 (0.900–0.982) **
PP score (-)	0.814 (0.606–1.094)	0.611 (0.461–0.811) ***	0.959 (0.621–1.481)	0.659 (0.443–0.980) *	0.994 (0.924–1.045)	0.935 (0.856–0.997) *
MNA score (-)	0.978 (0.716–1.336)	0.893 (0.695–1.147)	0.986 (0.613–1.586)	1.210 (0.717–2.045)	0.998 (0.922–1.052)	1.024 (0.950–1.073)
Financial condition [a]						
Not enough to cover the month	0.392 (0.082–1.870)	0.686 (0.165–2.851)	0.106 (0.010–1.125)	0.156 (0.012–1.949)	0.469 (0.070–1.015)	0.580 (0.086–1.070)
Health condition [b]						
Not good	0.969 (0.207–4.535)	0.323 (0.066–1.591)	0.692 (0.087–5.486)	0.063 (0.004–0.941) *	0.944 (0.417–1.123)	0.335 (0.031–0.992) *

No sarcopenia was chosen as the reference group for the outcome. [a] Reference category is "enough to cover the month"; [b] Reference category is "good". In the unadjusted model, each variable was independently tested as a predictor of sarcopenia status. In the adjusted model, all the variables were tested in the same model, controlling the effect of each other. The RR was estimated from the OR using the conversion formula from Zhang and Yu [32]. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.001$. OR, odds ratio; RR, relative risk; CI, confidence interval; BMI, body mass index; SMM, skeletal muscle mass; PP, physical performance, MNA, Mini Nutritional Assessment.

4. Discussion

This study aimed to identify the impact of different diagnostic criteria (the initial EWGSOP1 and the revised EWGSOP2) and the recommended and population-specific cut-points on sarcopenia and its conceptual stages in adults aged 60 years and older from a developing European country. Furthermore, it intended to evaluate the impact of socio-economic,

health-related, and lifestyle factors on sarcopenia status. The main findings identify the state of sarcopenia within the sample of ageing Kosovans being low, notwithstanding the used algorithms and diagnostic criteria (EWGSOP1, EWGSOP2, and the population-specific cut-points) and the used variable as a key characteristic (SMI vs. muscle strength). Furthermore, even with the lowering of the diagnostic thresholds in the revised EWGSOP2 guideline, no consequences were observed. While rather similar outcomes (0.8%, 2.9%, and 0.0%) were observed in the sarcopenic (EWGSOP1, EWGSOP2, and population-specific cut-points, respectively) and severe sarcopenic (2.5%, 1.3% and 2.5%, respectively) cases, differences were observed in the pre-sarcopenic/probable sarcopenic (2.1%, 5.4% and 22.9%) cases. With respect to this conceptual stage, the EWGSOP2 algorithm (applied in the EWGSOP2 and population-specific cut-points) was able to detect a generally higher percentage, particularly when using the population-specific cut-points. In all cases of pre-sarcopenia/probable sarcopenia (EWGSOP1, EWGSOP2 and population specific), females presented lower percentages (0.9%, 5.3% and 16.8%) in comparison to males (3.1%, 5.5% and 28.3%), and no algorithm could detect sarcopenic or severe sarcopenic females.

It is important to mention that aside from the availability of the various diagnostic cut-points for defining sarcopenia, there is a prevailing lack of general consensus on a robust definition that identifies sarcopenic individuals in different ethnic groups [12]. Furthermore, it has been suggested that until this global consensus is reached, prevalence data should be reported and interpreted within its own context [33]. Upon the publication of the revised EWGSOP2 consensus criteria [3], potentially different outcomes when comparing previous criteria have been investigated [34–39]. Reis et al. [34] were among the first to analyze the consequences of applying the new EWGSOP2 guideline instead of the former EWGSOP1 for sarcopenia case finding in older geriatric inpatients and described a substantial mismatch with a significant lowering of the number of men diagnosed with sarcopenia. The discord in prevalence from the lower numbers of the EWGSOP2 have been further explored and reported by other studies [35–37,39–41]. Besides the several distinguished differences between the two versions (lowering the diagnostic thresholds for isometric handgrip strength and muscle mass, the introduction of new suggested measurement methods for muscle strength, etc.), the shift towards muscle strength from muscle mass (as the major component) probably presents the greatest reason behind the differences.

Inconsistent results exist with respect to the association between sex and sarcopenia [42]; the lower number of (severe) sarcopenic females in this study could be attributed to several intermediate covariates, such as being younger and shorter, similar weight, and consequently a higher BMI. The very high percentage of overweight and obese cases observed in the total participants (43.3% and 41.7%, respectively) together with the higher levels of average BMI, especially among females, could explain the potential impact on the general sarcopenia results. A recent study investigating the potential co-occurrence of several age-related issues including sarcopenia, physical frailty, undernutrition, and obesity found a rather low coexistence between them all, suggesting for a need to assess them individually [43]. These concerning higher overweight and obesity levels could also be one of the determinants for the lower sarcopenic participants in the form that has been previously explained as the "obesity paradox" [44]. This became evident especially after having no cases of sarcopenic obesity. Regarding the prevalence of overweight and obese cases, the rates have been described to vary within European populations, from as low as 53.9% (20.9% male and 16.5% female) and 55.0% (38.7% male and 16.3% female) in Switzerland and Denmark, respectively, to up to 64.0% (40.9% male and 23.1% female) and 67.4% (46.5% male and 20.9% female) in Germany and Spain, respectively [45]. An interesting study from four different countries (Canada, Brazil, Colombia, and Albania) has shown that Albanian men and women aged 65–74 years presented the highest prevalence of overweight and obesity (46.7% and 36.3%) in comparison to their peers [46]. Despite coming from a very specific age group (65–74 years old), Albanian seniors may present the closest similar and comparable population to ours (Kosovo Albanians form the major ethnic group in the country at 95%) [17]; this provides grounded support for our findings.

The potential link between the lower number of sarcopenic females (within all conceptual stages) with higher percentages of overweight, obesity, and malnourishment (or being at risk for) might be explained by the significantly lower level of education and financial condition in females, as well as the higher medication intake and number of medications despite no higher level of chronic diseases compared with the males.

In contrast to the females, despite being older, males performed physically better in all physical performance tests except for the 30-s arm curl, where sex-specific weights were applied. The fact that males perform better than their female peers within the same age group in most performance measures has already been described [47], but one must consider that the males were significantly older than the females in the current study. Besides the differences in sex, the high percentages of obesity in females may serve as a further triggering factor, especially due to its inverse association with physical ability [48]. Despite the sex-specific differences in the sarcopenia-related components, overweight and obese instances might also be related and explained through genetic predisposing factors [49], notably through the suggested potential influence that a certain single nucleotide polymorphism (ACTN3 R577X) might exert on knee extensors' peak torque and BMI, particularly in females [50]. However, not only the gene × gene, but the gene × environment interactions must be further studied to better analyze the interaction.

The strengths of this study lay in the fact that it followed the revised 2019 version of EWGSOP(2); higher age, lower height, SMM, SMI, and ASMM present reliable parameters that significantly change between the different conceptual stages of sarcopenia, lowering as one observes from the non-sarcopenic group to the probable sarcopenic and sarcopenic/severe sarcopenic groups. With respect to the ASMI, it should be noted that it is directly dependent on the subject's height, which can become less reliable when having a significantly shorter, yet overweight or obese population. Furthermore, the EWGSOP2 are also accompanied by a lowering of physical performance parameters (gait speed, timed up and go, 30-s arm curl, 30-s chair stand, and 6-min walking test) and muscle strength measurements (handgrip strength), which are all significantly related to the occurrence of sarcopenia. Additionally, population-specific cut-off points were able to detect much higher rates of probable sarcopenia than the EWGSOP2-suggested cut-off points. However, this was made possible mainly due to the higher values for the population-specific cut-off points. While taking into consideration the suggestion that the cut-off points used for the muscle mass could affect the reported prevalence rates for sarcopenia [51], the revised EWGSOP2 guidelines' emphasis should be given with respect to muscle strength.

A recent study [34] raised the point that the importance of the lower number of SMM measurements (in this case using DXA) required in EWGSOP2 guidelines undoubtedly presented an advantage in terms of both availability and cost. While observing the same situation with respect to the Inbody measurements in the community-dwelling older adults while following the EWGSOP2 guidelines, using the suggested key characteristic of strength over muscle mass as a better indicator of the adverse clinical outcomes of mortality and low physical performance [3,52,53] should positively impact the case finding process, particularly in developing lower- and middle-income countries. Therefore, future updates and revisions of the diagnostic approaches (for different populations and population settings) should seriously consider following the EWGSOP2 pathway or attempt to adjust towards using the same logic in order to enhance and offer more robust diagnostic approaches.

Despite performing this study to the best of our scientific knowledge, some limitations emerged that were out of our reach. The first is in regards to the assessment method of muscle mass. Among the several methods available, the usage of BIA in this study instead of DXA, which presents a widely available non-invasive instrument [3,54] and has also been suggested as a reference standard [54], might have overestimated the muscle mass [55]. One study has even suggested the possibility of standing multi-frequency BIA (the same as used in this study) overestimating the fat mass percentage in women with higher BMIs in comparison to both the supine BIA and DXA methods [56]. With respect to the different forms of BIA, good inter-unit precision and no differences between the standing and supine

BIA were reported [56,57]. However, BIA as a technique was shown to have an acceptable accuracy [58,59], was recommended for use in research [3], presented a high degree of correlation with the DXA approach [20,60] and magnetic resonance imaging (MRI) [61] in the muscle mass assessment. This, with smaller differences within European populations in comparisons to other methods, all while being affordable, widely available, and portable. Of note, MRI and computerized tomography (CT) techniques, despite being considered the gold standard for a non-invasive assessment of muscle mass, are not commonly used in practice mainly due to their high cost, lack of portability, need for specialized experts [62], and not yet well-defined cut-off points [3]. Another potential limitation of the study was the multivariate regression formula that we used for the DXA estimation of BIA muscle mass measurements, which was initially used for a different population (Koreans) [20]. Lastly, the number of participating subjects might present a limitation for this study, representing less than 1% of the eligible population (within the region) and thus resulting in a margin of error of approximately 6.3% when considered to be a representative population. Nevertheless, besides the inverse relationship of the margin of error with the sample size, the upper threshold for an acceptable marginal error of an estimated sample size has been reported to be not more than 7% at the 97% confidence level [63]. In this context, we believe that the outcomes from this study may provide preliminary data and anticipate potential future directions to follow, according to the current state-of-the-art within the field of the age-related decline of muscle mass and functioning in ageing adults from a developing European country. Furthermore, there is a need to conduct bigger epidemiological studies within these populations in order to further explore and clarify the situation. This must be preceded by recruiting a representative sample of young participants in order to develop and determine more accurate population-specific cut-off points.

5. Conclusions

The revised EWGSOP2 algorithm (applied in the EWGSOP2 and population-specific diagnostic criteria) was able to detect generally higher percentages of the pre-sarcopenia/probable sarcopenia conceptual stages, particularly when using the population-specific cut-points. Males presented higher rates and better performance, despite their older age. Higher rates of overweight and obesity could be contributing to the lower number of sarcopenic/severe sarcopenic females, which could also be providing a preventative role in the total population. However, sarcopenia presents a health condition and a growing health-related concern with the ageing of the world population, particularly when taking into consideration the serious health implications that it unveils. To date, prevention and early detection present the strongest instruments within our reach to tackle this issue. Additionally, the introduction and promotion of sarcopenia together with its implications is mandatory for healthcare providers and others directly involved in dealing with older persons in developing countries. Additionally, population-specific diagnostic cut-off points should be developed for different populations to create a very accurate and versatile instrument for screening and diagnostic purposes. Further studies are required to examine the impact of weight on sarcopenia in the study's population.

The higher levels of overweight and obesity that were found in the study participants (Kosovan older adults) should be taken as a serious concern of public health, keeping in mind that the obesity epidemic in industrialized countries displays a very critical risk factor for chronic diseases and has a heavy impact on health, quality of life, and life expectancy [64]. Considering these facts, the very high level of overweight and obesity in our study population could be one of the direct factors triggering the main cause of death in Kosovo (cardiovascular diseases) [65], consequently resulting in the lower expected life expectancy at birth in this country.

Author Contributions: Conceptualization, A.B. (Arben Boshnjaku) and B.W.; data curation, A.B. (Arben Boshnjaku), E.K., and B.W.; formal analysis, A.B. (Abedin Bahtiri) and K.F.; funding acquisition, B.W.; investigation, A.B. (Arben Boshnjaku), A.B. (Abedin Bahtiri), K.F. and E.K.; methodology, A.B. (Arben Boshnjaku), H.T. and B.W.; project administration, B.W.; resources, A.B. (Arben Boshn-

jaku) and B.W.; supervision, B.W.; validation, A.B. (Abedin Bahtiri), K.F., E.K. and B.W.; visualization, B.W.; writing—original draft, A.B. (Arben Boshnjaku); writing—review and editing, A.B. (Arben Boshnjaku), A.B. (Abedin Bahtiri), K.F., E.K., H.T. and B.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Higher Education and Research in Applied Sciences (HERAS) under Grant No. K-02-2017 and by the Universi College Prishtina (Kosovo) under Grant No. 2802. A Ph.D. completion fellowship was provided to Arben Boshnjaku by the Vienna Doctoral School of Pharmaceutical, Nutritional, and Sport Sciences, University of Vienna (VDS PhaNuSpo). This article was supported by the Open Access Publishing Fund of the University of Vienna.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical-Professional Committee of the University Clinical Centre of Kosovo (protocol code 1246/14.09.2016).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: Open Access Funding by the University of Vienna.

Conflicts of Interest: The authors declare no conflict of interest.

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5.1.4 Publication 3: ACTN3 genotypes and their relationship with muscle mass and function of Kosovan adults

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Status:

Published by *International Journal of Environ Research and Public Health* on 30 August, 2021. 18(17):9135. Special Issue: "Sport, Exercise and Health across the Lifespan". https://doi.org/10.3390/ijerph18179135

Personal contribution

- Conceptualization;
- Data curation;
- Investigation;
- Methodology;
- Writing – original draft.

International Journal of
Environmental Research and Public Health

MDPI

Article

ACTN3 Genotypes and Their Relationship with Muscle Mass and Function of Kosovan Adults

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Abstract: Maintaining muscle mass and function is important throughout the lifestyle. While environmental factors such as physical activity and healthy nutrition are well investigated, the contribution of genetic factors is still controversial. Therefore, we aimed to investigate the impact of a common ACTN3 polymorphism (rs1815739) on body composition, handgrip strength, knee extensor peak torque, and physical performance (gait speed, 30-s arm curl, 30-s chair stand) in Kosovan adults. In total, 308 participants (160 females and 148 males, age range from 40 to 91 years) took part in this cross-sectional study. Genomic DNA was extracted from saliva and assessed for ACTN3 genotype distribution (41.5% of RR, 53.9% of RX and 4.6% of XX). Genotype allocation did not account for differences in any of the variables. Interestingly, female XX carriers were taller ($p = 0.025$) and had a higher isokinetic knee extension peak torque ($p = 0.024$) than the RX+RR group. In males, XX carriers were also taller ($p = 0.049$) and had a lower BMI ($p = 0.026$), but did not differ in any of the strength and performance parameters. These results indicate that the ACTN3 R577X polymorphism might exert a sex-specific impact on knee extensor peak torque and BMI.

Keywords: ageing; muscle strength; physical performance; genotypes; ACTN3 R577X

Citation: Boshnjaku, A.; Krasniqi, E.; Tschan, H.; Wessner, B. ACTN3 Genotypes and Their Relationship with Muscle Mass and Function of Kosovan Adults. *Int. J. Environ. Res. Public Health* **2021**, *18*, 9135. https://doi.org/10.3390/ijerph18179135

Academic Editor: Paul B. Tchounwou

Received: 30 July 2021
Accepted: 27 August 2021
Published: 30 August 2021

1. Introduction

Muscle mass and function have been shown to decline with increasing age, especially beyond 50 years, where a yearly decline of about 1–2% in muscle mass, 1.5–5% in muscle strength, and 3.5% in muscle power can be expected [1,2]. The importance of strength and power are emphasized particularly in maintaining the quality of life and independence for daily activities of life, as well as prohibiting falls, fractures, morbidity, and even mortality. Therefore, it is not surprising that these parameters are also used as diagnostic factors for sarcopenia, a progressive and generalized skeletal muscle disorder associated with ageing and immobility [3,4].

Many changes underlying the age-associated decay of the musculoskeletal system can be understood at the cellular and molecular level [5]. Muscle fiber numbers and size decrease with age with a disproportional shrinking of fast-twitch type II fibers being especially important for power-related muscle traits [6]. In addition, genetic factors have been studied for their potential relation with power-related muscle traits. However, relatively few of the investigated genotypes have been proven to significantly contribute towards a variation in muscle phenotypes [7]. The identification of genetic variants with the potential to influence muscle phenotypes is of interest not only for elite athletes, but also for its potential to identify persons at risk and to develop individualized therapeutic strategies [8,9]. With respect to muscle function, much attention has been paid to the ACTN3 gene, expressing one of two major components of the contractile apparatus at the Z-line (alpha-actinin-3

protein) and being found only in fast twitch type II fibers [10]. A common null polymorphism of ACTN3 (R577X-rs1815739) is responsible for the fact that individuals homozygous for the X allele are unable to express α-actinin-3 in contrast to those with the RX or RR genotype [11]. Despite the growing body of evidence suggesting the potential modifying effect of ACTN3 genotypes [12] on muscle strength [13–17], mass [13,18–20], power [13,21], and functional performance [17,22–24] in older populations, inconsistency exists amongst studies [7,19,25–28]. Thereby, several studies go in line with the physiological expectations and show an increased muscle mass [18,20], strength [14,16,23], power [22], or physical performance [22] in individuals carrying the R allele. However, these results are contrasted by studies revealing better results for XX individuals [17,21], studies with mixed results for different parameters [13] or with respect to trainability [16,19].

Furthermore, the frequency distribution of the ACTN3 XX genotype differs across different populations, while averaging from approximately 25% in Sweden, 21.6% in Italy, 18.3% in Greece, 10.4% in Lithuania, to as low as 1% in Kenya, 1% in Bantu, and 0% in Nigeria (Yoruba) [29]. Therefore, the main aim of this study was to acquire population-specific ACTN3 genotype distributions in Kosovo and to investigate their distinct characteristics and sex-based relationships to various parameters of muscle mass, strength, and function.

2. Materials and Methods

2.1. Participants

Males and females with Kosovan nationality aged 40 years and above and living in or in the surroundings of Prishtina, the largest of seven regions of Kosovo with a total of 477,312 inhabitants, were eligible to take part in the study [30]. Recruitment involved national broadcasting as well as written and verbal announcements in the Kosovo Pensioners' Association and Prishtina Nursing Homes in Prishtina and Fushë Kosova (neighboring municipalities). Participants were allowed to bring their partners. Exclusion criteria represented health conditions that would not allow to perform exercise as screened by physical activity readiness questionnaire (PAR-Q) [31], the presence of chronic diseases that could impair the measurements of physical performance such as serious cardiovascular diseases (i.e., decompensated chronic heart failure, severe or symptomatic aortic stenosis treated, pacemakers, unstable angina, cardiac arrhythmias, diabetic retinopathy) or any other similar conditions that could prohibit subjects performing measurements [32]. Inclusion and exclusion criteria were assessed by two health professionals at the entrance examination.

Following detailed explanations of the testing procedures, written informed consents were obtained from all participants. The study protocol was reviewed and approved by the Ethical-Professional Committee of the University Clinical Centre of Kosovo (no. 1246/15.09.2016), whereas all procedures were performed in accordance with the guidelines of the Declaration of Helsinki for Human Research.

Data were collected during two separate visits by trained assessors. At first health status, socioeconomic, nutritional, and physical activity levels together with anthropometric measurements, muscle strength, isokinetic peak torque and gait speed, were assessed. On the second visit, saliva samples for isolating DNA were collected from the study participants.

2.2. Anthropometric, Muscle Strength, Isokinetic Peak Torque, and Physical Performance

2.2.1. Data Collection and Anthropometric Assessments

Following the previously set protocol, data collection was organized at the Sports Medicine Laboratory in Universi College in Prishtina. Test–retest reliability of all the used assessments was evaluated and described previously [33]. Anthropometric measurements were performed in accordance to the International Standards for Anthropometric Assessment [34] by the same research team during the entire data collection period.

Height was measured barefoot wearing light indoor clothing, using a portable stadiometer and following the stretch stature method (DT05L, Kinlee, Zhongshan Jinli Electronic Weighing Equipment Co. Ltd., Guangdong, China). Weight and body composition

(including whole-body skeletal muscle mass (SMM) and fat mass) were measured using a segmental multifrequency bioelectrical impedance analyser (BIA) (Inbody 770 device, Biospace Co., Ltd., Seoul, Korea) with data output calculated by manufacturer's algorithm. This process required the participants to hold the hand electrodes while standing upright above the tactile foot electrodes. Furthermore, body mass index (BMI) was determined as body mass by height squared (expressed as kg/m^2) [35].

2.2.2. Handgrip Strength and Isokinetic Knee Extensor Peak Torque Assessments

Isometric handgrip strength was assessed in a sitting position through recording the best of two trials by maximally squeezing the adaptable dynamometer (JAMAR, Patterson Medical, Warrenville, IL, USA) with the self-reported dominant hand (1 min rest in between trials) [36]. Isokinetic dynamometry [37] was used to assess the absolute and relative knee extensors isokinetic concentric peak torque at 60°/s unilaterally on the self-reported dominant side (Biodex System 4 Pro, Biodex Medical Systems, Inc., Shirley, NY, USA). During the assessment, participants were fixed on the device's chair with straps over the shin, thigh, pelvic, and crossed above upper torso, while holding the supporting sideway handles [38].

2.2.3. Functional Performance Measurements

Physical performance was assessed through six-meter gait speed (m/s) for usual walking speed, 30-s chair stand test (reps) for lower body strength (endurance) and 30-s arm curl test (reps) for upper body strength (endurance) [36,39]. Gait speed was assessed over six meters on a course 10 m in length, using the first two meters for acceleration and the last two meters for deceleration. Time to pass the middle six meters was taken by means of a stopwatch. The usage of a cane was only allowed if a participant expressed the absolute necessity to use it while performing (n = 4). The 30-s chair-stand test was administered as described by Jones and Rikli [39] by instructing the participants to stand up and sit down as often as possible within 30 s from a 46 cm high armless chair placed against the wall. Similarly, the 30-s arm curl test was used to assess the number of repetitions for weight-loaded (5 pounds for woman and 8 pounds for men) elbow flexion and extension of the self-reported dominant arm, while the participants were sitting on an armless chair [39]. In both tests (30-s chair-stand and arm curl), the tester stood next to the participant while signaling the starting and ending time points and counting the number of successful repetitions, including the very last attempt if more than 50% of the range of motion was mastered before the timeline.

2.3. Genetic Analyses

Participants were asked to refrain from eating, drinking, smoking, brushing teeth, or chewing gum for 30 min before the sample collection. Every participant spit about 2 mL of saliva in a tube prefilled with 2 mL stabilization buffer. The tubes were thoroughly mixed and stored at −20 °C for not longer than three months. After finalizing sample collection, they were sent for further analyses to the Laboratory of Molecular Exercise Physiology at the Centre for Sport Sciences and University Sports, University of Vienna, Austria. Genomic DNA was extracted from the saliva samples using GeneFiX™ Saliva-Prep DNA Isolation Kit (Isohelix, Kent, UK) following the manufacturer's instructions. Quantity (58.07 ± 57.02 ng/mL) and quality (A_{260}/A_{280}: 1.77 ± 0.17) of DNA were checked on a NanoDrop spectrophotometer (Peqlab, Erlangen, Germany). From the isolated DNA samples, quantity, and quality of 282 samples were high enough to perform genotyping. Genotyping was performed using real time PCR (QuantStudio 7 Flex real-time PCR System, ThermoFisher Scientific, Vienna, Austria) and a commercially available TaqMan™ SNP genotyping assay to detect the ACTN3 R577X polymorphism (rs1815739, C_590093_1, ThermoFisher Scientific, Vienna, Austria). According to the manufacturer's instruction, a reaction mix was prepared containing 2.50 µL of 2X TaqMan™ Master Mix and 0.25 µL of 20X Assay Working Stock per well. Each DNA sample was diluted with DNase free water

to deliver 20 ng of DNA per well. Total reaction volume was set to 5 µL and reactions were performed on a MicroAmp™ Optical 384-Well Reaction Plate (ThermoFisher Scientific, Vienna, Austria). Samples were analyzed in triplicates and a no-template control with DNase-free water instead of a DNA sample was run on each plate. A pre-PCR plate read was performed to record the background fluorescence of each well. Cycling conditions involved 95 °C for 10 min for polymerase activation, and 40 cycles with alternating steps at 95 °C for 15 s followed by 60 °C for 1 min. Finally, fluorescence was detected at the post-PCR plate read and used to determine the genotypes of the samples.

2.4. Statistical Analyses

All statistical evaluations were performed by the SPSS statistical package version 26.0. As normal distribution was violated for many of the parameters, data were expressed as medians and interquartile ranges (75th minus 25th percentile) in case of continuous variables and as absolute numbers or percentages for frequencies of categorical variables. The non-parametric Mann–Whitney U test was used to determine potential differences between two groups, whereas Kruskal–Wallis test was used to evaluate differences between the three genotypes. p values below 0.05 were considered as being statistically significant.

Hardy–Weinberg equilibrium (HWE) was calculated using chi-squared (χ^2) statistics with one degree of freedom, comparing the observed distribution of genotypes with the distribution of genotypes expected from applying the Hardy–Weinberg equilibrium assumption (using the online application: https://ihg.gsf.de/cgi-bin/hw/hwa1.pl (accessed on 30 August 2019)).

3. Results

In total, 308 participants (160 females and 148 males, age range from 40 to 91 years) could be recruited via media (n = 68) or the Kosovo Pensioners' Association and Prishtina Nursing Homes (n = 240) between September 2016 and November 2017. From these participants, 299 persons of Caucasian descent agreed to provide a salivary DNA sample. Unfortunately, further 17 samples (5.7%) could not be genotyped based on the low quality of the DNA sample. Therefore, 282 participants (91.6%) were included in the final analyses. Participants' general characteristics for the total genotyped sample and separated by sex are summarized in Table 1.

Table 1. Participants' characteristics.

Variable (Units)	Genotyped Sample N = 282	Females N = 149	Males N = 133	p-Value
Age (years)	67.6 (60.7–72.7)	65.2 (57.6–70.2)	70.3 (66.4–74.3)	<0.001
Body weight (kg)	77.6 (70.8–86.5)	76.8 (68.4–83.9)	79.8 (73.4–88.1)	0.006
Height (m)	1.64 (1.58–1.72)	1.59 (1.54–1.63)	1.72 (1.66–1.75)	<0.001
BMI (kg/m^2)	28.98 (26.40–32.21)	30.16 (27.44–34.05)	27.53 (25.06–30.03)	<0.001
Skeletal muscle mass (kg)	25.8 (22.6–30.4)	23.3 (21.0–25.3)	30.7 (27.1–34.6)	<0.001
Fat mass (kg)	28.4 (21.6–35.7)	32.5 (26.7–39.4)	24.4 (18.8–29.1)	<0.001
Fat percentage (%)	36.9 (29.2–44.2)	43.3 (37.9–48.2)	30.1 (24.7–35.1)	<0.001
Handgrip strength (kg)	29.3 (24.1–37.5)	25.7 (21.7–28.6)	37.4 (30.9–42.8)	<0.001
Relative handgrip strength (kg/kg)	0.39 (0.31–0.48)	0.33 (0.28–0.39)	0.45 (0.39–0.53)	<0.001
Isokinetic knee extensors peak torque (60°/s)	69.0 (44.8–96.5)	55.3 (39.7–80.8)	87.8 (58.4–119.4)	<0.001
Relative knee extensors peak torque (60°/s/kg)	0.91 (0.59–1.26)	0.74 (0.52–1.05)	1.1 (0.78–1.44)	<0.001
Gait speed (m/s)	1.10 (0.94–1.24)	1.05 (0.89–1.2)	1.15 (0.99–1.28)	0.001
30-s arm curl (reps)	14 (12–17)	14 (12–17)	15 (12–17)	0.423
30-s chair stand (reps)	12 (9–14)	11 (9–14)	12 (10–14)	0.085

Notes: Data are expressed as medians (25th–75th percentile). Differences between males and females have been calculated by Mann–Whitney U test. Abbreviations: BMI, body mass index; reps, repetitions.

Differences between males and females in the genotyped participants were observed for age (U = 13667.5, Z = 5.499, $p < 0.001$), body weight (U = 11783.0, Z = 2.742, $p = 0.006$), height (U = 17689.5, Z = 11.402, $p < 0.001$), BMI (U = 6543.5, Z = −4.922, $p < 0.001$), SMM (U = 17413.0, Z = 11.149, $p < 0.001$), body fat mass (U = 4988.0, Z = −7.128, $p < 0.001$), body fat percentage (U = 2563.0, Z = −10.695, $p < 0.001$), handgrip strength (U = 16963.0,

Z = 10.319, $p < 0.001$), relative handgrip strength (U = 15954.5, Z = 8.844, $p < 0.001$), knee extensors peak torque (U = 14559.5, Z = 6.803, $p < 0.001$), relative knee extensors peak torque (U = 14007.5, Z = 5.996, $p < 0.001$), and gait speed (U = 12210.0, Z = 3.367, $p = 0.001$), whereby higher values were obtained for males except for BMI and fat mass. No differences were found for 30-s arm curl (U = 10454.0, Z = 0.801, $p = 0.423$) and 30-s chair stand test (U = 11079.0, Z = 1.721, $p = 0.085$).

ACTN3 genotype distribution of the total sample was 41.5% RR (n = 117), 53.9% RX (n = 152), 4.6% XX (n = 13) whereas the allelic frequencies were 0.68 and 0.32 for the R and X alleles, respectively. Noteworthy, the genotype distribution for the entire cohort did not meet HWE ($\chi^2 = 17.09$, $p < 0.001$), whereby the inbreeding factor (F) was detected to be negative (F = −0.248). Participants' characteristics for the ACTN3 genotypes (RR, RX, and XX), as well as the dominant (RR + RX) and recessive (RX + XX) models with the matched genotypes (XX and RR, respectively) are shown in Table 2 for total participants, Table 3 for females and Table 4 for males. The three genotype groups did not differ in any of the measured variables, neither in the total cohort nor separated by sex ($p > 0.05$) as evidenced by Kruskal–Wallis tests. For the dominant model, no differences were detected between RR+RX and XX for any of the measured parameters ($p > 0.05$) besides age with participants carrying the XX genotype being slightly older (U = 2323.5, Z = 2.002, $p = 0.045$). This difference was not confirmed when the total group was separated by sex (females: U = 763.0, Z = 1.676, $p = 0.094$; males: U = 444.5, Z = 1.473, $p = 0.141$). However, within the female subgroup, the XX carriers (n = 8, 5.4%) were also taller (U = 830.5, Z = 2.248, $p = 0.025$) and had a higher isokinetic knee extension peak torque (U = 832.5, Z = 2.261, $p = 0.024$). Furthermore, the median skeletal muscle mass was higher, though not significantly (U = 787.0, Z = 1.878, $p = 0.060$). In males, XX carriers (n = 5; 3.8%) were again taller (U = 486.5, Z = 1.972, $p = 0.049$) and had a lower BMI (U = 132.0, Z = −2.224, $p = 0.026$). However, these data need to be regarded with care given the very low number of XX carriers in the total population.

For the recessive model, RR carriers (N = 117, 41.5%) did not differ from RX+XX carriers in any of the variables, neither in the total cohort nor separated by sex ($p > 0.05$).

Table 2. Total participants' characteristics by ACTN3 genotypes.

Variable (Units)	RR (N = 117)	RX (N = 152)	XX (N = 13)	*p*-Value	RR + RX (N = 269)	XX (N = 13)	*p*-Value	RR (N = 117)	RX + XX (N = 165)	*p*-Value
Gender (f/m, [%f])	60/57 [51.3% f]	81/71 [53.3% f]	8/5 [61.5% f]	0.771	141/128 [52.4% f]	8/5 [61.5% f]	0.520	60/57 [51.3% f]	89/76 [53.9% f]	0.660
Age (years)	68.1 (62.4–71.8)	67.1 (59.4–73.0)	72.3 (67.5–75.3)	0.134	67.4 (60.5–72.4)	72.3 (67.5–75.3)	0.045 *	68.1 (62.4–71.8)	67.4 (59.8–73.4)	0.809
Body weight (kg)	78.2 (70.4–8.3)	77.2 (70.7–86.0)	80.4 (72.5–83.8)	0.662	77.3 (70.6–86.7)	80.4 (72.5–83.8)	0.563	78.2 (70.4–8.3)	77.3 (71.0–85.5)	0.561
Height (m)	1.64 (1.58–1.71)	1.64 (1.58–1.71)	1.65 (1.62–1.75)	0.309	1.64 (1.58–1.71)	1.65 (1.62–1.75)	0.128	1.64 (1.58–1.71)	1.64 (1.58–1.72)	0.640
BMI (kg/m^2)	29.3 (26.0–33.0)	28.8 (26.6–32.1)	27.5 (25.0–30.8)	0.598	29.0 (26.5–32.4)	27.45 (25.0–30.8)	0.406	29.3 (26.0–33.0)	28.8 (26.5–32.0)	0.468
Skeletal muscle mass (kg)	25.9 (22.7–30.4)	25.6 (22.1–30.2)	27.2 (24.7–32.4)	0.438	25.8 (22.5–30.3)	27.2 (24.7–32.4)	0.214	25.9 (22.7–30.4)	25.7 (22.6–30.6)	0.925
Fat mass (kg)	27.8 (20.8–36.2)	28.6 (22.3–35.2)	28.3 (19.5–34.1)	0.970	28.4 (21.6–35.9)	28.3 (19.5–34.1)	0.854	27.8 (20.8–36.2)	28.6 (22.4–35.0)	0.844
Body fat percentage (%)	36.4 (28.5–45.0)	37.4 (29.6–44.0)	38.7 (24.5–41.8)	0.957	36.8 (29.2–44.3)	38.7 (24.5–41.8)	0.766	36.4 (28.5–45.0)	37.5 (29.7–44.0)	0.964
Handgrip strength (kg)	29.0 (24.4–37.2)	29.7 (23.3–37.3)	32.0 (26.6–38.4)	0.673	29.2 (23.9–37.2)	32.0 (26.6–38.4)	0.396	29.0 (24.4–37.2)	30.0 (23.5–37.6)	0.673
Relative handgrip strength (kg/kg)	0.38 (0.31–0.48)	0.39 (0.31–0.47)	0.36 (0.32–0.50)	0.810	0.39 (0.31–0.47)	0.36 (0.32–0.50)	0.667	0.38 (0.31–0.48)	0.39 (0.31–0.47)	0.578
Isokinetic knee extensors peak torque (60°/s)	70.6 (44.5–94.0)	68.3 (43.8–97.1)	68.7 (49.7–98.8)	0.635	69.2 (44.1–96.3)	68.7 (49.7–98.8)	0.580	70.6 (44.5–94.0)	68.6 (45.0–97.4)	0.388
Relative isokinetic knee extensors peak torque (60°/s/kg)	0.89 (0.58–1.19)	0.94 (0.60–1.30)	0.88 (0.65–1.18)	0.537	0.91 (0.58–1.28)	0.88 (0.65–1.18)	0.817	0.89 (0.58–1.19)	0.92 (0.62–1.30)	0.265
Gait speed (m/s)	1.08 (0.94–1.23)	1.12 (0.94–1.25)	1.09 (1.00–1.33)	0.682	1.11 (0.94–1.24)	1.09 (1.00–1.33)	0.598	1.08 (0.94–1.23)	1.12 (0.94–1.25)	0.434
30-s biceps curl (reps)	14 (12–17)	15 (12–17)	14 (13–17)	0.933	15.00 (12–17)	14 (13–17)	0.961	14 (12–17)	15 (12–17)	0.711
30-s chair stand (reps)	14 (9–14)	12 (9–14)	12 (9–15)	0.958	12 (9–14)	12 (9–15)	0.982	14 (9–14)	12 (9–14)	0.770

Notes: Data are expressed as medians (25th–75th percentile), absolute numbers (percentages). Differences between genotypes were determined by Kruskal–Wallis or Mann–Whitney U test. Asterisks denote significant differences with * $p < 0.05$. Abbreviations: f, females; m, males; BMI, body mass index; reps, repetitions.

Table 3. Female participants' characteristics by ACTN3 genotypes.

Variable (Units)	RR (N = 60)	RX (N = 81)	XX (N = 8)	*p*-Value	RR + RX (N = 141)	XX (N = 8)	*p*-Value	RR (N = 60)	RX + XX (N = 89)	*p*-Value
Age (years)	64.5 (58.1–69.0)	65.6 (56.1–70.2)	71.3 (61.1–73.3)	0.210	64.7 (56.8–69.8)	71.3 (61.1–73.3)	0.094	64.5 (58.1–69.0)	66.2 (56.8–71.1)	0.380
Body mass (kg)	77.1 (68.2–87.2)	76.0 (68.1–80.9)	82.1 (72.6–87.9)	0.203	76.4 (68.2–83.5)	82.1 (72.6–87.9)	0.135	77.1 (68.2–87.2)	76.8 (68.5–82.2)	0.507
Height (m)	1.59 (1.54–1.62)	1.59 (1.53–1.63)	1.64 (1.60–1.65)	0.079	1.59 (1.53–1.62)	1.64 (1.60–1.65)	0.025 *	1.59 (1.54–1.62)	1.59 (1.54–1.63)	0.794
BMI (kg/m^2)	31.2 (26.8–34.8)	29.9 (27.5–33.8)	30.2 (28.0–32.9)	0.664	30.2 (27.4–34.2)	30.2 (28.0–32.9)	0.940	31.2 (26.8–34.8)	29.9 (27.5–33.6)	0.368
Skeletal muscle mass (kg)	23.4 (21.3–25.0)	22.9 (20.7–25.3)	24.9 (23.3–27.0)	0.153	23.1 (21.0–25.2)	24.9 (23.3–27.0)	0.060	23.4 (21.3–25.0)	23.3 (20.9–25.4)	0.920
Fat mass (kg)	35.0 (25.0–41.6)	31.6 (26.8–39.1)	33.2 (29.0–40.6)	0.514	32.4 (25.9–39.4)	33.2 (29.0–40.6)	0.649	35.0 (25.0–41.6)	31.7 (27.3–39.1)	0.342
Body fat percentage (%)	44.3 (37.3–48.7)	42.8 (37.9–47.8)	40.6 (39.1–46.6)	0.656	43.5 (37.8–48.3)	40.6 (39.1–46.6)	0.743	44.3 (37.3–48.7)	42.8 (38.0–47.6)	0.365
Handgrip strength (kg)	25.9 (22.5–28.6)	27.7 (21.1–28.5)	27.7 (22.7–31.4)	0.326	25.3 (21.7–28.6)	27.7 (22.7–31.4)	0.257	25.9 (22.5–28.6)	25.0 (21.3–28.8)	0.460
Relative handgrip strength (kg/kg)	0.34 (0.27–0.40)	0.32 (0.28–0.38)	0.34 (0.30–36)	0.871	0.33 (0.27–0.39)	0.34 (0.30–36)	0.880	0.34 (0.27–0.40)	0.32 (0.29–0.38)	0.642
Isokinetic knee extensors peak torque (60°/s)	51.5 (37.4–78.0)	55.3 (38.8–81.6)	76.5 (56.1–99.4)	0.075	54.8 (38.8–79.1)	76.5 (56.1–99.4)	0.024 *	51.5 (37.4–78.0)	55.6 (39.7–84.3)	0.493
Relative isokinetic knee extensors peak torque (60°/s/kg)	0.73 (0.51–1.03)	0.72 (0.49–1.07)	0.97 (0.79–1.19)	0.204	0.72 (0.50–1.04)	0.97 (0.79–1.19)	0.084	0.73 (0.51–1.03)	0.77 (0.52–1.10)	0.437
Gait speed (m/s)	1.04 (0.94–1.18)	1.06 (0.89–1.21)	1.23 (0.86–1.41)	0.537	1.05 (0.89–1.19)	1.23 (0.86–1.41)	0.272	1.04 (0.94–1.18)	1.06 (0.89–1.23)	0.687
30-s arm curl (reps)	15 (11–18)	14 (12–16)	15 (13–20)	0.597	14 (12–17)	15 (13–20)	0.368	15 (11–18)	14 (12–17)	0.777
30-s chair stand (reps)	11 (9–14)	11 (9–14)	13 (11–15)	0.406	11 (9–14)	13 (11–15)	0.210	11 (9–14)	12 (9–14)	0.817

Notes: Data are expressed as medians (25th–75th percentile). Differences between genotypes were determined by Kruskal–Wallis or Mann–Whitney U test. Asterisks denote significant differences with * $p < 0.05$. Abbreviations: f, females; m, males; BMI, body mass index; reps, repetitions.

Table 4. Male participants' characteristics by ACTN3 genotypes.

Variable (Units)	RR (N = 57)	RX (N = 71)	XX (N = 5)	*p*-Value	RR + RX (N = 128)	XX (N = 5)	*p*-Value	RR (N = 57)	RX + XX (N = 76)	*p*-Value
Age (years)	70.1 (67.3–73.7)	69.9 (64.8–74.6)	73.3 (69.8–78.9)	0.305	70.1 (66.2–74.1)	73.3 (69.8–78.9)	0.141	70.1 (67.3–73.7)	70.4 (65.6–74.7)	0.845
Body mass (kg)	78.6 (73.3–88.5)	80.2 (73.8–88.1)	78.5 (72.5–81.7)	0.778	80.0 (73.3–88.4)	78.5 (72.5–81.7)	0.489	78.6 (73.3–88.5)	80.0 (73.8–87.9)	0.973
Height (m)	1.71 (1.65–1.75)	1.72 (1.67–1.75)	1.76 (1.73–1.80)	0.082	1.71 (1.66–1.75)	1.76 (1.73–1.80)	0.049 *	1.71 (1.65–1.75)	1.72 (1.67–1.75)	0.169
BMI (kg/m^2)	28.6 (25.2–30.2)	27.4 (25.4–30.4)	24.8 (24.0–25.8)	0.077	28.1 (25.4–30.3)	24.8 (24.0–25.8)	0.026 *	28.6 (25.2–30.2)	27.3 (24.9–30.0)	0.428
Skeletal muscle mass (kg)	30.5 (27.0–34.4)	30.3 (26.8–34.6)	32.9 (31.4–35.9)	0.385	30.4 (27.0–34.5)	32.9 (31.4–35.9)	0.172	30.5 (27.0–34.4)	30.9 (27.4–34.8)	0.653
Fat mass (kg)	25.6 (19.5–29.0)	23.8 (17.9–29.9)	14.0 (12.9–24.9)	0.193	24.4 (18.9–29.4)	14.0 (12.9–24.9)	0.071	25.6 (19.5–29.0)	23.8 (16.8–29.6)	0.635
Body fat percentage (%)	30.2 (25.8–35.1)	30.4 (23.5–35.3)	18.97 (17.2–30.5)	0.179	30.4 (24.8–35.1)	19.0 (17.2–30.5)	0.067	30.2 (25.8–35.1)	30.1 (22.9–34.9)	0.540
Handgrip strength (kg)	36.6 (29.5–41.1)	36.9 (31.1–45.2)	38.2 (36.9–43.0)	0.208	36.8 (30.6–42.9)	38.2 (36.9–43.0)	0.381	36.6 (29.5–41.1)	37.5 (31.3–45.1)	0.095
Relative handgrip strength (kg/kg)	0.45 (0.34–0.52)	0.45 (0.40–0.54)	0.52 (0.46–0.57)	0.194	0.45 (0.39–0.53)	0.52 (0.46–0.57)	0.181	0.45 (0.34–0.52)	0.46 (0.41–0.54)	0.152
Isokinetic knee extensors peak torque (60°/s)	86.3 (54.9–116.6)	90.1 (62.4–123.4)	50.5 (46.8–115.1)	0.225	88.9 (62.1–119.6)	50.5 (46.8–115.1)	0.232	86.3 (54.9–116.6)	88.3 (62.1–122.6)	0.305
Relative isokinetic knee extensors peak torque (60°/s/kg)	1.06 (0.75–1.41)	1.12 (0.90–0.15)	0.66 (0.62–1.44)	0.225	1.10 (0.82–1.45)	0.66 (0.62–1.44)	0.282	1.06 (0.75–1.41)	1.11 (0.87–1.49)	0.252
Gait speed (m/s)	1.15 (0.93–1.28)	1.16 (0.97–1.29)	1.08 (1.06–1.14)	0.585	1.16 (0.97–1.29)	1.08 (1.06–1.14)	0.445	1.15 (0.93–1.28)	1.15 (1.04–1.29)	0.576
30-s arm curl (reps)	14 (12–16)	15 (12–18)	13 (12–15)	0.308	15 (12–17)	13 (12–15)	0.293	14 (12–16)	15 (12–18)	0.356
30-s chair stand (reps)	12 (10–14)	12 (10–15)	9 (6–13)	0.167	12 (10–14)	9 (6–13)	0.117	12 (10–14)	12 (10–15)	0.438

Notes: Data are expressed as medians (25th–75th percentile). Differences between genotypes were determined by Kruskal–Wallis or Mann–Whitney U test. Asterisks denote significant differences with * $p < 0.05$. Abbreviations: f, females; m, males; BMI, body mass index; reps, repetitions.

4. Discussion

This study aimed to evaluate the influence of ACTN3 genotypes (RR, RX, and XX) on muscle strength, mass, isokinetic knee extensor peak torque and functional performance in a population of mature adults from Kosovo, a developing lower income country. No significant differences could be detected between the three genotypes neither in the total population nor specifically for men and women. Notwithstanding, differences were observed in females when comparing the XX to the RR+RX group. In this dominant model, XX carriers were taller with a higher isokinetic knee extensors peak torque (60°/s) and a tendency to higher relative isokinetic knee extensor peak torque and muscle mass.

These findings support the hypothesis that the ACTN3 R577X polymorphism would influence force-generating capacity of knee extensors in a sex-specific manner. Similar to our findings, Delmonico and colleagues [21] have shown that older females with the XX genotype were in favor to have a higher absolute and relative knee extensor concentric peak power in comparison to the RR and RX groups while no differences in absolute or relative peak power were observed between ACTN3 genotype groups in men [21]. Based on the physiological role of ACTN3 in force transmission these results are unexpected. Irrespective of baseline values the authors showed an advantage for RR homozygotes in peak power response to a 10 weeks lasting strength training [21]. While female-specific associations between ACTN3 genotype and knee extension peak torque were confirmed in another study, the concrete outcome differed as α-actinin-3-deficient women displayed lower knee extensor shortening and lengthening peak torques compared to women with RX+RR genotypes. Furthermore, the women homozygous for XX had a lower level of total fat free mass and lower limb fat free mass [13]. However, differently to the BIA technique (by Inbody device) used in our study, Walsh et al. used the dual energy X-ray absorptiometry (DEXA) to measure the whole-body soft tissue composition. Nevertheless, both techniques have shown high correlation degree in muscle mass assessment [40] and are both recommended to be used in research [3]. Potential differences in sample size and age may eventually explain the divergent results within these studies. While age and sample size were similar between our study (308 participants, 160 females and 148 males, 40–91 y) and the one by Delmonico and colleagues (157 Caucasian participants, 71 males and 86 females, 50–85 y) [21], the study by Walsh and colleagues [13] included a higher number and also younger participants (848 volunteers, 454 males and 394 females, 22–90 y). Nevertheless, Walsh et al. [13] observed the same outcomes even after calculation in a subcohort of females aged above 50 years. Up to now no mechanistic explanation for these unexpected and dichotomous findings can be given although it might be important to discriminate between studies in highly trained athletes, where elite sprint athletes have significantly higher frequencies of the 577R allele than do controls [41] and those with rather untrained (and older) populations. In addition, it has been suggested that subjects possessing the ACTN3-deficient genotype (XX) had lower baseline creatine kinase levels, a marker of muscle damage, compared with the heterozygotes [42]. This might affect the response to strength training. If it also would affect general strength or power measures is still under investigation and to our knowledge no data exist in older adults.

Most studies showing ACTN3 R577X polymorphism's association with muscular strength and power primarily concentrate on physical performance and trainability rather than ageing and physical fitness of older people. Yet, the ACTN3 polymorphism has also been described as an evolutionary tool for improving the energy economy during locomotion [43]. A recent meta-analysis, estimating the association of ACTN3 R577X polymorphism with elite power sports, emphasized the importance of understanding the potential genetic impact together with the modifying effects of ACTN3 R577X genotype on organisms' phenotypes and consequently on conditions such as muscle deterioration (sarcopenia) [20]. Some studies [14,44] have shown an association between the ACTN3 genotype and sarcopenia-related declines in muscle phenotypes in older adults. Outcomes from a recent study suggest that active women aged 75 and above carrying the R allele

might have a higher risk for sarcopenia in comparison to the XX carriers [17], which somehow fits to the tendencies observed in our study. However, it is very likely that various genotypes would interact in modulating muscle-related phenotypes. A study investigated the impact of ACE I/D and ACTN3 R577X polymorphisms on maximal strength of the arm and leg muscles, muscle power performance (counter-movement jump) and functional capacity (sit-to-stand test) subsequent to 12 weeks of high-speed power training. Significant differences in all parameters in response to high-speed power training were detected between the power (ACTN3 RR + RX & ACE DD) and the 'non-power' muscularity-oriented genotypes (ACTN3 XX & ACE II + ID)] [45]. In this respect, the underlying cause of the obtained results might be a result of gene × gene or gene × environment interactions which need to be elucidated in further studies.

The rather low prevalence of ACTN3 XX genotype found in this Kosovan study population (4.6%) present a novelty within the world-wide human populations' prevalence based on the countries mean geographical latitude [29]. In fact, countries positioned in similar coordinates (Kosovo latitude 42.60°) have shown rather different prevalences, varying between 24% (Japan, 41.50°), 21.6% (Italy, 42.61°), 19.9% (Spain, 40.64°), and 18.3% (Greece, 40.00°) [29]. Nevertheless, our findings are consistent with a prevalence study from six neighboring Balkan countries. Even though a relatively small number of participants (total n = 483) have been genotyped, a generally lower representation of the ACTN3 XX genotype, varying from 6.0% in North Macedonia, 6.7% in Montenegro, 12.5% in Albania, 13.1% in Croatia, 13.6% in Serbia, and 17.7% in Bosnia and Herzegovina has been confirmed [46]. From these, North Macedonia, Montenegro, Albania, and Serbia are land bordered with Kosovo. This underrepresentation below the reference latitude populations opens a gap for further discussion within the field. This is important especially since the allelic frequencies from our study (0.68 and 0.32 for the R and X alleles, respectively) are comparable to other European (0.58 and 0.42, respectively) [47] and Balkan populations (from 0.59 and 0.41 in Serbia, 0.66 and 0.34 in Albania, to 0.79 and 0.21 in Montenegro) [46]. A factor that seems to be very specific for Kosovo and could be an important factor for genetic studies, is the fact that compared to neighboring countries—such as Albania, Macedonia, and Serbia—Kosovo has had among the lowest number of immigrants in South East Europe. It remained outside the so-called Balkan Route [48]. According to the 2011 Population Census data, in Kosovo there were 128,808 immigrants, which comprised 7.4% of the population. Around 97% of immigrants had Kosovan citizenship; therefore, the vast majority of immigrants in fact were returnees to their country. Taking into account the citizenship of its residents, Kosovo seems to be a fairly homogeneous country [49].

Another aspect that undoubtedly presents an issue of great scientific interest as well as a serious public health concern is the very high level of overweight (42.9%) and obesity (39.4%) found for the present study population. This necessarily needs to be thoroughly analyzed and further explored in subsequent studies taking potential co-variates (e.g., socio-economic status, educational level, health status) into consideration. However, the higher levels of overweight and obesity could be also connected to the low level of participants homogenous for the XX genotype as this variant may affect body metabolic phenotypes [50]. Amongst the few studies exploring this phenomenon, Houweling et al. [50] were suggesting that the XX genotype may be protective against obesity in knockout mouse model. Lower body weight and fat-free mass was also associated with the XX genotype in female elite ballerinas aged 18–39 years, but not in the control group which had a higher prevalence of the XX genotype (20.2% in contrast to 24.7%) [51]. Furthermore, it has been shown that women homozygous for XX displayed lower levels of weight, BMI, body fat free mass (FFM), lower limb FFM and fat mass, compared to other genotypes [13]. Therefore, findings from our study such as the lower BMI of the male XX carriers, together with the simultaneous low prevalence of XX homozygotes and high number of overweight and obese participants support an association between the ACTN3 genotype and obesity.

Another important issue to be mentioned is the fact that this study was not in HWE. This violation might be affected by several factors, starting from genotyping, inbreeding,

small group of participants, mutation, etc. [52]. The assay quality with the successful genotyped samples was satisfyingly high (94%), thereby excluding a methodological issue with the genotyping. For recruitment, a multidirectional approach has been used as described in the methods section. The negative inbreeding factor ($F = -0.248$) suggests an excess of heterozygotes [53]. Participants were allowed to invite their partners in the study, but the degree of heredity (family relations) between different participants was not assessed. Given the relatively low number of inhabitants in the administrative region of Prishtina (477,312) within a small country (1,739,825 inhabitants), and with a low percentage of people aged 60 [30] and above closer relationships could have contributed to the observed outcomes.

Although the study has been performed with utmost care, its limitations need to be mentioned. First, the low baseline allele frequency of 577X within our sampled population (4.6%) could reduce the effective power of the study, to potentially obscuring the genetic association with muscle mass and function. In fact, this encountered outcome (lower than the worldwide average of 18% [12]) was both interesting and intriguing. Another issue would be the very high percentage of overweight and obesity in general population, which could as well mask the outcome measures. Furthermore, the violation of HWE is another limitation that needs to be mentioned. Finally, future epidemiological studies are definitely needed, which could aim to balance the number of participants with respect to the different genotypes, in order to overcome the statistical limitations inherent with low sample sizes. Additional data with respect to sarcopenia prevalence or consequent risk of falls and associated illnesses would be very important to interpret the genetic data in the specific environmental context.

5. Conclusions

This study shows that from a convenient sample of Kosovo mature adults, there were no direct significant associations between the three ACTN3 genotypes (RR, RX, and XX) with muscle strength, mass, isokinetic peak torque and functional performance in neither total population, nor sex-specifically). Distinctive observations hint to sex-specific outcomes as female carriers of the XX genotype showed significantly higher isokinetic knee extensors peak torque ($60^\circ/s$) and male carriers of the XX genotype had a significantly lower BMI than the RR + RX genotype groups. However, this study also highlights the need for further studies as understanding the multifactorial relationship between genotypes, various covariates and the development of sarcopenia is the key for developing accurate diagnostic tools for an earlier detection of sarcopenia and similar related conditions.

Author Contributions: Conceptualization, A.B. and B.W.; Data curation, A.B. and E.K.; Formal analysis, B.W.; Funding acquisition, B.W.; Investigation, A.B. and E.K.; Methodology, A.B., B.W. and H.T.; Project administration, B.W.; Resources, B.W.; Supervision, B.W.; Writing–original draft, A.B.; Writing–review and editing, E.K., H.T., and B.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Higher Education and Research in Applied Sciences (HERAS) under the grant number K-02-2017 and the Research Platform Active Ageing, University of Vienna, Austria.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethical-Professional Committee of the University Clinical Centre of Kosovo (protocol code 1246/14.09.2016).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: Open Access Funding by the University of Vienna.

Conflicts of Interest: The authors declare no conflict of interest.

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6 Discussion

The contribution of this thesis extends within an ever-growing field of interest amongst the scientific community, in an under-explored population setting (developing upper middle-income European country), laid through a series of three studies.

Publication 1 [151] demonstrated the test-retest reliability in a range of field and laboratory based tests to be used for evaluating functional performance, strength, power related assessments, and the body composition components within the specific Kosovo population. Good reliability (ICC between 0.75 and 0.90 based on the 95% confident interval, as suggested by Koo and Li [179]) was found between the two testing sessions within the majority of assessed variables in both younger and older cohorts, including all the sarcopenia-related variables (muscle strength, mass and physical performance). Furthermore, a range between good and moderate reliability (ICC between 0.5 and 0.75) was observed amongst peak torque assessments, thus supporting the potential for these assessments to be used as measurement tools within the studied population and others similar (or as a reference if needed to calculate diagnostic cut-off points for other populations).

Publication 2 [161] described the state of sarcopenia and its conceptual stages in Kosovo older men and women based on different recommended algorithms and their suggested cut-off points (initial EWGSOP1 and revised EWGSOP2) together with the population specific cut-off points that were calculated deriving from the young population recruited in Study 1. The main findings from this study [161] present the lower numbers of sarcopenia no matter what diagnostic pathway is followed, and a distinctively higher number of cases in male participants. Additionally, differences between non-sarcopenic and sarcopenic groups, as well as the determinant factors behind the sarcopenia status were analyzed. Thus, sarcopenic participants were observed to have higher age and risk for malnourishment, lower height, body mass, BMI, SMM, physical performance score, educational level and nutritional status in comparison to the non sarcopenic group. Interestingly, besides sarcopenia and obesity, sex differences were also seen in medication usage, education level and financial condition.

Publication 3 [162] focused on extending the investigations towards other determinant factors potentially affecting the sarcopenia related components (muscle mass and function) such as the genetic influence through ACTN3 genotypes (RR, RX and XX). Although not finding a direct association between any of the evaluated genotypes and assessed parameters, extensive assessments including the comparisons between dominant and recessive models with the matched genotypes (XX and RR, respectively) suggested for a potential sex-specific influence of ACTN3 R577X polymorphism on extensor peak torque and BMI.

6.1 Reliability of measurement methods

Measurement methods present a component of significant importance in science, which have to be well executed and reliable if and when used. The form of reliability and/or validity used together with its outcomes determines the quality of research. Test-retest reliability that was used in this study as the instrument to assess the applicability of certain measurement techniques and parameters across time helped on a matter of estimating composed measurements from

sarcopenia diagnosis. Notwithstanding the fact that these single methods have already been shown to be valid and reliable to be used [49, 80], other means of reliability and/or validity should be considered to be proved for validation in future investigations.

Noteworthy, for assessing different age-related changes in human organism, certain assessment criteria are suggested for wider worldwide of particular region application (e.g. the sarcopenia assessments and their cut-off points suggested by the EWGSOP and EWGSOP2). However, it is of great importance that these methods are reliable and/or valid also for countries in a scientific lower state then that of the HICs, or countries where the approach towards contemporary technological advancements is often unreachable due to the lack of economic resources. In this context, findings from this dissertation contribute towards assessing of which of these assessments are feasible and reliable not only within the studies from this thesis, but also to be used in large epidemiological studies in other countries related from the region of western Balkans. After all, until 2-3 decades ago Kosovo used to belong to the same federalist country with 6 other countries of today (including North Macedonia, Montenegro, Serbia, Croatia, Bosnia and Herzegovina, and Slovenia) whereas with Albania share the same ancestry and ethnicity (95% of Kosovans are of Albanian ethnicity [76]).

The observed generally higher ICC values [151] in functional performance tests (including chair stand, arm curl, 6MWT, sit and reach, timed up and go, and the gait speed normal pace), isometric handgrip strength and the bioimpedance-measured parameters (SMM, SMI, ASMM and ASMI) represent tools with good reliability to be used in Kosovo population. This means that all the sarcopenia-related components that were previously shown to be valid and suggested to be used like muscle strength [95], mass [83] and performance [80], can be as well assessed within this population through these assessment tools. However, notwithstanding the importance, applicability and successful application shown of the EWGSOP2 diagnostic cut-off values, population-specific values are encouraged to be used when available [49, 180]. The usage of EWGSOP2 recommended grip strength in particular was suggested to have the potential to underdiagnose the sarcopenia probable conceptual stage by Bahat and colleagues [180] who offered cut-off values (35 kg and 20 kg in males and females, respectively) for older Turkish much higher than those suggested by the revised EWGSOP consensus criteria (27 kg and 16 kg, respectively) [80, 95] and to some extent similar to ours (32.8 kg and 19.6 kg, respectively).

Good and moderate reliability was also observed amongst the laboratory based assessments of peak torque and average power extension and flexion (at 60^0/s and 120^0/s), this way further facilitating and easing the assessments needed to be performed in older adults of this population or within reference or similar populations. Similar findings were reported previously in older subjects within the functional performance tests [181-184], BIA-measured parameters [185, 186] and isometric handgrip strength [187].

It should be noted that despite the detailed description of procedures and protocols given to each participant separately by the test administrators prior to each test, as well as a tryout trial as a means to understand the process more thoroughly, the significant differences found between the testing and retesting sessions in several performance assessments (chair stand and arm curl tests in older participants), handgrip strength (in younger participants) and power-related variables (average power flexion at 60^0/s and 120^0/s, average power extension at 120^0/s and peak torque

flexion at 60^0/s and 120^0/s in older, peak torque flexion at 60^0/s, average power flexion at 60^0/s and 120^0/s in younger participants) could be related to the participants' familiarization with the assessment techniques, especially since the retest was characterized to be higher in all cases. Understandably, this was not the case in the majority of body composition variables, excluding SMM and SMI in older adults (both cases $p < 0.05$).

6.2 Sarcopenia state in Kosovo and the comparison between diagnostic algorithms

Sarcopenia nowadays presents an issue of great interest amongst the scientific communities. It has been lately described for an association with a significantly higher mortality risk, notwithstanding the definition used [188]. Yet, many clinicians persist being unaware regarding this condition together with its suggested diagnostic tools and algorithms together with their specific cut-off points [189, 190]. On the other hand, the lack of a general agreement on the more robust definition with variables to be included and cut-off points to be followed in detecting sarcopenic people in different ethnic groups is yet to be reached [150, 191]. A publication from 2018 by Zengin and colleagues [150] emphasized the fact that most of the research data were coming from Caucasian populations from high income countries. Since then, compelling works raid to fill the gap [192-196], enlightening us with the prevalence of sarcopenia (and its conceptual stages) in different populations and ethnic groups as well. The assigned ICD-10-CM (M62.84) code for sarcopenia provided another significant breakthrough on the process of better understanding and establishing its position amongst both researchers and clinicians. This was described as a major step forward, which should lead towards the raise of available diagnostic tools and specific medications targeting sarcopenia [197].

Notwithstanding the ongoing progress towards providing comprehensive and comparable worldwide data on sarcopenia, especially coming from other non-European populations, the scarcity of information coming from developing European populations of Caucasian decent is obvious. Kosovo is one of these countries as part of the Balkan peninsula, classified as upper middle-income by OECD [75] with a lower expected life expectancy at birth (72.5 years in total, 74.8 years in females and 70.3 years in males) comparing to European and world averages (81.1 years in total, 83.8 years in females and 78.4 years in males, versus 72.7 years in total, 75.0 years in females and 70.6 years in males, respectively [77]). In this case, this study observed the prevalence to be low notwithstanding the followed algorithms, their recommended diagnostic criteria (EWGSOP1, EWGSOP2 and population specific cut-off points) together with the variable used as the key characteristic (SMI or muscle strength) [161]. It must be emphasized that to date the current state of art is continuously sharpening based on the different diagnostic guidelines followed, as well as the newer data coming from different population settings. Emerging evidence has been suggesting for a general incidence that increases with age [191]. The worldwide prevalence of sarcopenia in older adults has been estimated to range between 9.9 to 40.4% [198], 10% in female and 10% in males, while being higher in non-Asian populations [199], although depending on the definitions used [198] or the settings occurring [191]. A recent systematic review evidenced a prevalence of 17.7% (ranging between 6.2 and 35.3%) following the EWGSOP1 and 11% (ranging between 3.2 and 26.3%) following the revised EWGSOP2, as well as a general decrease in prevalence of ~ 7% when following the EWGSOP2 [200].

In fact, despite the lower diagnostic thresholds in the revised EWGSOP2 recommended guideline, no differences in the sarcopenia outcomes could be detected in our study [161]. The same situation was encountered even when following the population specific cut-off points, with sarcopenia varying between 0.8 %, 2.9 % and 0.0 % (EWGSOP1, EWGSOP2 and population specific cut-off points, respectively). With respect to the other two conceptual stages, severe sarcopenia was described to be in the same line, accounting for 2.5 %, 1.3 % and 2.5% (respectively). Nevertheless, it was pre-sarcopenia / sarcopenia probable that exposed the most observable differences between the followed guidelines (2.1 %, 5.4 % and 22.9 %, respectively). This way, the revised EWGSOP2 algorithm (used in both EWGSOP2 and population specific) detected the higher percentages in pre-sarcopenia / sarcopenia probable, with the population specific cut-off points striking towards the identification of the highest numbers (22.9%). These findings emphasize the importance of population specific cut-off points based on the particular characteristics and distinctions of the same population, in the detection of sarcopenia and its related components. Furthermore, they perfectly conform to the EWGSOP2 revised recommendations aims [49] when suggested to shift the key characteristic from muscle mass towards muscle strength as a measure to facilitate the swift sarcopenia identification.

The consequences of applying the newer EWGSOP2 guideline instead of the former EWGSOP1 started being investigated in the recent couple of years [201-206], upon the publication of the revised consensus criteria [49]. Amongst the first to report their findings in this context were Reiss and colleagues [201], suggesting for a substantial mismatch in between the two recommended guidelines, with a significant lower number of men diagnosed with sarcopenia following the EWGSOP2. These prevalence discordances with lower numbers in EWGSOP2 were supported and further explored by other studies as well [202-206]. The particularly lower prevalence in males following the EWGSOP2 guidelines were also described by Van Ancum JM and colleagues [207] in a study encompassing a range of different cohorts including community-dwelling older adults, geriatric outpatients and acute and subacute inpatients, attributing the low prevalence to the lower cut-off points for handgrip strength. With respect to the pre - sarcopenia / sarcopenia probable findings observed in our study[161], similar outcomes were also presented in a conference proceeding by Murphy C and colleagues [208] where community-dwelling older Irish adults were characterized by a lower sarcopenia prevalence following EWGSOP2 (5.5% in total, 1.6% sarcopenia and 3.9% severe sarcopenia), but also a rather high prevalence (23.4 %) of sarcopenia probable group. Alike to our study, participants from Murphy et al., (2020) also presented a generally lower sarcopenia prevalence. Regarding this conceptual stage (sarcopenia probable using EWGSOP2), interesting findings were also mentioned by Kim M and Won CW [202], where they described significantly different sex outcomes based on the used muscle strength assessment (handgrip strength or chair stand test). Nevertheless, it should be important to emphasize the fact that none of the other studies presented such a lower occurrence of sarcopenia and / or severe sarcopenia as our study. Therefore, as observed in our case, EWGSOP2 recommended algorithm through muscle strength as the key characteristic might also be the ultimate path to detect the potential for age-related decline in muscle mass and function in populations with generally lower numbers of sarcopenia (especially in the cases where there might be other reasons covering it)[161]. However, notwithstanding the advantage that EWGSOP2 might present in detecting pre – sarcopenia / sarcopenia probable, as

well as facilitating the case-findings in clinical settings, further research coming from different study settings and populations is required as a necessity to explore and detect the finest EWGSOP2 recommended cut-off values.

6.3 Sarcopenia and sex

Due to the differences in body composition between men and women, it is only fair to assume that there might be sex variances in a disease like sarcopenia, which is directly related to the decline of certain body components. However, data on the sarcopenia's association (or any of its conceptual stages) with sex are generally scarce and inconsistent [199], as well as often contradictory. A systematic review and meta-analysis from 2017 [199] analyzed the worldwide prevalence of sarcopenia based on the existing data, concluding for an estimated equal proportion between sexes (10% in men and 10% in women), while emphasizing the observed differences between studies based on the diagnostic tools and populations. Another comprehensive study from 2019 [198] which was concentrated in analyzing prevalence outcomes based on the different recommended diagnostic guidelines, observed higher sarcopenia prevalence in males following EWGSOP/AWGS, and ALM/height and ALM/BMI, and in females following FNIH and ALM/weight. Elsewhere, Du and colleagues [209] reported males being more likely to have sarcopenia in community-dwelling older east Chinese. They suggested that the mechanism behind this situation could be the age-related tendency of losing muscle mass and strength as a consequence to the decrease of testosterone and insulin-like growth factor-1 levels amongst males, a phenomenon shown to be insignificant or slightly significant amongst females [209, 210]. Likewise, higher prevalence of sarcopenia amongst men (11% vs. 2% in females) was also described by Petermann-Rocha F and colleagues [211] when following the EWGOP2 suggested criteria, which was not the case when following the IWGS criteria (12% in men vs. 17% in women). One recent study from Saudi Arabia reported something similar in terms of significantly higher prevalence of sarcopenia amongst older female community-dwellers [212], but with sarcopenia assessed only following the SARC-F questionnaire. Previous similar studies within the same context had already been reporting a higher risk for sarcopenia amongst community dwelling females [213, 214], thus providing supportive background for these findings. Nonetheless, it should be noted that the existing inconsistencies regarding sex and sarcopenia may originate from different grounds, such as population characteristics, socio-economic situation, cultural and racial traits, and other various covariates. Cultural aspect and its reflection on lifestyle and everyday life is an important factor that should be taken in consideration. Yet, many of these papers were published before the revised EWGSOP2 diagnostic guidelines, resulting in a lack for data based on this perspective as well.

The rate of muscle decline, as described previously to be lower in women [215], presents another striking factor that increases concern for the potential differences in sex based sarcopenia outcomes. The sarcopenia study (from this dissertation) was characterized by lower numbers of (severe) sarcopenic females [161]. This could be resulting from the lower estimated rate of muscle decline in females comparing to males [215, 216], but could be attributed to several intermediate covariates as well. Age amongst others, presents one of the potentially affecting factors when having in mind that females were significantly younger ($p < 0.001$), and the muscles annual decline increases exponentially with the advanced ages [28, 216], from 5 – 13% in the seventh decade, to 11 – 50% upon the years above 80 [80, 217]. Furthermore, height and body mass present the other potentially modifying factors, with males being significantly

taller ($p < 0.001$) but not heavier ($p = 0.504$)[161]. This directly results in significantly higher BMI amongst females ($p < 0.001$), which is consequently conveyed in higher overweight and obesity levels within this sex. The epilogue of such situation might be described with the interaction these covariates might play in the occurrence of sarcopenia, to some extent even masking the outcomes.

6.4 Overweight and obesity

Obesity presents an increasing phenomenon amongst older adults, as well as one serious multifactorial public health concern that is raising with the extending of longevity [218]. This is best described with the progressive decrease of physical activity with ageing, followed by a decline in fat free mass and total energy expenditure, reflecting this way in weight gain, by beginning with an increase in visceral abdominal fat [219]. Additionally, it has been described previously that by sharing the joint damage pathways, muscle and fat are together strongly connected in the pathological context [219].

That being sad, the sarcopenia study from this thesis [161] reported higher overweight and obesity prevalence within participants (85% in total, 43.3% and 41.7%, respectively) accompanied by higher levels of average BMI (29.7 ± 4.7), particularly in females (93.8% in total, 31% and 62.8%, respectively; 32.0 ± 4.3) comparing to males (77.1% in total, 54.3% and 22.8%, respectively; 27.7 ± 4.2). Females were also characterized by lower nutritional score (from MNA long form, $t238 = -3.911$, $p < 0.001$, $d = -0.507$) and higher malnourishment and risk for malnourishment prevalence's ($p < 0.001$). Such higher prevalence of obesity was previously reported in adults above 60 in the United States of America, being 42.8% [220]. Nevertheless, the higher prevalence of overweight and obesity in older populations has also been presented in European countries, with a range beginning from 53.9% (20.9% and 16.5%) in Switzerland, followed by 55.0% (38.7% and 16.3%), 59.0% (42.4% and 16.6%) and 59.6% (39.2% and 20.4%) in Denmark, Italy and Belgium respectively, peaking up to 62.9% (41.6% and 21.3%), 64.0% (40.9% and 23.1%) and 67.4% (46,5% and 20.9%) in Austria, Germany and Spain respectively [221]. Other countries like Turkey [222] and Iran [223] reported different data on older populations in overweight and obesity (13% and 21.4%, as well as 21.3 – 25.5% and 16.8 – 27.2%, respectively). It should be mentioned that another similar study to ours conducted by Peralta and colleagues [221] that compared differences between the European populations [224], but concentrating adults aged 18 and above, reported the highest prevalence of overweight and obesity in certain countries with a shared past (all post-communist countries and majority being in Eastern Europe) like Slovenia, Lithuania, Czech Republic and Hungary (58.9%, 59.6%, 60.1% and 61.6%). These results emphasized the tendency for higher prevalence especially in populations from countries that went through socio-economic transitions at a given point through their modern history (including the target generation of our interest). The most striking findings were seen in an intercontinental study including participants from Canada, Brazil, Colombia and Albania [225], where the highest overweight and obesity prevalence was observed in 65-74 year old Albanians, being 83.0% (46.7% 36.3%, respectively). These findings are important since Albanians present the most similar and compatible population to our study participants due to the fact that Albanians present the major ethnic group in Kosovo as well (95%) [226]. Therefore, despite the specific narrow

age-group at this study (65-74 year olds), the higher prevalence that we found presents undoubtedly a serious concern of public health in Kosovo that needs to be addressed with the outmost diligence and carefulness.

An emerging concept of "sarcopenic obesity" came up as a need to describe the cases of obese subjects that fulfill the diagnostic criteria of sarcopenia [227], and remarkably, presents an independent predictor of survival [216, 228, 229]. Despite the higher prevalence of overweight and obesity found in the sarcopenia study [161], no cases of sarcopenic obesity were evidenced. In contrary, it is possible that the generally lower occurrence of sarcopenia prevalence is directly affected by the higher overweight and obesity outcomes, emerging this way as a potential modifying factor. This novel phenomenon has been called "obesity paradox" and was described and analyzed previously [230]. Caan BJ and colleagues [231] proposed to term it as "BMI paradox" and suggested that body composition through visceral (central) adiposity may explain this paradox, mainly because general higher body adiposity may not necessarily be related to worse outcomes, until it is visceral. Nonetheless, even when analyzing this aspect in our study subjects using Waist Hip Ratio (WHR) as calculated from the Inbody device, outcomes were rather similar with BMI based measurements (Table 5). Unfortunately, the observed higher prevalence of visceral adiposity confirms the seriousness that this issue presents in the health of older population from a developing upper middle-income country such as Kosovo. Taking in consideration that the visceral fat is spread in the central body and surrounds internal organs, it consequently stimulates the development of different inflammatory processes, thus raising the risk potential for cardiovascular diseases and diabetes [230, 232, 233]. All this leads towards conclusion that it (higher levels of overweight and obesity) might just be one of the major reasons for the lower expected life expectancy in Kosovo [161] (72.5 years in total, 74.8 years in females and 70.3 years in males), lower than the world averages (72.7 years in total, 75.0 years in females and 70.6 years in males) and much lower than Europe (81.1 years in total, 83.8 years in females and 78.4 years in males) [77]. Furthermore, this might as well be playing the role of one of the major factors behind the triggering of cardiovascular diseases, officially qualified as the main cause of death in Kosovo [234].

6.5 Socioeconomic impact on ageing and sarcopenia

Socioeconomic status emerges within the different factors influencing or being interrelated to the ageing process. The influence of socioeconomic together with related social factors on health has been well documented, notwithstanding the lack of knowledge on the specific mechanisms involved [235]. Sarcopenia in particular has been described to be prone to socioeconomic impact and has been suggested to be classified amongst the chronic diseases influenced by social factors [236]. In the context of older Kosovans, socioeconomic situation has been reported to be rather challenging, particularly when combined with the current demographic trends such as urbanization, internal and external migration [237]. Within the few data on the mater from this respective population, Jerliu and colleagues provided a percentage of as high as 41% of respondents in their study perceiving themselves poor or extremely poor [237], roughly similar percentage (42%) being unable to access medical care and 82% reporting at least one chronic condition [238]. Furthermore, within the major problems emerging with ageing, chronic morbidity and multimorbidity have been described to be the most consistent correlates with older age and lack of medical care access [238]. Additionally, the oldest old (particularly females) as well as the poorer were observed to have the majority of chronic

conditions [238]. Likewise, a high percentage of self-reported chronic conditions was evidenced in our study as well (76.2%) [161], whereas about 30.8% of our study population (38.1% of females and 24.4% of males) reported a lack of financial means to cover the month. When considering that poor health or at least the presence of one chronic condition results in significantly lower health literacy [239], the combination of this poor health (57.1%) with higher occurrence of chronic conditions (76.2%) [161] could be translated only in despaired outcomes in terms of health literacy within our study population. Regarding the financial context, a positive association in between incomes and isometric handgrip strength in men has been previously described [240], which nevertheless does not seem to be the case for our study since males reported a significantly better ($p = 0.022$) financial condition comparing to females, despite the much higher number of individuals classified as either sarcopenic or part of one of sarcopenia conceptual stages [161]. Notwithstanding, the co-occurrence of lower financial status in females with lower education level ($p < 0.001$), medication consumption ($p = 0.021$), higher number of medications consumed, lower nutritional status and the higher number of individuals classified as either malnourished or at risk for ($p < 0.001$) should not be neglected. This might be amongst the cases when the complexity of socioeconomic interrelations might come from the higher influence that a third covariate could exert (e.g. higher levels of overweight and obesity that were observed particularly in females).

Another interesting finding that we observed was the rapid decline of the percentage of those without formal education (4.2% in total, 6.4 in females and 2.4 in males) in comparison to previous studies in Kosovo older population, ranging between 42.3%, 61.4% and 21.1% (in total, females and males, respectively) in 2009 [241] and 44.2%, 47.9% and 16.7% (total, females and males, respectively) in 2012 [237]. This decline presents a positive trend within this country since with the generation shift, this previously quite concerning phenomenon seems to be gradually in the process of eradication. Then, with the increase of the number of individuals with education in association with the increase of education level itself will influence the health literacy and collective health and wellbeing in general. On the other hand, higher education levels have been already reported to be associated with stronger isometric strength in both arm (handgrip) and leg, as well as higher muscle mass [236], providing a good ground for future's planning's and intervention strategies. Nonetheless, the potential positive research bias with better educated people being more likely to participate has been suggested [242] while observed in countries with rather heterogeneous population like the United States of America [243, 244], as well as in developing counties [245-247]. Thus, even though performing to the best of our knowledge, there is also the potential of having recruited higher percentages of those participants with higher education.

In line with the above mentioned information, socio-economic factors are undeniably important by being responsible (either directly or indirectly) on the occurrence of many non-communicable diseases, including the age-related conditions like sarcopenia. Furthermore, it should not be neglected the fact that healthcare priorities might differ in between different countries. Thus, in between the many acute and emergency medical issues, the expected life expectancy and the quality of life might just be too ambiguous concepts to be asking for in a developing countries with fragile healthcare systems [147]. This represents an obstacle that needs to be addressed as fast as possible amongst

the general healthcare communities of these places, and not just the inner circles of those engaged in recent scientific developments.

6.6 ACTN3 and sarcopenia related components (muscle mass and function)

ACTN3 XX genotype has been investigated for a while now, whereas it has been tipped for implication in muscle phenotypes and sarcopenia related components [248]. Notwithstanding, genetic study from this thesis [162] did not detect significant direct associations in any of the assessed variables of muscle strength, mass, power and functional performance between the three ACTN3 genotype groups (RR, RX and XX). Apart from a slightly higher ($p = 0.075$) isokinetic knee extensors peak torque (at 60^0/sec) in XX females, no differences were observed within each sex as well. However, the highlight findings support the ACTN3 R577X polymorphisms impact on females force generating capacities on isokinetic knee extensors peak torque. This way, XX female group were observed to have significantly higher ($p = 0.024$) isokinetic knee extensors peak torque (60^0/s), and slightly higher ($p = 0.084$) relative isokinetic knee extension peak torque (at 60^0/s) when compared to the dominant model (RR + RX) [162].

Similar female-specific findings were reported previously in two other studies, though with different outcomes between each other [126, 134]. At the baseline from a 10 weeks strength training intervention study in older adults, Delmonico and colleagues [134] described the XX female group having higher values in both absolute and relative peak power of the knee extensors [134]. Nevertheless, they showed that RR homozygotes provided an advantage within the peak power as a response to strength training. Walsh and colleagues [126] presented similar findings in terms of sex-specific associations between the ACTN3 XX genotype and isokinetic knee extensions peak torque in a study encompassing adults across the lifespan, though with lower peak torque values in XX group. In addition to that, these findings were accompanied with lower levels of whole body and lower limbs fat free mass. Even though our study's findings [162] provide ground support for one of these contradictive paths (in this case Delmonico et al [134]) by helping to establish the state of art through further elucidating the issue, further investigations are needed in order to better understand the underlying mechanisms behind.

It should be noted that differences in age, physical capacities, and ethnicity, besides the sex predominance, have also been suggested to affect the outcomes [249]. However, another study has presented the potential of ACTN3 XX genotype to increase risk for falling (as self-reported) in older females [140]. Similarly, Ma and colleagues [135] suggested for the ACTN3 XX genotype's association with physical performance decline while being age and sex specific. One recent study [128] described the association of XX genotype with a faster decrease of age-related muscle function. An interesting systematic review in older adults noted ACTN3 RR genotypes potential on possessing greater disability incidence protection for as long as they're habitually active, as well as having an improved adaptation of functional performance in the exercise interventions [139]. On this direction, other studies started connecting dots leading towards the obvious conclusion, while starting to emphasize the potential influence of ACTN3 genotypes on sarcopenia and its conceptual stages, as deriving from the impact it might exert on muscle phenotypes of older people [127, 128, 133, 250]. However, the dichotomy on the influencing allele is obvious and continues to date. While studies like Cho and colleagues [133] are suggesting for an association of X allele with sarcopenia through a predisposition

of X allele towards the decline of muscle strength, power, and physical function, other studies [128] are suggesting the opposite to it for a higher risk for sarcopenia amongst older females carrying the R allele. In this context, our findings [162] go in line with those from Romero-Blanco and colleagues [128] providing extra support for the ACTN3 XX genotype's modifying potential on power related phenotypes, as well as on R allele's potential relationship with sarcopenia-related conceptual stages. As observed above, ACTN3 R577X polymorphism presents a case of promising potential, for which further analyses are required. Therefore, it is of great importance to analyze individually and specifically the impact that this targeted SNP might assert in different population groups and settings. Additionally, population characteristics are necessary to be explored, since different covariates might also affect the outcomes. Amongst the distinctive population characteristics that might exert their influence in general outcomes are undoubtedly higher body mass levels, including the overweight and obesity. The very high levels of overweight (42.9%) and obesity (39.4%) in total population amongst the Kosovo participants could be also attributed to the lower prevalence of XX genotype homogeneous participants (4.6%) [162]. In fact the lower body mass recorded in XX genotypes have been already reported before [126, 232]. Furthermore, this variant has been described for its potential influence on the metabolic phenotypes, particularly for its suggested protectiveness against obesity in knockout mice [251].

An interesting issue to be addressed is the low percentage of ACTN3 R577X polymorphism found in the studied population in Kosovo (4.6%). Geographical latitude has been demonstrated to affect the prevalence of ACTN3 R577X polymorphism, which raises with the increase of gradient [146]. In our case (mean geographical latitude: 42.60^0) the prevalence was observed to be much lower than expected, with other similar geographically positioned countries ranging between 18.3%, 19.9%, 21.6% and 24% in Greece (40.00^0), Spain (40.64^0), Italy (42.61^0) and Japan (41.50^0) respectively [146]. However, relevant lower outcomes have been previously presented in another study [252] encompassing 6 different neighboring Balkan countries (though with a total number of only 483 participants), ranging between 6.0%, 6.7%, 12.5%, 13.1%, 13.6% and 17.7% in North Macedonia, Montenegro, Albania, Croatia, Serbia and Bosnia and Herzegovina, respectively. Four of these countries directly surround Kosovo. Interestingly enough from the genetic point of view, Kosovo has been reported to have a very homogeneous population [253] with the lowest of number of immigrants within the South East Europe in comparison to the other countries [254]. However, the predominant origin of populations in this western Balkan region is of Slavic background (an ethnically and linguistically distinct population), excluding Albania and Kosovo that are predominantly inhabited by ethnic Albanians (another ethnically and linguistically distinctive population), as well as an estimated 25.2% of population in North Macedonia [255] and 4.91% in Montenegro of Albanian origins [256]. Additionally, Kosovo populations allelic frequencies (0.68 for R and 0.32 for X) were observed to be in line with the other regional neighboring populations (ranging from 0.59 and 0.41, 0.66 and 0.34, to 0.79 and 0.21 in Serbia, Albania and Montenegro, respectively) [252] and European average (0.58 and 0.42, respectively) [257]. Therefore, the lower prevalence of ACTN3 R577X polymorphism in this region in comparison to the other reference populations opens a new topic in need for further investigation.

In line with the abovementioned arguments, genetics presents a novel yet solid and promising path to follow in the prevention of future health and mobility issues. This way genetic predisposition can be used in the future's individual-centered health-promotion and disease-preventive processes as a screening tool to identify in prior and predict the potential for certain individuals to develop age-related conditions together with consequences coming with them. For the time being it is of significant importance to proceed further investigating and establishing these associations in other populations and particularly through genome-wide studies. Additionally, the interaction between genetic predispositions with external influencing factors such as the socioeconomic, lifestyle and behavioral covariates should be better understood from the perspectives of different populations. The outcomes could be latter integrated within encompassing population-specific algorithms, taking in consideration not only the genetics but also its association with external factors, as a comprehensive instrument to better detect the potential for future age-related decline in muscle phenotypes. Upon the establishing of claims from these findings (the influence of ACTN3 R577X polymorphisms on females' force generating capacities) and others related, early genetic screening could be available from younger ages, and thus prevent the consequences before even happening.

7 Conclusion

Ageing presents a complex and multidimensional process of living organisms. The mechanisms behind, together with the multidirectional influences on this process need to be better understood and further explored. Understanding the age-related loss of muscle mass, strength and function intends to bring light in a topic of growing interest within the scientific community as well as a field of increasing need within the clinical settings. Its importance lays upon the novelty it brings by exploring this issue through the perspective of a European upper middle-income country. To our knowledge, it elaborates a rather novel topic in the studied and comparable populations, while being amongst the very first in a relatively under-explored population setting.

Functional performance, strength and power-related parameters, as well as muscle mass within the Kosovo population can be assessed through a range of reliable assessment methods for this specific population. The importance of such outcomes lay in the potential of this set of reliable tools to be used particularly when working with older populations. Additionally, the calculation of sex specific diagnostic cut-off points (valid for the Kosovan population) established appropriate diagnostic criteria to be used in clinical settings for the age-related disease such sarcopenia and its conceptual stages. This way, both researchers and healthcare practitioners involved with ageing, geriatrics and other related fields in Kosovo, will have more options on their screening and diagnostic process. Furthermore, these data can be used for other similar populations as well (surrounding Western Balkan countries), or as references if needed to calculate population specific cut-points.

Sarcopenia in current aging Kosovans seems to be an issue of rather low occurrence notwithstanding the algorithms followed, their recommended diagnostic criteria (EWGSOP1, EWGSOP2 or population specific cut-off points) and the variable used as the key characteristic (SMI or muscle strength). As much of a good news it might sound, when considering the demographic changes going through around the world with more than two thirds of older adults globally expected to be living in LMICs by the year 2050 [258], it should be nevertheless taken as a serious matter. The fact that older population in Kosovo is expected to increase to 26.8% by 2061 (from initial 8.1% in 2017) [259] further emphasizes the seriousness and importance this condition imposes. Even if for the time being sarcopenia is not a healthcare priority and also does not directly imply a great public health concern within the country of Kosovo and many other developing European countries [147], with the rapid development, technological advance and the increase of quality of life it is highly probable that it will eventually raise to prominence and come to haunt these populations. Furthermore, sarcopenia is turning up to be a significant age-related problem that is expected to catch health care experts from developing counties unprepared to deal with it [147], and thus it should be addressed with high priority amongst the general healthcare communiies of such countries. It should be strategically promoted within the experts dealing with it as well as the potential patients, and why not even added within the group lists with other non-communicable diseases. It should be noted that to date, the worldwide prevalence of sarcopenia remains a point of discussion, mainly due to the lack of cohesion in between studies, settings, assessment methods and techniques, recommended diagnostic guidelines and population characteristics. The revised EWGSOP2 “complicated” things further in this context, with its different recommended guidelines and diagnostic criteria, thus raising possibilities for

different outcomes. Anyhow, muscle strength decline presents a more versatile diagnostic key characteristic (for sarcopenia and its conceptual stages) than muscle mass, which when following the population specific cut-points provides the most versatile diagnostic pathway. Nevertheless, findings from our study [161] support the potential that EWGSOP2 recommended algorithm following the population specific diagnostic cut-off points (in particular) provide a more accurate tool to detect the first conceptual stage of sarcopenia (sarcopenia probable) in the form of age-related decline in muscle function (notably muscle strength) in populations with generally lower sarcopenia prevalence. This could be particularly important in cases when there might be a potential reason covering the general outcomes (e.g. overweight or obesity).

Factors associated to the occurrence of sarcopenia and its conceptual stages include the male sex, higher age, lower body stature, education level, BMI, body mass, fat mass and percentage, nutritional status and consequently higher risk for malnourishment together with higher percentages of underweight and normal weight subjects [161]. ACTN3 R577X polymorphism emerges as another potentially influencing factor on the isokinetic muscle power in females, though not directly observed in any of the sarcopenia-related components. This emphasizes the position of ACTN3 as a promising gene to be seriously considered and further explored for its influence on muscle phenotypes in older females, which may either directly or indirectly impact the sarcopenia related components. In the future, upon the establishing of such assertions, targeting certain groups for their particular predispositions (either internal or external) would facilitate detecting populations at risk and help integrate preventive strategies as a precaution for unfavorable scenarios

Higher overweight and obesity prevalence evidenced within our study population presents an observed issue of great concern. To some extent, these higher levels could be one of the reasons for the lower sarcopenia prevalence, having in mind the potentially masking effect they might exert on the matter. This was particularly observed in females, where no sarcopenia cases could be detected, even though 16.8% of them qualified for “probable sarcopenia” conceptual stage (with the most striking diagnostic criteria: EWGSOP2 algorithm, population-specific cut-off points). Therefore, the higher levels of overweight and obesity in the studied population presents an urgent need to be addressed as a public health emergency in Kosovo, risking for severe outcomes in populations health and well-being. Having in mind the serious implications they present in older adults health and wellbeing [218], it leads towards conclusion that this might also be one of the significant reasons for the lowest regional expected life expectancy in Kosovo, lower than worlds and much lower than Europe averages [161].

Finally, it should be a matter of high priority to address and further advocate the age-related decline of muscle mass and function within the populations of developing world amongst researchers and healthcare practitioners. Researchers could add more depth to the situation while analyzing it from different perspectives, whereas practitioners could gain knowledge and information on a matter of growing concern as well as an issue that interrelates to plenty of other health-related conditions.

8 Acknowledgements

I would like to wholeheartedly thank each and every one who helped me on this astonishing journey:

- Barbara – supervisor, friend, “the alchemist who introduced me to the philosopher’s stone”
- Harry – role model, godfather, “the wise men who showed me the river with drinking-water (and not only)”
- Ermira – my other half, my partner in crime, my perpetual source of energy, “the one person who believed in me even when I didn’t”
- Visar – my one true conscience and “fellow gladiator in this theater of life”
- Parents – the two firm stones beneath whom my heart lays deep
- Elvis – the exceptional, my fellow Gjakovar and compass in Vienna
- My friends – all those who helped me in one or other way
- My enemies – “may God not leave us without your kind”
- ♥ Moj e bukura More

9 List of further publications and manuscripts

Boshnjaku A. Is age-related sarcopenia a real concern for my developing country? Journal of Cachexia, Sarcopenia and Muscle. https://doi.org/10.1002/jcsm.13107

Krasniqi E, Boshnjaku A, Wagner KH, Wessner B. Association between Polymorphisms in Vitamin D Pathway-Related Genes, Vitamin D Status, Muscle Mass and Function: A Systematic Review. Nutrients 2021, 13(9), 3109; https://doi.org/10.3390/nu13093109

10 List of figures and tables

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13 Abbreviations

6MWT	6 minutes walking test
ACE	angiotensin I converting enzyme
ACTG1	actin gamma 1
ACTN3	alpha-actinin 3
ADAMTSL3	ADAMTS like 3
ALBM	appendicular lean body mass
APOE	apolipoprotein E,
ASMI	appendicular skeletal muscle index
ASMM	appendicular skeletal muscle mass
AWGS	Asian working group on sarcopenia
BIA	bioelectrical impedance analysis
BMI	Body mass index
BMQ1	brief medication questionnaire 1
CNTF/R	ciliary neurotrophic factor/receptor
CSA	cross sectional area
CT	computerized tomography
DNA	deoxyribonucleic acid
DXA	dual energy X-ray absorptiometry
EWGSOP	European working group on sarcopenia in older people
FNIH	foundation for the national institutes of health
FTO	FTO alpha-ketoglutarate dependent dioxygenase
GWAS	genome wide association studies
HIC	higher income countries
HSD17B11	hydroxysteroid 17-beta dehydrogenase 11
ICC	interclass correlation coefficient
ICD-10-MC	international classification of diseases and related health problems
IGF1/IGFBP3	insulin like growth factor 1/ insulin like growth factor binding protein,
IRS1	insulin receptor substrate 1,
IWGS	international working group on sarcopenia
KANSL1	regulatory NSL complex subunit 1
KAT8	lysine acetyltransferase 8
LMIC	lower middle income countries
LRPPRC	leucine rich pentatricopeptide repeat containing
MNA	mini nutritional assessment

MRI	magnetic resonance imaging
n / a	not available
OECD	organization for economic cooperation and development
PEX14	peroxisomal biogenesis factor 14
RNA	ribonucleic acid
SD	standard deviation
SMI	skeletal muscle index
SMM	skeletal muscle mass
SNP	single-nucleotide polymorphisms
SPPB	short physical battery test
SYT1	synaptotagmin 1
TGFA	transforming growth factor alpha
TNFα	tumour necrosis factor alpha-like,
TUG	timed up and go test
UCP2/3	uncoupling protein 2/3
UN	united nations
VCAN	versican
VDR	vitamin D receptor
WHR	waist hip ratio