Aus dem Institut für Humanernährung und Lebensmittelkunde
der Christian-Albrechts-Universität zu Kiel

Association between body composition and the endocrine crosstalk of tissues in older adults

Dissertation
zur Erlangung des Doktorgrades
der Agrar- und Ernährungswissenschaftlichen Fakultät
der Christian-Albrechts-Universität zu Kiel

vorgelegt von
M.Sc. Carina Ottilie Walowski
aus Ravensburg

Kiel, 2023

Dekan:	Prof. Dr. Georg Thaller
1. Berichterstatterin:	Prof. Dr. Dr. Anja Bosy-Westphal
2. Berichterstatter:	Prof. Dr. Gerald Rimbach
Tag der mündlichen Prüfung:	24.05.2023

Meinen Eltern Astrid und Dominik

und

meinem Bruder Christoph

VORBEMERKUNG

Die vorliegende Dissertation wurde in kumulativer Form angefertigt und beruht auf zwei veröffentlichten Manuskripten und auf einer zur Veröffentlichung eingereichten Abhandlung. Zusätzlich zur fortlaufenden Seitennummerierung der Arbeit sind die Seitenzahlen der publizierten Manuskripte abgedruckt. Das Inhaltsverzeichnis bezieht sich auf die fortlaufende Seitennummerierung der Arbeit.

Veröffentlicht mit Genehmigung der Agrar- und Ernährungswissenschaftlichen Fakultät.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

ASM	appendicular lean soft tissue mass
AT	adipose tissue
AWGS	Asian Working Group for Sarcopenia
BIA	bioelectrical impedance analysis
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
CT	computed tomography
DXA	dual X-ray absorptiometry
EWGSOP	European Working Group on Sarcopenia in Older People
FM	fat mass
FFM	fat-free mass
FMI	fat mass index
FNIH	Foundation for the National Institutes of Health
HGS	hand grip strength
IGF-1	insulin-like growth factor 1
MM	muscle mass
MRI	magnetic resonance imaging
RCT	randomized controlled trial
SAT	subcutaneous adipose tissue
SDs	standard deviations
SMI	skeletal muscle mass index
VAT	visceral adipose tissue
WHO	World Health Organization
4C	four-compartment

CHAPTER I

GENERAL INTRODUCTION AND OBJECTIVES

A healthy body composition facilitates healthy ageing

Improvements in healthcare, medicine and nutrition have led to a continuous increase in worldwide life expectancy at birth, and thus rapid manifestation of demographic ageing (*1, 2*). Between 1960 and 2020 lifespan had risen globally from 53 to 73 years (*3*). According to the first German mortality table (which covers the periods 1871/1881), average life expectancy was 35.6 years among men and 38.5 years among women (*4*). In 2019/2021, it has increased to 78.5 years (men) and 83.4 years (women) (*4*). This corresponds to an average life expectancy at birth that has approximately doubled for both sexes.

Globally, the proportion of the population aged 60 years or over is predicted to increase from one billion in 2020 to two point one billion in 2050 (*5*). Projections indicate that by 2050 one in every six persons could be aged 60 years or above (*5*).

Since life expectancy is rising, many older people can spend a longer period in good health (*6*). Nevertheless, the probability of decreased physical function grows (*7, 8*), and thus the risk of falls (*9*), hospitalization (*10*), co-morbidity (*11*) and mortality (*10*). In light of the demographic change, ever-increasing personal but also social and economic burdens (e.g. high health care and social protection systems costs) will be the consequence. Therefore, the aim in an ageing population is to age in a healthy way without disability and disease to maintain active participation in society, independence and quality of life. Research focusing on the mechanisms of healthy aging is imperative whereby body composition changes in older adults could provide a thematic focal point.

Age-related changes in body composition

The ageing process leads to a wide variety of physiological changes among which those affecting body composition are phenotypically the most apparent. During adolescence, muscle and bone develop and reach a peak in mass around the ages between 20 and 40, which is then maintained in midlife ((*12–18*), for reviews see (*19, 20*)). In the time of maintenance, the musculoskeletal system, that plays an essential role in human movement and regulation of whole body metabolism (for reviews see (*21–24*)), comprises approximately 55% of the body composition of a healthy adult without overweight or obesity (for a review see (*25*)). With advanced age, a progressive and generalized reduction of muscle quantity (i.e. muscle mass (MM)) and quality, defined as micro- and macroscopic changes in muscle architecture and composition (*26*) and muscle strength or power per unit of MM (for a review see (*27*)), occurs. Estimates of MM loss rates vary between 0.3% and 2.6% annually (for a review see (*28*)), while

the average rates of muscle strength loss are reported to be between 0.5% and 3.1% (*29–32*). A low muscle quantity and quality accompanied by a decline in muscle strength and physical performance are referred to as 'sarcopenia', which can be divided into primary (age-related) and secondary (disease-related) sarcopenia (*26*). In terms of human health, sarcopenia is associated with numerous negative outcomes including a higher risk of falls and fractures (for a review see (*33*)), impaired ability to perform activities of daily living (*34*), cognitive impairment (for a review see (*35), (36*)) and death (*34*).

Similarly, after achievement of the peak bone mass, an age-related progressive bone loss can be observed ((*17, 18*), for reviews see (*19, 20*)). Depending on the bone type and age, published rates of bone loss vary between 3% and 13% per decade (*37*). A low bone mass accompanied by a microarchitectural deterioration of bone tissue leading to greater bone fragility results in 'osteopenia' and/or 'osteoporosis' (*38*). These are silent diseases, in which the affected individual is often unaware of his or her condition until fractures occur causing secondary adverse outcomes like disability (*39, 40*), reductions in quality of life (*41, 42*) and death (*43, 44*). Osteoporosis affects a large number of persons, particularly older women (*45*). Using the World Health Organization's (WHO) definition, approximately 6.3% of men and 21.2% of women over the age of 50 years globally suffer from osteoporosis (*45*). In 2019, across Europe (i.e. European Union, Switzerland and United Kingdom) 32 million individuals aged over 50 years were estimated to be affected from osteoporosis, equivalent to nearly 25.5 million women and 6.5 million men (*45*).

Growing evidence indicates that sarcopenia and osteoporosis frequently co-occur (*46–49*). For example, in a population-based Finnish study, postmenopausal women with sarcopenia had 12.9 times higher odds of suffering from osteoporosis than women without sarcopenia (*47*). Similarly, in the 'Copenhagen Sarcopenia study', bone mineral density (BMD) was found to be lower in subjects with sarcopenia, increasing the risk of developing osteoporosis (*49*). The synergy of these two conditions is called 'osteosarcopenia' and leads to a higher risk of adverse clinical outcomes than sarcopenia or osteoporosis alone (*50, 51*).

In contrast to a reduction in muscle and bone tissue, the aging process is associated with an increase in fat mass (FM) and with a higher prevalence of overweight and obesity in both women and men (*6*) resulting in a higher risk of morbidity and mortality ((*52, 53*), for a review see (*54*)). Data from the 'German Health Update' (*6*) show that about 26.2% of women and 36.5% of men in the 18-to 29-year-old age group are affected by overweight (including obesity). These proportions rise to more than 56% (women) and 68% (men) among the 65 years old (*6*).

Thus, overweight and obesity are increasingly present in medical practice as people are getting older. Since the prevalence of both sarcopenia and obesity increases with advanced age, 'sarcopenic obesity' is often observed in older adults (*26*). The excess amount of adipose tissue (AT) in addition to a too low MM can exacerbate the negative effect of sarcopenia (*55*). In this context, a higher risk of incident disability and mortality in persons with sarcopenic obesity compared to subjects with sarcopenia alone has been reported (*56*).

The peak FM appears to be obtained between the sixth and seventh decades of life (*57–59*) and then it might plateau or decline in very old subjects (*60*). An increase in FM may mask a MM loss by a concomitant increase in connective tissue (i.e. fat-free mass (FFM) in AT). Further important changes during the aging process are (i) the redistribution from subcutaneous to intra-abdominal visceral fat depots (*61, 62*) enhancing the risk to develop low insulin sensitivity (*63*), type 2 diabetes (*64, 65*) and cardiovascular diseases (*66–68*), (ii) ectopic fat infiltrations within the liver, muscle and bone marrow (for a review see (*69*)) and (iii) the release of free fatty acids (for a review see (*70*)). These changes contribute to a decline of strength in both muscle and bone ((*71–73*), except liver fat). As underlying mechanism, the release of free fatty acids has been reported to exert a lipotoxic effect on osteocytes, osteoblasts and myocytes (*74, 75*). The abnormal secretion of pro-inflammatory mediators by AT and ectopic fat may further exacerbate the musculoskeletal decline in those individuals with obesity (*76, 77*).

Taken together, the age-related decline in muscle and bone mass with simultaneous increase in FM and redistribution of AT can lead to an 'obese osteosarcopenic' phenotype, a new syndrome which was introduced in 2014 by Ilich and colleagues (*78*). Up to that time point, the combination of only two tissues like muscle (sarcopenia) and bone (osteoporosis (*46, 47*), for a review see (*79*)) or muscle and AT (sarcopenic obesity (for reviews see (*80, 81*))) has been discussed. Osteosarcopenic obesity is likely to be associated with negative health outcomes such as a higher risk of frailty (*82*) and poor physical performance and functionality (*82–84*). When combined, it was reported that the triad of sarcopenia, osteoporosis and obesity was associated with worse outcomes compared to persons with sarcopenic obesity, sarcopenia or obesity alone (*83*). Consequently, the triad represents a high health burden for affected individuals. It is therefore important to recognize the co-existence and co-development of all three conditions with ageing and to expand knowledge and understanding of pathophysiological mechanisms helping to set up an improved therapy plan.

History of the diagnosis of obesity, osteoporosis and sarcopenia

In 1832, Adolphe Quetelet developed the body mass index (BMI, kg/m^2). Since Ancel Keys demonstrated that the BMI was a good indirect measure to evaluate total body fat (*85*), it is used until today to assess overweight and obesity in adults (*86*). According to the WHO, overweight in adults is defined as BMI higher or equal to 25 kg/m² and obesity as BMI greater than or equal to 30 kg/m² (*86*).

In 1993, an international consensus development conference statement defined osteoporosis as '[…] *a systematic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture*' (*38*). One year later, the WHO established diagnostic criteria defining osteopenia as one to two point five standard deviations (SDs) and osteoporosis as two point five SDs or more below the mean BMD or bone mineral content (BMC) of a young adult reference group (*87*), using dual X-ray absorptiometry (DXA).

The term sarcopenia derives from the Greek words '*sarx*' meaning flesh and '*penia*' meaning poverty (*88*). It was first defined by Irwin Rosenberg in 1989 referring to the age-related loss of MM (*89*). Seven years later, the new concept of sarcopenic obesity was introduced by Heber and colleagues (*90*). Since MM and strength are not directly correlated with each other (*91, 92*), Clark and Manini subsequently coined the term 'dynapenia' in 2008 to distinguish between the age-related loss of MM (sarcopenia) and the loss of muscle strength and power (dynapenia) (for a review see (*93*)). This knowledge was taken up by 'the European Working Group on Sarcopenia in Older People' (EWGSOP) that provides a working definition of sarcopenia as '[…] *a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength with a risk of adverse outcomes such as physical disability, poor quality of life and death*' (*88*).

Subsequently, various sets of operational criteria for sarcopenia have been developed by other institutional groups such as 'the European Society for Clinical Nutrition and Metabolism Special Interest Groups' (*94*), 'the International Working Group for the Study of Sarcopenia' (*95*), 'the Asian Working Group for Sarcopenia' (AWGS) (for a review see (*96*)), 'the AWGS 2019' (*97*), 'the Foundation for the National Institutes of Health Sarcopenia Project' (FNIH) (*98*) and the 'EWGSOP2' (*26*) whereas the sets by the two latter ones are the most widely used. Although the two definitions of FNIH and EWGSOP2 differ to some instances, both define sarcopenia as the loss of MM, decline in muscle strength (e.g. hand grip strength (HGS)) and physical performance (e.g. gait speed).

Since sarcopenia has been recognized as a specific disease entity, it was assigned the individual 'International Statistical Classification of Disease and Related Health Problems' code ICD-10-CM (M62.84) in 2016 *(99, 100)*. This may lead to an increased interest of physicians to diagnose sarcopenia and of pharmacological companies to develop drugs.

Limitations of the definition of sarcopenia

Whereas the diagnostic criteria and definitions of osteoporosis are internationally accepted, no universally adopted consensus on the operational definition of sarcopenia that is suitable for the use in research and clinical practice has been reached. As mentioned above, rather a variety of definitions exist that have been developed based on different reference populations. There is also a lack of standardized diagnostic criteria, the availability of techniques used to accurately determine parameters of sarcopenia and appropriate cut-off points. Missing guidelines lead to challenges in estimating the prevalence of sarcopenia, when comparing results from interventions directed against MM depletion and in diagnosis and treatment of sarcopenia for clinicians.

Due to heterogeneous methods and terminologies that lead to differences in diagnostic criteria among studies and consensus definitions, the assessment of a low MM for the diagnosis of primary and secondary sarcopenia remains difficult. The second chapter of this thesis therefore provides an overview of previously published cut-off points for a low MM applied in clinical and research settings considering the impact of the underlying methodological assumptions, limitations and normalization of MM parameters.

Endocrine relationship between muscle, bone and adipose tissue

The prevalence of each body composition component of osteosarcopenic obesity increases with advanced age and with shared risk factors (for a review see (*101*)). Thus, an overlap in these three conditions can be assumed. In **Figure 1** this overlap between muscle, bone and fat tissues in older adults and its associated risk factors is presented.

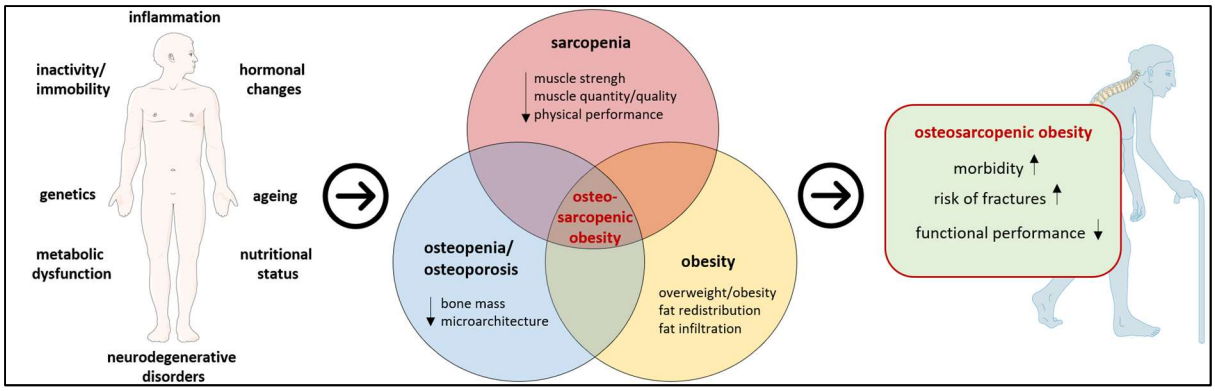


Figure 1. Risk factors for the development of an obese osteosarcopenic phenotype and its health consequences in older adults.

Besides endogenous factors (e.g. genetic, developmental, chronic and neurodegenerative disorders (for reviews see (*78, 102*))), exogenous factors are discussed (e.g. sedentary lifestyle, nutritional status (for reviews see (*101-103*))). The impact of endocrine determinants on body composition has led to an emerging interest since a variety of hormonal changes is observed during the ageing process. In addition to the increase in inflammatory cytokines, aging is characterized by a gradual decrease in anabolic hormones (e.g. sex and growth hormones (*104, 105*)) with a simultaneous increase in catabolic hormones (e.g. cortisol (*106*)). In this context, muscle, bone and AT are recognized as hormonal target tissues and hormonal tissues themselves producing various biologically active proteins (for reviews see (*25, 78, 107, 108*)). Increasing evidence suggests that these three tissues are in a close interrelationship acting through an endocrine crosstalk orchestrated via alterations in levels of myokines (derived from myocytes), osteokines (derived from bone cells) and adipokines (derived from adipocytes) (for reviews see (*25, 78*)). The fourth chapter of this thesis sets out to analyse this 'bone-muscle-fat crosstalk' in healthy community-dwelling older adults.

The adiponectin paradox

Adiponectin is one of the most prominent adipokines. Studies have shown beneficial effects of this hormone on diabetes, metabolic syndrome and cardiovascular diseases in young and middle-aged persons (for a review see (*109*)). It is therefore surprising that human studies suggested that adiponectin levels were negatively correlated with muscle (*110*) and bone tissue (*111*) and positively associated with all-cause and cardiovascular mortality in older adults (*112, 113*). This so called '*adiponectin paradox*' indicates that adiponectin may not exert salutary effects in advanced age. Although some explanations have been proposed for the *adiponectin paradox* (for reviews see (*114, 115*)), it remains unknown why higher adiponectin levels among

older adults are associated with adverse changes in body composition. In the third chapter of this thesis, possible causes for the *adiponectin paradox* were investigated in healthy older adults.

Objectives

Based on this background the present thesis analyses

(i) reference values for a 'normal' skeletal MM (CHAPTER II),

(ii) the paradoxical relationship between adiponectin and MM (CHAPTER III),

(iii) endocrine determinants of bone mass in healthy older adults (CHAPTER IV).

References

1. Sin, M.A., Askar, M., Beermann, S., Bertz, J., Buda, S., Busch, M., Du, Y., Duadareva-Vizule, S., Ellert, U., Fehr, A. *et al*. Gesundheitsberichterstattung des Bundes. Gemeinsam getragen von RKI und Destatis. Gesundheit in Deutschland **2015**, 1–516. Available online: https://www.destatis.de/DE/Themen/Gesellschaft-Umwelt/Gesundheit/Gesundheitszustand-Relevantes-Verhalten/Publikationen/Downloads-Gesundheitszustand/gesundheit-in-deutschland-publikation.pdf?__blob=publicationFile (accessed: 6 December 2022).
2. United Nations, 2. *United Nations Department of Economic and Social Affairs, Population Division (**2022**). World Population Prospects 2022: Summary of Results. UN DESA/POP/2022/TR/NO. 3*, 2022. Available online: https://www.un.org/development/desa/pd/sites/www.un.org.development.desa.pd/files/wpp2022_summary_of_results.pdf (accessed: 6 December 2022).
3. The World Bank. *Life expectancy at birth, total (years). Updated 2022.* Available online: https://data.worldbank.org/indicator/SP.DYN.LE00.IN (accessed: 6 December 2022).
4. DESTATIS. *Sterbefälle und Lebenserwartung. Entwicklung der Lebenserwartung in Deutschland seit 1871/1881. Updated 2022.* Available online: https://www.destatis.de/DE/Themen/Gesellschaft-Umwelt/Bevoelkerung/Sterbefaelle-Lebenserwartung/sterbetafel.html (accessed: 6 December 2022).
5. WHO. *Ageing and health - Fact sheet*. *Updated October 2022.* Available online: https://www.who.int/news-room/fact-sheets/detail/ageing-and-health (accessed: 11 December 2022).
6. Schienkiewitz, A.; Kuhnert, R.; Blume, M.; Mensink, G. B. Übergewicht und Adipositas bei Erwachsenen in Deutschland - Ergebnisse der Studie GEDA 2019/2020-EHIS **2022.** DOI: 10.25646/10292.
7. Milanovic, Z.; Jorgić, B.; Trajković, N.; Sporis; Pantelić, S.; James. Age-related decrease in physical activity and functional fitness among elderly men and women. *Clinical Interventions in ageing* **2013,** (8), 549-556. DOI: 10.2147/CIA.S44112.
8. Hall, K. S.; Cohen, H. J.; Pieper, C. F.; Fillenbaum, G. G.; Kraus, W. E.; Huffman, K. M.; Cornish, M. A.; Shiloh, A.; Flynn, C.; Sloane, R. *et al*. Physical Performance Across the Adult Life Span: Correlates With Age and Physical Activity. *The Journals of Gerontology* **2017,** *72* (4), 572-578. DOI: 10.1093/gerona/glw120.
9. Smee, D. J.; Anson, J. M.; Waddington, G. S.; Berry, H. L. Association between Physical Functionality and Falls Risk in Community-Living Older Adults. *Current Gerontology and Geriatrics Research* **2012,** *2012* (6), 1–6. DOI: 10.1155/2012/864516.
10. Guadalupe-Grau, A.; Carnicero, J. A.; Gómez-Cabello, A.; Gutiérrez Avila, G.; Humanes, S.; Alegre, L. M.; Castro, M.; Rodríguez-Mañas, L.; García-García, F. J. Association of regional muscle strength with mortality and hospitalisation in older people. *Age and Ageing* **2015,** *44* (5), 790–795. DOI: 10.1093/ageing/afv080.
11. Cesari, M.; Onder, G.; Russo, A.; Zamboni, V.; Barillaro, C.; Ferrucci, L.; Pahor, M.; Bernabei, R.; Landi, F. Comorbidity and Physical Function: Results from the Aging and Longevity Study in the Sirente Geographic Area (ilSIRENTE Study). *Gerontology* **2006,** *52* (1), 24–32. DOI: 10.1159/000089822.
12. Cheng, Q.; Zhu, X.; Zhang, X.; Li, H.; Du, Y.; Hong, W.; Xue, S.; Zhu, H. A cross-sectional study of loss of muscle mass corresponding to sarcopenia in healthy Chinese men and

women: Reference values, prevalence, and association with bone mass. *Journal of Bone and Mineral Metabalism* **2014,** *32* (1), 78–88. DOI: 10.1007/s00774-013-0468-3.

13. Coin, A.; Sarti, S.; Ruggiero, E.; Giannini, S.; Pedrazzoni, M.; Minisola, S.; Rossini, M.; Del Puente, A.; Inelmen, E. M.; Manzato, E. *et al*. Prevalence of Sarcopenia Based on Different Diagnostic Criteria Using DEXA and Appendicular Skeletal Muscle Mass Reference Values in an Italian Population Aged 20 to 80. *Journal of the American Medical Directors Association* **2013,** *14* (7), 507–512. DOI: 10.1016/j.jamda.2013.02.010.
14. Marwaha, R. K.; Garg, M. K.; Bhadra, K.; Mithal, A.; Tandon, N. Assessment of lean (muscle) mass and its distribution by dual energy X-ray absorptiometry in healthy Indian females. *Archives of Osteoporosis* **2014,** *9* (1), 129. DOI: 10.1007/s11657-014-0186-z.
15. Kwon, H.-J.; Ha, Y.-C.; Park, H.-M. The Reference Value of Skeletal Muscle Mass Index for Defining the Sarcopenia of Women in Korea. *Journal of Bone Metabolism* **2015,** *22* (2), 71-75. DOI: 10.11005/jbm.2015.22.2.71.
16. Shafiee, G.; Ostovar, A.; Heshmat, R.; Keshtkar, A.; Sharifi, F.; Shadman, Z.; Nabipour, I.; Soltani, A.; Larijani, B. Appendicular Skeletal Muscle Mass Reference Values and the Peak Muscle Mass to Identify Sarcopenia among Iranian Healthy Population. *International Journal of Preventive Medicine* **2018,** *9* (1), 25. DOI: 10.4103/ijpvm.IJPVM_295_17.
17. Recker, R.R, Davies, S.M., Hinders, S.M., Heaney, R.P., Stegman, M.R., Kimmel, D.B. Bone Gain in Young Adult Women. *JAMA* **1992,** *268* (17), 2403–2408.
18. Firooznia, H.; Golimbu, C.; Rafil, M.; Schwartz, M. S.; Alterman, E. R. Quantitative computed tomography assessment of spinal trabecular bone. I. Age-related regression in normal men and women. *Journal of Computed Tomography* **1984,** *8* (2), 91–97. DOI: 10.1016/0149-936X(84)90091-2.
19. Sambrook, P.; Kelly, P.; Eisman, J. Bone mass and ageing. *Baillière's Clinical Rheumatology* **1993,** *7* (3), 445–457. DOI: 10.1016/S0950-3579(05)80072-X.
20. Weaver, C. M.; Gordon, C. M.; Janz, K. F.; Kalkwarf, H. J.; Lappe, J. M.; Lewis, R.; O'Karma, M.; Wallace, T. C.; Zemel, B. S. The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: A systematic review and implementation recommendations. *Osteoporosis International* **2016,** *27* (4), 1281–1386. DOI: 10.1007/s00198-015-3440-3.
21. Wolfe, R. R. The underappreciated role of muscle in health and disease. *The American Journal of Clinical Nutrition* **2006,** *84* (3), 475–482. DOI: 10.1093/ajcn/84.3.475.
22. Ferron, M.; Lacombe, J. Regulation of energy metabolism by the skeleton: Osteocalcin and beyond. *Archives of Biochemistry and Biophysics* **2014,** *561*, 137–146. DOI: 10.1016/j.abb.2014.05.022.
23. Tieland, M.; Trouwborst, I.; Clark, B. C. Skeletal muscle performance and ageing. *Journal of Cachexia, Sarcopenia and Muscle* **2018,** *9* (1), 3–19. DOI: 10.1002/jcsm.12238.
24. Lombardi, G. Exercise-Dependent Modulation of Bone Metabolism and Bone Endocrine Function: New Findings and Therapeutic Perspectives. *Journal of Science in Sport and Exercise* **2019,** *1* (1), 20–28. DOI: 10.1007/s42978-019-0010-y.
25. Kirk, B.; Feehan, J.; Lombardi, G.; Duque, G. Muscle, Bone, and Fat Crosstalk: The Biological Role of Myokines, Osteokines, and Adipokines. *Current Osteoporosis Reports* **2020,** *18* (4), 388–400. DOI: 10.1007/s11914-020-00599-y.
26. Cruz-Jentoft, A. J.; Bahat, G.; Bauer, J.; Boirie, Y.; Bruyère, O.; Cederholm, T.; Cooper, C.; Landi, F.; Rolland, Y.; Sayer, A.A. *et al*. Sarcopenia: Revised European consensus on

definition and diagnosis. *Age and Ageing* **2019,** *48* (1), 16–31. DOI: 10.1093/ageing/afy169.

27. Barbat-Artigas, S.; Rolland, Y.; Zamboni, M.; Aubertin-Leheudre, M. How to assess functional status: A new muscle quality index. *Journal of Nutrition, Health and Aging* **2012,** *16* (1), 67–77. DOI: 10.1007/s12603-012-0004-5.
28. Mitchell, W. K.; Williams, J.; Atherton, P.; Larvin, M.; Lund, J.; Narici, M. Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength; a quantitative review. *Frontiers in Physiology* **2012,** *3. DOI:* 10.3389/fphys.2012.00260.
29. Daly, R. M.; Rosengren, B. E.; Alwis, G.; Ahlborg, H. G.; Sernbo, I.; Karlsson, M. K. Gender specific age-related changes in bone density, muscle strength and functional performance in the elderly: A-10 year prospective population-based study. *BMC Geriatrics* **2013,** *13* (1), 208. DOI: 10.1186/1471-2318-13-71.
30. Forrest, K.; Zmuda, J.; Cauley, J. Patterns and determinants of muscle strength change with aging in older men. *The Aging Male* **2005,** *8* (3), 151–156. DOI: 10.1080/13685530500137840.
31. Rantanen, T.; Masaki, K.; Foley, D.; Izmirlian, G.; White, L.; Guralnik, J. M. Grip strength changes over 27 yr in Japanese-American men. *Journal of Applied Physiology* **1998,** *85* (6), 2047–2053. DOI: 10.1152/jappl.1998.85.6.2047.
32. Bassey, E. J.; Harries, U. J. Normal values for handgrip strength in 920 men and women aged over 65 years, and longitudinal changes over 4 years in 620 survivors. *Clinical Science* **1993,** *84* (3), 331–337. DOI: 10.1042/cs0840331.
33. Yeung, S. S. Y.; Reijnierse, E. M.; Pham, V. K.; Trappenburg, M. C.; Lim, W. K.; Meskers, C. G. M.; Maier, A. B. Sarcopenia and its association with falls and fractures in older adults: A systematic review and meta-analysis. *Journal of Cachexia, Sarcopenia and Muscle* **2019,** *10* (3), 485–500. DOI: 10.1002/jcsm.12411.
34. Iida, H.; Seki, T.; Sakai, Y.; Watanabe, T.; Wakao, N.; Matsui, H.; Imagama, S. Low muscle mass affect hip fracture treatment outcomes in older individuals: A single-institution case-control study. *BMC Musculoskelet Disorders* **2021,** *22* (1), 259. DOI: 10.1186/s12891-021-04143-6.
35. Peng, T.-C.; Chen, W.-L.; Wu, L.-W.; Chang, Y.-W.; Kao, T.-W. Sarcopenia and cognitive impairment: A systematic review and meta-analysis. *Clinical Nutrition* **2020,** *39* (9), 2695–2701. DOI: 10.1016/j.clnu.2019.12.014.
36. Salinas-Rodríguez, A.; Palazuelos-González, R.; Rivera-Almaraz, A.; Manrique-Espinoza, B. Longitudinal association of sarcopenia and mild cognitive impairment among older Mexican adults. *Journal of Cachexia, Sarcopenia and Muscle* **2021,** *12* (6), 1848–1859. DOI: 10.1002/jcsm.12787.
37. Mazess, R. B. On aging bone loss. *Clinical orthopaedics and related research* **1982** (165), 239–252.
38. Peck, W.A., Burckhardt, P., Christiansen C. Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis. *The American Journal of Medicine* **1993,** *94* (6), 646–650. DOI: 10.1016/0002-9343(93)90218-e.
39. Magaziner, J. Changes in Functional Status Attributable to Hip Fracture: A Comparison of Hip Fracture Patients to Community-dwelling Aged. *American Journal of Epidemiology* **2003,** *157* (11), 1023–1031. DOI: 10.1093/aje/kwg081.

40. Fredman, L.; Magaziner, J.; Hawkes, W.; Hebel, J.; Fried, L.; Kasper, J.; Guralnik, J. Female hip fracture patients had poorer performance-based functioning than community-dwelling peers over 2-year follow-up period. *Journal of Clinical Epidemiology* **2005,** *58* (12), 1289–1298. DOI: 10.1016/j.jclinepi.2004.04.016.

41. Borgström, F.; Zethraeus, N.; Johnell, O.; Lidgren, L.; Ponzer, S.; Svensson, O.; Abdon, P.; Ornstein, E.; Lunsjö, K.; Thorngren, K. G. *et al*. Costs and quality of life associated with osteoporosis-related fractures in Sweden. *Osteoporosis International* **2006,** *17* (5), 637–650. DOI: 10.1007/s00198-005-0015-8.

42. Adachi, J. D.; Adami, S.; Gehlbach, S.; Anderson, F. A.; Boonen, S.; Chapurlat, R. D.; Compston, J. E.; Cooper, C.; Delmas, P.; Díez-Pérez, A. *et al*. Impact of Prevalent Fractures on Quality of Life: Baseline Results From the Global Longitudinal Study of Osteoporosis in Women. *Mayo Clinic Proceedings* **2010,** *85* (9), 806–813. DOI: 10.4065/mcp.2010.0082.

43. Johnell, O.; Kanis, J. A.; Odén, A.; Sernbo, I.; Redlund-Johnell, I.; Petterson, C.; Laet, C. de; Jönsson, B. Mortality after osteoporotic fractures. *Osteoporosis International* **2004,** *15* (1), 38–42. DOI: 10.1007/s00198-003-1490-4.

44. Brown, J. P.; Adachi, J. D.; Schemitsch, E.; Tarride, J.-E.; Brown, V.; Bell, A.; Reiner, M.; Oliveira, T.; Motsepe-Ditshego, P.; Burke, N. *et al*. Mortality in older adults following a fragility fracture: Real-world retrospective matched-cohort study in Ontario. *BMC Musculoskeletal Disorders* **2021,** *22* (1), 105. DOI: 10.1186/s12891-021-03960-z.

45. International Osteoporosis Foundation. *Epidemiology of osteoporosis and fragility and fractures. Updated 2022*. Available online*:* https://www.osteoporosis.foundation/facts-statistics/epidemiology-of-osteoporosis-and-fragility-fractures#ref_bottom_2 (accessed: 10 January 2023).

46. Verschueren, S.; Gielen, E.; O'Neill, T. W.; Pye, S. R.; Adams, J. E.; Ward, K. A.; Wu, F. C.; Szulc, P.; Laurent, M.; Claessens, F. *et al*. Sarcopenia and its relationship with bone mineral density in middle-aged and elderly European men. *Osteoporosis International* **2013,** *24* (1), 87–98. DOI: 10.1007/s00198-012-2057-z.

47. Sjöblom, S.; Suuronen, J.; Rikkonen, T.; Honkanen, R.; Kröger, H.; Sirola, J. Relationship between postmenopausal osteoporosis and the components of clinical sarcopenia. *Maturitas* **2013,** *75* (2), 175–180. DOI: 10.1016/j.maturitas.2013.03.016.

48. He, H.; Liu, Y.; Tian, Q.; Papasian, C. J.; Hu, T.; Deng, H.-W. Relationship of sarcopenia and body composition with osteoporosis. *Osteoporosis International* **2016,** *27* (2), 473–482. DOI: 10.1007/s00198-015-3241-8.

49. Nielsen, B. R.; Andersen, H. E.; Haddock, B.; Hovind, P.; Schwarz, P.; Suetta, C. Prevalence of muscle dysfunction concomitant with osteoporosis in a home-dwelling Danish population aged 65–93 years - The Copenhagen Sarcopenia Study. *Experimental Gerontology* **2020,** *138* (5), 110974. DOI: 10.1016/j.exger.2020.110974.

50. Chalhoub, D.; Cawthon, P. M.; Ensrud, K. E.; Stefanick, M. L.; Kado, D. M.; Boudreau, R.; Greenspan, S.; Newman, A. B.; Zmuda, J.; Orwoll, E. S.; Cauley, J. A. Risk of Nonspine Fractures in Older Adults with Sarcopenia, Low Bone Mass, or Both. *Journal of the American Geriatrics Society* **2015,** *63* (9), 1733–1740. DOI: 10.1111/jgs.13605.

51. Yoo, J.-I.; Kim, H.; Ha, Y.-C.; Kwon, H.-B.; Koo, K.-H. Osteosarcopenia in Patients with Hip Fracture Is Related with High Mortality. *Journal of Korean Medical Science* **2018,** *33* (4), 675. DOI: 10.3346/jkms.2018.33.e27.

52. Manson, J. E.; Willett, W. C.; Stampfer, M. J.; Colditz, G. A.; Hunter, D. J.; Hankinson, S. E.; Hennekens, C. H.; Speizer, F. E. Body Weight and Mortality among Women. The *new England Journal of Medicine* **1995,** *333* (11), 677–685. DOI: 10.1056/NEJM199509143331101.

53. Seidell, J. C.; Verschuren, W. M.; van, L. E. M.; Kromhout, D. Overweight, underweight, and mortality. A prospective study of 48,287 men and women. *Archives of International Medicine* **1996,** *156* (9), 958-963. DOI: 10.1001/archinte.156.9.958.

54. Abdelaal, M.; Le Roux, C. W.; Docherty, N. G. Morbidity and mortality associated with obesity. *Annals of Translational Medicine* **2017,** *5* (7), 161. DOI: 10.21037/atm.2017.03.107.

55. Ishimura, E.; Okuno, S.; Nakatani, S.; Mori, K.; Miyawaki, J.; Okazaki, H.; Sugie, N.; Norimine, K.; Yamakawa, K.; Tsujimoto, Y. *et al*. Significant Association of Diabetes With Mortality of Chronic Hemodialysis Patients, Independent of the Presence of Obesity, Sarcopenia, and Sarcopenic Obesity. *Journal of Renal Nutrition* **2022,** *32* (1), 94–101. DOI: 10.1053/j.jrn.2021.07.003.

56. Baumgartner, R. N.; Wayne, S. J.; Waters, D. L.; Janssen, I.; Gallagher, D.; Morley, J. E. Sarcopenic obesity predicts instrumental activities of daily living disability in the elderly. *Obesity Research* **2004,** *12* (12), 1995–2004. DOI: 10.1038/oby.2004.250.

57. Kyle, U. G.; Genton, L.; Hans, D.; Karsegard, L.; Slosman, D. O.; Pichard, C. Age-related differences in fat-free mass, skeletal muscle, body cell mass and fat mass between 18 and 94 years. *European Journal of Clinical Nutrition* **2001,** *55* (8), 663–672. DOI: 10.1038/sj.ejcn.1601198.

58. Jackson, A. S.; Janssen, I.; Sui, X.; Church, T. S.; Blair, S. N. Longitudinal changes in body composition associated with healthy ageing: Men, aged 20-96 years. *The British Journal of Nutrition* **2012,** *107* (7), 1085–1091. DOI: 10.1017/S0007114511003886.

59. Sornay-Rendu, E.; Karras-Guillibert, C.; Munoz, F.; Claustrat, B.; Chapurlat, R. D. Age determines longitudinal changes in body composition better than menopausal and bone status: The OFELY study. *Journal of Bone and Mineral research* **2012,** *27* (3), 628–636. DOI: 10.1002/jbmr.1469.

60. Kelly, T. L.; Wilson, K. E.; Heymsfield, S. B. Dual energy X-Ray absorptiometry body composition reference values from NHANES. *PLoS ONE* **2009,** *4* (9), e7038. DOI: 10.1371/journal.pone.0007038.

61. Schautz, B.; Later, W.; Heller, M.; Peters, A.; Müller, M. J.; Bosy-Westphal, A. Impact of age on leptin and adiponectin independent of adiposity. The *British Journal of Nutrition* **2012,** *108* (2), 363–370. DOI: 10.1017/S0007114511005605.

62. Geisler, C.; Braun, W.; Pourhassan, M.; Schweitzer, L.; Glüer, C.-C.; Bosy-Westphal, A.; Müller, M. J. Gender-Specific Associations in Age-Related Changes in Resting Energy Expenditure (REE) and MRI Measured Body Composition in Healthy Caucasians. *The Journals of Gerontology* **2016,** *71* (7), 941–946. DOI: 10.1093/gerona/glv211.

63. Kirchhoff, K.; Kantartzis, K.; Machann, J.; Schick, F.; Thamer, C.; Machicao, F.; Fritsche, A.; Häring, H.-U.; Stefan, N. Impact of different fat depots on insulin sensitivity: Predominant role of liver fat. *Journal of Diabetes Science and Technology* **2007,** *1* (5), 753–759. DOI: 10.1177/193229680700100521.

64. Boyko, E. J.; Fujimoto, W. Y.; Leonetti, D. L.; Newell-Morris, L. Visceral adiposity and risk of type 2 diabetes: A prospective study among Japanese Americans. *Diabetes Care* **2000,** *23* (4), 465–471. DOI: 10.2337/diacare.23.4.465.

65. Goodpaster, B. H.; Krishnaswami, S.; Resnick, H.; Kelley, D. E.; Haggerty, C.; Harris, T. B.; Schwartz, A. V.; Kritchevsky, S.; Newman, A. B. Association between regional adipose tissue distribution and both type 2 diabetes and impaired glucose tolerance in elderly men and women. *Diabetes Care* **2003,** *26* (2), 372–379. DOI: 10.2337/diacare.26.2.372.

66. Nicklas, B. J.; Penninx, B. W. J. H.; Cesari, M.; Kritchevsky, S. B.; Newman, A. B.; Kanaya, A. M.; Pahor, M.; Jingzhong, D.; Harris, T. B. Association of visceral adipose tissue with incident myocardial infarction in older men and women: The Health, Aging and Body Composition Study. *American Journal of Epidemiology* **2004,** *160* (8), 741–749. DOI: 10.1093/aje/kwh281.

67. Kouli, G.-M.; Panagiotakos, D. B.; Kyrou, I.; Georgousopoulou, E. N.; Chrysohoou, C.; Tsigos, C.; Tousoulis, D.; Pitsavos, C. Visceral adiposity index and 10-year cardiovascular disease incidence: The ATTICA study. *Nutrition, Metabolism, and Cardiovascular Diseases* **2017,** *27* (10), 881–889. DOI: 10.1016/j.numecd.2017.06.015.

68. Ballin, M.; Nordström, P.; Niklasson, J.; Nordström, A. Associations of Visceral Adipose Tissue and Skeletal Muscle Density With Incident Stroke, Myocardial Infarction, and All-Cause Mortality in Community-Dwelling 70-Year-Old Individuals: A Prospective Cohort Study. *Journal of the American Heart Association* **2021,** *10* (9), e020065. DOI: 10.1161/JAHA.120.020065.

69. Kuk, J. L.; Saunders, T. J.; Davidson, L. E.; Ross, R. Age-related changes in total and regional fat distribution. *Ageing Research Reviews* **2009,** *8* (4), 339–348. DOI: 10.1016/j.arr.2009.06.001.

70. Sepe, A.; Tchkonia, T.; Thomou, T.; Zamboni, M.; Kirkland, J. L. Aging and regional differences in fat cell progenitors - a mini-review. *Gerontology* **2011,** *57* (1), 66–75. DOI: 10.1159/000279755.

71. Goodpaster, B. H.; Carlson, C. L.; Visser, M.; Kelley, D. E.; Scherzinger, A.; Harris, T. B.; Stamm, E.; Newman, A. B. Attenuation of skeletal muscle and strength in the elderly: The Health ABC Study. *Journal of Applied Physiology* **2001,** *90* (6), 2157–2165. DOI: 10.1152/jappl.2001.90.6.2157.

72. Hilton, T. N.; Tuttle, L. J.; Bohnert, K. L.; Mueller, M. J.; Sinacore, D. R. Excessive adipose tissue infiltration in skeletal muscle in individuals with obesity, diabetes mellitus, and peripheral neuropathy: Association with performance and function. *Physical Therapy* **2008,** *88* (11), 1336–1344. DOI: 10.2522/ptj.20080079.

73. Schellinger, D.; Lin, C. S.; Hatipoglu, H. G.; Fertikh, D. Potential value of vertebral proton MR spectroscopy in determining bone weakness. *American Journal of Neuroradiology* **2001,** *22* (8), 1620–1627.

74. Al Saedi, A.; Bermeo, S.; Plotkin, L.; Myers, D. E.; Duque, G. Mechanisms of palmitate-induced lipotoxicity in osteocytes. *Bone* **2019,** *127* (35), 353–359. DOI: 10.1016/j.bone.2019.06.016.

75. Al Saedi, A.; A. Goodman, C.; E. Myers, D.; Hayes, A.; Duque, G.; Lamming, D. Rapamycin Affects Palmitate-Induced Lipotoxicity in Osteoblasts by Modulating Apoptosis and Autophagy. *The Journals of Gerontology* **2020,** *75* (1), 58–63. DOI: 10.1093/gerona/glz149.

76. Schaap, L. A.; Pluijm, S. M. F.; Deeg, D. J. H.; Visser, M. Inflammatory markers and loss of muscle mass (sarcopenia) and strength. *The American Journal of Medicine* **2006,** *119* (6), 526.e9-17. DOI: 10.1016/j.amjmed.2005.10.049.

77. Schaap, L. A.; Pluijm, S. M. F.; Deeg, D. J. H.; Harris, T. B.; Kritchevsky, S. B.; Newman, A. B.; Colbert, L. H.; Pahor, M.; Rubin, S. M.; Tylavsky, F. A. *et al*. Higher inflammatory marker levels in older persons: Associations with 5-year change in muscle mass and muscle strength. *The Journals of Gerontology* **2009,** *64* (11), 1183–1189. DOI: 10.1093/gerona/glp097.

78. Ilich, J. Z.; Kelly, O. J.; Inglis, J. E.; Panton, L. B.; Duque, G.; Ormsbee, M. J. Interrelationship among muscle, fat, and bone: Connecting the dots on cellular, hormonal, and whole body levels. *Ageing Research Reviews* **2014,** *15*, 51–60. DOI: 10.1016/j.arr.2014.02.007.

79. Kaji, H. Linkage between muscle and bone: common catabolic signals resulting in osteoporosis and sarcopenia. *Current Opinion in Clinical Nutrition and Metabolic Care* **2013,** *16* (3), 272–277. DOI: 10.1097/MCO.0b013e32835fe6a5.

80. Stenholm, S.; Harris, T. B.; Rantanen, T.; Visser, M.; Kritchevsky, S. B.; Ferrucci, L. Sarcopenic obesity: definition, cause and consequences. *Current Opinion in Clinical Nutrition and Metabolic Care* **2008,** *11* (6), 693–700. DOI: 10.1097/MCO.0b013e328312c37d.

81. Zamboni, M.; Mazzali, G.; Fantin, F.; Rossi, A.; Di Francesco, V. Sarcopenic obesity: a new category of obesity in the elderly. *Nutrition, Metabolism, and Cardiovascular Diseases* **2008,** *18* (5), 388–395. DOI: 10.1016/j.numecd.2007.10.002.

82. Szlejf, C.; Parra-Rodríguez, L.; Rosas-Carrasco, O. Osteosarcopenic Obesity: Prevalence and Relation With Frailty and Physical Performance in Middle-Aged and Older Women. *Journal of the American Medical Directors Association* **2017,** *18* (8), 733.e1-733.e5. DOI: 10.1016/j.jamda.2017.02.023.

83. Ilich, J. Z.; Inglis, J. E.; Kelly, O. J.; McGee, D. L. Osteosarcopenic obesity is associated with reduced handgrip strength, walking abilities, and balance in postmenopausal women. *Osteoporosis International* **2015,** *26* (11), 2587–2595. DOI: 10.1007/s00198-015-3186-y.

84. Ma, Q.; Cheng, X.; Hou, X.; Yang, Z.; Ma, D.; Wang, Z. Bone Marrow Fat Measured by a Chemical Shift-Encoded Sequence (IDEAL-IQ) in Patients With and Without Metabolic Syndrome. *Journal of Magnetic Resonance Imaging* **2021,** *54* (1), 146–153. DOI: 10.1002/jmri.27548.

85. Keys, A.; Fidanza, F.; Karvonen, M. J.; Kimura, N.; Taylor, H. L. Indices of relative weight and obesity. *Journal of Chronic Diseases* **1972,** *25* (6-7), 329–343. DOI: 10.1016/0021-9681(72)90027-6.

86. WHO, *Obesity*: *Preventing and managing the global epidemic*; WHO technical report series; Vol. 894; World Health Organization, 2000. Available online: https://apps.who.int/iris/handle/10665/42330 (accessed: 15 January 2023).

87. Kanis, J. A. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: Synopsis of a WHO report. *Osteoporosis International* **1994,** *4* (6), 368–381. DOI: 10.1007/BF01622200.

88. Cruz-Jentoft, A. J.; Baeyens, J. P.; Bauer, J. M.; Boirie, Y.; Cederholm, T.; Landi, F.; Martin, F. C.; Michel, J.-P.; Rolland, Y.; Schneider, S. M. *et al*. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age and Ageing* **2010,** *39* (4), 412–423. DOI: 10.1093/ageing/afq034.

89. Rosenberg, I. H. Summary comments. *The American Journal of Clinical Nutrition* **1989,** *50* (5), 1231–1233. DOI: 10.1093/ajcn/50.5.1231.

90. Heber, D.; Ingles, S.; Ashley, J. M.; Maxwell, M. H.; Lyons, R. F.; Elashoff, R. M. Clinical detection of sarcopenic obesity by bioelectrical impedance analysis. *The American Journal of Clinical Nutrition* **1996,** *64* (3), 472S-477S. DOI: 10.1093/ajcn/64.3.472S.

91. Goodpaster, B. H.; Park, S. W.; Harris, T. B.; Kritchevsky, S. B.; Nevitt, M.; Schwartz, A. V.; Simonsick, E. M.; Tylavsky, F. A.; Visser, M.; Newman, A. B. The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. *The Journals of Gerontology* **2006,** *61* (10), 1059–1064. DOI: 10.1093/gerona/61.10.1059.

92. Janssen, I.; Baumgartner, R. N.; Ross, R.; Rosenberg, I. H.; Roubenoff, R. Skeletal muscle cutpoints associated with elevated physical disability risk in older men and women. *American Journal of Epidemiology* **2004,** *159* (4), 413–421. DOI: 10.1093/aje/kwh058.

93. Clark, B. C.; Manini, T. M. Sarcopenia =/= dynapenia. *The Journals of Gerontology* **2008,** *63* (8), 829–834. DOI: 10.1093/gerona/63.8.829.

94. Muscaritoli, M.; Anker, S. D.; Argilés, J.; Aversa, Z.; Bauer, J. M.; Biolo, G.; Boirie, Y.; Bosaeus, I.; Cederholm, T.; Costelli, P. *et al*. Consensus definition of sarcopenia, cachexia and pre-cachexia: joint document elaborated by Special Interest Groups (SIG) "cachexia-anorexia in chronic wasting diseases" and "nutrition in geriatrics". *Clinical Nutrition* **2010,** *29* (2), 154–159. DOI: 10.1016/j.clnu.2009.12.004.

95. Fielding, R. A.; Vellas, B.; Evans, W. J.; Bhasin, S.; Morley, J. E.; Newman, A. B.; van Abellan Kan, G.; Andrieu, S.; Bauer, J.; Breuille, D. *et al*. Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. International working group on sarcopenia. *Journal of the American Medical Directors Association* **2011,** *12* (4), 249–256. DOI: 10.1016/j.jamda.2011.01.003.

96. Chen, L.-K.; Liu, L.-K.; Woo, J.; Assantachai, P.; Auyeung, T.-W.; Bahyah, K. S.; Chou, M.-Y.; Chen, L.-Y.; Hsu, P.-S.; Krairit, O. *et al*. Sarcopenia in Asia: consensus report of the Asian Working Group for Sarcopenia. *Journal of the American Medical Directors Association* **2014,** *15* (2), 95–101. DOI: 10.1016/j.jamda.2013.11.025.

97. Chen, L.-K.; Woo, J.; Assantachai, P.; Auyeung, T.-W.; Chou, M.-Y.; Iijima, K.; Jang, H. C.; Kang, L.; Kim, M.; Kim, S. *et al*. Asian Working Group for Sarcopenia: 2019 Consensus Update on Sarcopenia Diagnosis and Treatment. *Journal of the American Medical Directors Association* **2020,** *21* (3), 300-307.e2. DOI: 10.1016/j.jamda.2019.12.012.

98. Studenski, S. A.; Peters, K. W.; Alley, D. E.; Cawthon, P. M.; McLean, R. R.; Harris, T. B.; Ferrucci, L.; Guralnik, J. M.; Fragala, M. S.; Kenny, A. *et al*. The FNIH sarcopenia project: rationale, study description, conference recommendations, and final estimates. *The Journals of Gerontology* **2014,** *69* (5), 547–558. DOI: 10.1093/gerona/glu010.

99. ICD10Data. *2023 ICD-10-CM Diagnosis Code M62.84*. Available online: https:// www.icd10data.com/ICD10CM/Codes/M00-M99/M60-M63/M62-/M62.84 (accessed: 17 January 2023).

100. Vellas, B.; Fielding, R. A.; Bens, C.; Bernabei, R.; Cawthon, P. M.; Cederholm, T.; Cruz-Jentoft, A. J.; Del Signore, S.; Donahue, S.; Morley, J. *et al*. Implications of ICD-10 for Sarcopenia Clinical Practice and Clinical Trials: Report by the International Conference on Frailty and Sarcopenia Research Task Force. *The Journal of Frailty & Aging* **2018,** *7* (1), 2–9. DOI: 10.14283/jfa.2017.30.

101. Bauer, J. M.; Cruz-Jentoft, A. J.; Fielding, R. A.; Kanis, J. A.; Reginster, J.-Y.; Bruyère, O.; Cesari, M.; Chapurlat, R.; Al-Daghri, N.; Dennison, E. *et al*. Is There Enough Evidence

for Osteosarcopenic Obesity as a Distinct Entity? A Critical Literature Review. *Calcified Tissue International* **2019,** *105* (2), 109–124. DOI: 10.1007/s00223-019-00561-w.

102. Ormsbee, M. J.; Prado, C. M.; Ilich, J. Z.; Purcell, S.; Siervo, M.; Folsom, A.; Panton, L. Osteosarcopenic obesity: the role of bone, muscle, and fat on health. *Journal of Cachexia, Sarcopenia and Muscle* **2014,** *5* (3), 183–192. DOI: 10.1007/s13539-014-0146-x.

103. Kelly, O. J.; Gilman, J. C.; Boschiero, D.; Ilich, J. Z. Osteosarcopenic Obesity: Current Knowledge, Revised Identification Criteria and Treatment Principles. *Nutrients* **2019,** *11* (4), 747 DOI: 10.3390/nu11040747.

104. Morley, J. E.; Perry, H. M. Androgens and Women at the Menopause and Beyond. *The Journals of Gerontology* **2003,** *58* (5), M409-M416. DOI: 10.1093/gerona/58.5.M409.

105. Finkelstein, J. S.; Lee, H.; Burnett-Bowie, S.-A. M.; Pallais, J. C.; Yu, E. W.; Borges, L. F.; Jones, B. F.; Barry, C. V.; Wulczyn, K. E.; Thomas, B. J. *et al*. Gonadal Steroids and Body Composition, Strength, and Sexual Function in Men. The *new England Journal of Medicine* **2013,** *369* (11), 1011–1022. DOI: 10.1056/NEJMoa1206168.

106. Lamberts, S. W.; van den Beld, A. W.; van der Lely, A. J. The endocrinology of aging. *Science* **1997,** *278* (5337), 419–424. DOI: 10.1126/science.278.5337.419.

107. Gomarasca, M.; Banfi, G.; Lombardi, G. Myokines: The endocrine coupling of skeletal muscle and bone. *Advances in Clinical Chemistry* **2020,** *94*, 155–218. DOI: 10.1016/bs.acc.2019.07.010.

108. He, C.; He, W.; Hou, J.; Chen, K.; Huang, M.; Yang, M.; Luo, X.; Li, C. Bone and Muscle Crosstalk in Aging. *Frontiers in Cell and Developmental Biology* **2020,** *8*, 585644. DOI: 10.3389/fcell.2020.585644.

109. Di Chiara, T.; Argano, C.; Corrao, S.; Scaglione, R.; Licata, G. Hypoadiponectinemia: A Link between Visceral Obesity and Metabolic Syndrome. *Journal of Nutrition and Metabolism* **2012,** *2012* (12), 1–7. DOI: 10.1155/2012/175245.

110. Baker, J. F.; Newman, A. B.; Kanaya, A.; Leonard, M. B.; Zemel, B.; Miljkovic, I.; Long, J.; Weber, D.; Harris, T. B. The Adiponectin Paradox in the Elderly: Associations With Body Composition, Physical Functioning, and Mortality. *The Journals of Gerontology* **2019,** *74* (2), 247–253. DOI: 10.1093/gerona/gly017.

111. Michaëlsson, K.; Lind, L.; Frystyk, J.; Flyvbjerg, A.; Gedeborg, R.; Berne, C.; Zethelius, B.; Mallmin, H.; Söderberg, S.; Melhus, H. Serum adiponectin in elderly men does not correlate with fracture risk. *The Journal of Clinical Endocrinology and Metabolism* **2008,** *93* (10), 4041–4047. DOI: 10.1210/jc.2008-0617.

112. Menzaghi, C.; Trischitta, V. The Adiponectin Paradox for All-Cause and Cardiovascular Mortality. *Diabetes* **2018,** *67* (1), 12–22. DOI: 10.2337/dbi17-0016.

113. Obata, Y.; Yamada, Y.; Takahi, Y.; Baden, M. Y.; Saisho, K.; Tamba, S.; Yamamoto, K.; Umeda, M.; Furubayashi, A.; Matsuzawa, Y. Relationship between serum adiponectin levels and age in healthy subjects and patients with type 2 diabetes. *Clinical Endocrinology* **2013,** *79* (2), 204–210. DOI: 10.1111/cen.12041.

114. Woodward, L.; Akoumianakis, I.; Antoniades, C. Unravelling the adiponectin paradox: novel roles of adiponectin in the regulation of cardiovascular disease. *British Journal of Pharmacology* **2017,** *174* (22), 4007–4020. DOI: 10.1111/bph.13619.

115. Kalkman, H. O. An Explanation for the Adiponectin Paradox. *Pharmaceuticals* **2021,** *14* (12). DOI: 10.3390/ph14121266.

CHAPTER II

REFERENCE VALUES FOR SKELETAL MUSCLE MASS - CURRENT CONCEPTS AND METHODOLOGICAL CONSIDERATIONS

published in nutrients

Nutrients. 2020 Mar 12;12(3):755.
doi: 10.3390/nu12030755

nutrients

MDPI

Review

Reference Values for Skeletal Muscle Mass – Current Concepts and Methodological Considerations

Carina O. Walowski [1], Wiebke Braun [1], Michael J. Maisch [2], Björn Jensen [2], Sven Peine [3], Kristina Norman [4,5], Manfred J. Müller [1] and Anja Bosy-Westphal [1,*]

1 Institute for Human Nutrition and Food Science, Christian-Albrechts-University Kiel, 24105 Kiel, Germany; cwalowski@nutrition.uni-kiel.de (C.O.W.); wbraun@nutrition.uni-kiel.de (W.B.); mmueller@nutrfoodsc.uni-kiel.de (M.J.M.)
2 seca gmbh & co. kg., Hammer Steindamm 3-25, 22089 Hamburg, Germany; michael.maisch@seca.com (M.J.M.); bjoern.jensen@seca.com (B.J.)
3 Institute for Transfusion Medicine, University Hospital Hamburg-Eppendorf, 20246 Hamburg, Germany; s.peine@uke.de
4 Department of Nutrition and Gerontology, German Institute of Human Nutrition, Potsdam-Rehbruecke, 14558 Berlin, Germany; kristina.norman@dife.de
5 Charité-Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, 13347 Berlin, Germany
* Correspondence: abosyw@nutrition.uni-kiel.de; Tel.: +49-(0)431-880-5674

Received: 17 February 2020; Accepted: 9 March 2020; Published: 12 March 2020

Abstract: Assessment of a low skeletal muscle mass (SM) is important for diagnosis of ageing and disease-associated sarcopenia and is hindered by heterogeneous methods and terminologies that lead to differences in diagnostic criteria among studies and even among consensus definitions. The aim of this review was to analyze and summarize previously published cut-offs for SM applied in clinical and research settings and to facilitate comparison of results between studies. Multiple published reference values for discrepant parameters of SM were identified from 64 studies and the underlying methodological assumptions and limitations are compared including different concepts for normalization of SM for body size and fat mass (FM). Single computed tomography or magnetic resonance imaging images and appendicular lean soft tissue by dual X-ray absorptiometry (DXA) or bioelectrical impedance analysis (BIA) are taken as a valid substitute of total SM because they show a high correlation with results from whole body imaging in cross-sectional and longitudinal analyses. However, the random error of these methods limits the applicability of these substitutes in the assessment of individual cases and together with the systematic error limits the accurate detection of changes in SM. Adverse effects of obesity on muscle quality and function may lead to an underestimation of sarcopenia in obesity and may justify normalization of SM for FM. In conclusion, results for SM can only be compared with reference values using the same method, BIA- or DXA-device and an appropriate reference population. Limitations of proxies for total SM as well as normalization of SM for FM are important content-related issues that need to be considered in longitudinal studies, populations with obesity or older subjects.

Keywords: sarcopenia; sarcopenic obesity; skeletal muscle mass; skeletal muscle area; skeletal muscle mass index; appendicular skeletal muscle mass index; fat-free mass index

1. Introduction

Beyond the well-established role of ageing associated loss in skeletal muscle mass (SM) (primary sarcopenia) as a risk factor of frailty, morbidity and mortality in older people, a low SM is observed as a result of diseases like malignant cancer, chronic obstructive pulmonary disease, heart failure and

renal failure (secondary sarcopenia [1]) and is also an emerging prognostic marker in a number of diseases [2–12]. The etiology for sarcopenia as a risk factor might be partly explained by the correlation between SM and cardiac, respiratory or immune function but remains to be investigated further in order to understand the preventative and therapeutic potential of SM. Muscle not only functions as the major tissue for insulin-stimulated glucose uptake, amino acid storage and thermoregulation, but is also secreting a large number of myokines that regulate metabolism in muscle itself as well as in other tissues and organs including adipose tissue, the liver and the brain [13,14]. The recent popularity of SM outpaced the interest in fat mass (FM) that only has a limited and inconsistent impact on morbidity and mortality [15,16]. The assessment of SM by segmentation of continuous whole body magnetic resonance imaging (MRI) is considered as the gold standard [17]. However, this method is too cumbersome and expensive for clinical practice and is even rarely used in studies with larger sample sizes [17,18]. Instead, single slices at different reference sites measured by MRI or obtained from routine computed tomography (CT) examinations are taken as a proxy for the total tissue volume (e.g., L3 muscle cross-sectional area [17,19]). Most commonly, dual X-ray absorptiometry (DXA) is used to assess appendicular lean soft tissue (ASM, the sum of lean soft tissue from both arms and legs) or fat-free mass (FFM, total lean soft tissue plus bone mineral mass or body weight minus FM) as a proxy for SM. More simple and even non-invasive, the output of bioelectrical impedance analysis (BIA) depends on the reference method used to generate the BIA algorithm and can be FFM [20], ASM, e.g., [21–23] or even SM, e.g., [24–27].

To facilitate comparison between studies and to evaluate individual results for SM in patients, it is important to understand the differences between parameters and cut-offs for SM. These differences are not only method inherent but also depend on characteristics of the study population (e.g., ethnicity, age and disease). Device-specific characteristics by different manufacturers determine the validity and precision of parameters for SM. In addition, the available reference values differ with respect to parametric normalization (linear regression or indexing) to account for body size. Further complexity to the definition of a normal SM is derived from the concept of sarcopenic obesity [28]. Since high levels of FM may adversely affect the quality and function of SM [29,30], a normal SM may also depend on the amount of FM.

Different professional associations have published definitions of sarcopenia based on an estimate of SM and impaired muscle strength and/or physical performance [31–37], but no consensus definition has yet been reached. The aim of this review is not to provide an optimal diagnosis of sarcopenia but to compare current definitions of a low SM considering the impact of the underlying methodological assumptions, limitations and normalization of SM parameters for height, weight, body mass index (BMI) or FM.

2. Methods

In order to identify reference values for SM, seven consensus reports were reviewed [31–37]. Further studies were identified through reference lists and a search for relevant articles based on the keywords “sarcopenia”, “low muscle mass”, “cut-off sarcopenia”, “reference value sarcopenia”, “sarcopenic obesity”. Only parameters of SM normalized for height, weight, BMI or FM were considered. To be included in this article, studies were required to contain the following information: method of SM assessment (device), cut-off points for SM and description of the reference population including geographical location, sample size, distribution between sexes and age (range and/or standard deviation (SD) ± mean). Only English language articles were considered. Therefore, 64 studies were identified that met the inclusion criteria. Main reasons for the exclusion of articles were duplicate analyses conducted on the same reference population (only the first published paper was included), a missing normalization of reference values, a sample size <200 subjects (sample size <200 subjects will not be representative for both sexes, all ages and BMI-groups), the use of anthropometric measures to determine a low SM and the adoption of previously published cut-offs regarding SM and obesity.

Study Characteristics

Studies that met the inclusion criteria were published between 1998 and 2019 and were performed in 21 countries. The sample size of the individual studies ranged from 200 to 38,099 subjects with an age range between 18 and >90 years. In 36 studies, the authors clearly indicated that the reference population included healthy individuals.

3. Results

Published cut-off points for a low SM normalized by height are presented in Tables 1–3 stratified by DXA, BIA and CT. In the majority of studies (14 of 32), SM was measured by DXA using lean soft tissue from the arms and legs normalized by height^2 given as appendicular skeletal muscle mass index (ASMI) [22,38–50]. One study [40] used DXA-derived ASM to predict whole body SM measured by MRI using the equation by Kim et al. [51] that was validated in an ethnically diverse sample of healthy men and women. The range of published cut-off values for ASMI by DXA (without considering different classes of sarcopenia) was 5.86–7.40 kg/m^2 in men and 4.42–5.67 kg/m^2 in women.

With ten studies, the second most commonly used method underlying published SM reference values was BIA [21–26,52–55]. To measure SM by BIA, five studies have used the BIA-equation by Janssen et al. [56] to predict SM [24–26,53,55]. This BIA-equation was developed and cross-validated against whole body MRI in a sample of 269 Caucasian men and women aged 18 to 86 years with a BMI of 16-48 kg/m^2 using a model 101B BIA analyzer (RJL Systems, Detroit, MI, USA) [56]. The authors reported that the BIA-equation is applicable for Caucasian, African-American, and Hispanic populations but has not been validated for the estimation of SM in Asian populations. One study calculated SM by multiplying BIA-derived FFM with a constant factor (0.566) derived from comparison with SM estimates by 24 h creatinine excretion in healthy subjects [52]. The range of cut-offs for ASMI by BIA was 6.75–7.40 kg/m^2 in men and 5.07–5.80 kg/m^2 in women, whereas cut-offs for skeletal muscle mass index (SMI) by BIA validated against MRI ranged between 7.70 and 9.20 kg/m^2 in men and 5.67 and 7.40 kg/m^2 in women (without considering severity of sarcopenia).

Nine studies used standard diagnostic CT to determine SM cut-off points for single slices [57–65]. Skeletal muscle area (SMA) at the level of the third lumbar vertebra (L3 SMA; L3 SMI = L3 SMA/height^2, cm^2/m^2) was used in three studies on patients with cancer [62,64,65]. Cut-off points ranged between 36.00 and 43.20 cm^2/m^2 in men and 29.00 and 34.90 cm^2/m^2 in women. Six studies determined sex-specific cut-offs for SM by CT in healthy populations, thereof five in organ donors [57–61,63]. L3 SMI was used in four studies on healthy subjects [57–60] and three studies with a healthy reference group used CT imaging at the L3 level to measure the psoas muscle mass area (L3 PMA; L3 psoas muscle index (PMI) = L3 PMA/height^2, cm^2/m^2) [57,61,63]. In healthy populations, cut-off values for L3 SMI ranged between 36.54 and 45.40 cm^2/m^2 in men and 30.21 and 36.05 cm^2/m^2 in women, whereas thresholds for L3 PMI were 2.63-6.36 cm^2/m^2 for men and 1.48–4.00 cm^2/m^2 for women.

Table 1. Cut-off values and diagnostic criteria of a low muscle mass using dual X-ray absorptiometry (DXA).

Reference	Device/Software	Parameter/Cut-Off by Gender	Reference Group Characteristics (Mean ± SD)/*Diagnostic Criteria (→)*		
Alkahtani (2017)	Lunar iDXA General Electric machine, Healthcare	ASMI Class I and Class II sarcopenia men: 7.74 kg/m^2 and 6.51 kg/m^2	n = 232	Saudi Arabians	
				men	women
			n	232	0
			Age (y)	27.1 ± 4.2	
			BMI (kg/m^2)	28.1 ± 5.5	
			→ *Class I sarcopenia: 1 SD below the means for young, healthy adults*		
			→ *Class II sarcopenia: 2 SDs below the means for young, healthy adults*		
Imboden et al. (2017)	GE Lunar Prodigy or iDXA	(a) ASMI men: 6.35 kg/m^2 women: 4.92 kg/m^2	(a) n = 1246	US population	
				men	women
			n	488	758
			Age (y)	20 to 39	20 to 39
			BMI (kg/m^2)	NA	NA
			→ *2 SDs below the sex-specific means of young adults*		
		(b) ASMI men: 7.40 kg/m^2 women: 5.60 kg/m^2	(b) n = 351	US population	
				men	women
			n	168	183
			Age (year)	70 to 79	70 to 79
			BMI (kg/m^2)	NA	NA
			→ *sex-specific lowest 20% of study group*		
Kruger et al. (2015)	Hologic Discovery-W, software version 12.7 for Cape Town QDR-4500A, software version 12.5:7 for Soweto	(a) ASMI women: 4.93 kg/m^2	(a) n = 238	Black South Africans (Cape Town)	
				men	women
			n	0	238
			Age (year)		25.8 ± 5.9
			BMI (kg/m^2)		29.8 ± 8.0
			→ *2 SDs below the sex-specific means of young, healthy adults*		
		(b) ASMI women: 4.95 kg/m^2	(b) n = 371	Black South Africans (Soweto)	
				men	women
			n	0	371
			Age (year)		35.1 ± 3.2
			BMI (kg/m^2)		28.8 ± 6.2
			→ *2 SDs below the sex-specific means of young, healthy adults*		

Table 1. *Cont.*

Reference	Device/Software	Parameter/Cut-Off by Gender	Reference Group Characteristics (Mean ± SD)/*Diagnostic Criteria (→)*		
Alemán-Mateo & Ruiz Valenzuela (2014)	DPX-MD+, GE Lunar	ASMI men: 5.86 kg/m^2 women: 4.72 kg/m^2 SMI men: 6.63 kg/m^2 women: 5.22 kg/m^2 SM was predicted using Kim's equation (Kim et al., 2002)	n = 216	Mexicans	
				men	women
			n	136	80
			Age (year)	27.3 ± 5.0	28.2 ± 5.6
			BMI (kg/m^2)	25.7 ± 3.6	23.2 ± 3.1
			→ *2 SDs below the sex-specific means of young, healthy adults*		
Gould et al. (2014)	DPX-L scanner, software version 1.31; Lunar or Prodigy Pro, Lunar	ASMI men: 6.94 kg/m^2 women: 5.30 kg/m^2	n = 682	study performed in southeastern Australia	
				men	women
			n	374	308
			Age (year)	20 to 39	20 to 39
			BMI (kg/m^2)	NA	NA
			→ *2 SDs below the sex-specific means of young adults*		
Marwaha et al. (2014)	Prodigy Oracle, GE Lunar Corp.	(a) ASMI women: 4.42 kg/m^2	(a) n = 469	Indians	
				men	women
			n	0	469
			Age (year)		20 to 39
			BMI (kg/m^2)		NA
			→ *2 SDs below the sex-specific means of young adults*		
		(b) ASMI women: 5.11 kg/m^2	(b) n = 1045	Indians	
				men	women
			n	0	1045
			Age (year)		44.0 ± 17.1
			BMI (kg/m^2)		25.0 ± 5.2
			→ *sex-specific lowest 20% of study group*		
Yu et al. (2014)	Hologic Delphi W4500 densitometer, auto whole body version 12.4	ASMI men: 6.52 kg/m^2 women: 5.44 kg/m^2	n = 4000	Chinese (Hong Kong)	
				men	women
			n	2000	2000
			Age (year)	72.5 ± 5.2	72.5 ± 5.2
			BMI (kg/m^2)	23.7 ± 3.3	23.7 ± 3.3
			→ *lowest quintile*		

Table 1. *Cont.*

Reference	Device/Software	Parameter/Cut-Off by Gender	Reference Group Characteristics (Mean ± SD)/*Diagnostic Criteria (→)*		
Kim et al. (2012)	Hologic Discovery-W	ASMI Class I and Class II sarcopenia men: 7.50 kg/m^2 and 6.58 kg/m^2 women: 5.38 kg/m^2 and 4.59 kg/m^2	n = 2513	Koreans	
				men	women
			n	1245	1268
			Age (year)	31.0 ± 5.5	30.8 ± 5.6
			BMI (kg/m^2)	24.0 ± 3.4	22.1 ± 3.5
			→ *Class I sarcopenia: 1-2 SDs below the sex-specific means for young, healthy adults*		
			→ *Class II sarcopenia: 2 SDs below the sex-specific means for young, healthy adults*		
Oliveira et al. (2011)	DPX-L, Lunar Radiation Corporation	ASMI women: 5.0 kg/m^2	n = 349	Brazilians	
				men	women
			n	0	349
			Age (year)		29.0 ± 7.5
			BMI (kg/m^2)		23.5 ± 4.5
			→ *2 SDs below the sex-specific means of young, healthy adults*		
Sanada et al. (2010)	Hologic QDR-4500A scanner, software version 11.2:3	ASMI Class I and Class II sarcopenia men: 7.77 kg/m^2 and 6.87 kg/m^2 women: 6.12 kg/m^2 and 5.46 kg/m^2	n = 529	Japanese	
				men	women
			n	266	263
			Age (year)	28.2 ± 7.4	28.0 ± 7.0
			BMI (kg/m^2)	23.0 ± 3.0	20.8 ± 2.6
			→ *Class I sarcopenia: 1 SD below the sex-specific means for young, healthy adults*		
			→ *Class II sarcopenia: 2 SDs below the sex-specific means for young, healthy adults*		
Szulc et al. (2004)	Hologic 1000W	ASMI men: 6.32 kg/m^2	n = 845	study performed in France	
				men	women
			n	845	0
			Age (year)	64.0 ± 8.0	
			BMI (kg/m^2)	28.0 ± 3.7	
			→ *lowest quartile*		
Newman et al. (2003)	QDR 4500A, Hologic, Inc.	ASMI men: 7.23 kg/m^2 women: 5.67 kg/m^2 Values recommended by the International Working Group on Sarcopenia (Fielding et al., 2011)	n = 2984	study performed in USA (41% Blacks)	
				men	women
			n	1435	1549
			Age (year)	73.6 ± 2.9	73.6 ± 2.9
			BMI (kg/m^2)	27.4 ± 4.8	27.4 ± 4.8
			→ *sex-specific lowest 20% of study group*		

Table 1. *Cont.*

Reference	Device/Software	Parameter/Cut-Off by Gender	Reference Group Characteristics (Mean ± SD)/*Diagnostic Criteria (→)*		
Tankó et al. (2002)	QDR4500A scanner, Hologic, software version V8.10a:3 and DPX scanner, Lunar Radiation, software versions 3.1 and 3.2	(a) ASMI women: 6.10 kg/m^2 (b) ASMI women: 5.40 kg/m^2	*n* = 216 women	Danes	
				men	women
			n	0	216
			Age (year)		30.4 ± 5.3
			BMI (kg/m^2)		NA
			→ *(a) 1-2 SDs below the sex-specific means for young, healthy, premenopausal women*		
			→ *(b) 2 SDs below the sex-specific means for young, healthy, premenopausal women*		
Baumgartner et al. (1998)	Lunar DPX	ASMI men: 7.26 kg/m^2 women: 5.45 kg/m^2	*n* = 229	US population (non-Hispanic white men and women)	
				men	women
			n	107	122
			Age (year)	28.7 ± 5.1	29.7 ± 5.9
			BMI (kg/m^2)	24.6 ± 3.8	24.1 ± 5.4
			→ *2 SDs below the sex-specific means of young, healthy adults*		

ASMI, appendicular skeletal muscle mass index; BMI, body mass index; DXA, dual X-ray absorptiometry; NA, not available; SD, standard deviation; SM, skeletal muscle mass; SMI, skeletal muscle mass index.

Table 2. Cut-off values and diagnostic criteria of a low muscle mass using bioelectrical impedance analysis (BIA).

Reference	Device/Software	Parameter/Cut-Off by Gender	Reference Group Characteristics (Mean ± SD)/*Diagnostic Criteria (→)*		
Krzymińska-Siemaszko et al. (2019)	InBody 170 analyzer, Biospace Co.	ASMI men: 7.35 kg/m^2 (20–30 y), 7.38 kg/m^2 (18–40 y, 18–39 y, 20–35 y), 7.40 kg/m^2 (20–39 y, 20–40 y) women: 5.51 kg/m^2 (20–30 y), 5.56 kg/m^2 (18–40 y), 5.53 kg/m^2 (18–39 y), 5.59 kg/m^2 (20–39 y), 5.60 kg/m^2 (20–40 y), 5.58 kg/m^2 (20–35 y) Authors recommended the highest cut-off points, i.e., 5.60 kg/m^2 in women and 7.40 kg/m^2 in men	*n* = 1512	study performed in Poland (Caucasians)	
				men	women
			n	635	877
			Age (year)	24.2 ± 5.3	28.4 ± 6.8
			BMI (kg/m^2)	NA	NA
			total *n* for men and women depends on age range		
			→ *2 SDs below the sex-specific means of young, healthy adults*		
Alkahtani (2017)	Tanita MC-980MA, Tanita Corporation Inbody 770, Inbody Co.	ASMI Class I and Class II sarcopenia men: 8.68 kg/m^2 and 7.45 kg/m^2 ASMI Class I and Class II sarcopenia men: 7.29 kg/m^2 and 6.42 kg/m^2	*n* = 232	Saudi Arabians	
				men	women
			n	232	0
			Age (year)	27.1 ± 4.2	
			BMI (kg/m^2)	28.1 ± 5.5	
			→ *Class I sarcopenia: 1 SD below the means for young, healthy adults*		
			→ *Class II sarcopenia: 2 SDs below the means for young, healthy adults*		

Table 2. *Cont.*

Reference	Device/Software	Parameter/Cut-Off by Gender	Reference Group Characteristics (Mean ± SD)/*Diagnostic Criteria (→)*		
Bahat et al. (2016)	Tanita BC 532 model body analysis monitor	SMI men: 9.2 kg/m^2 women: 7.4 kg/m^2 SM (kg) = 0.566 x FFM	*n* = 301	study performed in Turkey	
				men	women
			n	187	114
			Age (year)	26.8 ± 4.5	25.9 ± 4.7
			BMI (kg/m^2)	25.5 ± 3.6	22.4 ± 3.4
			→ *2 SDs below the sex-specific means of young, healthy adults*		
Chang et al. (2013)	Tanita BC-418	ASMI men: 6.76 kg/m^2 women: 5.28 kg/m^2 SMI men: 7.70 kg/m^2 women: 5.67 kg/m^2 SM by Janssen et al. (2000) equation	*n* = 998	Taiwanese	
				men	women
			n	498	500
			Age (year)	23.1 ± 3.0	23.1 ± 2.7
			BMI (kg/m^2)	22.2 ± 3.1	20.2 ± 2.6
			→ *2 SDs below the sex-specific means of young, healthy adults*		
Yamada et al. (2013)	Inbody 720, Biospace Co.	ASMI men: 6.75 kg/m^2 women: 5.07 kg/m^2	*n* = 38,099	Japanese	
				men	women
			n	19,797	18,302
			Age (year)	18 to 40	18 to 40
			BMI (kg/m^2)	NA	NA
			→ *2 SDs below the sex-specific means of young adults*		
Masanés et al. (2012)	RJL Systems BIA 101	SMI men: 8.25 kg/m^2 women: 6.68 kg/m^2 SM by Janssen et al. (2000) equation	*n* = 230	study performed in Spain	
				men	women
			n	110	120
			Age (year)	28.6 ± 5.0	28.2 ± 6.0
			BMI (kg/m^2)	24.6 ± 2.6	21.9 ± 2.2
			→ *2 SDs below the sex-specific means of young, healthy adults*		
Tanimoto et al. (2012)	Tanita MC-190	ASMI men: 7.0 kg/m^2 women: 5.8 kg/m^2	*n* = 1719	Japanese	
				men	women
			n	838	881
			Age (year)	26.6 ± 6.7	28.5 ± 7.3
			BMI (kg/m^2)	22.4 ± 3.2	20.8 ± 2.9
			→ *2 SDs below the sex-specific means of young, healthy adults*		

Table 2. *Cont.*

Reference	Device/Software	Parameter/Cut-Off by Gender	Reference Group Characteristics (Mean ± SD)/*Diagnostic Criteria (→)*		
Chien et al. (2008)	Maltron BioScan 920	SMI men: 8.87 kg/m^2 women: 6.42 kg/m^2 SM by Janssen et al. (2000) equation	n = 200	Taiwanese	
				men	women
			n	100	100
			Age (year)	26.7 ± 5.7	27.6 ± 5.9
			BMI (kg/m^2)	23.2 ± 3.5	20.6 ± 2.5
			→ *2 SDs or more below the sex-specific means of young, healthy adults*		
Tichet et al. (2008)	Impedimed multifrequency analyser	SMI men: 8.60 kg/m^2 women: 6.20 kg/m^2 SM by Janssen et al. (2000) equation	n = 782	French people	
				men	women
			n	394	388
			Age (year)	30.2 ± 6.1	29.2 ± 6.3
			BMI (kg/m^2)	23.9 ± 3.0	22.5 ± 3.4
			→ *2 SDs below the sex-specific means of young, healthy adults*		
Janssen et al. (2004)	Valhalla 1990B Bio-Resistance Body Composition Analyzer	SMI moderate and severe sarcopenia men: 8.51–10.75 kg/m^2 and ≤8.50 kg/m^2 women: 5.76–6.75 kg/m^2 and ≤5.75 kg/m^2 SM by Janssen et al. (2000) equation	n = 4499	US population (non-Hispanic White, non-Hispanic Black and Mexican American)	
				men	women
			n	2223	2276
			Age (year)	70.0 ± 7.0	71.0 ± 8.0
			BMI (kg/m^2)	26.6 ± 4.3	27.0 ± 5.5
			→ *receiver operating characteristics*		

ASMI, appendicular skeletal muscle mass index; BIA, bioelectrical impedance analysis; BMI, body mass index; FFM, fat-free mass; NA, not available; SD, standard deviation; SM, skeletal muscle mass; SMI, skeletal muscle mass index.

Table 3. Cut-off values and diagnostic criteria of a low muscle mass using computed tomography (CT).

Reference	Device/Software	Parameter/Cut-Off by Gender	Reference Group Characteristics (Mean ± SD)/*Diagnostic Criteria (→)*		
Ufuk & Herek (2019)	lumbar CT images (16-detector row, Brilliance)	CT L3 SMI men: 44.98 cm^2/m^2 women: 36.05 cm^2/m^2 CT L3 PMI men: 2.63 cm^2/m^2 women: 2.02 cm^2/m^2	n = 270	healthy Turkish population	
				men	women
			n	134	136
			Age (year)	44.3 ± 11.2	45.0 ± 8.6
			BMI (kg/m^2)	26.4 ± 3.5	25.4 ± 3.6
			→ *2 SDs below the sex-specific means of young adults*		
Derstine et al. (2018)	lumbar CT images (GE Discovery or LightSpeed scanner)	(a) CT L3 SMI men: 45.4 cm^2/m^2 women: 34.4 cm^2/m^2	(a) n = 727	healthy US population	
				men	women
			n	317	410
			Age (year)	18 to 40	18 to 40
			BMI (kg/m^2)	NA	NA
			→ *2 SDs below the sex-specific means of young adults*		
		(b) CT T10 SMI men: 28.8 cm^2/m^2 women: 20.4 cm^2/m^2	(b) n = 278	healthy US population	
				men	women
			n	122	156
			Age (year)	18 to 40	18 to 40
			BMI (kg/m^2)	NA	NA
			→ *2 SDs below the sex-specific means of young adults*		
		(c) CT T11 SMI men: 27.6 cm^2/m^2 women: 19.2 cm^2/m^2	(c) n = 577	healthy US population	
				men	women
			n	241	366
			Age (year)	18 to 40	18 to 40
			BMI (kg/m^2)	NA	NA
			→ *2 SDs below the sex-specific means of young adults*		
		(d) CT T12 SMI men: 28.8 cm^2/m^2 women: 20.8 cm^2/m^2	(d) n = 700	healthy US population	
				men	women
			n	299	401
			Age (year)	18 to 40	18 to 40
			BMI (kg/m^2)	NA	NA
			→ *2 SDs below the sex-specific means of young adults*		
		(e) CT L1 SMI men: 34.6 cm^2/m^2 women: 25.9 cm^2/m^2	(e) n = 724	healthy US population	
				men	women
			n	315	409
			Age (year)	18 to 40	18 to 40
			BMI (kg/m^2)	NA	NA
			→ *2 SDs below the sex-specific means of young adults*		

Table 3. *Cont.*

Reference	Device/Software	Parameter/Cut-Off by Gender	Reference Group Characteristics (Mean ± SD)/*Diagnostic Criteria (→)*		
			(f) n = 726	healthy US population	
				men	women
		(f) CT L2 SMI	n	315	411
		men: 40.1 cm^2/m^2	Age (year)	18 to 40	18 to 40
		women: 30.4 cm^2/m^2	BMI (kg/m^2)	NA	NA
			→ *2 SDs below the sex-specific means of young adults*		
			(g) n = 704	healthy US population	
				men	women
		(g) CT L4 SMI	n	305	399
		men: 41.3 cm^2/m^2	Age (year)	18 to 40	18 to 40
		women: 34.2 cm^2/m^2	BMI (kg/m^2)	NA	NA
			→ *2 SDs below the sex-specific means of young adults*		
			(h) n = 506	healthy US population	
				men	women
		(h) CT L5 SMI	n	211	295
		men: 39.0 cm^2/m^2	Age (year)	18 to 40	18 to 40
		women: 30.6 cm^2/m^2	BMI (kg/m^2)	NA	NA
			→ *2 SDs below the sex-specific means of young adults*		
			n = 300	healthy Caucasian population	
				men	women
van der Werf et al. (2018)	lumbar CT images (64-row CT scanner, Sensation 64, Siemens or CT Brilliance 64, Philips)	CT L3 SMI men: 44.6 cm^2/m^2 women: 34.0 cm^2/m^2	n	126	174
			Age (y)	20 to 60	20 to 60
			BMI (kg/m^2)	NA	NA
			→ *5th percentile*		
			n = 275	healthy Asian Indians	
				men	women
Benjamin et al. (2017)	lumbar CT images (Discovery 750 HD 64-row spectral CT scanner)	CT L3 SMI men: 36.54 cm^2/m^2 women: 30.21 cm^2/m^2	n	139	136
			Age (year)	32.2 ± 9.8	32.2 ± 9.8
			BMI (kg/m^2)	24.2 ± 3.2	24.2 ± 3.2
			→ *2 SDs below the sex-specific means of young adults*		

Table 3. *Cont.*

Reference	Device/Software	Parameter/Cut-Off by Gender	Reference Group Characteristics (Mean ± SD)/*Diagnostic Criteria (→)*		
Kim et al. (2017)	lumbar CT images (64-slice multidetector CT scanner, Brilliance 64, Philips Healthcare)	CT L3 PMI men: 5.92 cm^2/m^2 (20–39 y), 4.74 cm^2/m^2 (40–49 y), 4.22 cm^2/m^2 (50–59 y), 3.74 cm^2/m^2 (60–69 y), 3.32 cm^2/m^2 (70–89 y) women: 4.0 cm^2/m^2 (20–39 y), 2.88 cm^2/m^2 (40–49 y), 2.43 cm^2/m^2 (50–59 y), 2.20 cm^2/m^2 (60–69 y), 1.48 cm^2/m^2 (70–89 y)	n = 1422	study performed in Korea	
				men	women
			n	550	872
			Age (year)	52.4 ± 12.0	53.3 ± 12.2
			BMI (kg/m^2)	24.5 ± 3.1	22.8 ± 3.2
			total n for men and women depends on age range → *2 SDs below the sex-specific means of young, healthy adults*		
Sakurai et al. (2017)	lumbar CT images	CT L3 SMI men: 43.2 cm^2/m^2 women: 34.6 cm^2/m^2	n = 569 patients with gastric cancer	study performed in Japan	
				men	women
			n	396	173
			Age (year)	66.7 ± 11.2	66.7 ± 11.2
			BMI (kg/m^2)	22.0 ± 3.4	22.0 ± 3.4
			→ *lowest sex-specific quartile*		
Hamaguchi et al. (2016)	lumbar CT images (Aquilion 64, Toshiba Medical Systems)	CT L3 PMI men: 6.36 cm^2/m^2 women: 3.92 cm^2/m^2	n = 230	healthy Asian population	
				men	women
			n	116	114
			Age (year)	20 to 49	20 to 49
			BMI (kg/m^2)	NA	NA
			→ *2 SDs below the sex-specific means of young adults*		
Zhuang et al. (2016)	lumbar CT images	CT L3 SMI men: 40.8 cm^2/m^2 women: 34.9 cm^2/m^2	n = 937 patients with gastric cancer	study performed in China	
				men	women
			n	730	207
			Age (year)	64.0 ± 15.0	64.0 ± 15.0
			BMI (kg/m^2)	21.9 ± 3.0	21.9 ± 3.0
			→ *optimal stratification*		
Iritani et al. (2015)	lumbar CT images	CT L3 SMI men: 36.0 cm^2/m^2 women: 29.0 cm^2/m^2	n = 217 patients with hepatocellular carcinoma	study performed in Japan	
				men	women
			n	146	71
			Age (year)	27 to 90	27 to 90
			BMI (kg/m^2)	13.4 to 35.9	13.4 to 35.9
			→ *optimal stratification*		

BMI, body mass index; CT, computed tomography; L, lumbar vertebra; L3, third lumbar vertebra; NA, not available; PMI, psoas muscle index; SD, standard deviation; SMI, skeletal muscle mass index; T, thoracic vertebra.

Combination of Measures for Muscle mass and Obesity

Table 4 shows reference values of 34 publications for a low SM in combination with different measures of obesity. Cut-offs for a low SM were mostly determined by DXA or BIA, whereas only a few studies reported CT-defined cut-offs in combination with obesity criteria. SM parameters were commonly normalized for height squared or given as % of body weight. In addition, two studies adjusted ASM for BMI [66,67]. Alternative parameters were FM/FFM ratio [68], visceral fat area/thigh muscle area ratio (VFA/TMA) [69] and fat mass index (FMI) in combination with fat-free mass index (FFMI) [70].

Prado et al. [71] published CT-derived SMI cut-offs determined in a population of obese (BMI ≥ 30 kg/m^2) Canadians with tumors of the respiratory or gastrointestinal tract. In 2013, this CT database was extended by Martin et al. [72] and low SM reference values were reported for subjects with normal weight and overweight according to BMI classifications. In both studies, optimal stratification was used to determine the threshold of mortality. Many studies adopted the criteria proposed by Prado et al. [71] and Martin et al. [72] (e.g., [73–75]). Only one further study developed BMI-dependent reference values for SM [76]. Although some studies referenced the cut-offs by Prado et al. [71], reported thresholds differ from the original work (e.g., [77,78]). These reported values were then cited in further studies [79].

In most studies, obesity was defined as BMI ≥ 30 kg/m^2 [71,76,80,81]. Alternative BMI thresholds were 27.5 kg/m^2 [82,83], 27 kg/m^2 [84], 25 kg/m^2 [72,85–90] or 23 kg/m^2 [91]. Furthermore, sex and ethnic-specific waist circumference (WC) thresholds for central obesity were considered [44,84,92–95]. Other criteria include %FM [50,81,96–101], visceral fat area [73] or fat-muscle ratios like visceral fat area (VFA) to total abdominal muscle area (TAMA) [74].

Table 5 displays cut-offs and average values for body composition stratified into groups of subjects with underweight, normal weight, overweight and obesity. Cut-offs for FMI_{DXA} were released by the National Health and Nutrition Examination Survey (NHANES; [102]) and respective BMI-dependent normal values for $FFMI_{DXA}$ were calculated as BMI minus FMI. For each given BMI displayed in Table 5, corresponding normal value for SMI_{MRI} were calculated using a stepwise regression analysis (SMI_{MRI}, men = 0.479 × $FFMI_{DXA}$ −0.017 × age + 0.683 and SMI_{MRI}, women = 0.348 × $FFMI_{DXA}$ − 0.011 × age + 1.971) in a healthy Caucasian population. In addition, respective values for SMI_{BIA} validated against MRI were generated based on a young and healthy Caucasian population using linear regression analysis (SMI_{BIA}, men = 0.168 × BMI + 5.49 (R^2 = 0.53, standard error of estimate (SEE) = 0.514) and SMI_{BIA}, women = 0.159 × BMI + 3.72 (R^2 = 0.61, SEE = 0.465)). Adjacent to the average SMI_{BIA} (median) for each BMI, cut-offs with two SDs below the sex-specific mean of the young and healthy population were shown.

Table 4. Cut-off values that combine measures of muscle mass and obesity.

Reference	Device/Software	Parameter/Cut-Off by Gender	Reference Group Characteristics (Mean ± SD)/*Diagnostic Criteria (→)*		
Prado et al. (2008)	CT images	CT L3 SMI: men: ≤52.4 cm^2/m^2 women: ≤38.5 cm^2/m^2 + BMI ≥ 30 kg/m^2	n = 250 obese patients with cancers of the respiratory tract and gastrointestinal locations	study performed in Canada	
				men	women
			n	136	114
			Age (year)	64.6 ± 10.2	63.2 ± 10.5
			BMI (kg/m^2)	33.9 ± 4.4	34.7 ± 4.3
			→ *optimal stratification*		
Martin et al. (2013)	CT images	CT L3 SMI: men: <43 cm^2/m^2 women: <41 cm^2/m^2 for BMI < 25 kg/m^2 men: <53 cm^2/m^2 for BMI ≥ 25 kg/m^2	n = 1473 patients with cancers of the respiratory tract and gastrointestinal locations	study performed in Canada	
				men	women
			n	828	645
			Age (year)	64.7 ± 11.2	64.8 ± 11.5
			BMI (kg/m^2)	26.0 ± 4.9	25.1 ± 5.8
			→ *optimal stratification*		
Muscariello et al. (2016)	BIA (RJL 101, Akern SRL)	(a) SMI + BMI < 25 kg/m^2 Class I and Class II sarcopenia women: 7.4 and 6.8 kg/m^2	(a) n = 313	study performed in Italy	
				men	women
			n	0	313
			Age (year)		28.5 ± 7.6
			BMI (kg/m^2)		24.1 ± 2.5
			→ *Class I sarcopenia: 1 SD below the sex-specific means of young adults*		
			→ *Class II sarcopenia: 2 SDs below the sex-specific means of young adults*		
		(b) SMI + BMI ≥ 30 kg/m^2 Class I and Class II sarcopenia women: 8.3 and 7.3 kg/m^2 SM by Janssen et al. (2000) equation	(b) n = 361	study performed in Italy	
				men	women
			n	0	361
			Age (year)		30.9 ± 7.9
			BMI (kg/m^2)		35.1 ± 4.6
			→ *Class I sarcopenia: 1 SD below the sex-specific means of young adults*		
			→ *Class II sarcopenia: 2 SDs below the sex-specific means of young adults*		
Nishigori et al. (2016)	CT images	CT L3 SMI (Prado et al. 2008): men: ≤52.4 cm^2/m^2 women: ≤38.5 cm^2/m^2 + visceral fat area (VFA) ≥100 cm^2 in both sexes	reference group characteristic CT L3 SMI see Prado et al. (2008)		

Table 4. *Cont.*

Reference	Device/Software	Parameter/Cut-Off by Gender	Reference Group Characteristics (Mean ± SD)/*Diagnostic Criteria (→)*		
Pecorelli et al. (2016)	CT images	(a) CT L3 SMI (Prado et al. 2008): men: ≤52.4 cm^2/m^2 women: ≤38.5 cm^2/m^2 + (b) visceral fat area/total abdominal muscle area ratio (VFA/TAMA) men & women: 3.2	(a) reference group characteristic CT L3 SMI see Prado et al. (2008) (b) n = 202 patients with resectable pancreas, periampullary	study performed in Italy	
				men	women
			n	108	94
			Age (year)	66.8 ± 10.7	66.8 ± 10.7
			BMI (kg/m^2)	23.6 ± 3.7	23.6 ± 3.7
			→ *optimal stratification*		
Kwon et al. (2017)	DXA (Discovery QDR 4500, Hologic)	ASM (as % of body weight) men: 30.98% women: 24.81% + BMI ≥ 25 kg/m^2 (based on the definition in the Asian-Pacific region)	n = 3550	Koreans	
				men	women
			n	1668	1882
			Age (year)	20 to 39	20 to 39
			BMI (kg/m^2)	NA	NA
			→ *1 SD below the sex-specific means of young adults*		
Chiles Shaffer et al. (2017)	DXA (Lunar Prodigy Advance with GE EnCore 2006 version 10.51.0006)	ASM adjusted for BMI men: <0.725 kg/m^2 women: <0.591 kg/m^2	n = 545	study performed in US	
				men	women
			n	287	258
			Age (year)	79.2 ± 7.2	77.7 ± 7.3
			BMI (kg/m^2)	27.2 ± 3.8	27.0 ± 5.2
			→ *CART analysis*		
An & Kim (2016)	DXA (Discovery-W, Hologic)	ASM (as % of body weight) men: 30.1% women: 21.2% + WC ≥ 90 cm in men WC ≥ 80 cm in women (sex-specific cut-off for Asians)	n = 5944	study performed in Korea	
				men	women
			n	2502	3334
			Age (year)	20 to 39	20 to 39
			BMI (kg/m^2)	NA	NA
			→ *1 SD below the sex-specific means of young adults*		
Cho et al. (2015)	(a) DXA (Discovery-W, Hologic)	(a) ASM (as % of body weight) men: 30.3% women: 23.8% + WC ≥ 90 cm in men WC ≥ 85 cm in women	(a) n = 4987	Koreans	
				men	women
			n	2123	2864
			Age (year)	20 to 39	20 to 39
			BMI (kg/m^2)	NA	NA
			→ *1 SD below the sex-specific means of young, healthy adults*		

Table 4. *Cont.*

Reference	Device/Software	Parameter/Cut-Off by Gender	Reference Group Characteristics (Mean ± SD)/*Diagnostic Criteria (→)*		
Oh et al. (2015)	DXA (Lunar Corp.)	ASM (as % of body weight) men: 44% women: 52% + BMI ≥ 25 kg/m^2	n = 1746	Koreans	
				men	women
			n	748	998
			Age (year)	20 to 39	20 to 39
			BMI (kg/m^2)	NA	NA
			→ *1 SD below the sex-specific means of young, healthy adults*		
Lee et al. (2015)	DXA (Discovery QDR 4500, Hologic)	ASM (as % of body weight) men: 32.2% women: 25.5% + BMI ≥ 25 kg/m^2 (based on the criteria of the Asian-Pacific region)	n = 2200	Koreans	
				men	women
			n	960	1240
			Age (year)	20 to 30	20 to 30
			BMI (kg/m^2)	NA	NA
			→ *1 SD below the sex-specific means of young, healthy adults*		
Baek et al. (2014)	DXA (Lunar Corp.)	ASMI men: 6.96 kg/m^2 women: 4.96 kg/m^2 ASM (as % of body weight) men: 30.65% women: 23.90% + BMI ≥ 25 kg/m^2 (IOTF-proposed classification of BMI for Asia)	n = 4192	Koreans	
				men	women
			n	1699	2493
			Age (year)	20 to 39	20 to 39
			BMI (kg/m^2)	NA	NA
			→ *1 SD below the sex-specific means of young, healthy adults*		
Cawthon et al. (2014)	DXA (QDR 4500, Hologic 2000, Lunar Prodigy)	ASM adjusted for BMI men: <0.789 women: <0.512 recommended by FNIH (Studenski et al., 2014)	n = 11,270	study performed in US	
				men	women
			n	7582	3688
			Age (year)	65 to 80	65 to 80
			BMI (kg/m^2)	NA	NA
			→ *CART analysis plus sensitivity analyses*		
Chung et al. (2013)	(a) DXA (fan-beam technology, Lunar Corp.)	(a) ASM (as % of body weight) men: 32.5% women: 25.7% + BMI ≥ 25 kg/m^2 (IOTF-proposed classification of BMI for Asia)	(a) n = 2781	study performed in Korea	
				men	women
			n	1155	1626
			Age (year)	20 to 39	20 to 39
			BMI (kg/m^2)	NA	NA
			→ *1 SD below the sex-specific means of young, healthy adults*		

Table 4. *Cont.*

Reference	Device/Software	Parameter/Cut-Off by Gender	Reference Group Characteristics (Mean ± SD)/*Diagnostic Criteria (→)*		
Hwang et al. (2012)	DXA (Discovery-W, Hologic)	ASM (as % of body weight) men: 29.53%women: 23.20% + WC ≥ 90 cm in men WC ≥ 85 cm in women (Korean abdominal obesity criteria; Lee et al., 2007)	n = 2269	Koreans	
				men	women
			n	1003	1266
			Age (year)	30.7 ± 5.5	31.0 ± 5.5
			BMI (kg/m^2)	24.1 ± 3.5	22.1 ± 3.6
			→ *2 SDs below the sex-specific means of young adults*		
Lee et al. (2012)	DXA (Discovery-W, Hologic)	ASM (as % of body weight) men: 26.8% women: 21.0% + BMI ≥ 27.5 kg/m^2	n = 2113	Koreans	
				men	women
			n	902	1211
			Age (year)	20 to 40	20 to 40
			BMI (kg/m^2)	NA	NA
			→ *2 SDs below the sex-specific means of young, healthy adults*		
Kim et al. (2012)	DXA (Discovery-W, Hologic)	ASM (as % of body weight) Class II sarcopenia men: 29.1% women: 23.0% ASMI Class II sarcopenia men: 6.58 kg/m^2 women: 4.59 kg/m^2 + WC ≥ 90 cm in men (Lee et al., 2007) WC ≥ 85 cm in women	n = 2513	Koreans	
				men	women
			n	1245	1268
			Age (year)	31.0 ± 5.5	30.8 ± 5.6
			BMI (kg/m^2)	24.0 ± 3.4	22.1 ± 3.5
			→ *2 SDs below the sex-specific means of young, healthy adults*		
Kim et al. (2011)	DXA (Lunar Corp.)	ASM (as % of body weight) men: 29.5% women: 23.2% + BMI ≥ 27.5 kg/m^2	n = 2392	study performed in Korea	
				men	women
			n	1054	1338
			Age (year)	20 to 40	20 to 40
			BMI (kg/m^2)	NA	NA
			→ *2 SDs below the sex-specific means of young, healthy adults*		

Table 4. *Cont.*

Reference	Device/Software	Parameter/Cut-Off by Gender	Reference Group Characteristics (Mean ± SD)/*Diagnostic Criteria (→)*		
Kim et al. (2009)	DXA (Discovery A, Hologic)	(a) ASMI men: 8.81 kg/m^2 women: 7.36 kg/m^2 + (b) FM men: 20.21% women: 31.71%	n = 526	Koreans	
				men	women
			n	198	328
			Age (year)	52.2 ± 14.4	51.2 ± 14.8
			BMI (kg/m^2)	25.2 ± 3.1	23.9 ± 3.7
			→ *(a) lower two quintiles* → *(b) two highest quintiles*		
Rolland et al. (2009)	(a) DXA (Lunar DPX, Lunar Corp.) (b) DXA (QDR 4500 W, Hologic)	(a) ASMI women: 5.45 kg/m^2 (Baumgartner et al., 1998) + (b) FM women: 40%	(a) n = 122	US population (non-Hispanic white men and women)	
				men	women
			n	0	122
			Age (year)		29.7 ± 5.9
			BMI (kg/m^2)		24.1 ± 5.4
			→ *2 SDs below the sex-specific means of young, healthy adults*		
			(b) n = 1308	study performed in France	
				men	women
			n	0	1308
			Age (year)		≥75
			BMI (kg/m^2)		NA
			→ *60th percentile of the healthy study sample*		
Baumgartner et al. (1998)	DXA (Lunar DPX, Lunar Corp.)	(a) ASMI men: 7.26 kg/m^2 women: 5.45 kg/m^2 + (b) FM men: 27% women: 38%	n = 229	US population (non-Hispanic white men and women)	
				men	women
			n	107	122
			Age (year)	28.7 ± 5.1	29.7 ± 5.9
			BMI (kg/m^2)	24.6 ± 3.8	24.1 ± 5.4
			(a) → *2 SDs below the sex-specific means of young, healthy adults* *(b)* → >sex-specific median		

Table 4. *Cont.*

Reference	Device/Software	Parameter/Cut-Off by Gender	Reference Group Characteristics (Mean ± SD)/*Diagnostic Criteria (→)*		
Bahat et al. (2016); Bahat et al. (2018)	BIA (Tanita-BC532)	(a) SMI men: 9.2 kg/m^2 women: 7.4 kg/m^2 SM (kg) = 0.566 × FFM + (b) FM men: 27.3% women: 40.7%	(a) n = 301	study performed in Turkey	
				men	women
			n	187	114
			Age (year)	26.8 ± 4.5	25.9 ± 4.7
			BMI (kg/m^2)	25.5 ± 3.6	22.4 ± 3.4
			→ *2 SDs below the sex-specific means of young, healthy adults*		
			(b) n = 992	study performed in Turkey	
				men	women
			n	308	684
			Age (year)	75.2 ± 7.2	75.2 ± 7.2
			BMI (kg/m^2)	27.7 ± 4.3	30.7 ± 5.6
			→ *above 60th percentile*		
Ishii et al. (2016)	(a) BIA (Tanita MC-190) (b) BIA (InBody 430, Biospace)	(a) ASMI men: 7.0 kg/m^2 women: 5.8 kg/m^2 + (b) FM men: 29.7% women: 37.2%	(a) n = 1719	Japanese	
				men	women
			n	838	881
			Age (year)	26.6 ± 6.7	28.5 ± 7.3
			BMI (kg/m^2)	22.4 ± 3.2	20.8 ± 2.9
			→ *2 SDs below the sex-specific means of young, healthy adults*		
			(b) n = 1731	Japanese	
				men	women
			n	875	856
			Age (year)	≥ 65	≥ 65
			BMI (kg/m^2)	NA	NA
			→ *highest quintile*		
Moreira et al. (2016)	BIA (InBody R20, Biospace)	ASMI women: 6.08 kg/m^2 + WC ≥ 88 cm in women (Brazilian obesity guidelines)	n = 491	study performed in Northeast Brazil (Whites, Blacks, Pardo)	
				men	women
			n	0	491
			Age (year)		50.0 ± 5.6
			BMI (kg/m^2)		29.0 ± 4.8
			→ *20th percentile*		

Table 4. *Cont.*

Reference	Device/Software	Parameter/Cut-Off by Gender	Reference Group Characteristics (Mean ± SD)/*Diagnostic Criteria (→)*		
Kemmler et al. (2016)	BIA (InBody 770, Biospace)	(a) ASMI women: 5.66 kg/m^2	(a) n = 689	study performed in Germany (Caucasians)	
				men	women
			n	0	689
			Age (year)		18 to 35
			BMI (kg/m^2)		NA
			→ *2 SDs below the sex-specific means of young, healthy adults*		
		(b) ASMI women: 5.99 kg/m^2 + BMI ≥ 30 kg/m^2 (NIH) FM ≥ 35% (WHO)	(b) n = 1325	study performed in Germany (Caucasians)	
				men	women
			n	0	1325
			Age (year)		76.4 ± 4.9
			BMI (kg/m^2)		26.7 ± 4.3
			→ *lowest quintile*		
Lee et al. (2016)	BIA (InBody 720, Biospace)	(a) SMI (as % of body weight) men: 38.2 % women: 32.2% SM by Janssen et al. (2000) equation +	(a) n = 273	study performed in Korea	
				men	women
			n	157	116
			Age (year)	25.5 ± 2.9	26.1 ± 4.6
			BMI (kg/m^2)	24.1 ± 3.0	20.7 ± 2.6
			→ *2 SDs below the sex-specific means of young, healthy adults*		
		(b) FM men: 25.8% women: 36.5%	(b) n = 309	study performed in Korea	
				men	women
			n	85	224
			Age (year)	70.7 ± 6.3	66.4 ± 7.2
			BMI (kg/m^2)	NA	NA
			→ *two highest quintiles*		
Biolo et al. (2015)	BIA (Human IM-Plus, DS, Dieto System, BIA 101, Akern Srl, Tanita BC418MA, Tanita Corp.)	FM/FFM ratio > 0.8	n = 200	study performed in Italy and Slovenia	
				men	women
			n	89	111
			Age (year)	48.0 ± 12.0	51.0 ± 12.0
			BMI (kg/m^2)	35.6 ± 6.2	35.5 ± 5.4

Table 4. *Cont.*

Reference	Device/Software	Parameter/Cut-Off by Gender	Reference Group Characteristics (Mean ± SD)/*Diagnostic Criteria (→)*		
De Rosa et al. (2015)	BIA (Human IM Plus II–DS Medical)	SMI moderate and severe sarcopenia men: 8.44–9.53 kg/m^2 and ≤8.43 kg/m^2 women: 6.49–7.32 kg/m^2 and ≤6.48 kg/m^2 SMI (as % of body weight) moderate and severe sarcopenia men: 28.8–35.6% and ≤28.7% women: 23.1–28.4% and ≤23.0% SM by Janssen et al. (2000) equation + BMI ≥ 30 kg/m^2	n = 500	Italians	
				men	women
			n	100	400
			Age (year)	27.0 ± 7.0	25.0 ± 6.0
			BMI (kg/m^2)	25.8 ± 5.7	25.2 ± 5.7
			→ *moderate sarcopenia: within 1 to 2 SDs below the sex-specific means of young, healthy adults*		
			→ *severe sarcopenia: 2 SDs below the sex-specific means of young, healthy adults*		
Atkins et al. (2014)	BIA (Bodystat 500, Bodystat Ltd.)	FFMI men: ≤16.7 kg/m^2 FFM (equation by Deurenberg et al., 1991) + FMI > 11.1 kg/m^2	n = 4045	study performed in UK (> 99 % white Europeans)	
				men	women
			n	4045	0
			Age (year)	60 to 79	
			BMI (kg/m^2)	NA	
			→ *lowest two-fifths of FFMI*		
Baek et al. (2013)	BIA (InBody 520, Biospace)	ASMI men: 10.70 kg/m^2 women: 8.60 kg/m^2 + BMI > 25 kg/m^2 (WHO definition)	n = 1150	study performed in Korea	
				men	women
			n	618	532
			Age (year)	43.6 ± 11.5	43.6 ± 11.5
			BMI (kg/m^2)	24.6 ± 3.3	24.6 ± 3.3
			→ *50th percentile of healthy study sample*		
Gomez-Cabello et al. (2011)	BIA (Tanita BC 418-MA)	(a) SMI men: 8.61 kg/m^2 women: 6.19 kg/m^2 (b) FM men: 30.33% women: 40.9% SM by Janssen et al. (2000) equation	n = 3136	Spaniards	
				men	women
			n	678	2198
			Age (year)	72.4 ± 5.5	72.1 ± 5.2
			BMI (kg/m^2)	NA	NA
			→ *(a) two lower quintiles*		
			→ *(b) two highest quintiles*		

Table 4. *Cont.*

Reference	Device/Software	Parameter/Cut-Off by Gender	Reference Group Characteristics (Mean ± SD)/*Diagnostic Criteria (→)*		
Lou et al. (2017)	CT images	CT L3 SMI (Zhuang et al., 2016) men: ≤40.8 cm^2/m^2 women: ≤34.9 cm^2/m^2 + BMI ≥ 23 kg/m^2 (WHO definition for Asians)	Predefined cut-off values for sarcopenia and obesity		
Ramachandran et al. (2012)	CT images (Somatom Sensation 10 CT scanner)	adjusted thigh muscle area: men: 110.7 cm^2 women: 93.8 cm^2 + (1) BMI ≥ 27 kg/m^2 (2) WC ≥ 102 cm for men WC ≥ 88 cm for women	n = 539	study performed in US	
				men	women
			n	280	259
			Age (year)	71.1 ± 0.4	71.1 ± 0.4
			BMI (kg/m^2)	NA	NA
			→ *lowest sex-specific tertile*		
Lim et al. (2010)	CT images (Brilliance 64, Philips)	Visceral fat area (VFA)/thigh muscle area (TMA) men: 0.93 women: 0.90	n = 264	Koreans	
				men	women
			n	126	138
			Age (year)	20 to 88	20 to 88
			BMI (kg/m^2)	NA	NA
			→ *VFA/TMA median higher 50th percentile of the healthy study sample*		

ASM, appendicular skeletal muscle mass; ASMI, appendicular skeletal muscle mass index; BMI, body mass index; BIA, bioelectrical impedance analysis; CART, classification and regression tree analysis; CT, computed tomography; DXA, dual X-ray absorptiometry; FFM, fat-free mass; FFMI, fat-free mass index; FM, fat mass; FMI, fat mass index; FNIH, Foundation for the National Institutes of Health; IOTF, International Obesity Taskforce; L3, third lumbar vertebra; NA, not available; NIH, National Institutes of Health; SD, standard deviation; SM, skeletal muscle mass; SMI, skeletal muscle mass index; TAMA, total abdominal muscle area; TMA, thigh muscle area; VFA, visceral fat area; WC, waist circumference; WHO, World Health Organization.

Table 5. Generation of cut-offs for SMI (corresponding to BMI thresholds) based on FFMI.

	BMI (kg/m^2)	FMI_{DXA} (kg/m^2) (Kelly et al., 2009)	$FFMI_{DXA}$ (kg/m^2) (Modified according to Kelly et al., 2009)	SMI_{MRI} (kg/m^2) (1.5 T Siemens Avanto MRI Scanner)	SMI_{BIA_median} (kg/m^2) (mBCA 515, Seca)	SMI_{BIA_-2SDs} (kg/m^2) (mBCA 515, Seca)
Caucasian men	<18.5	<2.9	15.6		8.6	>7.6
	>25	>6.0	19.0	9.85	9.7	>8.7
	>30	>8.9	21.1	10.71	10.5	>9.5
	>35	>11.9	23.1	12.15	11.4	>10.3
	>40	>15.0	25.0	13.67	12.2	>11.2
Caucasian women	<18.5	<4.9	13.6	6.65	6.7	>5.7
	>25	>9.2	15.8	7.49	7.7	>6.8
	>30	>12.9	17.1	8.15	8.5	>7.6
	>35	>16.8	18.2	8.99	9.3	>8.4
	>40	>20.6	19.4	9.74	10.1	>9.2

BMI, body mass index; FMI_{DXA}, fat mass index by dual X-ray absorptiometry (QDR 4500A fan beam densitometer (Hologic, Inc., Bedford, MA, Hologic Discovery software version 12.1)); $FFMI_{DXA}$, fat-free mass index by dual X-ray absorptiometry; SMI_{MRI}, skeletal muscle mass index by magnetic resonance imaging calculated by stepwise regression analysis (n = 410, 219 women (age: 38 ± 13 years, BMI: 27.7 ± 6.5 kg/m^2) and 191 men (age: 41 ± 14 years, BMI: 27.7 ± 5.0 kg/m^2) (detailed description of the segmentation procedure given elsewhere (Schautz et al., 2012)); SMI_{BIA_median}, skeletal muscle mass index by bioelectrical impedance analysis given as median calculated by linear regression analysis (n = 529, 264 women (27 ± 6 years, BMI: 23.9 ± 3.6 kg/m^2) and 265 men (28 ± 6 years, BMI: 25.2 ± 3.2 kg/m^2) (detailed description of the BIA measurement procedure given elsewhere (Bosy-Westphal et al., 2017)); SMI_{BIA_-2SDs}, skeletal muscle mass index by bioelectrical impedance analysis given as 2 SDs below the sex-specific mean calculated as linear regression analysis.

4. Discussion

SM has evolved as the most promising body composition parameter associated with health risk in ageing and many chronic diseases [1]. Evaluation of SM is complicated by a variety of available methods that provide different outcome parameters as a proxy for total body SM. Therefore, it is important to have accurate reference values that apply to the patient or population under study as well as to the respective body composition method. In this review, we identified multiple published reference values for discrepant parameters of SM (Tables 1–4), discussed the differences in the underlying assumptions and limitations as well as different concepts for normalization of SM parameters for height, weight, BMI or FM.

Imaging technologies are thought to provide the best assessment of SM. Briefly, segmentation of transversal images by special software (e.g., SliceOmatic Tomovision, version 4.3; Montreal, Québec, Canada) results in muscle areas that are multiplied by the correspondent slice thickness to calculate muscle volume [27] that is transformed to SM by assuming a constant density (1.04 kg/L) of adipose tissue-free SM [103]. Muscles at the head, hands and feet are commonly neglected in this approach. The precision of whole body SM_{MRI} is high (intra-observer coefficient of variation = 1.8% [104]). Reference data for total SM based on the gold standard whole body MRI (Table 5) are scarce due to high costs and cumbersome image-segmentation [17,18]. However, whole body MRI was integrated in the assessment of current large and representative national databases like the UK biobank [105] or the national cohort (NAKO) in Germany [106]. Future evaluation of these databases will provide the basis of statistically derived normal values whereas prospective investigation of mortality or correlation with frailty, fracture risk, glucose or amino acid metabolism would allow to establish even more meaningful disease-specific cut-offs.

Instead of whole body imaging, reference values for L3 single slices are frequently published (Tables 3 and 4), especially in patients where CT images are routinely applied for cancer staging. The use of these cut-offs may be specific for the population studied and transferability of the results to other patient groups needs to be investigated. Radiation exposure is a major limitation that confines the application of CT to individual transversal images or the secondary analysis of routine clinical measurements. As a further drawback, clinical CT protocols for L3 are not standardized across hospital sites. SMA at L1, L2, L4, L5, and the thoracic vertebra T12, T11, and T10 were reported to be suitable alternatives to SMA measured at L3 [58]. Nonetheless, there are also advantages of CT images with a high resolution and precision of the measurement. Most studies report the precision of single slice CT scan analysis to range between 1% and 2% [107]. Thus, automated segmentation is facilitated by using a characteristic range of Hounsfield units for fat-free muscle tissue [107,108]. CT can also differentiate individual muscle or muscle groups and can thus for example investigate the impact of pectoralis muscle area for survival at the Intensive Care Unit [12] because respiratory musculature may determine weaning from mechanical ventilation. On the other hand, characteristic changes in the Hounsfield distribution of muscle can reveal qualitative changes of the tissue (e.g., fatty infiltration or edema) that have been found to be of prognostic value [71].

DXA is the most commonly used method for assessment of SM (Table 1). Lean soft tissue at the arms and legs (ASM) is highly correlated with muscle volume derived from imaging studies (correlation coefficients ranging from 0.77 to 0.97 for both, whole body and regional scans [51,109–115]). However, only 44% of total lean soft tissue is derived from extremities (unpublished results) and only part of total lean soft tissue is SM. Therefore, SM measured by DXA is considerably higher when compared with muscle volume measured by imaging technologies [27,116]. Precision errors for total ASM are reported to be low (1–3%), device specific and depend on population characteristics like age or prevalence of obesity [117].

BIA can assess SM, ASM or FFM, depending on the reference method used to generate the BIA-algorithm. The choice of the BIA-algorithm not only depends on the desired target-parameter but also on the agreement between the BIA-device or reference population used to generate the BIA-algorithm and the BIA-device and patient characteristics to be evaluated [118]. However, in two

studies, the equation by Janssen et al. [56] that is not suitable for Asians was used to predict SM in Asian populations [53,55] with only one study providing a validation in 41 Taiwanese people (age: 20–99 years; BMI: 17.6–34.6 kg/m^2) [55]. Except for the study by Masanés et al. [26], all other studies used different BIA devices than Janssen et al. [56] (Table 2). Validity and precision of BIA results differ between manufacturers and depend on the hardware as well as the appropriate validation of the BIA-algorithm [119]. Discrepancies in the assumptions of the homogeneous bioelectrical model that lead to a higher measurement error occur with changes in hydration (e.g., edema) and with differences in body shape that are associated with aging (decreasing limb relative to trunk diameter), obesity (apple and pear shape of body fat distribution) and ethnicity (trunk to leg length, regional adiposity and muscularity). Therefore, segmental BIA that can measure the relative contribution of trunk and extremities to total body conductivity may help to reduce assumptions on body shape leading to an improved prediction compared with conventional wrist-ankle measurements [27]. The accuracy of phase-sensitive segmental BIA compared with MRI as a reference is clinically acceptable when whole body SM was assessed (two SDs: 11–12% for different ethnicities) but it was low when small compartments of the body were assessed (e.g., two SDs: 20–29% for the arms) [27].

4.1. Limitations of Proxies for Total Skeletal Muscle

Single SMA at L3 level turned out to be the best compromise site to assess volumes of total SM together with visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) (r = 0.832–0.986; $p < 0.01$ [17]). Furthermore, SMA at L3 is considered as a valid proxy for whole body FFM (r = 0.940; $p < 0.001$ [120]). Other authors reported high correlations between single abdominal SMA at L4-L5 intervertebral space and total SM (r = 0.710–0.920 [121]), whereas the use of PMI to determine whole body SM is controversial because psoas is a relatively small muscle. A good correlation between PMI and SMI measured by BIA in healthy 35 Asian liver donors (r = 0.737; $p < 0.001$) and a moderate correlation in 137 living donor liver transplantation recipients (r = 0.682; $p < 0.001$) were found [63]. Other authors argue that L3 PMA is not representative of total SM [122,123]. Despite acceptable correlations, the accuracy of single images is limited in individual cases. Likewise, it is well established that the correlation between BMI and FM is fairly good at the population level whereas at the individual level BMI is only a poor indicator of adiposity [124]. In addition, validity of the assessment of changes in SM during follow-up is limited by the use of individual images from L3 or mid-thigh. These images cannot be used as pars pro toto because of regional differences in changes of muscle volume with age or obesity (e.g., the contribution of SM_{MRI} at the arms and legs to ASM tended to decrease at higher adiposity in both genders [104]).

Similarly, ASM has limitations to assess the change in total SM with ageing or overweight and obesity. Since lean soft tissue from the extremities also contains lean compartments from connective tissue (e.g., skin and adipose tissue), SM accounts for only about 50% of FFM in obesity [116]. ASM was therefore shown to overestimate appendicular SM assessed by MRI with increasing BMI [27]. In line with this finding, DXA was also shown to underestimate the age-related loss of thigh muscle mass in comparison with MRI [125]. Furthermore, DXA measures of change in lean mass before and 10-week after resistance training were only modestly associated with MRI measures of change in muscle volume [126].

In summary, the random error of single images or ASM as a proxy for total SM limits the applicability of these substitutes in individual cases and together with the systematic error limit the accurate detection of changes in SM.

4.2. Normalization of Skeletal Muscle Mass for Body Size and Obesity

Normalization of lean mass for weight is inappropriate because two people with the same %FFM who differ in height have a different nutritional status, with the taller person having a lower muscularity [127]. FFM has been shown to scale to height with a power of around two in different

ethnicities, ranging from 1.86 in non-Hispanic white women to 2.32 in non-Hispanic black men [128]. Consequently, appropriate normalization of total SM, SM-area, ASM and FFM is performed for height^2.

In addition to the physiologic increase in SM with height, there is also an increase in SM with weight gain that depends on the initial amount of FM [129]. The evaluation of SM may thus also depend on the amount of FM. With increasing obesity, adverse effects on myocyte metabolism, muscle tissue composition and peak force generation can be mediated via paracrine signaling of proinflammatory immune cells in intermuscular adipose tissue [30]. The same SM at a higher FM may also lead to a limitation of strength and increased disability because at the same work load, energy expenditure and muscle force are higher for a person with obesity [130]. In line with these mechanisms, patients with a low SM and a concomitant high FM were shown to have a higher morbidity and mortality when compared to patients with a high FM only (for review see [131]). However, it remains unclear whether the risk of a low SM and a high FM is additive or if the risk of a high FM is disproportionally higher at a concomitantly low SM.

Published definitions of sarcopenic obesity use BMI to assess overweight and obesity in combination with fixed cut-offs for a low SM that are derived from subjects with normal weight and/or overweight [72,76]. To the best of our knowledge, all current definitions disregard the relationship between fat and lean mass that can be investigated by applying the Forbes rule (energy partitioning, i.e., the fraction of energy lost or gained as protein, is a nonlinear function of FM [129]) or the Hattori chart (two dimensional plot of FMI vs. FFMI [132]). Table 5 provides novel BMI-dependent SMI cut-offs.

The combination of FFMI with FMI [133], %FM [6,8] or BMI [134] facilitate to investigate the proportional contribution of fat and lean compartments to health risk as well as their presumable interaction. An attractive alternative to the simultaneous use of two indices is integration of information on fat and lean compartments in one index as FM/FFM^2. This index was proposed by Wells and Victoria who determined the appropriate power by which to raise the denominator from regressing FM on FFM [135]. The usefulness of this index needs to be investigated in future studies because it depends on a linear correlation between FM and FFM^2, as well as on absence of heteroscedasticity.

Beyond diverse methods of normalization (e.g., appendicular lean mass (ALM) adjusted by BMI [66,67], FFM normalized for body surface area (FFM_{BSA} = (weight [kg]$^{0.425}$ × height [m]$^{0.725}$) × 0.007184 [20])) heterogeneous outcome parameters (ASMI, SMI, L3 SMI, L3 PMI, FFMI) and a discrepant nomenclature for the same outcome parameter as well as different ways of reporting reference values hinder the comparison between studies. ASMI (i.e., appendicular skeletal muscle mass/height^2) and SMI (total skeletal muscle mass/height^2) were the most commonly used denominations within publications and therefore consistently applied in Tables 1–5. A great variety of different notations for the same outcome parameter were found for (a) SMI: e.g., skeletal muscle mass index, SMMI [52], muscle mass index, MMI [25,26], total skeletal muscle index, TSMI [53], total body skeletal muscle mass index, TBSMI [40] and also (b) ASMI: e.g., appendicular skeletal muscle mass index, ASMMI [136], appendicular muscle mass index, AMI (appendicular muscle mass (AMM)/height^2) [54], relative appendicular skeletal muscle index, RASM [47,137], relative skeletal muscle mass index [138] and appendicular lean mass index (ALM/height^2) [21]. In contrast to the heterogeneous nomenclature, some studies apply the same term "SMI" for different outcome parameters: e.g., ALM/BMI [66,67], ASM/height^2 [46,139,140], ALM/height^2 [141], ASM/body weight [53] and SM/body weight × 100 [25,137,142–144]. In cancer studies, SMI is normally defined as SMA/height^2 [62,71,72]. Thus, a consistent nomenclature for proxies of SM is needed in order to facilitate comparison between studies.

Moreover, suitable reference values require an appropriate sample size ideally comprised of healthy or "normal" subjects (normative approach) or derive cut-offs from an older population or a group of patients (stratification approach). In addition, reference values can be reported using parametric methods, like Z-scores or 2 SDs below the mean, that rely on normal distribution of the data, on the absence of residual associations, and on constant variance of the normalized measurements throughout the entire sample (absence of heteroscedasticity, logarithmic transformation of the dependent variables

or weighted regression models). In Tables 1–4, most studies used cut-off thresholds for low SM on the basis of young healthy adults' reference groups according to the recommendations proposed by the European Working Group on Sarcopenia in Older People [32]. The majority of these studies used two SDs below the means of healthy young subjects as a cut-off, e.g., [21,39,40,44,45,50] whereas other studies defined a low SM as one SD below the mean, e.g., [85,90,94,95]. Six articles stratified the cut-offs according to severity of a low SM [22,44,46,49,76,80]. One SM threshold was based on the fifth percentile [59] or on the 20th percentile [92] or on the 50th percentile [89]. Other studies used the sex-specific lowest quintiles [43], quartiles [47,62], tertiles [84], the lower two quintiles of the study population [98,100] or the lowest 20% of the distribution [38,42,48]. In one study, receiver operating characteristics analysis was used to develop SM cut-offs associated with physical disability [24]. In four studies, optimal stratification was used to determine the SM threshold of mortality risk in cancer patients [64,65,71,72]. Further diagnostic criteria applied classification and regression tree analysis [66,67].

5. Conclusions and Recommendations

In summary, published reference values for SM differ widely dependent on the outcome parameter and reference population. Results should consider the limitation of all proxies for total SM with respect to application in individual cases as well as for measurement of changes in SM. To facilitate comparison between results of different studies, authors should use a unified nomenclature for outcome parameters and indicate the device and software version of the body composition analyzer. In addition, the choice of body composition method should depend on the aim of the study. For assessment of changes in SM and evaluation of individual patients, a high precision is required that is, for instance, not fulfilled when segmental bioelectrical impedance is used to assess limb SM. The adverse effects of obesity on muscle quality and function may lead to an underestimation of sarcopenia in obesity and therefore requires normalization of SM for FM.

Author Contributions: Conceptualization, A.B.-W. and W.B.; methodology, C.O.W., A.B.-W. and W.B.; formal analysis, C.O.W., B.J. and W.B.; data curation (Table 5), B.J., S.P. and W.B..; writing—original draft preparation, A.B.-W., C.O.W. and W.B.; writing—review and editing, B.J., M.J.M. (Michael J. Maisch), M.J.M. (Manfred J. Müller), K.N. and S.P. All authors have read and agreed to the published version of the manuscript.

Funding: We acknowledge financial support by Land Schleswig-Holstein within the funding programme Open Access Publikationsfonds.

Conflicts of Interest: Michael Maisch and Björn Jensen are employed by seca gmbh & co. kg., Anja Bosy-Westphal serves a consultant for seca gmbh & co. kg. The other authors declare no conflict of interest.

Abbreviation

ALM	appendicular lean mass
ASM	appendicular skeletal muscle mass
ASMI	appendicular skeletal muscle mass index
BIA	bioelectrical impedance analysis
BMI	body mass index
BSA	body surface area
CART	classification and regression tree analysis
CT	computed tomography
DXA	dual X-ray absorptiometry
FFM	fat-free mass
FFMI	fat-free mass index
FM	fat mass
FMI	fat mass index
FNIH	Foundation for the National Institutes of Health
IOTF	International Obesity Taskforce

L	lumbar vertebra
L3	third lumbar vertebra
MRI	magnetic resonance imaging
NA	not available
NAKO	German National Cohort
NHANES	National Health and Nutrition Examination Survey
NIH	National Institutes of Health
PMA	psoas muscle area
PMI	psoas muscle index
SAT	subcutaneous adipose tissue
SD	standard deviation
SEE	standard error of estimate
SM	skeletal muscle mass
SMI	skeletal muscle mass index
SMA	skeletal muscle area
T	thoracic vertebra
TAMA	total abdominal muscle area
TMA	thigh muscle area
VAT	visceral adipose tissue
VFA	visceral fat area
WC	waist circumference
WHO	World Health Organization

References

1. Bauer, J.; Morley, J.E.; Schols, A.M.W.J.; Ferrucci, L.; Cruz-Jentoft, A.J.; Dent, E.; Baracos, V.E.; Crawford, J.A.; Doehner, W.; Heymsfield, S.B.; et al. Sarcopenia: A Time for Action. An SCWD Position Paper. *J. Cachexia Sarcopenia Muscle* **2019**, *10*, 956–961. [CrossRef] [PubMed]
2. Hanai, T.; Shiraki, M.; Nishimura, K.; Ohnishi, S.; Imai, K.; Suetsugu, A.; Takai, K.; Shimizu, M.; Moriwaki, H. Sarcopenia impairs prognosis of patients with liver cirrhosis. *Nutrition* **2015**, *31*, 193–199. [CrossRef] [PubMed]
3. Lin, T.-Y.; Lim, P.-S.; Hung, S.-C. Impact of Misclassification of Obesity by Body Mass Index on Mortality in Patients with CKD. *Kidney Int. Rep.* **2018**, *3*, 447–455. [CrossRef] [PubMed]
4. Caan, B.J.; Cespedes Feliciano, E.M.; Prado, C.M.; Alexeeff, S.; Kroenke, C.H.; Bradshaw, P.; Quesenberry, C.P.; Weltzien, E.K.; Castillo, A.L.; Olobatuyi, T.A.; et al. Association of Muscle and Adiposity Measured by Computed Tomography with Survival in Patients with Nonmetastatic Breast Cancer. *JAMA Oncol.* **2018**, *4*, 798–804. [CrossRef] [PubMed]
5. Hopkins, J.J.; Reif, R.L.; Bigam, D.L.; Baracos, V.E.; Eurich, D.T.; Sawyer, M.B. The Impact of Muscle and Adipose Tissue on Long-term Survival in Patients with Stage I to III Colorectal Cancer. *Dis. Colon Rectum* **2019**, *62*, 549–560. [CrossRef]
6. Huang, B.-T.; Peng, Y.; Liu, W.; Zhang, C.; Huang, F.-Y.; Wang, P.-J.; Zuo, Z.-L.; Liao, Y.-B.; Chai, H.; Huang, K.-S.; et al. Lean mass index, body fat and survival in Chinese patients with coronary artery disease. *QJM Int. J. Med.* **2015**, *108*, 641–647. [CrossRef]
7. Medina-Inojosa, J.R.; Somers, V.K.; Thomas, R.J.; Jean, N.; Jenkins, S.M.; Gomez-Ibarra, M.A.; Supervia, M.; Lopez-Jimenez, F. Association Between Adiposity and Lean Mass with Long-Term Cardiovascular Events in Patients with Coronary Artery Disease: No Paradox. *J. Am. Heart Assoc.* **2018**, *7*. [CrossRef]
8. Lavie, C.J.; De Schutter, A.; Patel, D.A.; Romero-Corral, A.; Artham, S.M.; Milani, R.V. Body Composition and Survival in Stable Coronary Heart Disease: Impact of lean mass index and body fat in the "obesity paradox". *J. Am. Coll. Cardiol.* **2012**, *60*, 1374–1380. [CrossRef]
9. Toledo, D.O.; Carvalho, A.M.; Oliveira, A.M.R.R.; Toloi, J.M.; Silva, A.C.; Francisco de Mattos Farah, J.; Prado, C.M.; Silva, J.M. The use of computed tomography images as a prognostic marker in critically ill cancer patients. *Clin. Nutr. ESPEN* **2018**, *25*, 114–120. [CrossRef]

10. Kou, H.-W.; Yeh, C.-H.; Tsai, H.-I.; Hsu, C.-C.; Hsieh, Y.-C.; Chen, W.-T.; Cheng, H.-T.; Yu, M.-C.; Lee, C.-W. Sarcopenia is an effective predictor of difficult-to-wean and mortality among critically ill surgical patients. *PLoS ONE* **2019**, *14*, e0220699. [CrossRef]
11. Moisey, L.L.; Mourtzakis, M.; Cotton, B.A.; Premji, T.; Heyland, D.K.; Wade, C.E.; Bulger, E.; Kozar, R.A.; Nutrition and Rehabilitation Investigators Consortium (NUTRIC). Skeletal muscle predicts ventilator-free days, ICU-free days, and mortality in elderly ICU patients. *Crit. Care* **2013**, *17*, R206. [CrossRef]
12. Jaitovich, A.; Khan, M.M.H.S.; Itty, R.; Chieng, H.C.; Dumas, C.L.; Nadendla, P.; Fantauzzi, J.P.; Yucel, R.M.; Feustel, P.J.; Judson, M.A. ICU Admission Muscle and Fat Mass, Survival, and Disability at Discharge: A Prospective Cohort Study. *Chest* **2019**, *155*, 322–330. [CrossRef]
13. Carson, B.P. The Potential Role of Contraction-Induced Myokines in the Regulation of Metabolic Function for the Prevention and Treatment of Type 2 Diabetes. *Front. Endocrinol.* **2017**, *8*, 97. [CrossRef]
14. Lee, J.H.; Jun, H.-S. Role of Myokines in Regulating Skeletal Muscle Mass and Function. *Front. Physiol.* **2019**, *10*, 42. [CrossRef]
15. Bigaard, J.; Frederiksen, K.; Tjønneland, A.; Thomsen, B.L.; Overvad, K.; Heitmann, B.L.; Sørensen, T.I.A. Body Fat and Fat-Free Mass and All-Cause Mortality. *Obes. Res.* **2004**, *12*, 1042–1049. [CrossRef]
16. Lee, D.H.; Giovannucci, E.L. Body composition and mortality in the general population: A review of epidemiologic studies. *Exp. Biol. Med.* **2018**, *243*, 1275–1285. [CrossRef]
17. Schweitzer, L.; Geisler, C.; Pourhassan, M.; Braun, W.; Glüer, C.-C.; Bosy-Westphal, A.; Müller, M.J. What is the best reference site for a single MRI slice to assess whole-body skeletal muscle and adipose tissue volumes in healthy adults? *Am. J. Clin. Nutr.* **2015**, *102*, 58–65. [CrossRef]
18. Heymsfield, S.B.; Gonzalez, M.C.; Lu, J.; Jia, G.; Zheng, J. Skeletal muscle mass and quality: Evolution of modern measurement concepts in the context of sarcopenia. *Proc. Nutr. Soc.* **2015**, *74*, 355–366. [CrossRef]
19. Jung Lee, S.; Janssen, I.; Heymsfield, S.B.; Ross, R. Relation between whole-body and regional measures of human skeletal muscle. *Am. J. Clin. Nutr.* **2004**, *80*, 1215–1221. [CrossRef]
20. Bahat, G.; Saka, B.; Tufan, F.; Akin, S.; Sivrikaya, S.; Yucel, N.; Erten, N.; Karan, M.A. Prevalence of sarcopenia and its association with functional and nutritional status among male residents in a nursing home in Turkey. *Aging Male* **2010**, *13*, 211–214. [CrossRef]
21. Krzymińska-Siemaszko, R.; Fryzowicz, A.; Czepulis, N.; Kaluźniak-Szymanowska, A.; Dworak, L.B.; Wieczorowska-Tobis, K. The impact of the age range of young healthy reference population on the cut-off points for low muscle mass necessary for the diagnosis of sarcopenia. *Eur. Rev. Med. Pharmacol. Sci.* **2019**, *23*, 4321–4332. [CrossRef]
22. Alkahtani, S.A. A cross-sectional study on sarcopenia using different methods: Reference values for healthy Saudi young men. *BMC Musculoskelet. Disord.* **2017**, *18*, 119. [CrossRef]
23. Yamada, M.; Nishiguchi, S.; Fukutani, N.; Tanigawa, T.; Yukutake, T.; Kayama, H.; Aoyama, T.; Arai, H. Prevalence of Sarcopenia in Community-Dwelling Japanese Older Adults. *J. Am. Med. Dir. Assoc.* **2013**, *14*, 911–915. [CrossRef]
24. Janssen, I.; Baumgartner, R.N.; Ross, R.; Rosenberg, I.H.; Roubenoff, R. Skeletal Muscle Cutpoints Associated with Elevated Physical Disability Risk in Older Men and Women. *Am. J. Epidemiol.* **2004**, *159*, 413–421. [CrossRef]
25. Tichet, J.; Vol, S.; Goxe, D.; Salle, A.; Berrut, G.; Ritz, P. Prevalence of sarcopenia in the French senior population. *J. Nutr. Health Aging* **2008**, *12*, 202–206. [CrossRef]
26. Masanés, F.; Culla, A.; Navarro-Gonzalez, M.; Navarro-Lopez, M.; Sacanella, E.; Torres, B.; Lopez-Soto, A. Prevalence of sarcopenia in healthy community-dwelling elderly in an urban area of Barcelona (Spain). *J. Nutr. Health Aging* **2012**, *16*, 184–187. [CrossRef]
27. Bosy-Westphal, A.; Jensen, B.; Braun, W.; Pourhassan, M.; Gallagher, D.; Müller, M.J. Quantification of whole-body and segmental skeletal muscle mass using phase-sensitive 8-electrode medical bioelectrical impedance devices. *Eur. J. Clin. Nutr.* **2017**, *71*, 1061–1067. [CrossRef]
28. Prado, C.M.; Siervo, M.; Mire, E.; Heymsfield, S.B.; Stephan, B.C.; Broyles, S.; Smith, S.R.; Wells, J.C.; Katzmarzyk, P.T. A population-based approach to define body-composition phenotypes. *Am. J. Clin. Nutr.* **2014**, *99*, 1369–1377. [CrossRef]
29. Akhmedov, D.; Berdeaux, R. The effects of obesity on skeletal muscle regeneration. *Front. Physiol.* **2013**, *4*. [CrossRef]

30. Wu, H.; Ballantyne, C.M. Skeletal muscle inflammation and insulin resistance in obesity. *J. Clin. Invest.* **2017**, *127*, 43–54. [CrossRef]
31. Cederholm, T.; Jensen, G.L.; Correia, M.I.T.D.; Gonzalez, M.C.; Fukushima, R.; Higashiguchi, T.; Baptista, G.; Barazzoni, R.; Blaauw, R.; Coats, A.; et al. GLIM criteria for the diagnosis of malnutrition—A consensus report from the global clinical nutrition community. *Clin. Nutr.* **2019**, *38*, 1–9. [CrossRef] [PubMed]
32. Cruz-Jentoft, A.J.; Baeyens, J.P.; Bauer, J.M.; Boirie, Y.; Cederholm, T.; Landi, F.; Martin, F.C.; Michel, J.-P.; Rolland, Y.; Schneider, S.M.; et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing* **2010**, *39*, 412–423. [CrossRef]
33. Cruz-Jentoft, A.J.; Bahat, G.; Bauer, J.; Boirie, Y.; Bruyère, O.; Cederholm, T.; Cooper, C.; Landi, F.; Rolland, Y.; Sayer, A.A.; et al. Sarcopenia: Revised European consensus on definition and diagnosis. *Age Ageing* **2019**, *48*, 16–31. [CrossRef] [PubMed]
34. Chen, L.-K.; Liu, L.-K.; Woo, J.; Assantachai, P.; Auyeung, T.-W.; Bahyah, K.S.; Chou, M.-Y.; Chen, L.-Y.; Hsu, P.-S.; Krairit, O.; et al. Sarcopenia in Asia: Consensus Report of the Asian Working Group for Sarcopenia. *J. Am. Med. Dir. Assoc.* **2014**, *15*, 95–101. [CrossRef]
35. Chen, L.-K.; Lee, W.-J.; Peng, L.-N.; Liu, L.-K.; Arai, H.; Akishita, M. Recent Advances in Sarcopenia Research in Asia: 2016 Update From the Asian Working Group for Sarcopenia. *J. Am. Med. Dir. Assoc.* **2016**, *17*, 767.e1–767.e7. [CrossRef]
36. Studenski, S.A.; Peters, K.W.; Alley, D.E.; Cawthon, P.M.; McLean, R.R.; Harris, T.B.; Ferrucci, L.; Guralnik, J.M.; Fragala, M.S.; Kenny, A.M.; et al. The FNIH Sarcopenia Project: Rationale, Study Description, Conference Recommendations, and Final Estimates. *J. Gerontol. A Biomed. Sci. Med Sci.* **2014**, *69*, 547–558. [CrossRef] [PubMed]
37. Fielding, R.A.; Vellas, B.; Evans, W.J.; Bhasin, S.; Morley, J.E.; Newman, A.B.; Abellan van Kan, G.; Andrieu, S.; Bauer, J.; Breuille, D.; et al. Sarcopenia: An Undiagnosed Condition in Older Adults. Current Consensus Definition: Prevalence, Etiology, and Consequences. International Working Group on Sarcopenia. *J. Am. Med. Dir. Assoc.* **2011**, *12*, 249–256. [CrossRef] [PubMed]
38. Imboden, M.T.; Swartz, A.M.; Finch, H.W.; Harber, M.P.; Kaminsky, L.A. Reference standards for lean mass measures using GE dual energy x-ray absorptiometry in Caucasian adults. *PLoS ONE* **2017**, *12*, e0176161. [CrossRef]
39. Kruger, H.S.; Micklesfield, L.K.; Wright, H.H.; Havemann-Nel, L.; Goedecke, J.H. Ethnic-specific cut-points for sarcopenia: Evidence from black South African women. *Eur. J. Clin. Nutr.* **2015**, *69*, 843–849. [CrossRef]
40. Alemán-Mateo, H.; Ruiz Valenzuela, R.E. Skeletal Muscle Mass Indices in Healthy Young Mexican Adults Aged 20–40 Years: Implications for Diagnoses of Sarcopenia in the Elderly Population. *Sci. World J.* **2014**, *2014*. [CrossRef]
41. Gould, H.; Brennan, S.L.; Kotowicz, M.A.; Nicholson, G.C.; Pasco, J.A. Total and Appendicular Lean Mass Reference Ranges for Australian Men and Women: The Geelong Osteoporosis Study. *Calcif. Tissue Int.* **2014**, *94*, 363–372. [CrossRef] [PubMed]
42. Marwaha, R.K.; Garg, M.K.; Bhadra, K.; Mithal, A.; Tandon, N. Assessment of lean (muscle) mass and its distribution by dual energy X-ray absorptiometry in healthy Indian females. *Arch. Osteoporos.* **2014**, *9*, 186. [CrossRef] [PubMed]
43. Yu, R.; Wong, M.; Leung, J.; Lee, J.; Auyeung, T.W.; Woo, J. Incidence, reversibility, risk factors and the protective effect of high body mass index against sarcopenia in community-dwelling older Chinese adults: Sarcopenia incidence and its risk factors. *Geriatr. Gerontol. Int.* **2014**, *14*, 15–28. [CrossRef] [PubMed]
44. Kim, Y.-S.; Lee, Y.; Chung, Y.-S.; Lee, D.-J.; Joo, N.-S.; Hong, D.; Song, G.; Kim, H.-J.; Choi, Y.J.; Kim, K.-M. Prevalence of Sarcopenia and Sarcopenic Obesity in the Korean Population Based on the Fourth Korean National Health and Nutritional Examination Surveys. *J. Gerontol. A Biomed. Sci. Med. Sci.* **2012**, *67*, 1107–1113. [CrossRef]
45. Oliveira, R.J.; Bottaro, M.; Júnior, J.T.; Farinatti, P.T.V.; Bezerra, L.A.; Lima, R.M. Identification of sarcopenic obesity in postmenopausal women: A cutoff proposal. *Braz. J. Med. Biol. Res.* **2011**, *44*, 1171–1176. [CrossRef]
46. Sanada, K.; Miyachi, M.; Tanimoto, M.; Yamamoto, K.; Murakami, H.; Okumura, S.; Gando, Y.; Suzuki, K.; Tabata, I.; Higuchi, M. A cross-sectional study of sarcopenia in Japanese men and women: Reference values and association with cardiovascular risk factors. *Eur. J. Appl. Physiol.* **2010**, *110*, 57–65. [CrossRef]
47. Szulc, P.; Duboeuf, F.; Marchand, F.; Delmas, P.D. Hormonal and lifestyle determinants of appendicular skeletal muscle mass in men: The MINOS study. *Am. J. Clin. Nutr.* **2004**, *80*, 496–503. [CrossRef]

48. Newman, A.B.; Kupelian, V.; Visser, M.; Simonsick, E.; Goodpaster, B.; Nevitt, M.; Kritchevsky, S.B.; Tylavsky, F.A.; Rubin, S.M.; Harris, T.B.; et al. Sarcopenia: Alternative Definitions and Associations with Lower Extremity Function. *J. Am. Geriatr. Soc.* **2003**, *51*, 1602–1609. [CrossRef]
49. Tankó, L.B.; Movsesyan, L.; Mouritzen, U.; Christiansen, C.; Svendsen, O.L. Appendicular lean tissue mass and the prevalence of sarcopenia among healthy women. *Metabolism* **2002**, *51*, 69–74. [CrossRef]
50. Baumgartner, R.N.; Koehler, K.M.; Gallagher, D.; Romero, L.; Heymsfield, S.B.; Ross, R.R.; Garry, P.J.; Lindeman, R.D. Epidemiology of Sarcopenia among the Elderly in New Mexico. *Am. J. Epidemiol.* **1998**, *147*, 755–763. [CrossRef]
51. Kim, J.; Wang, Z.; Heymsfield, S.B.; Baumgartner, R.N.; Gallagher, D. Total-body skeletal muscle mass: Estimation by a new dual-energy X-ray absorptiometry method. *Am. Clin. Nutr.* **2002**, *76*, 378–383. [CrossRef] [PubMed]
52. Bahat, G.; Tufan, A.; Tufan, F.; Kilic, C.; Akpinar, T.S.; Kose, M.; Erten, N.; Karan, M.A.; Cruz-Jentoft, A.J. Cut-off points to identify sarcopenia according to European Working Group on Sarcopenia in Older People (EWGSOP) definition. *Clin. Nutr.* **2016**, *35*, 1557–1563. [CrossRef] [PubMed]
53. Chang, C.-I.; Chen, C.-Y.; Huang, K.-C.; Wu, C.-H.; Hsiung, C.A.; Hsu, C.-C.; Chen, C.-Y. Comparison of three BIA muscle indices for sarcopenia screening in old adults. *Eur. Geriatr. Med.* **2013**, *4*, 145–149. [CrossRef]
54. Tanimoto, Y.; Watanabe, M.; Sun, W.; Sugiura, Y.; Tsuda, Y.; Kimura, M.; Hayashida, I.; Kusabiraki, T.; Kono, K. Association between sarcopenia and higher-level functional capacity in daily living in community-dwelling elderly subjects in Japan. *Arch. Gerontol. Geriatr.* **2012**, *55*, e9–e13. [CrossRef]
55. Chien, M.-Y.; Huang, T.-Y.; Wu, Y.-T. Prevalence of Sarcopenia Estimated Using a Bioelectrical Impedance Analysis Prediction Equation in Community-Dwelling Elderly People in Taiwan. *J. Am. Geriatr. Soc.* **2008**, *56*, 1710–1715. [CrossRef]
56. Janssen, I.; Heymsfield, S.B.; Baumgartner, R.N.; Ross, R. Estimation of skeletal muscle mass by bioelectrical impedance analysis. *J. Appl. Physiol.* **2000**, *89*, 465–471. [CrossRef]
57. Ufuk, F.; Herek, D. Reference Skeletal Muscle Mass Values at L3 Vertebrae Level Based on Computed Tomography in Healthy Turkish Adults. *Int. J. Geront.* **2019**, *13*, 221–225.
58. Derstine, B.A.; Holcombe, S.A.; Ross, B.E.; Wang, N.C.; Su, G.L.; Wang, S.C. Skeletal muscle cutoff values for sarcopenia diagnosis using T10 to L5 measurements in a healthy US population. *Sci. Rep.* **2018**, *8*, 11369. [CrossRef]
59. van der Werf, A.; Langius, J.A.E.; de van der Schueren, M.A.E.; Nurmohamed, S.A.; van der Pant, K.A.M.I.; Blauwhoff-Buskermolen, S.; Wierdsma, N.J. Percentiles for skeletal muscle index, area and radiation attenuation based on computed tomography imaging in a healthy Caucasian population. *Eur. J. Clin. Nutr.* **2018**, *72*, 288–296. [CrossRef]
60. Benjamin, J.; Shasthry, V.; Kaal, C.R.; Anand, L.; Bhardwaj, A.; Pandit, V.; Arora, A.; Rajesh, S.; Pamecha, V.; Jain, V.; et al. Characterization of body composition and definition of sarcopenia in patients with alcoholic cirrhosis: A computed tomography based study. *Liver Int.* **2017**, *37*, 1668–1674. [CrossRef]
61. Kim, J.S.; Kim, W.Y.; Park, H.K.; Kim, M.C.; Jung, W.; Ko, B.S. Simple Age Specific Cutoff Value for Sarcopenia Evaluated by Computed Tomography. *Ann. Nutr. Metab.* **2017**, *71*, 157–163. [CrossRef] [PubMed]
62. Sakurai, K.; Kubo, N.; Tamura, T.; Toyokawa, T.; Amano, R.; Tanaka, H.; Muguruma, K.; Yashiro, M.; Maeda, K.; Hirakawa, K.; et al. Adverse Effects of Low Preoperative Skeletal Muscle Mass in Patients Undergoing Gastrectomy for Gastric Cancer. *Ann. Surg. Oncol.* **2017**, *24*, 2712–2719. [CrossRef] [PubMed]
63. Hamaguchi, Y.; Kaido, T.; Okumura, S.; Kobayashi, A.; Hammad, A.; Tamai, Y.; Inagaki, N.; Uemoto, S. Proposal for new diagnostic criteria for low skeletal muscle mass based on computed tomography imaging in Asian adults. *Nutrition* **2016**, *32*, 1200–1205. [CrossRef] [PubMed]
64. Zhuang, C.-L.; Huang, D.-D.; Pang, W.-Y.; Zhou, C.-J.; Wang, S.-L.; Lou, N.; Ma, L.-L.; Yu, Z.; Shen, X. Sarcopenia is an Independent Predictor of Severe Postoperative Complications and Long-Term Survival After Radical Gastrectomy for Gastric Cancer: Analysis from a Large-Scale Cohort. *Medicine* **2016**, *95*, e3164. [CrossRef] [PubMed]
65. Iritani, S.; Imai, K.; Takai, K.; Hanai, T.; Ideta, T.; Miyazaki, T.; Suetsugu, A.; Shiraki, M.; Shimizu, M.; Moriwaki, H. Skeletal muscle depletion is an independent prognostic factor for hepatocellular carcinoma. *J. Gastroenterol.* **2015**, *50*, 323–332. [CrossRef]

66. Chiles Shaffer, N.; Ferrucci, L.; Shardell, M.; Simonsick, E.M.; Studenski, S. Agreement and Predictive Validity Using Less-Conservative Foundation for the National Institutes of Health Sarcopenia Project Weakness Cutpoints. *J. Am. Geriatr. Soc.* **2017**, *65*, 574–579. [CrossRef]
67. Cawthon, P.M.; Peters, K.W.; Shardell, M.D.; McLean, R.R.; Dam, T.-T.L.; Kenny, A.M.; Fragala, M.S.; Harris, T.B.; Kiel, D.P.; Guralnik, J.M.; et al. Cutpoints for Low Appendicular Lean Mass That Identify Older Adults with Clinically Significant Weakness. *J. Gerontol. A Biomed. Sci. Med. Sci.* **2014**, *69*, 567–575. [CrossRef]
68. Biolo, G.; Di Girolamo, F.G.; Breglia, A.; Chiuc, M.; Baglio, V.; Vinci, P.; Toigo, G.; Lucchin, L.; Jurdana, M.; Pražnikar, Z.J.; et al. Inverse relationship between "a body shape index" (ABSI) and fat-free mass in women and men: Insights into mechanisms of sarcopenic obesity. *Clin. Nutr.* **2015**, *34*, 323–327. [CrossRef]
69. Lim, K.I.; Yang, S.J.; Kim, T.N.; Yoo, H.J.; Kang, H.J.; Song, W.; Baik, S.H.; Choi, D.S.; Choi, K.M. The association between the ratio of visceral fat to thigh muscle area and metabolic syndrome: The Korean Sarcopenic Obesity Study (KSOS). *Clin. Endocrinol.* **2010**, *73*, 588–594. [CrossRef]
70. Atkins, J.L.; Whincup, P.H.; Morris, R.W.; Lennon, L.T.; Papacosta, O.; Wannamethee, S.G. Sarcopenic Obesity and Risk of Cardiovascular Disease and Mortality: A Population-Based Cohort Study of Older Men. *J. Am. Geriatr. Soc.* **2014**, *62*, 253–260. [CrossRef]
71. Prado, C.M.; Lieffers, J.R.; McCargar, L.J.; Reiman, T.; Sawyer, M.B.; Martin, L.; Baracos, V.E. Prevalence and clinical implications of sarcopenic obesity in patients with solid tumours of the respiratory and gastrointestinal tracts: A population-based study. *Lancet Oncol.* **2008**, *9*, 629–635. [CrossRef]
72. Martin, L.; Birdsell, L.; MacDonald, N.; Reiman, T.; Clandinin, M.T.; McCargar, L.J.; Murphy, R.; Ghosh, S.; Sawyer, M.B.; Baracos, V.E. Cancer Cachexia in the Age of Obesity: Skeletal Muscle Depletion Is a Powerful Prognostic Factor, Independent of Body Mass Index. *J. Clin. Oncol.* **2013**, *31*, 1539–1547. [CrossRef] [PubMed]
73. Nishigori, T.; Tsunoda, S.; Okabe, H.; Tanaka, E.; Hisamori, S.; Hosogi, H.; Shinohara, H.; Sakai, Y. Impact of Sarcopenic Obesity on Surgical Site Infection after Laparoscopic Total Gastrectomy. *Ann. Surg. Oncol.* **2016**, *23*, 524–531. [CrossRef] [PubMed]
74. Pecorelli, N.; Carrara, G.; De Cobelli, F.; Cristel, G.; Damascelli, A.; Balzano, G.; Beretta, L.; Braga, M. Effect of sarcopenia and visceral obesity on mortality and pancreatic fistula following pancreatic cancer surgery. *Br. J. Surg.* **2016**, *103*, 434–442. [CrossRef]
75. Cushen, S.J.; Power, D.G.; Murphy, K.P.; McDermott, R.; Griffin, B.T.; Lim, M.; Daly, L.; MacEneaney, P.; O' Sullivan, K.; Prado, C.M.; et al. Impact of body composition parameters on clinical outcomes in patients with metastatic castrate-resistant prostate cancer treated with docetaxel. *Clin. Nutr. ESPEN* **2016**, *13*, e39–e45. [CrossRef] [PubMed]
76. Muscariello, E.; Nasti, G.; Siervo, M.; Di Maro, M.; Lapi, D.; D'Addio, G.; Colantuoni, A. Dietary protein intake in sarcopenic obese older women. *Clin. Interv. Aging* **2016**, *133*. [CrossRef]
77. Antoun, S.; Baracos, V.E.; Birdsell, L.; Escudier, B.; Sawyer, M.B. Low body mass index and sarcopenia associated with dose-limiting toxicity of sorafenib in patients with renal cell carcinoma. *Ann. Oncol.* **2010**, *21*, 1594–1598. [CrossRef]
78. Barret, M.; Antoun, S.; Dalban, C.; Malka, D.; Mansourbakht, T.; Zaanan, A.; Latko, E.; Taieb, J. Sarcopenia Is Linked to Treatment Toxicity in Patients with Metastatic Colorectal Cancer. *Nutr. Cancer* **2014**, *66*, 583–589. [CrossRef]
79. Huillard, O.; Mir, O.; Peyromaure, M.; Tlemsani, C.; Giroux, J.; Boudou-Rouquette, P.; Ropert, S.; Delongchamps, N.B.; Zerbib, M.; Goldwasser, F. Sarcopenia and body mass index predict sunitinib-induced early dose-limiting toxicities in renal cancer patients. *Br. J. Cancer* **2013**, *108*, 1034–1041. [CrossRef]
80. De Rosa, E.; Santarpia, L.; Marra, M.; Sammarco, R.; Amato, V.; Onufrio, M.; De Simone, G.; Contaldo, F.; Pasanisi, F. Preliminary evaluation of the prevalence of sarcopenia in obese patients from Southern Italy. *Nutrition* **2015**, *31*, 79–83. [CrossRef]
81. Kemmler, W.; von Stengel, S.; Engelke, K.; Sieber, C.; Freiberger, E. Prevalence of sarcopenic obesity in Germany using established definitions: Baseline data of the FORMOsA study. *Osteoporos. Int.* **2016**, *27*, 275–281. [CrossRef] [PubMed]
82. Kim, M.K.; Baek, K.H.; Song, K.-H.; Il Kang, M.; Park, C.Y.; Lee, W.Y.; Oh, K.W. Vitamin D Deficiency Is Associated with Sarcopenia in Older Koreans, Regardless of Obesity: The Fourth Korea National Health and Nutrition Examination Surveys (KNHANES IV) 2009. *J. Clin. Endocrinol. Metab.* **2011**, *96*, 3250–3256. [CrossRef]

83. Lee, S.; Kim, T.-N.; Kim, S.-H. Sarcopenic obesity is more closely associated with knee osteoarthritis than is nonsarcopenic obesity: A cross-sectional study. *Arthritis Rheum.* **2012**, *64*, 3947–3954. [CrossRef] [PubMed]
84. Ramachandran, R.; Gravenstein, K.S.; Metter, E.J.; Egan, J.M.; Ferrucci, L.; Chia, C.W. Selective Contribution of Regional Adiposity, Skeletal Muscle, and Adipokines to Glucose Disposal in Older Adults. *J. Am. Geriatr. Soc.* **2012**, *60*, 707–712. [CrossRef] [PubMed]
85. Kwon, S.S.; Lee, S.-G.; Lee, Y.; Lim, J.-B.; Kim, J.-H. Homeostasis model assessment of insulin resistance in a general adult population in Korea: Additive association of sarcopenia and obesity with insulin resistance. *Clin. Endocrinol.* **2017**, *86*, 44–51. [CrossRef]
86. Lee, Y.; Jung, K.S.; Kim, S.U.; Yoon, H.; Yun, Y.J.; Lee, B.-W.; Kang, E.S.; Han, K.-H.; Lee, H.C.; Cha, B.-S. Sarcopaenia is associated with NAFLD independently of obesity and insulin resistance: Nationwide surveys (KNHANES 2008–2011). *J. Hepatol.* **2015**, *63*, 486–493. [CrossRef]
87. Oh, C.; Jho, S.; No, J.-K.; Kim, H.-S. Body composition changes were related to nutrient intakes in elderly men but elderly women had a higher prevalence of sarcopenic obesity in a population of Korean adults. *Nutr. Res.* **2015**, *35*, 1–6. [CrossRef]
88. Chung, J.-Y.; Kang, H.-T.; Lee, D.-C.; Lee, H.-R.; Lee, Y.-J. Body composition and its association with cardiometabolic risk factors in the elderly: A focus on sarcopenic obesity. *Arch. Gerontol. Geriatr.* **2013**, *56*, 270–278. [CrossRef]
89. Baek, J.; Park, D.; Kim, I.; Won, J.-U.; Hwang, J.; Roh, J. Autonomic dysfunction of overweight combined with low muscle mass. *Clin. Auton. Res.* **2013**, *23*, 325–331. [CrossRef]
90. Baek, S.J.; Nam, G.E.; Han, K.D.; Choi, S.W.; Jung, S.W.; Bok, A.R.; Kim, Y.H.; Lee, K.S.; Han, B.D.; Kim, D.H. Sarcopenia and sarcopenic obesity and their association with dyslipidemia in Korean elderly men: The 2008–2010 Korea National Health and Nutrition Examination Survey. *J. Endocrinol. Investig.* **2014**, *37*, 247–260. [CrossRef]
91. Lou, N.; Chi, C.-H.; Chen, X.-D.; Zhou, C.-J.; Wang, S.-L.; Zhuang, C.-L.; Shen, X. Sarcopenia in overweight and obese patients is a predictive factor for postoperative complication in gastric cancer: A prospective study. *Eur. J. Surg. Oncol.* **2017**, *43*, 188–195. [CrossRef] [PubMed]
92. Moreira, M.A.; Zunzunegui, M.V.; Vafaei, A.; da Câmara, S.M.A.; Oliveira, T.S.; Maciel, Á.C.C. Sarcopenic obesity and physical performance in middle aged women: A cross-sectional study in Northeast Brazil. *BMC Public Health* **2016**, *16*, 43. [CrossRef] [PubMed]
93. Hwang, B.; Lim, J.-Y.; Lee, J.; Choi, N.-K.; Ahn, Y.-O.; Park, B.-J. Prevalence Rate and Associated Factors of Sarcopenic Obesity in Korean Elderly Population. *J. Korean Med. Sci.* **2012**, *27*, 748–755. [CrossRef] [PubMed]
94. Cho, Y.; Shin, S.-Y.; Shin, M.-J. Sarcopenic obesity is associated with lower indicators of psychological health and quality of life in Koreans. *Nutr. Res.* **2015**, *35*, 384–392. [CrossRef] [PubMed]
95. An, K.O.; Kim, J. Association of Sarcopenia and Obesity with Multimorbidity in Korean Adults: A Nationwide Cross-Sectional Study. *J. Am. Med. Dir. Assoc.* **2016**, *17*, 960.e1–960.e7. [CrossRef]
96. Bahat, G.; Kilic, C.; Topcu, Y.; Aydin, K.; Karan, M.A. Fat percentage cutoff values to define obesity and prevalence of sarcopenic obesity in community-dwelling older adults in Turkey. *Aging Male* **2018**, 1–7. [CrossRef]
97. Ishii, S.; Chang, C.; Tanaka, T.; Kuroda, A.; Tsuji, T.; Akishita, M.; Iijima, K. The Association between Sarcopenic Obesity and Depressive Symptoms in Older Japanese Adults. *PLoS ONE* **2016**, *11*, e0162898. [CrossRef]
98. Kim, T.N.; Yang, S.J.; Yoo, H.J.; Lim, K.I.; Kang, H.J.; Song, W.; Seo, J.A.; Kim, S.G.; Kim, N.H.; Baik, S.H.; et al. Prevalence of sarcopenia and sarcopenic obesity in Korean adults: The Korean sarcopenic obesity study. *Int. J. Obes.* **2009**, *33*, 885–892. [CrossRef]
99. Lee, J.; Hong, Y.; Shin, H.J.; Lee, W. Associations of Sarcopenia and Sarcopenic Obesity with Metabolic Syndrome Considering Both Muscle Mass and Muscle Strength. *J. Prev. Med. Public Health* **2016**, *49*, 35–44. [CrossRef]
100. Gomez-Cabello, A.; Pedrero-Chamizo, R.; Olivares, P.R.; Luzardo, L.; Juez-Bengoechea, A.; Mata, E.; Albers, U.; Aznar, S.; Villa, G.; Espino, L.; et al. Prevalence of overweight and obesity in non-institutionalized people aged 65 or over from Spain: The elderly EXERNET multi-centre study: Adiposity and lifestyle in Spanish elderly. *Obes. Rev.* **2011**, *12*, 583–592. [CrossRef]

101. Rolland, Y.; Lauwers-Cances, V.; Cristini, C.; van Kan, G.A.; Janssen, I.; Morley, J.E.; Vellas, B. Difficulties with physical function associated with obesity, sarcopenia, and sarcopenic-obesity in community-dwelling elderly women: The EPIDOS (EPIDemiologie de l'OSteoporose) Study. *Am. J. Clin. Nutr.* **2009**, *89*, 1895–1900. [CrossRef] [PubMed]
102. Kelly, T.L.; Wilson, K.E.; Heymsfield, S.B. Dual Energy X-Ray Absorptiometry Body Composition Reference Values from NHANES. *PLoS ONE* **2009**, *4*, e7038. [CrossRef] [PubMed]
103. Snyder, W.S.C.; Cook, M.J.; Nasset, E.S.; Karhansen, L.R.; Howells, G.P.; Tipton, I.H. *Report of the Task Group on Reference Men*; Pergamon Press: Oxford, UK, 1975.
104. Schautz, B.; Later, W.; Heller, M.; Müller, M.J.; Bosy-Westphal, A. Total and regional relationship between lean and fat mass with increasing adiposity—Impact for the diagnosis of sarcopenic obesity. *Eur. J. Clin. Nutr.* **2012**, *66*, 1356–1361. [CrossRef] [PubMed]
105. Linge, J.; Borga, M.; West, J.; Tuthill, T.; Miller, M.R.; Dumitriu, A.; Thomas, E.L.; Romu, T.; Tunón, P.; Bell, J.D.; et al. Body Composition Profiling in the UK Biobank Imaging Study. *Obesity* **2018**, *26*, 1785–1795. [CrossRef] [PubMed]
106. Bamberg, F.; Kauczor, H.-U.; Weckbach, S.; Schlett, C.L.; Forsting, M.; Ladd, S.C.; Greiser, K.H.; Weber, M.-A.; Schulz-Menger, J.; Niendorf, T.; et al. Whole-Body MR Imaging in the German National Cohort: Rationale, Design, and Technical Background. *Radiology* **2015**, *277*, 206–220. [CrossRef] [PubMed]
107. MacDonald, A.J.; Greig, C.A.; Baracos, V. The advantages and limitations of cross-sectional body composition analysis. *Curr. Opin. Support. Palliat. Care* **2011**, *5*, 342–349. [CrossRef] [PubMed]
108. Prado, C.M.M.; Heymsfield, S.B. Lean Tissue Imaging: A New Era for Nutritional Assessment and Intervention. *J. Parenter. Enter. Nutr.* **2014**, *38*, 940–953. [CrossRef]
109. Visser, M.; Fuerst, T.; Lang, T.; Salamone, L.; Harris, T.B. Validity of fan-beam dual-energy X-ray absorptiometry for measuring fat-free mass and leg muscle mass. Health, Aging, and Body Composition Study–Dual-Energy X-ray Absorptiometry and Body Composition Working Group. *J. Appl. Physiol.* **1999**, *87*, 1513–1520. [CrossRef]
110. Hansen, R.D.; Williamson, D.A.; Finnegan, T.P.; Lloyd, B.D.; Grady, J.N.; Diamond, T.H.; Smith, E.U.; Stavrinos, T.M.; Thompson, M.W.; Gwinn, T.H.; et al. Estimation of thigh muscle cross-sectional area by dual-energy X-ray absorptiometry in frail elderly patients. *Am. J. Clin. Nutr.* **2007**, *86*, 952–958. [CrossRef]
111. Zhao, X.; Wang, Z.; Zhang, J.; Hua, J.; He, W.; Zhu, S. Estimation of Total Body Skeletal Muscle Mass in Chinese Adults: Prediction Model by Dual-Energy X-Ray Absorptiometry. *PLoS ONE* **2013**, *8*, e53561. [CrossRef]
112. Freda, P.U.; Shen, W.; Reyes-Vidal, C.M.; Geer, E.B.; Arias-Mendoza, F.; Gallagher, D.; Heymsfield, S.B. Skeletal Muscle Mass in Acromegaly Assessed by Magnetic Resonance Imaging and Dual-Photon X-Ray Absorptiometry. *J. Clin. Endocrinol. Metab.* **2009**, *94*, 2880–2886. [CrossRef] [PubMed]
113. Bridge, P.; Pocock, N.A.; Nguyen, T.; Munns, C.; Cowell, C.T.; Forwood, N.; Thompson, M.W. Validation of Longitudinal DXA Changes in Body Composition From Pre- to Mid-Adolescence Using MRI as Reference. *J. Clin. Densitom.* **2011**, *14*, 340–347. [CrossRef] [PubMed]
114. Bredella, M.A.; Ghomi, R.H.; Thomas, B.J.; Torriani, M.; Brick, D.J.; Gerweck, A.V.; Misra, M.; Klibanski, A.; Miller, K.K. Comparison of DXA and CT in the Assessment of Body Composition in Premenopausal Women with Obesity and Anorexia Nervosa. *Obesity* **2010**, *18*, 2227–2233. [CrossRef] [PubMed]
115. Bilsborough, J.C.; Greenway, K.; Opar, D.; Livingstone, S.; Cordy, J.; Coutts, A.J. The accuracy and precision of DXA for assessing body composition in team sport athletes. *J. Sports Sci.* **2014**, *32*, 1821–1828. [CrossRef] [PubMed]
116. Jensen, B.; Braun, W.; Geisler, C.; Both, M.; Klückmann, K.; Müller, M.J.; Bosy-Westphal, A. Limitations of Fat-Free Mass for the Assessment of Muscle Mass in Obesity. *Obes. Facts* **2019**, *12*, 307–315. [CrossRef] [PubMed]
117. Buckinx, F.; Landi, F.; Cesari, M.; Fielding, R.A.; Visser, M.; Engelke, K.; Maggi, S.; Dennison, E.; Al-Daghri, N.M.; Allepaerts, S.; et al. Pitfalls in the measurement of muscle mass: A need for a reference standard. *J. Cachexia Sarcopenia Muscle* **2018**, *9*, 269–278. [CrossRef]
118. Bosy-Westphal, A.; Schautz, B.; Later, W.; Kehayias, J.J.; Gallagher, D.; Müller, M.J. What makes a BIA equation unique? Validity of eight-electrode multifrequency BIA to estimate body composition in a healthy adult population. *Eur. J. Clin. Nutr.* **2013**, *67*, S14–S21. [CrossRef]

119. Dehghan, M.; Merchant, A.T. Is bioelectrical impedance accurate for use in large epidemiological studies? *Nutr. J.* **2008**, *7*, 26. [CrossRef]
120. Mourtzakis, M.; Prado, C.M.M.; Lieffers, J.R.; Reiman, T.; McCargar, L.J.; Baracos, V.E. A practical and precise approach to quantification of body composition in cancer patients using computed tomography images acquired during routine care. *Appl. Physiol. Nutr. Metab.* **2008**, *33*, 997–1006. [CrossRef]
121. Shen, W.; Punyanitya, M.; Wang, Z.; Gallagher, D.; St.-Onge, M.-P.; Albu, J.; Heymsfield, S.B.; Heshka, S. Total body skeletal muscle and adipose tissue volumes: Estimation from a single abdominal cross-sectional image. *J. Appl. Physiol.* **2004**, *97*, 2333–2338. [CrossRef]
122. Rutten, I.J.G.; Ubachs, J.; Kruitwagen, R.F.P.M.; Beets-Tan, R.G.H.; Olde Damink, S.W.M.; Van Gorp, T. Psoas muscle area is not representative of total skeletal muscle area in the assessment of sarcopenia in ovarian cancer. *J. Cachexia Sarcopenia Muscle* **2017**, *8*, 630–638. [CrossRef]
123. Baracos, V.E. Psoas as a sentinel muscle for sarcopenia: A flawed premise. *J. Cachexia Sarcopenia Muscle* **2017**, *8*, 527–528. [CrossRef]
124. Müller, M.J.; Braun, W.; Enderle, J.; Bosy-Westphal, A. Beyond BMI: Conceptual Issues Related to Overweight and Obese Patients. *Obes. Facts* **2016**, *9*, 193–205. [CrossRef]
125. Maden-Wilkinson, T.M.; Degens, H.; Jones, D.A.; McPhee, J.S. Comparison of MRI and DXA to measure muscle size and age-related atrophy in thigh muscles. *J. Musculoskelet. Neuronal Interact.* **2013**, *13*, 320–328.
126. Tavoian, D.; Ampomah, K.; Amano, S.; Law, T.D.; Clark, B.C. Changes in DXA-derived lean mass and MRI-derived cross-sectional area of the thigh are modestly associated. *Sci. Rep.* **2019**, *9*, 10028. [CrossRef]
127. Bosy-Westphal, A.; Müller, M.J. Identification of skeletal muscle mass depletion across age and BMI groups in health and disease—There is need for a unified definition. *Int. J. Obes.* **2015**, *39*, 379–386. [CrossRef]
128. Heymsfield, S.B.; Heo, M.; Thomas, D.; Pietrobelli, A. Scaling of body composition to height: Relevance to height-normalized indexes. *Am. J. Clin. Nutr.* **2011**, *93*, 736–740. [CrossRef]
129. Forbes, G.B. Lean Body Mass-Body Fat Interrelationships in Humans. *Nutr. Rev.* **2009**, *45*, 225–231. [CrossRef]
130. Hulens, M.; Vansant, G.; Claessens, A.L.; Lysens, R.; Muls, E. Predictors of 6-min walk test results in lean, obese and morbidly obese women. *Scand. J. Med. Sci. Sports* **2003**, *13*, 98–105. [CrossRef]
131. Baracos, V.E.; Arribas, L. Sarcopenic obesity: Hidden muscle wasting and its impact for survival and complications of cancer therapy. *Ann. Oncol.* **2018**, *29*, ii1–ii9. [CrossRef]
132. Hattori, K.; Tatsumi, N.; Tanaka, S. Assessment of body composition by using a new chart method. *Am. J. Hum. Biol.* **1997**, *9*, 573–578. [CrossRef]
133. Schutz, Y.; Kyle, U.; Pichard, C. Fat-free mass index and fat mass index percentiles in Caucasians aged 18–98 y. *Int. J. Obes. Relat. Metab. Disord.* **2002**, *26*, 953–960. [CrossRef]
134. Feliciano, E.M.C.; Kroenke, C.H.; Meyerhardt, J.A.; Prado, C.M.; Bradshaw, P.T.; Kwan, M.L.; Xiao, J.; Alexeeff, S.; Corley, D.; Weltzien, E.; et al. Association of Systemic Inflammation and Sarcopenia with Survival in Nonmetastatic Colorectal Cancer: Results From the C SCANS Study. *JAMA Oncol.* **2017**, *3*, e172319. [CrossRef] [PubMed]
135. Wells, J.C.K.; Victora, C.G. Indices of whole-body and central adiposity for evaluating the metabolic load of obesity. *Int. J. Obes.* **2005**, *29*, 483–489. [CrossRef]
136. Coin, A.; Sarti, S.; Ruggiero, E.; Giannini, S.; Pedrazzoni, M.; Minisola, S.; Rossini, M.; Del Puente, A.; Inelmen, E.M.; Manzato, E.; et al. Prevalence of Sarcopenia Based on Different Diagnostic Criteria Using DEXA and Appendicular Skeletal Muscle Mass Reference Values in an Italian Population Aged 20 to 80. *J. Am. Med. Dir. Assoc.* **2013**, *14*, 507–512. [CrossRef]
137. Lee, W.-J.; Liu, L.-K.; Peng, L.-N.; Lin, M.-H.; Chen, L.-K. Comparisons of Sarcopenia Defined by IWGS and EWGSOP Criteria Among Older People: Results From the I-Lan Longitudinal Aging Study. *J. Am. Med. Dir. Assoc.* **2013**, *14*, 528.e1–528.e7. [CrossRef]
138. Han, P.; Kang, L.; Guo, Q.; Wang, J.; Zhang, W.; Shen, S.; Wang, X.; Dong, R.; Ma, Y.; Shi, Y.; et al. Prevalence and Factors Associated with Sarcopenia in Suburb-dwelling Older Chinese Using the Asian Working Group for Sarcopenia Definition. *J. Gerontol. A Biomed. Sci. Med. Sci.* **2016**, *71*, 529–535. [CrossRef]
139. Yuki, A.; Ando, F.; Otsuka, R.; Matsui, Y.; Harada, A.; Shimokata, H. Epidemiology of sarcopenia in elderly Japanese. *J. Phys. Fit. Sports Med.* **2015**, *4*, 111–115. [CrossRef]
140. Ishii, S.; Tanaka, T.; Shibasaki, K.; Ouchi, Y.; Kikutani, T.; Higashiguchi, T.; Obuchi, S.P.; Ishikawa-Takata, K.; Hirano, H.; Kawai, H.; et al. Development of a simple screening test for sarcopenia in older adults. *Geriatr. Gerontol. Int.* **2014**, *14*, 93–101. [CrossRef]

141. Cheng, Q.; Zhu, X.; Zhang, X.; Li, H.; Du, Y.; Hong, W.; Xue, S.; Zhu, H. A cross-sectional study of loss of muscle mass corresponding to sarcopenia in healthy Chinese men and women: Reference values, prevalence, and association with bone mass. *J. Bone Miner. Metab.* **2014**, *32*, 78–88. [CrossRef]
142. Janssen, I.; Heymsfield, S.B.; Ross, R. Low Relative Skeletal Muscle Mass (Sarcopenia) in Older Persons Is Associated with Functional Impairment and Physical Disability. *J. Am. Geriatr. Soc.* **2002**, *50*, 889–896. [CrossRef]
143. Wen, X.; Wang, M.; Jiang, C.-M.; Zhang, Y.-M. Are current definitions of sarcopenia applicable for older Chinese adults? *J. Nutr. Health Aging* **2011**, *15*, 847–851. [CrossRef]
144. Zoico, E.; Di Francesco, V.; Guralnik, J.M.; Mazzali, G.; Bortolani, A.; Guariento, S.; Sergi, G.; Bosello, O.; Zamboni, M. Physical disability and muscular strength in relation to obesity and different body composition indexes in a sample of healthy elderly women. *Int. J. Obes. Relat. Metab. Disor.* **2004**, *28*, 234–241. [CrossRef]

CHAPTER III

ANALYSIS OF THE ADIPONECTIN PARADOX IN HEALTHY OLDER PEOPLE

published in Journal of Cachexia, Sarcopenia and Muscle

J Cachexia Sarcopenia Muscle. 2023 Feb;14(1):270-278.
doi: 10.1002/jcsm.13127

ORIGINAL ARTICLE

Journal of Cachexia, Sarcopenia and Muscle (2022)
Published online in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/jcsm.13127

Analysis of the adiponectin paradox in healthy older people

Carina O. Walowski[1], Catrin Herpich[2,3,4], Janna Enderle[1], Wiebke Braun[1], Marcus Both[5], Mario Hasler[6], Manfred J. Müller[1], Kristina Norman[2,3,4,7] & Anja Bosy-Westphal[1*]

[1]*Institute for Human Nutrition and Food Science, Christian-Albrechts-University, Kiel, Germany;* [2]*Institute of Nutritional Science, University of Potsdam, Nuthetal, Germany;* [3]*Department of Geriatrics and Medical Gerontology, Charité Universitätsmedizin Berlin, Corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany;* [4]*Department of Nutrition and Gerontology, German Institute of Human Nutrition, Potsdam-Rehbrücke, Nuthetal, Germany;* [5]*Department of Radiology and Neuroradiology, University Medical Center Schleswig-Holstein, Kiel, Germany;* [6]*Applied Statistics, Faculty of Agricultural and Nutritional Sciences, Christian-Albrechts-University, Kiel, Germany;* [7]*German Center for Cardiovascular Research (DZHK), Partner Site Berlin, Berlin, Germany*

Abstract

Background It remains unknown why adiponectin levels are associated with poor physical functioning, skeletal muscle mass and increased mortality in older populations.

Methods In 190 healthy adults (59–86 years, BMI 17–37 kg/m^2, 56.8% female), whole body skeletal muscle mass (normalized by height, SMI, kg/m^2), muscle and liver fat were determined by magnetic resonance imaging. Bone mineral content (BMC) and density (BMD) were assessed by dual X-ray absorptiometry (n = 135). Levels of insulin-like growth factor 1 (IGF-1), insulin, inflammation markers, leptin and fibroblast growth factor 21 were measured as potential determinants of the relationship between adiponectin and body composition.

Results Higher adiponectin levels were associated with a lower SMI ($r = -0.23$, $P < 0.01$), BMC ($r = -0.17$, $P < 0.05$) and liver fat ($r = -0.20$, $P < 0.05$) in the total population and with higher muscle fat in women ($r = 0.27$, $P < 0.01$). By contrast, IGF-1 showed positive correlations with SMI ($r = 0.33$), BMD ($r = 0.37$) and BMC ($r = 0.33$) (all $P < 0.01$) and a negative correlation with muscle fat ($r = -0.17$, $P < 0.05$). IGF-1 was negatively associated with age ($r = -0.21$, $P < 0.01$) and with adiponectin ($r = -0.15$, $P < 0.05$). Stepwise regression analyses revealed that IGF-1, insulin and leptin explained 18% of the variance in SMI, and IGF-1, leptin and age explained 16% of the variance in BMC, whereas adiponectin did not contribute to these models.

Conclusions Associations between higher adiponectin levels and lower muscle or bone mass in healthy older adults may be explained by a decrease in IGF-1 with increasing adiponectin levels.

Keywords Adiponectin paradox; Older adults; Skeletal muscle mass; Muscle quality; Bone; Liver fat

Received: 18 May 2022; Revised: 4 October 2022; Accepted: 25 October 2022
**Correspondence to: Anja Bosy-Westphal, Christian-Albrechts-Universität zu Kiel, Institut für Humanernährung und Lebensmittelkunde, Düsternbrooker Weg 17, 24105 Kiel, Germany. Email: abosyw@nutrition.uni-kiel.de*

Introduction

Adiponectin is an abundant peptide hormone primarily secreted by adipose tissue (AT), and to a lesser extent by skeletal muscle (SM) and bone marrow adipocytes (for a review, see Fazeli et al.[1]). It is well-known for improving insulin sensitivity as well as for anti-inflammatory and anti-atherogenic properties (for a review, see Robinson et al.[2]). Several metabolic and cardiovascular disorders including type 2 diabetes, metabolic syndrome (for a review, see Di Chiara et al.[3]), atherosclerosis[4] and non-alcoholic fatty liver disease[5] are thus accompanied by hypoadiponectinaemia. As a myokine, adiponectin acts as a myogenic factor through the participation in muscle differentiation and tissue regeneration, and influencing the behaviour of muscle cells (for a review, see Gamberi et al.[6]). Accumulating evidence also suggests that

adiponectin promotes osteoblastogenesis, while simultaneously inhibiting osteoclastogenesis (for a review, see Lewis et al.[7]). It is therefore causally enigmatic why adiponectin levels are negatively associated with SM, muscle density, physical functioning[8] or bone mineral density (BMD)[9] among older adults. Adiponectin levels were shown to increase with age[10] and are even associated with higher rates of cardiovascular and all-cause mortality in older populations.[11] The so-called *adiponectin paradox* therefore suggests that adiponectin may not exert beneficial effects in older adults.

Different explanations for the *adiponectin paradox* in older people have been discussed including adiponectin resistance,[12] compensatory effects of adiponectin to subclinical pathologies, impaired renal function and decreased hepatic clearance of adiponectin (for a review, see Kalkman[13]). In addition, adiponectin was proposed to be a biomarker of adverse catabolic processes (i.e., a low SM due to sarcopenia related to aging,[8,14] or cachexia associated with chronic inflammation and pre-existing illness[15,16]). It remains unknown if higher adiponectin levels in older adults are a cause of a low muscle mass by mediating catabolic processes or rather a consequence of catabolic processes that lead to a lower SM. The relationship between adiponectin and detailed body composition analysis therefore needs to be investigated in healthy older adults without catabolic disease or impaired renal function.

The aim of the present study was therefore (i) to investigate the association between adiponectin levels and SM (by whole body magnetic resonance imaging, (MRI)), muscle fat and strength or bone mass and bone density in healthy community-dwelling older adults and (ii) to identify potential endocrine determinants of adiponectin levels.

Methods

Study population

The present study was conducted at the 'German Reference Center for Body Composition' (Institute of Human Nutrition and Food Science at the University of Kiel, Germany) between 2019 and 2020. Exclusion criteria were oedema, acute diseases, heart failure, renal failure, intake of diuretics, paralysis (e.g., after a stroke), neurodegenerative diseases, tumours in treatment, amputation of limbs, electrical and metallic implants, current alcohol abuse, not removable piercings and large tattoos on the arms or legs (because of possible interference with MRI examinations) as well as medication, which could influence body composition. Subjects were recruited using notice board postings and local advertisements. Written informed consent was obtained from each participant. The study protocol was approved by the medical ethics committee of the Christian-Albrechts-University of Kiel, Germany, and followed the guidelines based on the 'Declaration of Helsinki'. The primary aim of the study was to validate measures of bioelectrical impedance analysis vs. reference methods in older adults. The trial was registered at ClinicalTrials.gov as NCT04028648. The study population was expanded by a subgroup of 40 healthy Caucasian older participants as described in detail elsewhere.[17] From the included 190 participants, data of 173 adults were analysed (data from 15 participants were excluded because of motion artefacts or incorrect patient positioning in MRI. Further data from two subjects were excluded due to missing endocrine parameters).

Body composition analysis

Body weight was measured to the nearest 0.01 kg by an electronic Tanita scale (Tanita, Tokyo, Japan) coupled to the BOD POD® Body Composition System (Cosmed srl, Rome, Italy) with subjects in underwear. Height was determined without shoes using a stadiometer (SECA, Modell 285, Hamburg, Germany). Fat mass (FM) and fat-free mass (FFM) were determined via air-displacement plethysmography (BOD POD® Cosmed srl, Rome, Italy) as previously described.[18] FM was calculated using the equation by Siri et al.[19] FFM was calculated from the difference between body weight and FM. On the basis of FM and FFM, FM-Index (FMI) and FFM-Index (FFMI) were calculated as FM (kg)/height (m^2) and FFM (kg)/height (m^2).

SM and AT were measured using whole body MRI as described in detail elsewhere.[20] Briefly, subjects were examined in a supine position with arms extended above their heads. For scans in abdominal and thoracic regions participants were required to hold their breath. Images were obtained using a 1.5 T scanner (Magnetom Avanto, Siemens Medical Systems, Erlangen, Germany) with a T1-weighted-gradient echo sequence (repetition time (TR): 157 ms; echo time (TE): 4 ms for scans of arms, legs and abdominal region). The whole body was scanned from wrist to ankle using continuous axial images with a slice thickness of 8 mm and 2 mm interslice gaps for arms, legs and trunk. Volumes of SM, subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) were manually segmented using SliceOmatic 4.3 software (Tomovision, Montreal, Canada). VAT was evaluated from the top of the liver to femoral heads. Volumes of total SM (excluding head and neck muscles, hands and feet), SAT and VAT were determined from the sum of tissue areas (cm^2) multiplied by the slice thickness. Tissue volumes were then converted into masses using the assumed densities of 1.04 g cm^{-3} for SM and 0.92 g cm^{-3} for SAT and VAT.[21] SM was normalized to height squared to calculate skeletal muscle mass index (SMI, (kg)/height (m^2)).

In a subgroup of 135 subjects, whole body bone mineral content (BMC), BMD and T-Score were quantified using dual energy X-ray absorptiometry (DXA) (HOLOGIC Discovery

A (S/N 82686), Inc., Bedford, MA, USA). Before daily measurements, a spine phantom calibration was performed. Scans were analysed using manufacturer's software (version 12.6.1:3, Hologic, Inc.). Results were summed up for both arms and legs as well as the head.

Liver fat was determined by MRI (Magnetom Avanto, Siemens Medical Systems, Erlangen, Germany) based on the two-point Dixon method with a volumetric interpolated breath-hold examination sequence as described in detail elsewhere.[22] Briefly, a T1-weighted gradient-echo sequence with in-phase and out-of-phase imaging was conducted (TR: 10.4 ms; TE: 4.76 (in-phase) and 7.14 (opposed-phase) ms; flip angle, 10° matrix, 80 × 128; and field of view, 440 mm; slice thickness/interslice gap: 5/1 mm; total scan time: 19 s). From in-phase and opposed-phase images, the water-only (WO) and fat-only (FO)-images were calculated:

$$\mathrm{WO}: \frac{1}{2} \times (\text{in-phase} + \text{opposed-phase}) \quad (1)$$

$$\mathrm{FO}: \frac{1}{2} \times (\text{in-phase} + \text{opposed-phase}) \quad (2)$$

WO and FO-images were than analysed using ImageJ software (US NIH, Bethesda, MD, USA[22]) to determine hepatic fat fraction (HFF). In each of five adjacent HFF images, a single continuous region of interest (ROI) was defined (20.62 × 20.62) and was placed in the liver parenchyma, avoiding vascular structures. The quantity of liver fat was averaged for the five HFF images and was determined as percentage of the total liver core.

Intermuscular adipose tissue (IMAT) in a single mid-thigh MRI slice and muscle fat by the two-point Dixon method were used to assess muscle quality in 173 and 172 subjects, respectively (Figures 1 and 2).

IMAT was defined as visible AT between muscle groups that is located within a muscle and beneath the muscle fascia. It was assessed in single cross-sectional MRI images at the level of the mid-thigh. The midpoint of the thigh was defined as half way between the femoral head and the tibial plateau. Masses of IMAT were manually determined by using a semi-automatic segmentation software (SliceOmatic 4.3, Tomovision, Montreal, Canada).

Muscle fat was determined based on the Dixon method using ImageJ software (US NIH, Bethesda, MD, USA[22]). In lumbar region of multifidus and erector spinae muscles, a single continuous ROI was defined (10.75 × 10.75) in each of five adjacent muscle fat fraction images and was placed in the same area for all repeated measurements. Muscle fat was quantified as percentage of the total muscle area and was averaged for the five muscle fat fraction images. All procedures were conducted by the same observer.

In 173 subjects, hand grip strength (HGS) was measured using a hydraulic SAEHAN® handgrip dynamometer (SH5001, Masan, South Korea). 135 subjects conducted the test in a standing position and 38 in a sitting position and the elbow was flexed at 90 degrees with the shoulder attached to the torso. In 135 subjects, HGS of the left and right hand was determined three times and the greatest value of the dominant hand was included in the analysis, whereas in 38 subjects, HGS was determined only twice.

Endocrine parameters

Serum and plasma blood samples were taken from an antecubital vein after >10 h overnight fast. The subjects were instructed to refrain from vigorous exercise and alcohol intake for 24 h prior to blood sampling. After collection, se-

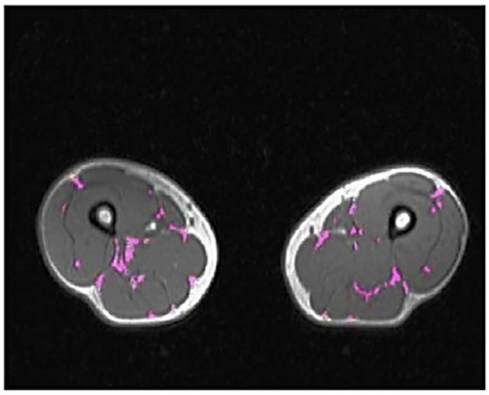


Figure 1 Intermuscular adipose tissue (IMAT) in a single mid-thigh magnetic resonance imaging (MRI) slice segmented in purple using SliceOmatic software, acquired as axial T1-weighted gradient-echo sequence. Result for total IMAT was 9.30 g.

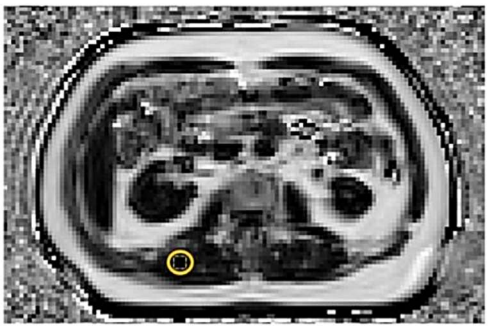


Figure 2 Lumbar muscle fat in the region of multifidus and erector spinae muscles with a yellow 10.75 × 10.75 region of interest (ROI) determined using ImageJ software, acquired as axial T1-weighted gradient-echo Dixon sequence. Result for the percentage of muscle fat was 9.54%.

rum samples were stored at room temperature in an upright position for 30 min for complete clot formation. Plasma and serum were obtained by centrifugation at 2000 g for 10 min at 20°C and stored at −40°C. Sample analyses were performed at the 'German Institute of Human Nutrition', Potsdam-Rehbrücke, Department of Nutrition and Gerontology, Nuthetal, Germany and a laboratory in Kiel, Germany.

In 172 subjects, leptin (intra-assay CV: 4.2–7.6%, inter-assay CV: 4.4–6.7%; BioVendor, Brno, Czech Republic), adiponectin (intra-assay CV: 2.8–3.9%, inter-assay CV: 5.9–6.4%; Immundiagnostik AG, Bensheim, Germany) as well as insulin-like growth factor 1 (IGF-1) (intra-assay CV: 5.1–6.7%, inter-assay CV: 5.5–6.6%; BioVendor, Brno, Czech Republic) were measured by commercial ELISA kits. As adiponectin expression is regulated by the hepatic hormone fibroblast growth factor 21 (FGF-21), also FGF-21 concentrations were quantified by ELISA (intra-assay CV: 1.6–2.4%, inter-assay CV: 3.1–3.5%; BioVendor, Brno, Czech Republic) in a subgroup of 135 subjects. As inflammatory parameters, interleukin 6 (IL-6) (intra-assay CV: 4.2–5.1%, inter-assay CV: 4.7–5.0%; BioVendor, Brno, Czech Republic) was determined in 135 participants using commercial ELISA kit, and high-sensitivity C-reactive protein (hsCRP) (intra-assay CV: 0.73–5.73%, inter-assay CV: 1.50–5.76%; BECKMAN COULTER, Brea, CA, USA) was measured using an immuno-turbidimetric test. Insulin was determined by chemiluminescent microparticle immunoassay (intra-assay CV: 1.4–2.1%, inter-assay CV: 1.5–2.2%; Abbott, Wiesbaden, Germany). Inflammation was based on hsCRP >3 mg/L and elevated insulin levels were set at >25.0 mU/L.

Statistical analysis

Statistical analyses were performed using SPSS statistical software (SPSS 28.0, Inc., Chicago, IL, USA). All data are given as means ±SD. Differences between independent samples were analysed using unpaired t-test. Evaluation of normality was performed using the Shapiro–Wilk test and residual analysis. Pearson's and Spearman's correlation coefficients were calculated to identify bivariate associations between and within body composition, endocrine and functional parameters. Partial correlations were used to adjust for various confounders. Stepwise multiple regression analyses were performed to assess factors independently associated with SMI, BMC and lumbar muscle fat. All tests were two-sided and level of significance was set at $P < 0.05$.

Results

In total, 173 older adults (101 women and 72 men) aged 59–86 years with a BMI between 18 and 37 kg/m^2 were included in the study. Descriptive characteristics are summarized in Table 1. Men were significantly older and had a higher BMI, FFMI, SM, SMI, IMAT, VAT, HGS as well as BMC, BMD and T-Score compared with women. According to WHO criteria, 26.6% of women and 30.6% of men were overweight or obese. In a subpopulation of 135 subjects with DXA results, the prevalence for a reduced muscle mass was 7.40% according to the recommended FFMI thresholds of the 'Global Leadership Initiative on Malnutrition' (FFMI cut-offs: <15 and <17 kg/m^2 in women and men, respectively[23]).

Potential endocrine determinants of adiponectin levels are summarized in Table 2. Adiponectin levels were lower in men compared with women. Insulin and IGF-1 levels were higher and leptin levels were lower in men compared with women. Prevalence for elevated hsCRP and insulin levels were 20.2% and 2.3%, respectively.

Correlations between adiponectin or IGF-1 levels and body composition parameters are presented in Table 3. In

Table 1 Characteristics of the study population

	All subjects	Women	Men
n	173	101	72
Age (years)	70.7 ± 5.3	70.0 ± 4.9*	71.7 ± 5.7
Height (m)	1.68 ± 0.10	1.62 ± 0.06***	1.77 ± 0.1
Weight (kg)	73.3 ± 15.2	65.1 ± 11.0***	84.7 ± 12.7
BMI (kg/m^2)	25.7 ± 3.8	24.9 ± 4.0***	27.0 ± 3.2
FMI (kg/m^2)	9.2 ± 3.4	9.9 ± 3.6***	8.1 ± 2.7
SAT (kg)	17.4 ± 6.5	18.8 ± 6.8***	15.5 ± 5.5
VAT (kg)	2.2 ± 1.6	1.4 ± 0.9***	3.3 ± 1.7
FFMI (kg/m^2)	16.6 ± 2.3	15.0 ± 1.2***	18.8 ± 1.2
SM (kg)	22.2 ± 5.9	18.0 ± 2.5***	28.0 ± 4.1
SMI (kg/m^2)	7.7 ± 1.4	6.9 ± 0.8***	8.9 ± 1.1
BMC (kg)	2.07 ± 0.54	1.71 ± 0.27***	2.61 ± 0.35
BMD (g/cm^2)	1.00 ± 0.14	0.92 ± 0.10***	1.12 ± 0.10
T-Score	−1.5 ± 1.2	−2.1 ± 1.1***	−0.7 ± 1.0
Liver fat (%)	8.4 ± 4.2	8.1 ± 4.5	8.8 ± 3.8
Lumbar muscle fat (%)	9.5 ± 3.7	9.3 ± 3.8	9.6 ± 3.7
IMAT single mid-thigh (g)	4.5 ± 2.3	3.4 ± 2.1***	5.4 ± 2.3
HGS (kg)	31.9 ± 10.3	25.0 ± 5.0***	41.4 ± 7.9

Abbreviations: BMC, bone mineral content; BMD, bone mineral density; BMI, body mass index; FFMI, fat-free mass index; FMI, fat mass index; HGS, hand grip strength; IMAT, intermuscular adipose tissue; SAT, subcutaneous adipose tissue; SM, skeletal muscle; SMI, skeletal muscle mass index; VAT, visceral adipose tissue.
Note: Values are means ± SD; *n*, no. of subjects.
*$P < 0.05$.
***$P < 0.001$ sex differences by *t*-test, two-sided.

Table 2 Adiponectin levels and its potential endocrine determinants

	All subjects	Women	Men
n	173	101	72
Adiponectin (mg/L)	19.8 ± 16.4	22.8 ± 19.3**	15.5 ± 9.8
IGF-1 (μg/L)	151.3 ± 56.8	140.3 ± 52.5**	166.7 ± 59.4
FGF-21 (pg/mL)	212.2 ± 212.2	233.5 ± 255.3	180.3 ± 117.3
Leptin (ng/mL)	11.2 ± 11.0	14.2 ± 12.7***	6.6 ± 5.5
IL-6 (pg/mL)	10.11 ± 24.52	6.72 ± 6.47	15.20 ± 37.58
hsCRP (mg/L)	2.31 ± 3.07	2.12 ± 2.60	2.58 ± 3.63
Insulin (μU/L)	9.8 ± 6.1	8.9 ± 4.3*	10.9 ± 7.8

Abbreviations: FGF-21, fibroblast growth factor 21; hsCRP, high-sensitivity C-reactive protein; IGF-1, insulin-like growth factor 1; IL-6, interleukin 6.
Note: Values are means ± SD; *n*, no. of subjects.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$ sex differences by *t*-test, two-sided.

accordance with the *adiponectin paradox*, higher adiponectin levels were associated with a lower SMI and BMC in the total study population. No correlation was observed between adiponectin and HGS. Lower adiponectin levels were associated with a higher BMI ($r = -0.34$, $P < 0.01$) and FMI in men and with greater VAT in the total population. The association between adiponectin and SMI remained significant after adjustment for VAT ($r = -0.16$, $P < 0.04$). No relationships between adiponectin and FGF-21, leptin, IL-6 or hsCRP and insulin were found.

In contrast to adiponectin, IGF-1 levels were positively associated with SMI in the total population and in the subgroups of men and women. Furthermore, IGF-1 concentrations were correlated with BMC, BMD and T-Score in the total population and BMD and T-Score in women. IGF-1 levels were also positively associated with HGS ($r = 0.31$, $P < 0.05$, all; $r = 0.35$, $P < 0.01$, men) and negatively with age ($r = -0.21$, $P < 0.01$, all; $r = -0.20$, $P < 0.05$, women; $r = -0.33$, $P < 0.01$, men) and adiponectin levels ($r = -0.15$, $P < 0.05$). To test, if the paradoxical association between higher adiponectin and lower SMI could be explained by lower IGF-1 levels with higher age, partial correlation analysis between adiponectin and SMI adjusted for IGF-1 was performed. The negative correlation between SMI and adiponectin in the total study population persisted, but was slightly weakened ($r = -0.20$, $P = 0.01$).

Stepwise regression analyses with SMI or BMC as dependent variables and adiponectin, IGF-1, insulin, IL-6, hsCRP, leptin and age (for SMI) and adiponectin, IGF-1, IL-6, leptin and age (for BMC), respectively as independent variables were performed (Table 4). Insulin, IGF-1 and leptin explained 18.4% of the variance in SMI. After considering sex as further

Table 3 Correlations between adiponectin or IGF-1 and body composition parameters

	All subjects		Women		Men	
	Adiponectin (mg/L)	IGF-1 (μg/L)	Adiponectin (mg/L)	IGF-1 (μg/L)	Adiponectin (mg/L)	IGF-1 (μg/L)
SMI (kg/m^2)	−0.23**	0.33**	-	0.22*	-	0.39**
BMC (kg)	−0.17*	0.33**	-	-	-	-
BMD (g/cm^2)	-	0.37**	-	0.22*	-	-
T-Score	-	0.34**	-	0.22*	-	-
FMI (kg/m^2)	-	-	-	-	−0.32**	-
VAT (kg)	−0.21**	-	-	-	-	-
Liver fat (%)	−0.20*	-	-	-	−0.26*	-
Lumbar muscle fat (%)	-	−0.17*	0.27**	−0.30**	-	-
IMAT single mid-thigh (g)	-	-	-	-	-	-

Abbreviations: BMC, bone mineral content; BMD, bone mineral density; FMI, fat mass index; IGF-1, insulin-like growth factor 1; IMAT, intermuscular adipose tissue; SMI, skeletal muscle mass index; VAT, visceral adipose tissue.
*$P < 0.05$.
**$P < 0.01$.

Table 4 Stepwise multiple regression analyses with SMI, BMC and lumbar muscle fat as dependent variables

Dependent variables and predictors	β coefficient	R^2	SEE	*P*-value	VIF
SMI (kg/m^2)					
Model[a]					
Step 1: insulin (μU/L)	0.073	0.101	0.018	<0.001	1.135
Step 2: IGF-1 (μg/L)	0.005	0.150	0.002	0.015	1.053
Step 3: leptin (ng/mL)	−0.023	0.184	0.010	0.021	1.104
BMC (kg)					
Model[b]					
Step 1: IGF-1 (μg/L)	3.232	0.098	0.794	<0.001	1.044
Step 2: leptin (ng/mL)	−8.500	0.135	3.715	0.024	1.009
Step 3: age (y)	16.918	0.164	7.949	0.035	1.040
Lumbar muscle fat (%)					
Model[c]					
Step 1: FMI (kg/m^2)	0.56	0.296	0.100	0.100	1.020
Step 2: IGF-1 (μg/L)	−0.02	0.343	0.007	0.007	1.020

Abbreviations: BMC, bone mineral content; FMI, fat mass index; IGF-1, insulin-like growth factor 1; SEE, standard error of estimation; SMI, skeletal muscle mass index; VIF, variance inflation factor.
[a]Model: independent variables: adiponectin, IGF-1, insulin, IL-6, hsCRP, leptin and age.
[b]Model: independent variables: adiponectin, IGF-1, leptin, IL-6 and age.
[c]Model: independent variables: adiponectin, IGF-1, leptin, insulin, IL-6, hsCRP, FMI and age.

covariate, sex and insulin together explained 65.1% of the variance in SMI. IGF-1, leptin and age explained 16.4% of the variance in BMC. After additional adjustment for sex, only sex was entered in the equation and explained 68.5% of the variance in BMC.

After excluding subjects with elevated hsCRP levels, the negative association between adiponectin concentrations and BMC in the total population was no longer significant ($P = 0.08$), whereas negative correlations between adiponectin levels and insulin ($r = -0.18$, $P < 0.05$), BMI ($r = -0.18$, $P < 0.05$) and HGS ($r = -0.18$, $P < 0.05$) could be observed. The negative association between IGF-1 and adiponectin levels in the total population persisted ($r = -0.18$, $P < 0.05$).

Concerning ectopic fat, higher adiponectin levels were associated with lower liver fat in the total study population and in the subgroup of men, whereas adiponectin levels were paradoxically positively correlated with lumbar muscle fat in women (Table 3). By contrast, IGF-1 showed negative correlations with lumbar muscle fat in women and in the total study population. The negative association between adiponectin and lumbar muscle fat in women weakened after adjustment for IGF-1 ($r = 0.19$, $P = 0.05$). Stepwise regression analysis with lumbar muscle fat as the dependent variable and adiponectin, IGF-1, leptin, insulin, IL-6, hsCRP, FMI and age as independent variables, revealed that only FMI and IGF-1 independently explained 34.3% of the variance (Table 4).

Insulin, leptin, IL-6 and hsCRP were positively correlated with ectopic fat deposition in liver and muscle, whereas higher FGF-21 levels were positively correlated with liver fat content. Furthermore, ectopic liver and muscle fat showed consistent positive associations with FMI and VAT ranging between $r = 0.31$ and $r = 0.61$ (all $P < 0.05$).

Discussion

Our data confirm the *adiponectin paradox* in older adults without inflammatory diseases. Higher adiponectin levels were associated with lower SMI and BMC in the total study population and higher lumbar muscle fat in women. These observations may explain the positive association between adiponectin and mortality that was found in previous studies even independent of comorbid conditions like history of cancer,[8] hypertension, diabetes, cardiovascular disease, congestive heart failure[8,24] and chronic kidney disease.[24]

However, adiponectin may not be causally related to an unhealthy body composition because of the negative association between adiponectin and IGF-1. IGF-1 has well-known anabolic effects on muscle and bone (for a review, see Gomarasca et al.[25]). In line with our hypothesis, stepwise multiple regression analyses revealed that adiponectin was no significant predictor of SMI, BMC and lumbar muscle fat when considering IGF-1 as a dependent variable (Table 4). Similar to our results in healthy people, adiponectin levels were negatively related to serum IGF-1 in acromegaly[26] as well as in men with type 2 diabetes.[27] This correlation was independent of BMI,[26,27] renal function and age,[27] suggesting that IGF-1 might inhibit the expression of adiponectin. In vitro experiments in cultured 3T3-L1 adipocytes have indeed shown decreased adiponectin mRNA levels by IGF-1 or insulin[28] and a study in rats demonstrated that infusion of recombinant human IGF-1 decreased plasma levels of adiponectin.[29] By contrast, IGF-1 supplementation in patients with growth hormone deficiency did not affect serum adiponectin levels.[30]

In our study, IGF-1 levels were negatively correlated with age and may therefore explain higher adiponectin levels in an older population. The negative association between adiponectin and IGF-1 in patients with type 2 diabetes[27] may be due to impaired insulin secretion and thus lower IGF-1 levels in these patients,[31] whereas in patients with acromegaly, overproduction of IGF-1 may contribute to lower adiponectin concentrations. There was a lack of association between adiponectin and IGF-1 levels in patients with morbid obesity after weight loss induced by bariatric surgery[32] whereas in young women with non-diabetic obesity a positive association between IGF-1 and adiponectin was found.[33] In the latter study, a significant negative correlation between IGF-1 and CRP was observed indicating that inflammation may be causal for the positive association between IGF-1 and adiponectin because inflammation may impair the expression of both hormones[33–35] (for a review, see Kirk et al.[36]).

In contrast to the association between IGF-1 and muscle mass, muscle fat or bone mass, to the best of our knowledge there is no direct causal effect of IGF-1 on VAT or liver fat. Therefore, the plausible negative correlations between adiponectin and VAT (total population), FMI and liver fat (especially in men) were evident in our heathy older population (Table 3).

An alternative explanation of the *adiponectin paradox* is that higher adiponectin levels in people with a lower SM may be interpreted as a starvation signal (i.e. as a consequence of poor nutritional status[37] or history of weight loss among older people[8]). In line with this argument, weight loss leads to an increase in adiponectin levels not only in people with obesity[32] but also in healthy lean subjects.[38] In the present study, partial correlation between adiponectin and SMI adjusted for IGF-1 revealed, that the negative correlation between SMI and adiponectin was only slightly weakened. Because our data are cross-sectional, we cannot exclude the possibility that a low SMI is causal for higher adiponectin levels. Adiponectin is also secreted by myocytes, therefore the impact of energy availability on this effect needs to be investigated. The interpretation of higher adiponectin levels as a starvation signal is however unlikely in a healthy non-malnourished study population of community-dwelling older people.

The present study has several strengths. First, the sample of older community-dwelling Caucasians was healthy, thus confounders caused by disease can be excluded. In addition, the population was well characterized using whole body MRI, which is considered as the gold standard method of assessment of SM and muscle fat. Body composition was complemented with HGS as a functional parameter. Nevertheless, our findings should be considered in the context of some limitations. First, adiponectin circulates in blood in multiple isoforms with different physiologic functions.[39,40] As only total adiponectin concentrations were measured, the effects of the different isoforms cannot be examined. Finally, our results need to be confirmed using a longitudinal study design. For example, nutrition interventions might affect adiponectin levels mediated by IGF-1. It has been demonstrated that IGF-1 levels decrease in response to fasting and short-term caloric restriction or protein restriction[41] (for a review, see Thissen et al.[42]). The effect of protein supplementation in healthy older people on adiponectin levels remains to be investigated.

In conclusion, our results suggest that adiponectin is not causally related to impaired mass and function of the musculoskeletal system in healthy older people but the associations are mediated by an age-related decline in IGF-1 levels that contribute to an increase in adiponectin. These results support the hypothesis of a functional interplay between IGF-1 and adiponectin that had been proposed by Orrù et al.[43]

Acknowledgements

The study protocol was approved by the medical ethics committee of the Christian-Albrechts-University of Kiel, Germany, and followed the guidelines based on the 'Declaration of Hel-

sinki'. Written informed consent was obtained from each participant. The authors certify that they comply with the ethical guidelines for authorship and publishing in the *Journal of Cachexia, Sarcopenia and Muscle*.[44] This work was supported by a grant for seca gmbh & co.kg., Germany (2019/2020) and the Danone Institute-Nutrition for Health, Germany (2013/14). We thank Britta Jux and the Clinic for Diagnostic Radiology, University medical Center Schleswig-Holstein, Kiel (Germany) for the help with MRI scanning.

Open Access funding enabled and organized by Projekt DEAL.

Conflict of interest

Anja Bosy-Westphal serves a consultant for seca gmbh & co. kg. The other authors declare no conflict of interest.

References

1. Fazeli PK, Horowitz MC, MacDougald OA, Scheller EL, Rodeheffer MS, Rosen CJ, et al. Marrow fat and bone--new perspectives. *J Clin Endocrinol Metab* 2013;**98**: 935–945.
2. Robinson K, Prins J, Venkatesh B. Clinical review: Adiponectin biology and its role in inflammation and critical illness. *Crit Care* 2011;**15**:221.
3. Di Chiara T, Argano C, Corrao S, Scaglione R, Licata G. Hypoadiponectinemia: A Link between Visceral Obesity and Metabolic Syndrome. *J Nutr Metab* 2012;**2012**:175245.
4. Hui E, Xu A, Chow W-S, Lee PCH, Fong CHY, Cheung SCW, et al. Hypoadiponectinemia as an independent predictor for the progression of carotid atherosclerosis: A 5-year prospective study. *Metab Syndr Relat Disord* 2014;**12**:517–522.
5. Engl J, Sturm W, Sandhofer A, Kaser S, Tschoner A, Tatarczyk T, et al. Effect of pronounced weight loss on visceral fat, liver steatosis and adiponectin isoforms. *Eur J Clin Invest* 2008;**38**:238–244.
6. Gamberi T, Magherini F, Fiaschi T. Adiponectin in Myopathies. *Int J Mol Sci* 2019; **20**:1544.
7. Lewis JW, Edwards JR, Naylor AJ, McGettrick HM. Adiponectin signalling in bone homeostasis, with age and in disease. *Bone Res* 2021;**9**:1.
8. Baker JF, Newman AB, Kanaya A, Leonard MB, Zemel B, Miljkovic I, et al. The Adiponectin Paradox in the Elderly: Associations With Body Composition, Physical Functioning, and Mortality. *J Gerontol A Biol Sci Med Sci* 2019;**74**:247–253.
9. Michaëlsson K, Lind L, Frystyk J, Flyvbjerg A, Gedeborg R, Berne C, et al. Serum adiponectin in elderly men does not correlate with fracture risk. *J Clin Endocrinol Metab* 2008;**93**:4041–4047.
10. Obata Y, Yamada Y, Takahi Y, Baden MY, Saisho K, Tamba S, et al. Relationship between serum adiponectin levels and age in healthy subjects and patients with type 2 diabetes. *Clin Endocrinol (Oxf)* 2013;**79**: 204–210.
11. Menzaghi C, Trischitta V. The Adiponectin Paradox for All-Cause and Cardiovascular Mortality. *Diabetes* 2018;**67**:12–22.
12. Uetani E, Tabara Y, Kawamoto R, Onuma H, Kohara K, Osawa H, et al. CDH13 Genotype–Dependent Association of High–Molecular Weight Adiponectin With All-Cause Mortality: The J-SHIPP Study. *Diabetes Care* 2014;**37**:396–401.
13. Kalkman HO. An Explanation for the Adiponectin Paradox. *Pharmaceuticals (Basel)* 2021;**14**:1266.
14. Sattar N, Nelson SM. Adiponectin, Diabetes, and Coronary Heart Disease in Older Persons: Unraveling the Paradox. *J Clin Endocrinol Metab* 2008;**93**:3299–3301.
15. Szabó T, Scherbakov N, Sandek A, Kung T, von Haehling S, Lainscak M, et al. Plasma adiponectin in heart failure with and without cachexia: Catabolic signal linking catabolism, symptomatic status, and prognosis. *Nutr Metab Cardiovasc Dis* 2014;**24**: 50–56.
16. Baker JF, von Feldt JM, Mostoufi-Moab S, Kim W, Taratuta E, Leonard MB. Insulin-like Growth Factor 1 and Adiponectin and Associations with Muscle Deficits, Disease Characteristics, and Treatments in Rheumatoid Arthritis. *J Rheumatol* 2015;**42**: 2038–2045.
17. Schweitzer L, Geisler C, Johannsen M, Glüer C-C, Müller MJ. Associations between body composition, physical capabilities and pulmonary function in healthy older adults. *Eur J Clin Nutr* 2017;**71**:389–394.
18. Bosy-Westphal A, Mast M, Eichhorn C, Becker C, Kutzner D, Heller M, et al. Validation of air-displacement plethysmography for estimation of body fat mass in healthy elderly subjects. *Eur J Nutr* 2003;**42**: 207–216.
19. Siri WE. Body composition from fluid spaces and density: Analysis of methods 1961. *Nutrition* 1993;**9**:480–491.
20. Hübers M, Geisler C, Plachta-Danielzik S, Müller MJ. Association between individual fat depots and cardio-metabolic traits in normal- and overweight children, adolescents and adults. *Nutr Diabetes* 2017;**7**: e267.
21. Snyder WS, Cook MJ, Nasset ES, Karhausen LR, Howells GP, Tipton IH. Report of the Task Group on Reference Man. (ICRP 23). Oxford: Pergamon Press, 1975. *Ann ICRP* 1979;**3**:iii–iii.
22. Ma J. Dixon techniques for water and fat imaging. *J Magn Reson Imaging* 2008;**28**: 543–558.
23. Cederholm T, Jensen GL, Correia MITD, Gonzalez MC, Fukushima R, Higashiguchi T, et al. GLIM criteria for the diagnosis of malnutrition – A consensus report from the global clinical nutrition community. *Clin Nutr* 2019;**38**:1–9.
24. Poehls J, Wassel CL, Harris TB, Havel PJ, Swarbrick MM, Cummings SR, et al. Association of adiponectin with mortality in older adults: The Health, Aging, and Body Composition Study. *Diabetologia* 2009;**52**:591–595.
25. Gomarasca M, Banfi G, Lombardi G. Myokines: The endocrine coupling of skeletal muscle and bone. *Adv Clin Chem* 2020; **94**:155–218.
26. Fukuda I, Hizuka N, Ishikawa Y, Itoh E, Yasumoto K, Murakami Y, et al. Serum adiponectin levels in adult growth hormone deficiency and acromegaly. *Growth Horm IGF Res* 2004;**14**:449–454.
27. Kanazawa I, Yamaguchi T, Sugimoto T. Serum insulin-like growth factor-I is negatively associated with serum adiponectin in type 2 diabetes mellitus. *Growth Horm IGF Res* 2011;**21**:268–271.
28. Lam KS-L, Xu A, Tan KC-B, Wong L-C, Tiu S-C, Tam S. Serum adiponectin is reduced in acromegaly and normalized after correction of growth hormone excess. *J Clin Endocrinol Metab* 2004;**89**:5448–5453.
29. Yamaza H, Komatsu T, To K, Toyama H, Chiba T, Higami Y, et al. Involvement of Insulin-Like Growth Factor-1 in the Effect of Caloric Restriction: Regulation of Plasma Adiponectin and Leptin. *J Gerontol A Biol Sci Med Sci* 2007;**62**:27–33.
30. Schmid C, Bianda T, Zwimpfer C, Zapf J, Wiesli P. Changes in insulin sensitivity induced by short-term growth hormone (GH) and insulin-like growth factor I (IGF-I) treatment in GH-deficient adults are not associated with changes in adiponectin levels. *Growth Horm IGF Res* 2005;**15**: 300–303.
31. Suda K, Matsumoto R, Fukuoka H, Iguchi G, Hirota Y, Nishizawa H, et al. The influence of type 2 diabetes on serum GH and IGF-I levels in hospitalized Japanese patients. *Growth Horm IGF Res* 2016;**29**:4–10.
32. Pardina E, Ferrer R, Baena-Fustegueras JA, Lecube A, Fort JM, Vargas V, et al. The relationships between IGF-1 and CRP, NO, leptin, and adiponectin during weight loss in the morbidly obese. *Obes Surg* 2010; **20**:623–632.
33. Sirbu A, Gologan S, Arbanas T, Copaescu C, Martin S, Albu A, et al. Adiponectin, body mass index and hepatic steatosis are inde-

pendently associated with IGF-I status in obese non-diabetic women. *Growth Horm IGF Res* 2013;**23**:2–7.
34. Savastano S, Di Somma C, Pizza G, de Rosa A, Nedi V, Rossi A, et al. Liver-spleen axis, insulin-like growth factor-(IGF)-I axis and fat mass in overweight/obese females. *J Transl Med* 2011;**9**:136.
35. Ouchi N, Kihara S, Funahashi T, Nakamura T, Nishida M, Kumada M, et al. Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. *Circulation* 2003;**107**:671–674.
36. Kirk B, Feehan J, Lombardi G, Duque G. Muscle, Bone, and Fat Crosstalk: The Biological Role of Myokines, Osteokines, and Adipokines. *Curr Osteoporos Rep* 2020;**18**: 388–400.
37. Hyun YY, Lee K-B, Oh K-H, Ahn C, Park SK, Chae DW, et al. Serum adiponectin and protein-energy wasting in predialysis chronic kidney disease. *Nutrition* 2017;**33**: 254–260.
38. Müller MJ, Enderle J, Pourhassan M, Braun W, Eggeling B, Lagerpusch M, et al. Metabolic adaptation to caloric restriction and subsequent refeeding: The Minnesota Starvation Experiment revisited. *Am J Clin Nutr* 2015;**102**:807–819.
39. Waki H, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, et al. Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. *J Biol Chem* 2003;**278**:40352–40363.
40. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A Novel Serum Protein Similar to C1q, Produced Exclusively in Adipocytes. *J Biol Chem* 1995;**270**: 26746–26749.
41. Smith WJ, Underwood LE, Clemmons DR. Effects of caloric or protein restriction on insulin-like growth factor-I (IGF-I) and IGF-binding proteins in children and adults. *J Clin Endocrinol Metab* 1995;**80**: 443–449.
42. Thissen JP, Ketelslegers JM, Underwood LE. Nutritional regulation of the insulin-like growth factors. *Endocr Rev* 1994;**15**: 80–101.
43. Orrù S, Nigro E, Mandola A, Alfieri A, Buono P, Daniele A, et al. A Functional Interplay between IGF-1 and Adiponectin. *Int J Mol Sci* 2017;**18**:2145.
44. von Haehling S, Coats AJS, Anker SD. Ethical guidelines for publishing in the Journal of Cachexia, Sarcopenia and Muscle: Update 2021. *J Cachexia Sarcopenia Muscle* 2021;**12**:2259–2261.

CHAPTER IV

DETERMINANTS OF BONE MASS IN HEALTHY OLDER ADULTS DERIVED FROM THE CROSSTALK WITH MUSCLE AND ADIPOSE TISSUE

submitted to scientific reports

Determinants of bone mass in healthy older adults derived from the crosstalk with muscle and adipose tissue

Carina O. Walowski[1], Catrin Herpich[2,3,4], Janna Enderle[1], Wiebke Braun[1], Marcus Both[5], Mario Hasler[6], Manfred J. Müller[1], Kristina Norman[2,3,4,7] and Anja Bosy-Westphal[1*]

[1]Institute for Human Nutrition and Food Science, Christian-Albrechts-University, Kiel, Germany
[2]Institute of Nutritional Science, University of Potsdam, Potsdam, Germany
[3]Charité Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Geriatrics and Medical Gerontology, Berlin, Germany
[4]German Institute of Human Nutrition, Potsdam-Rehbrücke, Department of Nutrition and Gerontology, Nuthetal, Germany
[5]Department of Radiology and Neuroradiology, University Medical Center Schleswig-Holstein, Campus Kiel, Germany
[6]Applied Statistics, Faculty of Agricultural and Nutritional Sciences, Christian-Albrechts-University, Kiel, Germany
[7]German Center for Cardiovascular Research (DZHK), Partner Site Berlin, Berlin, Germany

Disclosure Statement: The authors have nothing to disclose.

***Corresponding author:**

Prof. Anja Bosy-Westphal, PhD, MD

Christian-Albrechts-Universität zu Kiel

Institut für Humanernährung und Lebensmittelkunde

Düsternbrooker Weg 17

24105 Kiel

Tel.: ++49 (0)431/880-5674

Fax.: ++49 (0) 431/880-5679

Email: abosyw@nutrition.uni-kiel.de

Key words: bone, muscle, adipose tissue, crosstalk, osteosarcopenic obesity, older adults

ABSTRACT

Lower bone mass in older adults may be mediated by the endocrine crosstalk between muscle, adipose tissue and bone. In 150 healthy community-dwelling adults (59-86 years, BMI 17-37kg/m^2; 58.7% female), skeletal muscle mass index, adipose tissue and fat mass index (FMI) were determined. Levels of myokines, adipokines, osteokines, inflammation markers and insulin were measured as potential determinants of bone mineral content (BMC) and density (BMD). FMI was negatively associated with BMC and BMD after adjustment for mechanical loading effects of body weight (r-values between -0.37 and -0.71, all p<0.05). Higher FMI was associated with higher leptin levels in both sexes, with hsCRP in women and with lower adiponectin levels in men. In addition to weight and FMI, sclerostin, osteocalcin, leptin x sex and adiponectin were independent predictors of BMC in a stepwise multiple regression analysis. Muscle mass, but not myokines, showed positive correlations with bone parameters that were weakened after adjusting for body weight (r-values between 0.27 and 0.58, all p<0.01). Whereas the anabolic effect of muscle mass on bone in healthy older adults may be partly explained by mechanical loading, the adverse effect of obesity on bone is possibly mediated by low-grade inflammation, higher leptin and lower adiponectin levels.

INTRODUCTION

Aging is characterized by the progressive decline of bone mass that is responsible for adverse outcomes like fracture risk and mortality [1]. Numerous studies indicate that a higher skeletal muscle mass is associated with a higher bone mineral density (BMD) in older adults [2-4] whereas sarcopenia [5] and high levels of fat mass (FM, [6-8]) exert a negative impact on bone. Therefore, the age-related decrease in muscle mass and increase in FM as well as fat infiltration in the musculoskeletal system may contribute to the impairment of bone mass leading to an obese osteosarcopenic phenotype and resulting in poorer overall strength and functionality [9]. As an underlying mechanism, the endocrine and paracrine crosstalk between bone and muscle or adipose tissue is suggested (for reviews see [10-12]). Due to the clinical importance of age-related musculoskeletal diseases, this ‘bone-muscle-fat crosstalk’ may reveal new targets to prevent or mitigate bone degradation.

The role of adipokines in the regulation of bone mass in older adults remains controversial. A pro-osteogenic effect has been shown for adiponectin that was found to promote osteoblastogenesis and inhibit osteoclastogenesis in *in vitro* and *in vivo* models [13,14] whereas

results from studies in older people often demonstrate a negative relationship between adiponectin and bone [15,16]. Likewise, leptin was reported to be positively as well as negatively associated with bone parameters in humans [17]. Accordingly, both osteogenic and osteolytic effects of leptin have been found in cell and animal models (for a review see [18]). Myokines, like irisin and myostatin, could be useful markers for the assessment of disorders of the muscle-bone unit and metabolic bone diseases or even therapeutic targets for the treatment of sarcopenia and osteoporosis. Irisin was found to be positively associated with bone mineral content (BMC) [19] and negatively with the prevalence of fracture risk [19,20]. By contrast, myostatin negatively regulates bone mineralization while concurrently enhancing bone resorption by inhibiting osteoblast differentiation and promoting osteoclast differentiation [21,22].

Vice versa, osteokines may exert anabolic or catabolic effects on muscle and adipose tissue (for reviews see [10-12]). Sclerostin, a negative regulator of bone growth secreted by osteocytes (for a review see [23]), has been shown to inhibit myogenesis *in vitro* and *ex vivo* [24] and was observed to be negatively associated with muscle mass in humans [25]. Sclerostin is also thought to increase FM by promoting adipogenesis and lipid accumulation in pre-adipocyte cell lines [26,27], in primary mesenchymal stromal cells from mice and humans [26] and in animal experiments [28]. These findings are supported by positive correlations between sclerostin and FM in some [29,30], but not all clinical trials [31]. In a recent study, sclerostin was identified as a putative new myokine that was found to impair the functional maturation of osteoblasts [32]. By contrast, osteocalcin, an osteoblast-derived marker of bone formation (for a review see [23]), has been shown to exert positive effects on muscle *in vitro* [33], *in vivo* [34] and in humans [36,36] and to protect from obesity in men [37] and women [38].

These findings indicate that mechanistic experiments *in vitro* and *in vivo* do not always agree with results from human studies. Discrepant findings may be due to confounding effects of ageing associated diseases like metabolic impairment (i.e. insulin resistance, chronic inflammation) or decreased kidney function. The aim of the present study was therefore to identify potential determinants of bone mass and bone density derived from the crosstalk with skeletal muscle and adipose tissue in healthy community-dwelling older adults with a wide BMI-range.

SUBJECTS AND METHODS

Study population

Data of 150 Caucasian men and women were collected at the 'German Reference Center for Body Composition' (Institute of Human Nutrition and Food Science at the University of Kiel, Germany) between 2019 and 2020 as described in detail elsewhere [39]. The primary aim of the study was to develop prediction equations for two seca medical bioelectrical impedance analysis devices for older adults**.** The trial was registered at clinicaltrials.gov as NCT04028648. Exclusion criteria were edema, chronic diseases, heart failure, renal failure, paralysis (e.g. after a stroke), neurodegenerative diseases, tumors in treatment, amputation of limbs, electrical and metallic implants, current alcohol abuse, not removeable piercings and large tattoos on the arms or legs (because of possible interference with magnetic resonance imaging (MRI) examinations) as well as medication which could influence body composition. The recruitment was realized using local advertisements and notice board postings. The study protocol was authorized by the medical ethic committee of the Christian-Albrechts-University of Kiel, Germany, and conducted according to the guidelines laid down in the 'Declaration of Helsinki'. Written informed consent was received from each subject before participation [39].

Body composition analysis

Body weight was measured with subjects in underwear to the nearest 0.01 kg by an electronic scale (Tanita, Tokyo, Japan) connected to the BOD POD® Body Composition System (Cosmed srl, Rome, Italy). Height was assessed without shoes using a stadiometer (SECA, Modell 285, Hamburg, Germany). Air-displacement plethysmography (BOD POD® Cosmed srl, Rome, Italy) was performed to determine FM and fat-free mass (FFM) as previously described [40]. Absolute FM (kg) was calculated from body density using the equation by Siri et al. [41]. FFM (kg) was then calculated as the difference between body weight and absolute FM. FM-Index (FMI) and FFM-Index (FFMI) were calculated as FM (kg)/height (m^2) and FFM (kg)/height (m^2). Measurements of skeletal muscle mass, subcutaneous and visceral adipose tissue (SAT and VAT) were performed using whole body MRI with a 1.5 T scanner (Magnetom Avanto, Siemens Medical Systems, Erlangen, Germany) [42,43]. Subjects were examined in a supine position with arms extended above their heads and were required to hold their breath during scans in abdominal and thoracic regions. The whole body was scanned from wrist to ankle using continuous axial images of 8 mm slice thickness and 2 mm interslice gaps for arms, legs and trunk. Images were obtained using a T1-weighted-gradient echo sequence (repetition time: 157

ms; echo time: 4 ms for scans of arms, legs and abdominal region). Volumes of skeletal muscle mass, SAT and VAT were manually determined by using segmentation software (SliceOmatic 4.3, Tomovision, Montreal, Canada). VAT was defined as intra-abdominal fat between the top of the liver and femur heads. Volumes of total skeletal muscle (excluding head and neck muscles, hands and feet), VAT and SAT were determined from the sum of tissue areas (cm^2) multiplied by the slice thickness. Volume data were then converted into tissue masses using the assumed densities of 1.04 g cm^{-3} for muscle and 0.92 g cm^{-3} for SAT and VAT [44]. Skeletal muscle mass was normalized to height squared using skeletal muscle mass index (SMI, (kg)/height (m^2)).

Whole body BMC, BMD and T-Score were measured by dual X-ray absorptiometry (HOLOGIC Discovery A (S/N 82686), Inc., Bedford, MA, USA). Before daily measurements, a spine phantom calibration was performed. Manufacturer's software (version 12.6.1:3, Hologic, Inc.) was used for analysis. Results were summed up for both arms and legs as well as the head.

Hand grip strength (HGS) was measured using a hydraulic SAEHAN® handgrip dynamometer (SH5001, Masan, South Korea). Subjects conducted the test in a standing position. The elbows were flexed at 90 degrees with the shoulder attached to the torso. HGS of the left and right hand was determined three times and the greatest value of the dominant hand was included in the analysis.

Endocrine parameters

After a minimum 10-h overnight fast, serum and plasma blood samples were taken from an antecubital vein and analyzed as in detail described elsewhere [39]. Briefly, the participants were instructed to refrain from vigorous exercise and alcohol intake on the day prior to blood sampling. Serum was stored at room temperature in an upright position for 30 min for complete coagulation. Plasma and serum were obtained by centrifugation at 2000 g for 10 min at 20 °C and stored at − 40 °C. Blood sample analyses were performed at the 'German Institute of Human Nutrition', Potsdam-Rehbrücke, Department of Nutrition and Gerontology, Nuthetal, Germany and a laboratory in Kiel, Germany [39].

To investigate the interaction between and within bone, muscle and adipose tissue and its effects on body composition in advanced age, numerous humoral cytokines and growth factors were measured via commercial ELISA kits: the osteokines sclerostin (intra-assay CV: ≤ 7 %, inter-assay CV: ≤ 10 %; BIOMEDICA, Vienna, Austria) and osteocalcin (n = 93; intra-assay CV:

3.0-4.6 %, inter-assay CV: 3.4-5.5 %; BioVendor, Brno, Czech Republic), the myokines myostatin (intra-assay CV: 1.8-5.4 %, inter-assay CV: 3.1-6 %; bio-techne, NE, Minneapolis, MN, USA) and irisin (intra-assay CV: 4.9-8.2 %, inter-assay CV: 8.0-9.7 %; BioVendor, Brno, Czech Republic) and the adipokines leptin (intra-assay CV: 4.2-7.6 %, inter-assay CV: 4.4-6.7 %; BioVendor, Brno, Czech Republic) and adiponectin (intra-assay CV: 2.8-3.9 %, inter-assay CV: 5.9-6.4 %; Immundiagnostik AG, Bensheim, Germany). As growth factor insulin-like growth factor 1 (IGF-1) (intra-assay CV: 5.1-6.7 %, inter-assay CV: 5.5-6.6 %; BioVendor, Brno, Czech Republic) was measured. The inflammation markers interleukin 6 (IL-6) (intra-assay CV: 4.2-5.1 %, inter-assay CV: 4.7-5.0 %; BioVendor, Brno, Czech Republic) and high-sensitivity C-reactive protein (hsCRP) (intra-assay CV: 0.73-5.73 %, inter-assay CV: 1.50-5.76 %; BECKMAN COULTER, Brea, CA, USA) were determined via commercial ELISA kit and immuno-turbidimetric test, respectively. Levels of insulin were measured by a chemiluminescent microparticle immunoassay (intra-assay CV: 1.4-2.1 %, inter-assay CV: 1.5-2.2 %; Abbott, Wiesbaden, Germany). Elevated insulin levels were set at > 25.0 mU/l and inflammation was based on hsCRP > 3 mg/l [39].

Statistical analysis

Statistical analyses were carried out with SPSS statistical software (SPSS 28.0, Inc., Chicago, IL, USA). All data are presented as means ±SD. Differences between independent samples were tested by unpaired t-test. Shapiro-Wilk test and residual analysis were used to verify normality [39]. Pearson's and Spearman's correlation coefficients were calculated to identify bivariate associations between and within body composition, hormones, growth factors, inflammation markers and HGS. Partial correlations were used to adjust for various confounders. Effects of mechanical loading on bone parameters were assessed by adjusting for body weight. Stepwise regression analyses were performed to access factors independently associated with BMC, BMD and T-Score. The qualitative factor sex (male or female) was coded numerically (1 or 2). All tests were two-sided and the level of significance was set at $p < 0.05$.

RESULTS

From the included 150 participants, data of 117 adults (71 women and 46 men) aged 60-82 years with a BMI between 18 and 37 kg/m^2 were analysed. Data from 18 participants were excluded due to creatinine levels and estimated glomerular filtration rates exceeding the reference range. Further data from 15 subjects were excluded because of motion artefacts or

incorrect patient positioning in MRI. Descriptive characteristics of the study population are summarized in **Table 1**.

Table 1 Characteristics of the study population

	all subjects	women	men
n	117	71	46
age (y)	70.2 ±5.0	69.6 ±4.7	71.2 ±5.8
height (m)	1.68 ±0.10	1.62 ±0.06***	1.77 ±0.1
weight (kg)	73.1 ±16.1	64.4 ±10.5***	86.6 ±13.6
BMI (kg/m^2)	25.6 ±3.9	24.4 ±3.7***	27.4 ±3.5
FMI (kg/m^2)	9.3 ±3.2	9.8 ±3.3*	8.5 ±2.8
SAT (kg)	17.5 ±6.2	18.3 ±6.2	16.3 ±6.1
VAT (kg)	2.1 ±1.5	1.4 ±1.0***	3.2 ±1.6
FFMI (kg/m^2)	16.3 ±2.4	14.7 ±1.0***	18.8 ±1.4
Skeletal muscle mass (kg)	22.6 ±6.1	18.4 ±2.6***	29.0 ±3.7
SMI (kg/m^2)	7.8 ±1.4	7.0 ±0.8***	9.2 ±1.0
BMC (kg)	2.05 ±0.53	1.70 ±0.28***	2.58 ±0.36
BMD (g/cm^2)	1.0 ±0.1	0.9 ±0.1***	1.1 ±0.1
T-Score	-1.6 ±1.2	-2.1 ±1.1***	-0.8 ±1.0
HGS (kg)	31.4 ±10.5	24.7 ±5.0***	41.8 ±8.1

Values are means ±SD.; BMI, body mass index; FMI, fat mass index; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; FFMI, fat-free mass index; SMI, skeletal muscle mass index; BMC, bone mineral content; BMD, bone mineral density; HGS, hand grip strength. *$p < 0.05$, ***$p < 0.001$ sex differences by *t*-test.

Men had a higher BMI, FFMI, VAT, skeletal muscle mass, SMI, BMC, BMD, T-Score and HGS and a lower FMI compared with women.

Levels of hormones, growth factors and inflammation markers of the study population are summarized in **Table 2**.

Table 2 Levels of hormones, growth factors and inflammation markers in the total population and in the subgroups of men and women

	all subjects	women	men
n	117	71	46
insulin (µU/ml)	9.8 ±6.7	8.7 ±4.5*	11.6 ±8.9
leptin (ng/ml)	11.9 ±11.0	14.5 ±12.6***	8.0 ±6.2
adiponectin (mg/l)	18.8 ±14.1	21.3 ±16.0**	15.0 ±9.5
IL-6 (pg/ml)	10.38 ±26.06	6.09 ±4.88*	17.00 ±40.50
hsCRP (mg/l)	2.28 ±3.30	2.09 ±2.81	2.58 ±3.97
myostatin (ng/ml)	2.2 ±0.8	2.2 ±0.8	2.4 ±0.8
irisin (µg/ml)	6.6 ±4.7	6.7 ±4.7	6.5 ±4.7
sclerostin (pmol/l)	48.3 ±23.2	43.0 ±14.6**	56.5 ±30.8
osteocalcin (ng/ml)	14.8 ±5.4	16.0 ±5.4**	12.9 ±4.9
IGF-1 (µg/l)	163.9 ±57.8	147.4 ±52.2***	189.2 ±57.3

Values are means ±SD. IL-6, interleukin 6; hsCRP, high sensitivity C-reactive protein; IGF-1, insulin-like growth factor 1. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ sex differences by *t*-test.

Insulin, IL-6, IGF-1 and sclerostin levels were higher and leptin, adiponectin as well as osteocalcin levels were lower in men compared with women. Prevalence for elevated hsCRP and insulin levels was 18.8 % and 2.6 %, respectively.

Effects of muscle on bone parameters

In the total population, muscle mass and SMI were positively associated with bone parameters (for muscle mass, kg: BMC r = 0.82, BMD r = 0.63, T-Score r = 0.49, all $p < 0.001$ and for SMI, kg/m^2: BMC r = 0.67, BMD r = 0.55, T-Score r = 0.41, all $p < 0.001$). In the subgroup of women, muscle mass was only positively correlated with BMC (r = 0.49, $p < 0.001$) whereas in men, muscle mass was associated with higher BMC, BMD and T-Score (BMC r = 0.53, BMD r = 0.37, T-Score r = 0.37, $p < 0.01$ - $p < 0.001$). In the total population, the positive association between SM or SMI and bone parameters persisted after adjustment for weight (except for the correlation between SMI and T-Score) but were slightly weakened (data not shown). In the subgroups of men and women, the correlations between muscle mass and bone parameters remained no longer significant after adjusting for body weight. No relationships were found between myostatin or irisin and muscle mass, SMI or bone parameters.

Effects of adipose tissue on bone parameters

After adjustment for body weight, FMI showed consistent negative associations with BMC, BMD and T-Score ranging between -0.37 and -0.71 in men and women (all $p < 0.01$). Concerning different fat compartments, VAT was negatively correlated with bone parameters in men when controlling for weight (BMC r = -0.43, $p < 0.01$, BMD r = -0.33, $p < 0.05$). Negative relationships were also observed between SAT and bone parameters after accounting for weight (men: BMC r = -0.52, $p < 0.01$, BMD r = -0.35, $p < 0.05$, T-Score r = -0.42, $p < 0.01$, women: BMC -0.32, $p < 0.001$).

Body fat compartments (SAT and VAT) and FMI were positively associated with leptin concentrations in both sexes and with inflammation markers in women whereas FMI was negatively correlated with adiponectin in men (**Table 3**).

Table 3 Correlation coefficients between BMI or fat compartments and inflammation markers, leptin or adiponectin in the subgroups of men and women

	women				*men*			
	hsCRP	IL-6	leptin	adiponectin	hsCRP	IL-6	leptin	adiponectin
BMI	0.55**	-	0.77**	-	-	-	0.76**	-
FMI	0.57**	-	0.80**	-	-	-	0.78**	-0.29*
VAT	0.54**	0.25*	0.62**	-	-	-	0.74**	-
SAT	0.54**	0.25*	0.77**	-	-	-	0.70**	-

hsCRP, high sensitivity C-reactive protein; IL-6, interleukin 6; BMI, body mass index; FMI, fat mass index; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue. *$p < 0.05$, **$p < 0.01$.

IL-6 levels were negatively associated with bone parameters in men (BMC r = -0.34, $p < 0.05$, BMD r = -0.34, $p < 0.05$, T-Score r = -0.32, $p < 0.05$). Correlations remained significant after excluding two subjects with elevated IL-6 levels of 146 and 243 pg/ml. No relationship was observed between hsCRP or leptin and bone parameters. To test if an effect of leptin on bone could be masked by weight, partial correlation analyses adjusted for weight between leptin and bone parameters were performed and a significant negative association between leptin and BMC was found in men (r = -0.40, $p < 0.01$). Regarding inflammation markers, leptin levels were positively correlated with hsCRP (men: r = 0.36, $p < 0.05$, women: r = 0.54, $p < 0.001$) and with levels of IL-6 in women (r = 0.33, $p < 0.01$). Leptin showed consistent positive correlations with skeletal muscle mass and SMI (ranging between 0.38 and 0.44 in men and women (all $p < 0.01$)).

Effects of bone on bone parameters

Sclerostin levels were positively associated with bone parameters in the total population and in men whereas in women only a positive correlation between sclerostin and BMC was found (**Table 4**).

Table 4 Correlations coefficients between sclerostin or osteocalcin levels and bone parameters

	all subjects		*women*		*men*	
	sclerostin	osteocalcin	sclerostin	osteocalcin	sclerostin	osteocalcin
BMC (kg)	0.38**	-0.31**	0.31**	-	0.38**	-
BMD (g/cm^2)	0.36**	-0.33**	-	-	0.43**	-
T-Score	0.36**	-0.28**	-	-0.30**	0.43**	-

BMC, bone mineral content; BMD, bone mineral density. **$p < 0.01$.

Relationships between sclerostin and bone parameters remained significant after accounting for body weight or SMI as potential confounders in partial correlation analyses. In contrast to sclerostin, higher osteocalcin levels were associated with lower bone parameters in the total population and higher osteocalcin concentrations correlated with a lower T-Score in women. A negative association was observed between osteocalcin and sclerostin levels (all: r = -0.25, $p < 0.05$, men: r = -0.49, $p < 0.01$).

IGF-1 levels were positively correlated with BMC, BMD and T-Score in the total population (BMC r = 0.33, BMD r = 0.37, T-Score r = 0.33, all $p < 0.001$).

Multiple stepwise regression analyses with BMC, BMD or T-Score as dependent variables and weight, FMI, SMI, leptin, leptin x sex, adiponectin, sclerostin, osteocalcin, IL-6, hsCRP, IGF-1, age and sex as independent variables were performed (**Table 5**).

Table 5 Stepwise multiple regression analyses with BMC, BMD and T-Score as dependent variables

Dependent variables and predictors	β coefficient	R^2	*SEE*	*p*-value	*VIF*
BMC (kg)					
Model[a]					
Step 1: weight	32.644	0.526	1.413	< 0.001	1.618
Step 2: FMI	-128.564	0.833	9.795	< 0.001	2.681
Step 3: sclerostin	2.608	0.864	0.877	0.004	1.239
Step 4: osteocalcin	-13.799	0.875	3.569	< 0.001	1.223
Step 5: leptin x sex	4.040	0.890	1.197	0.001	2.142
Step 6: adiponectin	2.764	0.896	1.199	0.024	1.079
BMD (g/cm²)					
Model[a]					
Step 1: sex	-0.156	0.409	0.021	< 0.001	1.056
Step 2: sclerostin	0.001	0.458	0.000	0.006	1.056
T-Score					
Model[a]					
Step 1: sex	-0.625	0.173	0.242	0.012	1.255
Step 2: sclerostin	0.017	0.240	0.005	0.001	1.101
Step 3: IGF-1	0.005	0.286	0.002	0.019	1.200

BMC, bone mineral content; FMI, fat mass index; IGF-1, insulin-like growth factor 1; BMD, bone mineral density; SEE, standard error of estimation; VIF, variance inflation factor.

[a]Model: independent variables: weight, FMI, SMI, leptin, leptin x sex, adiponectin, sclerostin, osteocalcin, IL-6, hsCRP, IGF-1, age and sex.

Using BMC as independent variable, the analysis revealed that weight and FMI were the main predictors, but the osteokines sclerostin and osteocalcin independently explained 4.2 % of the variance and both adipokines leptin and adiponectin further explained 2.1 % of the variance. Concerning BMD and T-Score, sclerostin explained 4.7 % or 6.7 % of the variance in addition to the main predictor sex. As a further predictor for the T-Score, IGF-1 entered in the equation.

Regarding muscle, sclerostin levels were positively associated with SMI in the total population ($r = 0.23$, $p < 0.05$) and with HGS (women: $r = 0.26$, $p < 0.05$; all: $r = 0.27$, $p < 0.01$) whereas osteocalcin levels showed a negative correlation with SMI (all: $r = -0.32$, $p < 0.01$).

In **Figure 1** an overview of the regulation of bone mass or bone density derived from the results of the present study is given.

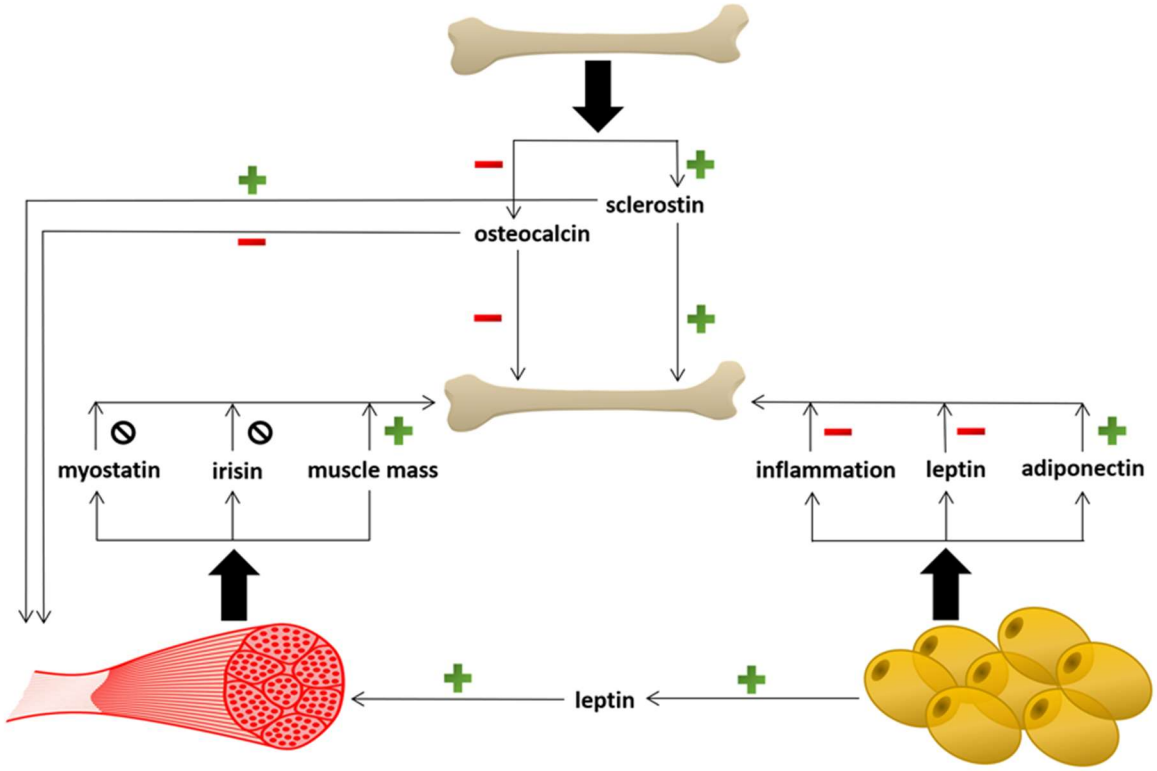


Figure 1 Overview of the regulation of bone mass or bone density.

positive correlation

negative correlation

no correlation.

DISCUSSION

Crosstalk between bone and adipose tissue

The present data indicate that beside FMI both, subcutaneous and visceral obesity are strong predictors and critical risk factors of a low bone mass and density in older adults when controlling for the mechanical loading effects of total body weight on bone mass (see results). These results are confirmed by previous findings that identified FM [8,45-47] or VAT [48-50] as independent negative determinants of bone mass or bone density. The etiology of impaired bone health in obesity is multifactorial and a number of mechanistic explanations have been proposed to explain the inverse association between fat and bone (for a review see [51]). Beyond vitamin D deficiency, insulin resistance and reduced mobility, altered adipokine secretion and chronic pro-inflammatory status were identified as risk factors. An increased release of pro-inflammatory mediators has been shown to stimulate bone resorption (for a

review see [52]). In line with this mechanism, the present data demonstrated negative associations between IL-6 and BMC, BMD or T-Score in men (who had higher IL-6 levels compared to women, **Table 2**). A higher expression of IL-6 mRNA has been found in bone samples from postmenopausal women with osteoporotic fractures compared to women with normal BMD [53]. Consistent with this clinical observation, knockout of the IL-6 gene in ovariectomized mice has been shown to prevent bone loss by upregulating mRNA expression of osteoblast-related genes and downregulating osteoclast-related mRNA [54].

The negative association between FMI and BMC might also be explained by lower adiponectin levels with increasing FMI [55] which we found in the subgroup of men (see results). Stepwise multiple regression analysis revealed that adiponectin was a significant positive predictor of BMC (**Table 5**). By contrast, previous human studies have demonstrated a negative relationship between adiponectin levels and BMD especially in advanced age [15,56]. The contradictory results may arise from distinct adiponectin concentrations or isoforms assessed by different ELISA kits as well as heterogenous study populations. In line with the findings of the present study, adiponectin has been demonstrated to promote proliferation, differentiation and mineralization in human osteoblasts [57]. With respect to the underlying mechanisms, transcription, translation and secretion of adiponectin as well as expression of its receptors were found in bone-forming cells [58] and a pro-osteogenic role for adiponectin with increased osteoblastogenesis or lower osteoclastogenesis was found in *in vitro* and *in vivo* studies [13,59].

Stepwise multiple regression analysis also revealed leptin x sex as a positive predictor of BMC (**Table 5**). Population based cross-sectional studies also found positive relationships between leptin and BMC and/or BMD adjusted for various confounders (e.g. age, %fat or BMI) in postmenopausal women [60-63] and in men [63] and reduced leptin levels in patients with vertebral fractures [61]. As an underlying mechanism, *in vitro* studies suggested that leptin exerts direct osteogenic effects mediated by its receptors in osteoblasts and osteoclasts [64-66]. Published data regarding the effects of leptin on bone parameters are however contradictory showing both anti-osteogenic as well as anabolic effects on bone formation (for a review see [67]). In the present study, partial correlation analyses revealed a significant negative association between leptin and BMC in men after adjusting for weight (with BMD and T-Score showing a trend towards significance) confirming a sex-specific effect of this hormone. It has been suggested that leptin may exert diverging effects depending on whether central (via hypothalamus) or peripheral (via osteoblasts) mechanisms are operating [68-70]. The response

of bone to leptin signaling might also differ between different skeletal sites (i.e. appendicular vs. axial) and bone structures (i.e. cortical vs. trabecular) [70-72].

Crosstalk between bone and muscle

The present data indicate positive effects of muscle mass on bone parameters in the total population. These findings are consistent with previously published results [3]. The bone-protective properties of muscle mass may be explained by increased weight bearing and mechanical effects due to muscle contraction whereas no relationships were found between myostatin or irisin and bone parameters (see results). In line with this supposition, the correlations between muscle mass and bone parameters weakened after adjusting for body weight (see results). In contrast to these findings, myostatin has been demonstrated as a negative regulator of bone in older subjects [73] whereas irisin was shown to be associated with reduced risk of osteoporosis in postmenopausal women (for a review see [74]). Previous *in vitro* and *in vivo* studies have demonstrated higher myostatin concentrations in the context of a sedentary lifestyle [75], obesity [76,77] and pro-inflammatory environments [78]. Since the study sample comprises healthy community-dwelling, mobile older adults, myostatin levels might be too low to exert a negative effect on bone tissue.

Higher skeletal muscle mass, kg and SMI, kg/m^2 were correlated with higher leptin levels in men and women (see results). These findings are confirmed by various studies among older subjects [79-81] and might be explained by an anabolic effect of leptin on muscle. In line with this supposition, *in vitro* experiments in cultured primary myoblasts have shown increased expression of myogenic genes by leptin treatment [82] and an *in vivo* study in aged mice demonstrated that leptin administration increased the expression of microRNAs involved in myogenesis [83].

Autocrine effects on bone

The present data demonstrated positive associations between sclerostin and all bone parameters (**Table 4**). Multiple stepwise regression analyses also revealed that sclerostin was a positive predictor of BMC, BMD and T-Score (**Table 5**). These findings are in line with previous studies that reported a positive relationship between sclerostin and BMD in pre- and postmenopausal women as well as in men [29,84,85]. Further studies also demonstrated that postmenopausal women with osteoporosis exhibit lower levels of sclerostin than healthy controls [84,86,87] and an increase in circulating sclerostin levels after risedronate treatment in patients with osteoporosis [86]. By contrast, levels of sclerostin have also been observed to be negatively

associated with BMD in patients with hemodialysis [88]. However, end-stage renal disease impairs bone mass by other factors like secondary hyperparathyroidism. In recent mendelian randomization studies, evidence for bidirectional causal relationship between circulating sclerostin concentrations and BMD was proposed, with a positive effect of BMD on sclerostin levels, and a negative effect of sclerostin on BMD [89,90]. These findings suggest that the measurements of sclerostin may include both bioactive molecules and biomarkers of osteocyte activity (for reviews see [23], [90]). Therefore, a reasonable explanation for the paradoxically positive association of sclerostin and bone parameters observed in our population may be due to the determination of total sclerostin. However, sclerostin is synthesized by osteocytes. Thus, the higher bone mass (i.e. more osteocytes), the higher is the overall synthesis and secretion of sclerostin.

In the present study, sclerostin levels were positively associated with SMI in the total population and with HGS in the total sample and in women. In agreement with these findings, higher serum sclerostin levels were shown to be associated with a lower risk of sarcopenia, low muscle mass and weak muscle strength in Korean older adults independent of age, sex and BMI [91]. In a recent study by Magarò et al. [32], sclerostin was discovered in muscle cells *in vitro* and in muscles from variably aged mice suggesting sclerostin as a putative new myokine. Since muscle was positively correlated with bone parameters and sclerostin levels in the present study, the positive association between sclerostin and bone parameters might be explained by muscle tissue. Partial correlation analyses between sclerostin and BMC, BMD and T-Score adjusted for SMI and skeletal muscle mass however revealed, that the positive associations between sclerostin and bone parameters persisted.

Due to the effect of sclerostin in inhibiting bone formation, osteocalcin as a marker of bone formation, would be expected to be negatively correlated with sclerostin levels. This hypothesis is supported by the present data. The negative correlation between sclerostin and osteocalcin levels may therefore explain the unexpected negative relationship between osteoclacin and BMC, BMD and T-Score (**Table 4**). By contrast, multiple regression analyses revealed that osteocalcin was a negative predictor for BMC independent of sclerostin (**Table 5**). Negative effects of osteocalcin on bone health, that have been proposed from studies in mice and *in vitro* experiments [92-94] can therefore not be excluded.

Strengths and limitations

The study population of older community-dwelling Caucasians was healthy, with normal renal function, thus confounders caused by disease or relevant medication can be excluded. The

population was also well characterized using whole body MRI, which is considered as the gold standard method of the assessment of skeletal muscle mass. Nevertheless, the present findings should be considered in the context of some limitations. First, adiponectin circulates in blood in multiple (iso-)forms with different physiologic functions [95,96]. As only total concentrations were measured, the effects of the different (iso-)forms could not be examined. Second, since the primary aim of the study was to validate measures of bioelectrical impedance analysis vs. reference methods, lifestyle factors affecting bone mass were not assessed (e.g. smoking habits, sports, vitamin D supplementation). Third, the quality of the kits for the measurement of the various hormones might influence the results. Finally, the findings need to be confirmed using a longitudinal study design.

In conclusion, in contrast to skeletal muscle mass, our results suggest that FMI and different body fat compartments are strongly related to lower bone mass and density in healthy older adults and possibly mediated by low-grade inflammation, higher leptin and lower adiponectin levels. In line with other human studies and in contrast to animal and cell culture experiments the present study reveals sclerostin as an important positive predictor of bone mass and density. Further investigations are needed to clarify this paradox.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

[1] Dong, Y. *et al*. Global, Regional, and national burden of low bone mineral density from 1990 to 2019. Results from the global burden of disease study 2019. *Front. Endocrinol.* **13**, 870905; 10.3389/fendo.2022.870905 (2022).

[2] Nakaoka, D. *et al*. Determinants of bone mineral density and spinal fracture risk in postmenopausal japanese women. *Osteoporos. Int.* **12**, 548–554; 10.1007/s001980170075 (2001).

[3] Kim, K. M., Lee, E. Y., Lim, S., Jang, H.-C. & Kim, C.-O. Favorable effects of skeletal muscle on bone are distinguished according to gender and skeletal sites. *Osteoporos. Sarcopenia.* **3**, 32–36; 10.1016/j.afos.2016.11.001 (2017).

[4] Ito, K. *et al*. Skeletal muscle mass index is positively associated with bone mineral density in hemodialysis patients. *Front. Med.* **7**, 187; 10.3389/fmed.2020.00187 (2020).

[5] Ma, X.-Y. *et al*. A bi-directional mendelian randomization study of the sarcopenia-related traits and osteoporosis. *Aging.* **14**, 5681–5698; 10.18632/aging.204145 (2022).

[6] Khosla, S., Atkinson, E. J., Riggs, B. L. & Melton, L. J. Relationship between body composition and bone mass in women. *J. Bone Miner. Res.* **11**, 857–863; 10.1002/jbmr.5650110618 (1996).

[7] El Hage, R., Jacob, C., Moussa, E. & Baddoura, R. Relative importance of lean mass and fat mass on bone mineral density in a group of lebanese postmenopausal women. *J. Clin. Densitom.* **14**, 326–331; 10.1016/j.jocd.2011.04.002 (2011).

[8] Ijuin, M. *et al*. Difference in the effects of body composition on bone mineral density between pre- and postmenopausal women. *Maturitas* **43**, 239–244; 10.1016/s0378-5122(02)00273-6 (2002).

[9] Ilich, J. Z., Inglis, J. E., Kelly, O. J. & McGee, D. L. Osteosarcopenic obesity is associated with reduced handgrip strength, walking abilities, and balance in postmenopausal women. *Osteoporos. Int.* **26**, 2587–2595; 10.1007/s00198-015-3186-y (2015).

[10] Ilich, J. Z. *et al*. Interrelationship among muscle, fat, and bone. Connecting the dots on cellular, hormonal, and whole body levels. *Ageing Res. Rev.* **15**, 51–60; 10.1016/j.arr.2014.02.007 (2014).

[11] He, C. *et al*. Bone and muscle crosstalk in aging. *Front. Cell Dev. Biol.* **8**, 585644; 10.3389/fcell.2020.585644 (2020).

[12] Kirk, B., Feehan, J., Lombardi, G. & Duque, G. Muscle, bone, and fat crosstalk. The biological role of myokines, osteokines, and adipokines. *Curr Osteoporos Rep.* **18**, 388–400; 10.1007/s11914-020-00599-y (2020).

[13] Oshima, K. *et al*. Adiponectin increases bone mass by suppressing osteoclast and activating osteoblast. *Biochem. Biophys. Res. Commun.* **331**, 520–526; 10.1016/j.bbrc.2005.03.210 (2005).

[14] Williams, G. A. *et al*. In vitro and in vivo effects of adiponectin on bone. *Endocrinology.* **150**, 3603–3610; 10.1210/en.2008-1639 (2009).

[15] Richards, J. B., Valdes, A. M., Burling, K., Perks, U. C. & Spector, T. D. Serum adiponectin and bone mineral density in women. *J. Clin. Endocrinol. Metab.* **92**, 1517–1523; 10.1210/jc.2006-2097 (2007).

[16] Wu, N., Wang, Q.-P., Li, H., Wu, X.-P., Sun, Z.-Q. & Luo, X.-H. Relationships between serum adiponectin, leptin concentrations and bone mineral density, and bone biochemical markers in Chinese women. *Clin. Chim. Acta.* **411**, 771–775; 10.1016/j.cca.2010.02.064 (2010).

[17] Liu, K., Liu, P., Liu, R., Wu, X. & Cai, M. Relationship between serum leptin levels and bone mineral density. A systematic review and meta-analysis. *Clin. Chim. Acta.* **444**, 260–263; 10.1016/j.cca.2015.02.040 (2015).

[18] Rinonapoli, G. *et al*. Obesity and bone. A complex relationship. *Int. J Mol. Sci.* **22**, 10.3390/ijms222413662 (2021).

[19] Yan, J., Liu, H.-J., Guo, W.-C. & Yang, J. Low serum concentrations of irisin are associated with increased risk of hip fracture in chinese older women. *Joint Bone Spine.* **85**, 353–358; 10.1016/j.jbspin.2017.03.011 (2018).

[20] Anastasilakis, A. D. *et al*. Circulating irisin is associated with osteoporotic fractures in postmenopausal women with low bone mass but is not affected by either teriparatide or denosumab treatment for 3 months. *Osteoporos. Int.* **25**, 1633–1642; 10.1007/s00198-014-2673-x (2014).

[21] Dankbar, B. *et a*l. Myostatin is a direct regulator of osteoclast differentiation and its inhibition reduces inflammatory joint destruction in mice. *Nat. Med.* **21**, 1085–1090; 10.1038/nm.3917 (2015).

[22] Qin, Y. *et al*. Myostatin inhibits osteoblastic differentiation by suppressing osteocyte-derived exosomal microRNA-218. A novel mechanism in muscle-bone communication. *J. Biol. Chem.* **292**, 11021–11033; 10.1074/jbc.M116.770941 (2017).

[23] Wang, J. S., Mazur, C. M. & Wein, M. N. Sclerostin and osteocalcin. Candidate bone-produced hormones. *Front. Endocrinol.* **12**, 584147; 10.3389/fendo.2021.584147 (2021).

[24] Huang, J. *et al*. Crosstalk between MLO-Y4 osteocytes and C2C12 muscle cells is mediated by the Wnt/β-catenin pathway. *JBMR Plus.* **1**, 86–100; 10.1002/jbm4.10015 (2017).

[25] Kim, J. A. *et al*. Association of serum sclerostin levels with low skeletal muscle mass. The korean sarcopenic obesity study (KSOS). *Bone.* **128**, 115053; 10.1016/j.bone.2019.115053 (2019).

[26] Fairfield, H. *et al*. The skeletal cell-derived molecule sclerostin drives bone marrow adipogenesis. *J. Cell. Physiol.* **233**, 1156–1167; 10.1002/jcp.25976 (2018).

[27] Ukita, M., Yamaguchi, T., Ohata, N. & Tamura, M. Sclerostin enhances adipocyte differentiation in 3T3-L1 cells. *J. Cell. Biochem.* **117**, 1419–1428; 10.1002/jcb.25432 (2016).

[28] Kim, S. P. *et al*. Sclerostin influences body composition by regulating catabolic and anabolic metabolism in adipocytes. *Proc. Natl. Acad. Sci. U S A.* **114**, E11238-E11247; 10.1073/pnas.1707876115 (2017).

[29] Urano, T., Shiraki, M., Ouchi, Y. & Inoue, S. Association of circulating sclerostin levels with fat mass and metabolic disease--related markers in japanese postmenopausal women. *J. Clin. Endocrinol. Metab.* **97**, E1473-7; 10.1210/jc.2012-1218 (2012).

[30] Confavreux, C. B. *et al*. Has sclerostin a true endocrine metabolic action complementary to osteocalcin in older men? *Osteoporos. Int.* **27**, 2301–2309; 10.1007/s00198-016-3540-8 (2016).

[31] Grethen, E. *et al*. Serum leptin, parathyroid hormone, 1,25-dihydroxyvitamin D, fibroblast growth factor 23, bone alkaline phosphatase, and sclerostin relationships in obesity. *J. Clin. Endocrinol. Metab.* **97**, 1655–1662; 10.1210/jc.2011-2280 (2012).

[32] Magarò, M. S.*et al.* Identification of sclerostin as a putative new myokine involved in the muscle-to-bone crosstalk. *Biomedicines.* **9**, 10.3390/biomedicines9010071 (2021).

[33] Liu, S. *et al*. Osteocalcin induces proliferation via positive activation of the PI3K/Akt, P38 MAPK pathways and promotes differentiation through activation of the GPRC6A-ERK1/2 pathway in C2C12 myoblast cells. *Cell. Physiol. Biochem.* **43**, 1100–1112; 10.1159/000481752 (2017).

[34] Mera, P., Laue, K., Wei, J., Berger, J. M. & Karsenty, G. Osteocalcin is necessary and sufficient to maintain muscle mass in older mice. *Mol. Metab.* **5**, 1042–1047; 10.1016/j.molmet.2016.07.002 (2016).

[35] Levinger, I. *et al*. Undercarboxylated osteocalcin, muscle strength and indices of bone health in older women. *Bone.* **64**, 8–12; 10.1016/j.bone.2014.03.008 (2014).

[36] Harslof, T., Sikjaer, T., Mosekilde, L., Langdahl, B. L. & Rejnmark, L. Correlations between changes in undercarboxylated osteocalcin and muscle function in hypoparathyroidism. *Int. J. Endocrinol. Metab.* **14**, e38440; 10.5812/ijem.38440 (2016).

[37] Chin, K.-Y. *et al*. Serum osteocalcin is significantly related to indices of obesity and lipid profile in Malaysian men. *Int. J. Med. Sci.* **11**, 151–157; 10.7150/ijms.7152 (2014).

[38] Lee, S. W., Jo, H. H., Kim, M. R., Kim, J. H. & You, Y. O. Association between osteocalcin and metabolic syndrome in postmenopausal women. *Arch. Gynecol. Obstet.* **292**, 673–681; 10.1007/s00404-015-3656-7 (2015).

[39] Walowski, C. O. *et al*. Analysis of the adiponectin paradox in healthy older people. *J. Cachexia Sarcopenia Muscle*. 10.1002/jcsm.13127 (2022).

[40] Bosy-Westphal, A. *et al*. Value of body fat mass vs anthropometric obesity indices in the assessment of metabolic risk factors. *Int. J. Obes.* **30**, 475–483; 10.1038/sj.ijo.0803144 (2006).

[41] Siri, W. E. Body composition from fluid spaces and density. Analysis of methods. 1961. *Nutrition (Burbank, Los Angeles County, Calif.)* **9**, 480-91; discussion 480, 492

[42] Hübers, M., Geisler, C., Plachta-Danielzik, S. & Müller, M. J. Association between individual fat depots and cardio-metabolic traits in normal- and overweight children, adolescents and adults. *Nutr. Diabetes.* **7**, e267; 10.1038/nutd.2017.20 (2017).

[43] Bosy-Westphal, A. *et al*. Measurement site for waist circumference affects its accuracy as an index of visceral and abdominal subcutaneous fat in a Caucasian population. *J. Nutr.* **140**, 954–961; 10.3945/jn.109.118737 (2010).

[44] Snyder, W.S. *et al*. Report of the task group on reference man. *Ann. ICRP.* **3**, iii-iii; 10.1016/0146-6453(79)90123-4 (1979).

[45] Zhao, L.-J., Liu, Y.-J., Liu, P.-Y., Hamilton, J., Recker, R. R. & Deng, H.-W. 2007 Relationship of obesity with osteoporosis. *The Journal of clinical endocrinology and metabolism* **92**, 1640–1646.

[46] Reid, I. R. *et al*. Determinants of total body and regional bone mineral density in normal postmenopausal women--a key role for fat mass. *J. Clin. Endocrinol. Metab.* **75**, 45–51; 10.1210/jcem.75.1.1619030 (1992).

[47] Reid, I. R., Evans, M. C. & Ames, R. W. Volumetric bone density of the lumbar spine is related to fat mass but not lean mass in normal postmenopausal women. *Osteoporos. Int.* **4**, 362–367; 10.1007/BF01622199 (1994).

[48] Bredella, M. A. *et al*. Vertebral bone marrow fat is positively associated with visceral fat and inversely associated with IGF-1 in obese women. Obesity. **19**, 49–53; 10.1038/oby.2010.106 (2011).

[49] Katzmarzyk, P. T. *et al*. Relationship between abdominal fat and bone mineral density in white and african american adults. *Bone.* **50**, 576–579; 10.1016/j.bone.2011.04.012 (2012).

[50] Sharma, D. K., Anderson, P. H., Morris, H. A. & Clifton, P. M. Visceral fat Is a negative determinant of bone health in obese postmenopausal women. *Int. J. Environ. Res Public Health.* **17**, 3996; 10.3390/ijerph17113996 (2020).

[51] Piñar-Gutierrez, A., García-Fontana, C., García-Fontana, B. & Muñoz-Torres, M. Obesity and bone health. A complex relationship. *Int. J. Mol Sci.* **23**, 8303; 10.3390/ijms23158303 (2022).

[52] Terkawi, M. A. *et al*. Interplay between inflammation and pathological bone resorption. Insights into recent mechanisms and pathways in related diseases for future perspectives. *Int. J. Mol. Sci.* **23**, 1786; 10.3390/ijms23031786 (2022).

[53] Ralston, S. H. Analysis of gene expression in human bone biopsies by polymerase chain reaction. Evidence for enhanced cytokine expression in postmenopausal osteoporosis. *J. Bone Miner. Res.* **9**, 883–890; 10.1002/jbmr.5650090614 (1994).

[54] Zhu, S. *et al*. Ovariectomy-induced bone loss in TNFα and IL6 gene knockout mice is regulated by different mechanisms. *J. Mol. Endocrinol.* **60**, 185–198; 10.1530/JME-17-0218 (2018).

[55] Reneau, J. *et al.* Effect of adiposity on tissue-specific adiponectin secretion. *PloS One.* **13**, e0198889; 10.1371/journal.pone.0198889 (2018).

[56] Zoico, E. *et al*. Relation between adiponectin and bone mineral density in elderly post-menopausal women. Role of body composition, leptin, insulin resistance, and dehydroepiandrosterone sulfate. *J. Endocrinol. Invest.* **31**, 297–302; 10.1007/BF03346361 (2008).

[57] Luo, X.-H. *et al*. Adiponectin stimulates human osteoblasts proliferation and differentiation via the MAPK signaling pathway. *Exp. Cell Res.* **309**, 99–109;10.1016/j.yexcr.2005.05.021 (2005).

[58] Berner, H. S. *et al*. Adiponectin and its receptors are expressed in bone-forming cells. *Bone.* **35**, 842–849; 10.1016/j.bone.2004.06.008 (2004).

[59] Lee, H. W. *et al*. Adiponectin stimulates osteoblast differentiation through induction of COX2 in mesenchymal progenitor cells. *Stem Cells.* **27**, 2254–2262; 10.1002/stem.144 (2009).

[60] Weiss, L. A., Barrett-Connor, E., Mühlen, D. von & Clark, P. 2006 Leptin predicts BMD and bone resorption in older women but not older men. The Rancho Bernardo study. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research* **21**, 758–764.

[61] Yamauchi, M. *et al*. Plasma leptin concentrations are associated with bone mineral density and the presence of vertebral fractures in postmenopausal women. *Clin. Endocrinol.* **55**, 341–347; 10.1046/j.1365-2265.2001.01361.x (2001).

[62] Blain, H. *et al*. Serum leptin level is a predictor of bone mineral density in postmenopausal women. *J. Clin. Endocrinol. Metab.* **87**, 1030–1035; 10.1210/jcem.87.3.8313 (2002).

[63] Zoico, E. *et al*. Relationship between leptin levels and bone mineral density in the elderly. *Clin. Endocrinol.* **59**, 97–103; 10.1046/j.1365-2265.2003.01808.x (2003).

[64] Cornish, J.*et al.* Leptin directly regulates bone cell function in vitro and reduces bone fragility in vivo. *J. Endocrinol.* **175**, 405–415; 10.1677/joe.0.1750405 (2002).

[65] Gordeladze, J. O., Drevon, C. A., Syversen, U. & Reseland, J. E. Leptin stimulates human osteoblastic cell proliferation, de novo collagen synthesis, and mineralization. Impact on differentiation markers, apoptosis, and osteoclastic signaling. *J. Cell. Biochem.* **85**, 825–836; 10.1002/jcb.10156 (2002).

[66] Holloway, W. R. *et al*. Leptin inhibits osteoclast generation. *J. Bone Miner. Res.* **17**, 200–209; 10.1359/jbmr.2002.17.2.200 (2002).

[67] Khosla, S. Leptin-central or peripheral to the regulation of bone metabolism? *Endocrinology.* **143**, 4161–4164; 10.1210/en.2002-220843 (2002).

[68] Steppan, C. M., Crawford, D.T., Chidsey-Frink, K. L., Ke, H. & Swick, A. G. Leptin is a potent stimulator of bone growth in ob/ob mice. *Regul. Pept.* **92**, 73–78; 10.1016/s0167-0115(00)00152-x (2000).

[69] Hamrick, M. W. *et al*. Leptin treatment induces loss of bone marrow adipocytes and increases bone formation in leptin-deficient ob/ob mice. *J. Bone Miner. Res.* **20**, 994–1001; 10.1359/JBMR.050103 (2005).

[70] Ducy, P. *et al*. Leptin inhibits bone formation through a hypothalamic relay. *Cell.* **100**, 197–207; 10.1016/s0092-8674(00)81558-5 (2000).

[71] Hamrick, M. W., Pennington, C., Newton, D., Xie, D. & Isales, C. Leptin deficiency produces contrasting phenotypes in bones of the limb and spine. *Bone.* **34**, 376–383; 10.1016/j.bone.2003.11.020 (2004).

[72] Iwaniec, U. T., Boghossian, S., Lapke, P. D., Turner, R. T. & Kalra, S. P. Central leptin gene therapy corrects skeletal abnormalities in leptin-deficient ob/ob mice. *Peptides.* **28**, 1012–1019; 10.1016/j.peptides.2007.02.001 (2007).

[73] Wu, L.-F. *et al.* Relative abundance of mature myostatin rather than total myostatin is negatively associated with bone mineral density in Chinese. *J. Cell. Mol. Med.* **22**, 1329–1336; 10.1111/jcmm.13438 (2018).

[74] Kornel, A., Den Hartogh, D. J., Klentrou, P. & Tsiani, E. Role of the myokine irisin on bone homeostasis. Review of the current evidence. *Int. J. Mol. Sci.* **22**, 9136; 10.3390/ijms22179136 (2021).

[75] Hittel, D. S. *et al*. Myostatin decreases with aerobic exercise and associates with insulin resistance. *Med. Sci. Sports Exerc.* **42**, 2023–2029; 10.1249/MSS.0b013e3181e0b9a8 (2010).

[76] Hittel, D. S., Berggren, J. R., Shearer, J., Boyle, K. & Houmard, J. A. Increased secretion and expression of myostatin in skeletal muscle from extremely obese women. *Diabetes.* **58**, 30–38; 10.2337/db08-0943 (2009).

[77] Amor, M. *et al*. Serum Myostatin is upregulated in obesity and correlates with insulin resistance in humans. *Exp. Clin. Endocrinol. Diabetes.* **127**, 550–556; 10.1055/a-0641-5546 (2019).

[78] Zhang, L. *et al.* Pharmacological inhibition of myostatin suppresses systemic inflammation and muscle atrophy in mice with chronic kidney disease. *FASEB J.* **25**, 1653–1663; 10.1096/fj.10-176917 (2011).

[79] Kao, T.-W. *et al*. Higher serum leptin levels are associated with a reduced risk of sarcopenia but a higher risk of dynapenia among older adults. *J. Inflamm. Res.* **14**, 5817–5825; 10.2147/JIR.S335694 (2021).

[80] Hubbard, R. E., O'Mahony, M. S., Calver, B. L. & Woodhouse, K. W. Nutrition, inflammation, and leptin levels in aging and frailty. *J. Am. Geriatr. Soc.* **56**, 279–284; 10.1111/j.1532-5415.2007.01548.x (2008).

[81] Lin, Y.-L., Wang, C.-H., Lai, Y.-H., Kuo, C.-H., Syu, R.-J. & Hsu, B.-G. Negative correlation between leptin serum levels and sarcopenia in hemodialysis patients. *International journal of clinical and experimental pathology* **11**, 1715–1723

[82] Arounleut, P. *et al*. Absence of functional leptin receptor isoforms in the POUND (Lepr(db/lb)) mouse is associated with muscle atrophy and altered myoblast proliferation and differentiation. *PloS One.* **8**, e72330; 10.1371/journal.pone.0072330 (2013)

[83] Hamrick, M. W. *et al*. The adipokine leptin increases skeletal muscle mass and significantly alters skeletal muscle miRNA expression profile in aged mice. *Biochem. Biophys. Res. Commun.* **400**, 379–383; 10.1016/j.bbrc.2010.08.079 (2010).

[84] Reppe, S. *et al.* Methylation of bone SOST, its mRNA, and serum sclerostin levels correlate strongly with fracture risk in postmenopausal women. *J. Bone Miner. Res.* **30**, 249–256; 10.1002/jbmr.2342 (2015).

[85] Amrein, K. *et al*. Sclerostin and its association with physical activity, age, gender, body composition, and bone mineral content in healthy adults. *J. Clin. Endocrinol. Metab.* **97**, 148–154; 10.1210/jc.2011-2152 (2012).

[86] Polyzos, S. A. *et al*. Serum sclerostin levels positively correlate with lumbar spinal bone mineral density in postmenopausal women--the six-month effect of risedronate and teriparatide. *Osteoporos. Int.* **23**, 1171–1176; 10.1007/s00198-010-1525-6 (2012).

[87] Sheng, Z. *et al*. Serum sclerostin levels were positively correlated with fat mass and bone mineral density in central south chinese postmenopausal women. *Clin. Endocrinol.* **76**, 797–801; 10.1111/j.1365-2265.2011.04315.x (2012).

[88] Lu, J.-W. *et al*. Serum sclerostin level is negatively associated with bone mineral density in hemodialysis patients. *Medicina.* **58**, 385; 10.3390/medicina58030385 (2022).

[89] Zheng, J. *et al*. Use of mendelian randomization to examine causal inference in osteoporosis. *Front. Endocrinol.* **10**, 807; 10.3389/fendo.2019.00807 (2019).

[90] Zheng, J. *et al.* 2019 Mendelian randomization analysis reveals a causal influence of circulating sclerostin levels on bone mineral density and fractures. *J. Bone Miner Res.* **34**, 1824–1836; 10.1002/jbmr.3803 (2019).

[91] Ahn, S. H. *et al.* Decreased serum level of sclerostin in older adults with sarcopenia. *Endocrinol. Metab.* **37**, 487–496; 10.3803/EnM.2022.1428 (2022).

[92] Ducy, P. *et al.* 1996 Increased bone formation in osteocalcin-deficient mice. *Nature.* **382**, 448–452; 10.1038/382448a0 (1996).

[93] Bodine, P.V.N. & Komm, B.S. Evidence that conditionally immortalized human osteoblasts express an osteocalcin receptor. *Bone.* **25**, 535–543; 10.1016/s8756-3282(99)00213-6 (1999).

[94] Neve, A., Corrado, A. & Cantatore, F. P. Osteocalcin. Skeletal and extra-skeletal effects. *J. Cell. Physiol.* **228**, 1149–1153; 10.1002/jcp.24278 (2013).

[95] Waki, H. *et al.* Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. *J. Biol. Chem.* **278**, 40352–40363; 10.1074/jbc.M300365200 (2003).

[96] Scherer, P.E. *et al.* A novel serum protein similar to C1q, produced in adipocytes. *J. Biol. Chem.* **270**, 26746-26749; 10.1074/jbc.270.45.26746 (1995).

Acknowledgements

We thank Britta Jux and the Clinic for Diagnostic Radiology, University medical Center Schleswig-Holstein, Kiel (Germany) for the help with MRI scanning.

Author contributions

Data acquisition (COW, WB, ABW, MJM, MB), hormone analyses (CH, KN), data analysis (COW, MH), discussion of data (COW, JE, CH, MB, KN, MJM, ABW), writing of the manuscript (COW, ABW); all authors read and approved the final manuscript.

Funding: This work was supported by a grant for seca gmbh & co.kg., Germany (2019/2020).

Competing interest

Anja Bosy-Westphal serves a consultant for seca gmbh & co.kg. The other authors declare no competing interests.

CHAPTER V

GENERAL DISCUSSION

Summary of the study rationale and objectives

The aging process is accompanied by a simultaneous deterioration of muscle and bone mass and an excessive fat accumulation that lead to an *obese osteosarcopenic* phenotype resulting in adverse health outcomes (*1-3*). Beside a high personal burden, an increase in social and economic costs will be the consequence. With regard to the demographic change and the growing number of older people there is thus a need to respond. More research on (i) how to develop accurate reference values for a 'normal' MM and (ii) how to define reliable cut-off points for individuals is needed in order to diagnose and treat sarcopenia at an early stage. Recognizing the co-existence and co-development of sarcopenia, osteoporosis and obesity, knowledge about the pathophysiology and underlying endocrine mechanisms that explains the interplay between muscle, bone and fat tissues has to be expanded deriving the best screening and treatment methods for these conditions.

Therefore, the present thesis first summarized and analysed previously published cut-offs for a 'normal' MM and discussed the impact of the underlying methodological assumptions and limitations (CHAPTER II).

Secondly, taken different hormones into account, we focused on adiponectin to investigate why increased adiponectin levels are paradoxically correlated with poor physical functioning, MM or BMD in older age despite its widely known beneficial properties. Associations between the hormone adiponectin and muscle or bone tissue were investigated and determinants of its concentrations were identified (CHAPTER III).

Finally, potential endocrine determinants of bone mass and density derived from the crosstalk with MM and AT as well as autocrine effects of bone-secreted osteokines were analysed in healthy older adults (CHAPTER IV).

Reference Values for Skeletal Muscle Mass - Current Concepts and Methodological Considerations

Main findings

Although the loss in MM is a major predictor of falls, fractures (for a review see *(4)*) and mortality (*5*) in older age, no broadly accepted clinical definition of sarcopenia has been reached so far. This is due to the use of variable combinations of body composition indices and discrepancies in the methods of normal range-definitions and cut-offs. Published reference values for a 'normal' MM thus vary dependent on the outcome parameter, measurement techniques and reference populations (for a review see (*6*), CHAPTER II). To prevent difficulties in comparisons between different research findings and to facilitate the interpretation of study results, it is recommended (i) to use a unified nomenclature for outcome parameters with specification of the respective device and software version of the body composition tools, (ii) to take into account the limitations of all proxies for the total body MM and (iii) to check, if the most suitable body composition method for the objective of the study was chosen (e.g. use of magnetic resonance imaging (MRI) for the assessment of MM changes due to its high precision) (for a review see (*6*), CHAPTER II). Since the evaluation of MM is complicated by a variety of available methods providing different outcome parameters as a proxy for MM, it is imperative to have accurate reference values using cut-off points that apply to the respective individual under study and body composition method. For example, MM cut-offs derived from a single slice at the level of the third lumbar vertebra are especially published in patients, in which computed tomography (CT) is routinely applied for cancer staging. The usage of these cut-off values might be specific for the population studied and the transferability of the results to other patient groups or healthy subjects needs to be investigated (for a review see (*6*), CHAPTER II).

State of research & critical reflection

Up to now, conventional reference values use sex-specific cut-offs for a low MM set at two SDs below the mean or percentiles of healthy normal weight young adult's reference groups to facilitate comparisons of study results (normative approach) (*7*). It may be yet questionable whether this approach is of advantage, since (i) the expected MM of a 70-year-old individual should be lower than that of a 50-year-old person and (ii) since an individual with obesity is likely to have a higher MM compared to a person with overweight. Contrary to the normative approach, many publications use a simple stratification of their own study populations based on cut-offs (e.g. lowest 20%, quartile, quintile) (stratification approach) (for a review see (*6*), CHAPTER II). Drawbacks of this method are the hampered transferability of cut-off points to

another study population of different ethnicity or age. Regardless of the approaches, diagnostic criteria or reference populations, cut-off points can still be criticised as arbitrary.

Reference values are used to determine whether an individual's MM is statistically 'normal' and considered as healthy or not. However, the theoretical linkage between reference values, normality and health has to be questioned as it is only based on statistics. For developing internationally accepted valid reference values of 'normal' MM, cut-offs should be correlated with the risk of health problems at population level (e.g. at which MM exists an increased risk of fractures?). The development of validated cut-off points will therefore depend on their predictive values for hard end-points - a challenge for research studies (*7*). The establishment of ever new population-based reference and cut-off values that are not related to a health problem is obsolete, since there is a risk of the existence of only 'normal' individuals.

The above mentioned examples illustrate the particular challenge in developing a reference value for a 'normal' MM and thus the diagnosis of sarcopenia. Besides the difficulty in determining the amount of MM that is associated with diseases, MM and also muscle quality are technically not easy to measure accurately and impracticable for epidemiological use (*7*). These limitations recently led to an updated consensus definition of the EWGSOP2 considering sarcopenia as a muscle disease, with poor muscle strength (as the most reliable measure of muscle function) overtaking the role of low MM as the primary determinant (*7*). This change could facilitate the assessment of sarcopenia in practice, particularly because a low HGS is a good predictor of poor patient outcomes such as increased functional impairments, reduced quality of life (*8*) and mortality (*9, 10*). However, in older adults with sarcopenic obesity the relatively low MM might be masked by a higher amount of FM, which in turn would require some kind of body composition analysis.

Given the importance of MM in clinical medicine, there is a need to establish accurate reference values and cut-offs for a low skeletal MM that are not strictly presented in the form of reference tables categorized according to sex, age and BMI.

Outlook

A pragmatic and easy-to-use concept to determine a person's 'normal' MM could be a valuable tool for assessing the risk of sarcopenia. As a possible approach, an online calculator as open source tool based on mathematical modeling would be conceivable. Due to a better reflection of the age-related dynamic and gradual changes of body composition, a continuous model is preferred as compared to a static model (e.g. a simple linear regression) that considers sex, age, height, FM, BMI and ethnicity as variables ((*11*), in revision). The advantage of using such a

model becomes apparent considering for example the age-related development of muscle tissue that first increases until reaching a peak mass, maintains in midlife and then begins slowly to decrease with an increasing declining rate with advanced age (*12, 13*). In the following paragraphs, explanations for the parameters that need to be considered in the continuous model are given:

(1) *age*

Reference values for a 'normal' MM related to age are needed due to the above mentioned physiological development of musculature within the ageing process. Besides, an increased contribution of connective tissue to lean mass in advanced age involves the risk of masking the MM depletion (*14, 15*). The latter aspect will be described in more detail under *(4) FM and BMI*.

(2) *sex*

Sex differences in age-associated decline of MM exist with a greater absolute (*16*) and higher relative reduction (*17*) of skeletal MM in men compared to women. Longitudinal studies reported a MM loss at a rate of 0.64-0.70% per year in women and 0.80-0.98% per year in men aged 75 years (*18*). These sex differences may be attributed to the greater initial MM in men (*19*).

(3) *height*

Height is associated with a physiologic increase in MM (for a review see (*15*)). Two individuals with the same %FFM differing in height can have a different nutritional status, with the taller person having a lower muscularity (for a review see (*15*)).

(4) *FM and BMI*

Body weight, fat and muscle are in a close relationship confirmed by the observation that an increase in weight and FM is normally related to a gain in MM expecting that subjects with overweight and obesity have a higher MM compared to persons with normal BMI (for a review see (*20*)). However, it should be noticed that age can be an important confounder affecting the relationship between FM and MM, since an increase in body weight and FM may also mask a loss of MM due to an increase in connective tissue (i.e. FFM in AT), especially with ageing-associated weight gain ((*11)*, in revision, (*12, 14*)). In addition, the same MM is worth less at a higher FM ((*11)*, in revision). That is likely due to fatty infiltrations (both intramyocellular and intermuscular), muscle collagen increases and alterations in fiber size, -number and -type (for a review see (*15*)) that together result in a lower MM quality and function in individuals with overweight or obesity (*21*).

(5) *ethnicity*

Both, the amount of MM and the risk of MM loss vary between ethnicities. In a cross-sectional study with a large multi-ethnic sample it was observed that African Americans tend to have higher values of MM followed by Caucasians, Hispanics and Asians (*22*). Furthermore, African American women showed the greatest MM loss, whereas Hispanic women had the least. Hispanic men tended to show a higher negative association of MM with age followed by African Americans and Whites (*22*).

In conclusion, a 'normal' MM should be defined sex- and ethnicity-specific and estimated on the basis of the parameters age, FM and BMI. Otherwise, advancing age, obesity or weight gain could lead to a misleading interpretation of MM due to an increased contribution of connective tissue to lean mass (for a review see (*15*)). Instead of rigid age- and sex- or BMI-specific cut-offs for the determination of a 'normal' MM, continuously modelled reference values are preferred, since they (i) are more sensitive to dynamical changes of body composition depending on age, BMI and FM and (ii) are more user-friendly avoiding the use of complex tables ((*11),* in revision).

Analysis of the adiponectin paradox in healthy older people

Main findings

Adiponectin levels showed negative correlations with skeletal muscle mass index (SMI, skeletal MM normalized by height, kg/m^2) and BMC (all) and a positive association with lumbar muscle fat (women) ((*23*), CHAPTER III). The present thesis thus confirms the *adiponectin paradox* in older adults without inflammatory diseases. In contrast to adiponectin, insulin-like growth factor 1 (IGF-1) levels were positively correlated with SMI (all, men, women), with BMC, BMD and T-Score in the total population and BMD and T-Score in women. Furthermore, IGF-1 concentrations were negatively associated with muscle fat (all, women), age (men, women) and adiponectin levels (all). These findings suggest that adiponectin might not be causally related to an unhealthy body composition in older subjects. The association is rather mediated by an age-related decrease in IGF-1 levels that might contribute to higher adiponectin concentrations in advanced age ((*23*), CHAPTER III).

State of research & critical reflection

Up to now, different explanations are discussed for the *adiponectin paradox* including renal dysfunction, impaired hepatic clearance, weight loss, and a compensatory increase in adiponectin levels due to subclinical risks (reverse causality) (*24*). Furthermore, previous studies have demonstrated that a low MM due to sarcopenia was linked to higher adiponectin concentrations (*25*). It is therefore possible that higher levels of adiponectin are a consequence of catabolic processes. Since our study design was cross-sectional, we cannot make any causal inference. But apart from the IGF-1 mediated approach contributing to the epiphenomenon of higher adiponectin levels, it is possible that higher adiponectin concentrations resulted from a low MM and might be thus be understood as a starvation signal. However, this interpretation seems to be unlikely in a healthy non-malnourished study population ((*23*), CHAPTER III).

Even though the question of causality cannot be sufficiently clarified due to the cross-sectional study design, for better understanding the relationship between adiponectin, IGF-1 and body composition, a mediation analysis could be conducted as a next step. This model is a useful statistical tool that is becoming more and more prominent in medical research (*26*). It tries to identify and explain the underlying mechanism of an observed relationship between an independent variable (adiponectin) and a dependent variable (MM or BMC) by the inclusion of a mediator variable (IGF-1) (**Figure 2**). Mediation has to be tested by identifying four regression coefficients: a, the effect of adiponectin on IGF-1, b, the effect of IGF-1 on MM or BMC, (c), the total effect of adiponectin on MM or BMC and c', the direct effect of adiponectin

on MM or BMC. A partial mediation is given, if the associations a, b, (c) and c′ are significant. If there is no significant effect c′, the mediation is assumed to be completed.

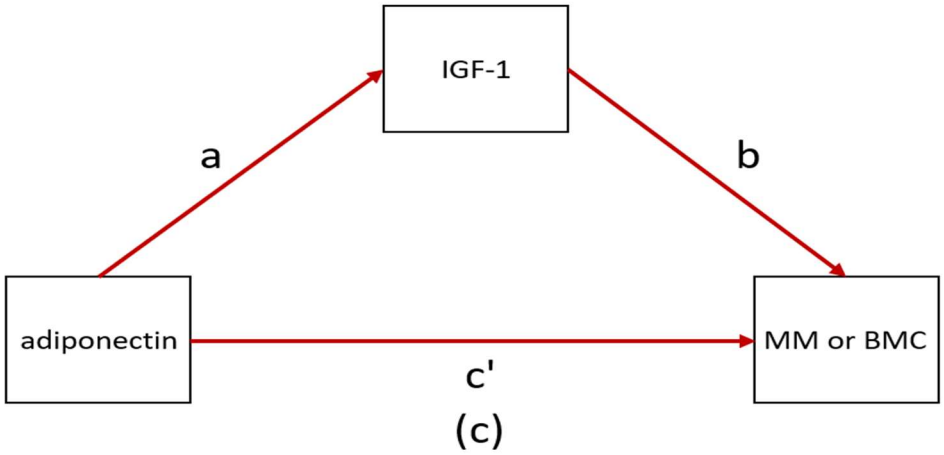


Figure 2. Conceptual framework of the mediation analysis. IGF-1, insulin-like growth factor 1; MM, muscle mass; BMC, bone mineral content; **a**, regression coefficient: the effect of adiponectin on IGF-1; **b**, regression coefficient: the effect of IGF-1 on MM or BMC; **(c)**, regression coefficient: total effect of adiponectin on MM or BMC; **c'**, direct effect of adiponectin on MM or BMC.

Results of this statistical approach have also to be interpreted with caution because there are many other alternative models that would explain the observed relationships equally well (e.g. adiponectin is the mediator of IGF-1 to MM or BMC or IGF-1 and MM or BMC cause adiponectin) (*26*). Thus, in many situations it is difficult to distinguish these alternatives without more information. Plausible mechanistic explanations derived from cell and animal studies could provide more certainty. In this context, our suggestion that the associations between higher adiponectin levels and lower MM or BMC in healthy older people might be explained by a decline in IGF-1 with increasing adiponectin levels is supported by mechanistic explanations showing decreased adiponectin mRNA levels by IGF-1 or insulin in *in vitro* experiments in cultured 3T3-L1 adipocytes (*27*) and decreased plasma levels of adiponectin in rats with infusion of recombinant human IGF-1 (*28*). Conducting a longitudinal study would be the best way to confirm our result. Examples are given in CHAPTER III (*23*).

Outlook

There is a great variety of techniques that can be used to measure MM but due to high costs and limited availability some techniques are more suitable for research than for clinical practice (*29*). The imaging technologies MRI and CT are gold standard methods for the assessment of MM because of their high accuracy (for a review see (*30*)) and precision (for a review see (*31*),

(*32*)). Even if these methods were used in clinical practice, large reference databases on sex-specific MRI or CT estimates of MM do not exist presently. DXA and bioelectrical impedance analysis (BIA) have been and are still being used in research and clinical settings. DXA is an alternative method which is used to determine appendicular lean soft tissue mass (ASM). However, it should be kept in mind that ASM is the sum of the bone-free fat-free mass plus skin and connective tissue resulting in a higher MM measured by DXA compared to muscle volume assessed by imaging technologies (*33*). The overestimation of MM increases with advanced age and obesity due to a higher contribution of connective tissue that can mask a reduction in MM at unchanged total ASM (for a review see (*15*)). These limitations may lead to an underestimation of the diagnosis of sarcopenia. With regards to BIA, available devices differ dependent on the reference methods that are used for the validation (e.g. MRI, DXA, four-compartment (4C) model) and thus in the BIA-algorithms and BIA muscle output parameters (e.g. MM, ASM or FFM) (for a review see (*6*), CHAPTER II). The choice of the reference methods can lead to significant differences in the measurement accuracy of distinct types of BIA-devices. For example, when compared with 4C model DXA was shown to provide systematically higher estimates of FFM (*34*). Thus, in order to allow better comparison of different study results, authors should indicate the manufacture, the device, the validation method and the software version of the BIA-device used. Body composition changes, and thus the risk of a low MM, are most prominent in older age. It is therefore an important advance that the BIA scale company *seca gmbh & co. kg.* (Hamburg, Germany) conducted a study to validate measures of BIA vs. reference methods in older adults. Nevertheless, for the diagnosis of sarcopenia in clinical practice, a user-friendly and easily realizable method would be preferable. Since in the present thesis results have suggested that the association between adiponectin and impaired mass of the musculoskeletal system is mediated by an age-related decrease in the anabolic hormone IGF-1 ((*23*), CHAPTER III), the implementation of IGF-1 as a biomarker for an adverse body composition in older people could be used for developing an attractive risk screening tool. At the admission to hospital, a blood test obtaining IGF-1 levels can be easily performed and thus supersede the technical difficulties in the assessment of MM and bone parameters. The availability of valid cut-offs for IGF-1 levels that are associated with a low muscle and bone mass is a prerequisite for this practical approach. If the cut-off value is reached by a person, further testing for sarcopenia can be applied like measuring HGS as a simple and inexpensive functional parameter, as recommended by the EWGSOP2 (*7*), or using BIA equipment that is affordable, portable and user-friendly (*7*).

Endocrine determinants of bone mass in healthy older adults derived from the crosstalk with muscle and adipose tissue

Main findings

EFFECTS OF AT ON BONE. Beside fat mass index (FMI, FM (kg)/height (m^2)), both subcutaneous and visceral AT (SAT, VAT) were strongly associated with lower bone mass and density in healthy community-dwelling adults. The negative effects of FM might be mediated by low-grade inflammation, higher leptin and lower adiponectin concentrations ((*35*), submitted, CHAPTER IV).

EFFECTS OF MM ON BONE. In contrast to AT, MM showed positive correlations with bone parameters. The osteo-protective effect of MM may be partly explained by increased mechanical loading ((*35*), submitted, CHAPTER IV). No associations were found between myokines and muscle or bone parameters.

AUTOCRINE EFFECTS OF BONE. Sclerostin levels showed positive correlations with bone mass and density, while osteocalcin concentrations were negatively associated with BMC, BMD and T-Score. Between sclerostin and osteocalcin a negative relationship was found that might explain the negative correlations between osteocalcin and bone parameters. In multiple regression analyses, osteocalcin was however a negative predictor of BMC independent of sclerostin ((*35*), submitted, CHAPTER IV).

State of research & critical reflection

EFFECTS OF AT ON BONE. There are many publications on the relationship between obesity and bone. Despite of a large variety of previously published study results showing a protective effect of obesity on bone (*36-39*), the traditional notion of an osteo-protective impact of obesity has come into question (for a review see (*40*)). Published epidemiological and clinical studies have indicated that high levels of FM exert negative effects on bone and bone health (*41-43*). For example, in a large cross-sectional study including 13,000 subjects, a higher risk of osteoporosis, osteopenia (defined by hip BMD) and non-spine fractures in individuals with a higher percentage of body fat was revealed after controlling for body weight, physical activity and age (*41*). Without adjustment for weight as confounder, a positive association between FM and bone mass was reported. In the same study, across 5 kg strata of body weight, FM was shown to be negatively associated with whole body BMC. These results implied that FM negatively affects BMD and BMC in contrast with the positive effect of total body weight itself (*41*), which could be confirmed in our study findings ((*35*), submitted, CHAPTER IV). The higher

bone mass in people with obesity can thus be explained by a larger body weight that induces greater mechanical loading effects on bone (*44, 45*), especially on the cortical elements (for a review see (*46*)). As underlying mechanisms, *in vitro* experiments have shown that in response to mechanical stimulation osteocytes inhibited osteoclast formation, released soluble factors that signal osteoclasts to decrease bone resorption (*47*) and stimulated osteoblastic differentiation (*48*). Moreover, an increased prevalence of osteoblast and osteocyte apoptosis was observed in mechanical unloaded mice, followed by bone resorption and loss of BMD and strength (*49*). No controlling for the mechanical loading effect of total body weight can therefore lead to biased results and to contradictory findings with regard to the relation between obesity (based on excessive fat accumulation) and bone (*41, 50, 51*).

In accordance to our findings highlighting a negative association between VAT and bone parameters ((*35*), submitted, CHAPTER IV), several studies have been published showing VAT as an independent negative predictor of BMD in obesity (*52-55*). Up to now, potential mechanisms by which VAT mediates a negative effect on bone are not fully elucidated. Some authors reported higher VAT levels to be correlated with lower bone formation markers (e.g. osteocalcin) (*56-58*) and decreased levels of the anabolic hormone IGF-1 in pre- and/or postmenopausal women (*59*) whereas in middle-aged men, higher amounts of VAT were associated with reduced growth hormone or testosterone concentrations (*53*). A study by Bredella and colleagues has demonstrated a positive association between VAT and vertebral bone marrow fat in premenopausal women with obesity (*59*). The authors also reported that higher vertebral bone marrow fat was associated with both lower vertebral BMD and serum IGF-1 levels. Latter correlated negatively with VAT and were a significant predictor of BMD and procollagen type 1 amino-terminal propeptide (*52*). These findings indicate that VAT exerts harmful effects on BMD through increased bone marrow fat and that IGF-I mediates the negative impact of VAT and marrow fat on bone via reduced bone formation markers (*52, 57, 59*). This hypothesis was supported by Cohen and colleagues (*57*). Our findings in both postmeno- and andropausal Caucasians with a wide BMI-range could not confirm the observed associations between VAT and osteocalcin, IGF-1 or BMD, possibly due to the heterogenous study populations and different adjustments made in the cited studies ((*35*), submitted, CHAPTER IV).

The negative relationships between SAT and bone parameters observed in our study ((*35*), submitted, CHAPTER IV) are in line with previous findings showing SAT to be independently and negatively related to BMD in postmenopausal women with severe obesity (*60*) and in white

and African American adults aged from 18 to 74 years (*54*). Contrary to this result, some authors showed a positive association but no results when controlling for body weight or lean body mass (e.g. (*61, 62*)). In a variety of CT studies, which distinguished between fat compartments, VAT was observed to be most strongly correlated with lower BMD (*52, 54, 63*). This is not in line with our findings demonstrating stronger negative associations between SAT and different bone parameters than VAT ((*35*), submitted, CHAPTER IV). Further research is needed to better understand the underlying mechanisms.

Regardless of FM, VAT or SAT, further mechanisms by which AT negatively affects bone health are (i) the release of pro-inflammatory cytokines from adipocytes (for a review see (*64*)), which play an important role in bone resorption by stimulating osteoclast activity (for a review see (*65*)) and (ii) the altered secretion of adipokines (*66, 67*). These findings are in line with our observations demonstrating that FMI and body fat compartments were positively associated with interleukin 6 and/or leptin levels which in turn showed a negative relationship to bone parameters in men ((*35*), submitted, CHAPTER IV). We also confirmed lower adiponectin concentrations with higher FMI and revealed adiponectin as a positive predictor of BMC ((*35*), submitted, CHAPTER IV). However, opposite results regarding the effect of adiponectin (*68, 69*) and leptin (*70*) on bone have also been reported in previously published studies. Possible explanations for the discrepancy as well as further examples by which AT can influence bone density and bone mass are given in CHAPTER IV ((*35*), submitted).

In our study, whole body BMD, BMC and T-Score were assessed by DXA (HOLOGIC Discovery A (S/N 82686), Inc., Bedford, MA, USA, software version 12.6.1:3, Hologic, Inc.). Up to now, DXA is considered the gold standard method for the measurement of BMD in clinical and research settings and is the most widely used technique for the diagnosis of osteoporosis and the evaluation of fracture risk (for reviews see (*30, 31*)). In addition to bone parameters, DXA systems provide whole body and regional estimates of lean soft mass and FM and are accepted as a non-invasive method for body composition analysis (for reviews see (*30, 31*)). The advantages of DXA include minimal training of operators, easy patient set-up, low radiation exposure (<10 µSv) (for a review see (*71*)), short scan time and a high precision (for a review see (*72*)). Besides high equipment costs, lack of portability and the rely on algorithm, the susceptibility to biased results due to fat accumulation is the major disadvantage of DXA (*73-77*). For example, Javed and colleagues showed that BMD of a beef femur progressively increased with increased thickness of fat surrounding the bone (*73*). When surrounded by 3 kg fat, the assessed BMD was overestimated by 20.5% in comparison to when bone was not

surrounded by fat (*73*). Increased BMD and BMC induced by exogenously added fat slices (lard) were also observed in 11 healthy subjects (BMD: +3.2% with 11.1 kg lard or +5.5% with 22.3 kg lard; BMC: +2.2% with 22.3 kg lard) (*74*). These results were in line with a previously conducted study among six adults showing a seven percent increase of total body BMC when 8.8 kg lard was positioned on the body (*75*). Contrary to this set of studies, exogenous fat layering was shown to significantly reduce DXA spine BMD (*76*) and whole body BMD (*77*) in humans. Differences between study results may be explained by the use of distinct DXA-devices, software versions, phantom configurations as well as the choice of measured sites (e.g. lumbar spine, whole body) and investigated target objects (human, spine phantom, cadaver). An additional source of error that might prevent accurate BMD measurements by DXA is the non-uniform distribution of extraosseous body fat and lean tissue (*78, 79*). Bolotin and colleagues showed that already small extraosseous soft tissue inhomogeneities within the region of interest of DXA scans lead to an increase in BMD inaccuracies that can be as large as 20-50%, especially in patients with osteopenia, osteoporosis and higher age (*78*). In summary, extraosseous fat (i) limits the accurate assessment of BMD and BMC in humans, animal models and phantom studies and (ii) leads to impeded reliable evaluations of bone status. Whether the percentage error includes an overestimation or an underestimation of BMD and BMC measurements in the case of overweight or obesity is still a continuing debate (*80*). Physicians and researchers should therefore be cautious in interpreting DXA results in patients with overweight or obesity. Moreover, to facilitate comparison between different study results, scientists should indicate the exact DXA device and software version. Since our study population also included subjects with overweight or obesity, we cannot exclude the possibility of biased measurements of BMD and BMC values and therefore of distorted results when bivariate correlations between AT and bone parameters were calculated.

EFFECTS OF MM ON BONE. Both, MM and SMI were positively associated with bone parameters ((*35*), submitted, CHAPTER IV). Our results are thereby in line with previous findings showing moderate to strong correlations (*81-83*). Up to now, there are conflicting results if MM or FM is the stronger predictor for bone density (for a review see (*46*)). A variety of previously published studies indicate that the effect of mechanical loading on bone is determined by lean mass rather than by FM (*84-86*). These observations are contrary to our findings showing stronger correlations between FMI and bone parameters in men and women after controlling for body weight ((*35*), submitted, CHAPTER IV). The discrepancy could be due to the different measurement approaches of body composition. In our study, MM and FM were assessed by whole body MRI ((*35*), submitted, CHAPTER IV), whereas in other analyses DXA was

performed. As mentioned above, the use of DXA is not without limitation, since DXA measures not the pure MM but lean mass as a proxy for MM. This results in an overestimation of skeletal MM when compared to muscle volume assessed by MRI (*33*). Thus, further studies, in which MRI is used for body composition analysis, are needed to confirm our results. Additional explanations for the inconsistent findings may be the inadequately powered study (*86*), the heterogenous study populations (age, ethnic groups, sex) as well as the measurement sites (*87*).

AUTOCRINE EFFECTS OF BONE. In cell culture (*88, 89*) and animal studies (*90-92*) sclerostin was demonstrated to be a negative regulator of bone metabolism, while osteocalcin was shown to exert positive or negative effects on bone (for reviews see (*93-95*)). The observed negative effects of sclerostin as well as the positive effects of osteocalcin are not in line with our results ((*35*), submitted, CHAPTER IV) and also with previous findings in pre- and postmenopausal women and men (*96-98*) showing exactly the opposite effects of sclerostin and osteocalcin on BMD and BMC. Differences in results could be explained by first, the choice of distinct assays for measuring circulating sclerostin and osteocalcin. Second, only total hormone concentrations were measured. In the case of sclerostin, hormone measurement might include both active protein and biomarkers of osteocyte activity (for a review see (*95*)). Third, the discrepancy in results might be further explained by the cross-sectional study design. Since there is no evidence for causal inference between the observed correlations, this type of study constitutes a potential hazard for distorted results. The best way to prove causality in research is by conducting an experiment, e.g. by randomized controlled trials (RCT), but ethical or practical reasons can complicate their realization (for a review see (*99*)). Knowledge must then be acquired via observational studies. Throughout the past decades, advances have been made in theory and methods of causal inference, which have contributed to a better understanding and avoidance of bias in cross-sectional study types (for a review see (*100*)). A number of factors distorting the results of observational studies exists, whereby distortion by '*confounding bias*' is the most familiar problem in research (*101*). Confounding bias occurs *'[…] when an apparently causal relationship between an exposure […] and an outcome is, in reality, distorted by the effect of a third variable (the confounder)'* (*101*). The bias can lead to an under- or overestimation of the exposure effect and to a reversal of the apparent direction of the effect. Hence, (i) confounding factors have to be identified and eliminated early when studies are planned or (ii) adjustments for potential confounders need to be made after the data gathering process reducing the bias effects from the final results. Prerequisite for this approach is the knowledge of possible confounders, which can pose a challenge to researchers. As epidemiological data demonstrate that higher body weight is correlated with higher bone density (*102*) and that body weight

reductions might cause bone loss (*103*), all significant correlations between bone parameters and muscle tissue, fat tissue or endocrine determinants were controlled for total body weight in our data analysis ((*35*), submitted, CHAPTER IV). Since our analysis was a secondary analysis, lifestyle factors that affect bone health were not determined. Physical activity, calcium and protein intake, vitamin D supplementation, smoking and drinking habits are, however, relevant factors for bone metabolism and thus potential confounders. Future analyses of the interactions within and between bone, muscle, AT and endocrine determinants with ageing should therefore consider dietary and exercise patterns for adjustments. A second factor leading to distorted results in cross-sectional studies is the so-called '*collider bias*' (for a review see (*100*)). The term '*collider*' describes *'[...] a variable that is caused by at least two other variables (the causing variables 'collide' in the collider)'* (for a review see (*100*)). An example with regard to our study 'Analysis of the adiponectin paradox in healthy older people' (CHAPTER III, (*23*)) is represented in **Figure 3**. If adiponectin would be negatively affected by both IGF-1 (exposure) and MM (outcome), adiponectin would be a collider for the association between IGF-1 and MM and not a confounder. The differentiation between confounder and collider is essential because methods correcting for confounding (e.g. regression analysis) can lead to bias if they are applied to colliders (for a review see (*100*)).

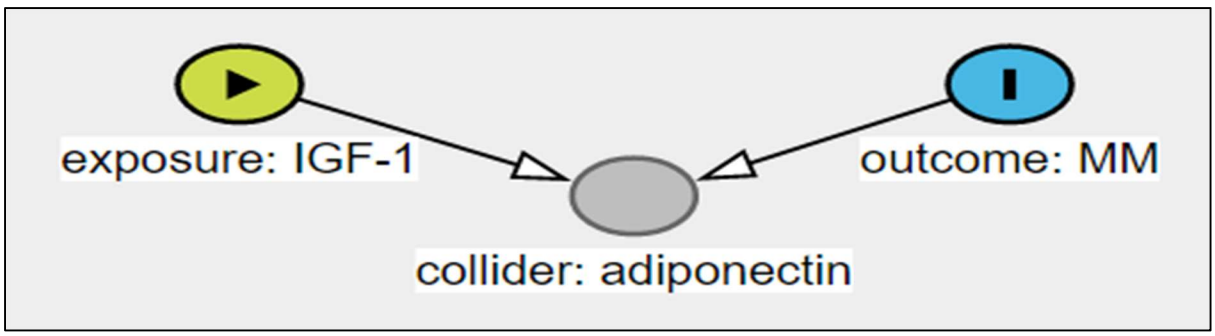


Figure 3. Graph depicting the hypothetical collider adiponectin caused by IGF-1 and MM. Arrows: causal relationships between the variables. IGF-1, insulin-like growth factor 1; MM, muscle mass. Graph was created by the software DAGitty, available free at www.dagitty.net.

While evidence exists for the causal negative relationship between IGF-1 and adiponectin *in vitro* (*27*) and *in vivo* (*28*), it is possible that higher adiponectin levels result from a low MM and might be therefore interpreted as a starvation signal. Since our study population was healthy and non-malnourished, we considered this assumption to be unlikely (CHAPTER III, (*23*)). Thus, we were able to reject the hypothesis of adiponectin as a collider. While confounding is a well-known problem that is habitually considered in the study analysis, bias due to colliders has

received little attention in research up to now (for a review see (*100*)). This circumstance can lead to wrong or paradoxical associations between variables. For a better understanding, a hypothetical example is given in **Figure 4**. There is a direct negative causal association between age and MM and an indirect causal relationship mediated by higher adiponectin levels that is due to increased age. Lower IGF-1 concentrations are associated with higher adiponectin levels and lower MM. Although there is no relation between IGF-1 and age, this relationship is evoked when conditioning is introduced on the collider 'adiponectin', since both IGF-1 and age influence adiponectin levels (example was inspired by: (*100*)).

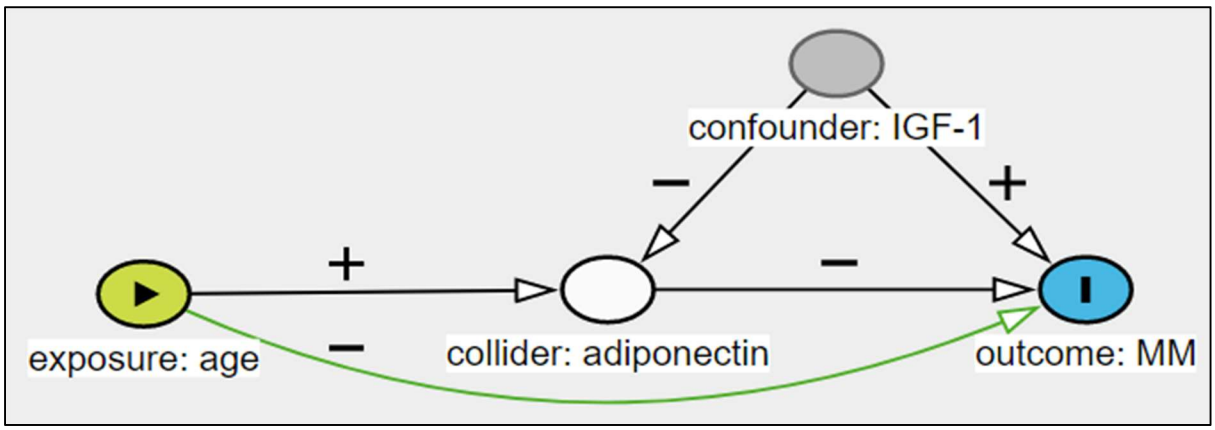


Figure 4. Graph depicting confounder and collider bias (hypothetical). Arrows: causal relationships between the variables. +, positive association; -, negative association. Green arrow: direct causal relationship between exposure and outcome. IGF-1, insulin-like growth factor 1; MM, muscle mass. Graph was created by the software DAGitty, available free at www.dagitty.net.

Up to now, no universal statistical methods correcting collider bias exist (for a review see (*100*)). It is therefore all the more important to identify potential sources for a collider at an early stage so that these can be considered during the data collection process (for a review see (*100*)). In this regard, creating graphs via software (as illustrate in **Figure 4**) representing causal relationships between variables could be a helpful tool (one example for such a software: DAGitty, available free at www.dagitty.net, for a review see (*100*)).

Outlook

First, as already stated, more lifestyle factors that are known to influence bone density and bone mass should be taken into account in further analyses. Second, instead of measuring total hormone concentrations, different (iso-)forms have to be considered, since they possibly exert different physiological functions on target tissues. Third, the investigation of a longitudinal relationship between changes in body composition and selected endocrine determinants is

preferred over a single measurement. This approach allows to determine the extent to which concentrations of hormones influence bone, muscle and AT. Finally, well-controlled longitudinal studies will be necessary to reveal reliable results of endocrine determinants of bone mass derived from the crosstalk with muscle and adipose tissue. The implementation of an antibody-based intervention study could be a useful strategy. Only recently, a novel potential human monoclonal antibody called 'bimagrumab' (BYM338; Novartis) was developed (*104*). Bimagrumab blocks activin type II receptors and prevents binding and activity of negative muscle regulators, e.g. the muscle-derived myostatin, and is thus considered as myostatin inhibitor (*104*). In preclinical studies, single and multiple doses of bimagrumab have been observed to increase MM (*105*) and lean mass (*106-109*) while decreasing FM (*107-109*). The results indicate that myostatin might be a potential target in the treatment of both sarcopenia and obesity (*107*). Myostatin not only plays a role in muscle and fat, but has also been observed to negatively affect bone metabolism *in vitro* ((*110-112*), for a review see (*113*)) and in animals (*110, 111, 114, 115*), whereas findings on the negative relationship between myostatin and bone in humans are limited (for a review see (*113*)). Therefore, as a complementation to our cross-sectional study and also to previous studies that only investigated the effect of bimagrumab on MM and FM, a double-masked, placebo-controlled RCT in community-dwelling adults with osteosarcopenic obesity aged 65 or older could be conducted, where adults receive either bimagrumab or placebo in a given period of time 'x'. As primary end points the change from baseline to week 'x' in bone density and bone mass could be measured by DXA. As secondary endpoints MM, FM (via MRI), body weight and hormone levels changes as well as the prevalence of fractures could be determined. This study design could allow to clarify the role of myostatin in the 'bone-muscle-fat crosstalk' providing a new pathway for the pharmacological management of osteosarcopenic obesity. Besides bimagrumab, anti-sclerostin antibodies like 'romosozumab' (AMG 785) could be examined in future RCTs to investigate the observed paradoxical association between sclerostin and bone parameters in our study ((*35*), submitted, CHAPTER IV). Romosozumab was the first human monoclonal antibody against sclerostin demonstrating to increase bone formation and BMD in animal and human studies (for reviews see (*116, 117*)).

References

1. Szlejf, C.; Parra-Rodríguez, L.; Rosas-Carrasco, O. Osteosarcopenic Obesity: Prevalence and Relation With Frailty and Physical Performance in Middle-Aged and Older Women. *Journal of the American Medical Directors Association* **2017,** *18* (8), 733.e1-733.e5. DOI: 10.1016/j.jamda.2017.02.023.
2. Ilich, J. Z.; Inglis, J. E.; Kelly, O. J.; McGee, D. L. Osteosarcopenic obesity is associated with reduced handgrip strength, walking abilities, and balance in postmenopausal women. *Osteoporosis International* **2015,** *26* (11), 2587–2595. DOI: 10.1007/s00198-015-3186-y.
3. Ma, X.-Y.; Liu, H.-M.; Lv, W.-Q.; Qiu, C.; Xiao, H.-M.; Deng, H.-W. A bi-directional Mendelian randomization study of the sarcopenia-related traits and osteoporosis. *Aging* **2022,** *14* (14), 5681–5698. DOI: 10.18632/aging.204145.
4. Yeung, S. S. Y.; Reijnierse, E. M.; Pham, V. K.; Trappenburg, M. C.; Lim, W. K.; Meskers, C. G. M.; Maier, A. B. Sarcopenia and its association with falls and fractures in older adults: A systematic review and meta-analysis. *Journal of Cachexia, Sarcopenia and Muscle* **2019,** *10* (3), 485–500. DOI: 10.1002/jcsm.12411.
5. Iida, H.; Seki, T.; Sakai, Y.; Watanabe, T.; Wakao, N.; Matsui, H.; Imagama, S. Low muscle mass affect hip fracture treatment outcomes in older individuals: A single-institution case-control study. *BMC Musculoskeletal Disorders* **2021,** *22* (1), 259. DOI: 10.1186/s12891-021-04143-6.
6. Walowski, C. O.; Braun, W.; Maisch, M. J.; Jensen, B.; Peine, S.; Norman, K.; Müller, M. J.; Bosy-Westphal, A. Reference Values for Skeletal Muscle Mass - Current Concepts and Methodological Considerations. *Nutrients* **2020,** *12* (3). DOI: 10.3390/nu12030755.
7. Cruz-Jentoft, A. J.; Bahat, G.; Bauer, J.; Boirie, Y.; Bruyère, O.; Cederholm, T.; Cooper, C.; Landi, F.; Rolland, Y.; Sayer, A.A. *et al*. Sarcopenia: Revised European consensus on definition and diagnosis. *Age and Ageing* **2019,** *48* (1), 16–31. DOI: 10.1093/ageing/afy169.
8. Sayer, A. A.; Syddall, H. E.; Martin, H. J.; Dennison, E. M.; Roberts, H. C.; Cooper, C. Is grip strength associated with health-related quality of life? Findings from the Hertfordshire Cohort Study. *Age and Ageing* **2006,** *35* (4), 409–415. DOI: 10.1093/ageing/afl024.
9. Gale, C. R.; Martyn, C. N.; Cooper, C.; Sayer, A. A. Grip strength, body composition, and mortality. *International Journal of Epidemiology* **2007,** *36* (1), 228–235. DOI: 10.1093/ije/dyl224.
10. Leong, D. P.; Teo, K. K.; Rangarajan, S.; Lopez-Jaramillo, P.; Avezum, A.; Orlandini, A.; Seron, P.; Ahmed, S. H.; Rosengren, A.; Kelishadi, R. *et al*. Prognostic value of grip strength: findings from the Prospective Urban Rural Epidemiology (PURE) study. *Lancet* **2015,** *386* (9990), 266–273. DOI: 10.1016/S0140-6736(14)62000-6.
11. Enderle, J.; Reljic, D.; Jensen, B.; Peine, S.; Zopf, Y.; Bosy-Westphal, A. Normal Values for Body Composition in Adults are better represented by Continuous Reference Ranges dependent on Age and BMI (*in revision*, **2023***)*.
12. Prado, C. M. M.; Siervo, M.; Mire, E.; Heymsfield, S. B.; Stephan, B. C. M.; Broyles, S.; Smith, S. R.; Wells, J. C. K.; Katzmarzyk, P. T. A population-based approach to define body-composition phenotypes. *The American Journal of Clinical Nutrition* **2014,** *99* (6), 1369–1377. DOI: 10.3945/ajcn.113.078576.

13. Volpi, E.; Nazemi, R.; Fujita, S. Muscle tissue changes with aging. *Current Opinion in Clinical Nutrition and Metabolic Care* **2004,** *7* (4), 405–410. DOI: 10.1097/01.mco.0000134362.76653.b2.

14. Kyle, U. G.; Genton, L.; Hans, D.; Karsegard, L.; Slosman, D. O.; Pichard, C. Age-related differences in fat-free mass, skeletal muscle, body cell mass and fat mass between 18 and 94 years. *European Journal of Clinical Nutrition* **2001,** *55* (8), 663–672. DOI: 10.1038/sj.ejcn.1601198.

15. Bosy-Westphal, A.; Müller, M. J. Identification of skeletal muscle mass depletion across age and BMI groups in health and disease--there is need for a unified definition. *International Journal of Obesity* **2015,** *39* (3), 379–386. DOI: 10.1038/ijo.2014.161.

16. Gallagher, D.; Ruts, E.; Visser, M.; Heshka, S.; Baumgartner, R. N.; Wang, J.; Pierson, R. N.; Pi-Sunyer, F. X.; Heymsfield, S. B. Weight stability masks sarcopenia in elderly men and women. *American Journal of Physiology, Endocrinology and Metabolism* **2000,** *279* (2), E366-375. DOI: 10.1152/ajpendo.2000.279.2.E366.

17. Gallagher, D.; Visser, M.; Meersman, R. E. de; Sepúlveda, D.; Baumgartner, R. N.; Pierson, R. N.; Harris, T.; Heymsfield, S. B. Appendicular skeletal muscle mass: effects of age, gender, and ethnicity. *Journal of Applied Physiology* **1997,** *83* (1), 229–239. DOI: 10.1152/jappl.1997.83.1.229.

18. Mitchell, W. K.; Williams, J.; Atherton, P.; Larvin, M.; Lund, J.; Narici, M. Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength; a quantitative review. *Frontiers in Physiology* **2012,** *3. DOI:* 10.3389/fphys.2012.00260.

19. Janssen, I.; Heymsfield, S. B.; Wang, Z.; Ross, R. Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. *Journal of Applied Physiology* **2000,** *89* (1), 81–88. DOI: 10.1152/jappl.2000.89.1.81.

20. Heymsfield, S. B.; Gonzalez, M. C. C.; Shen, W.; Redman, L.; Thomas, D. Weight loss composition is one-fourth fat-free mass: a critical review and critique of this widely cited rule. *Obesity Reviews* **2014,** *15* (4), 310–321. DOI: 10.1111/obr.12143.

21. Delmonico, M. J.; Harris, T. B.; Visser, M.; Park, S. W.; Conroy, M. B.; Velasquez-Mieyer, P.; Boudreau, R.; Manini, T. M.; Nevitt, M.; Newman, A. B.; Goodpaster, B. H. Longitudinal study of muscle strength, quality, and adipose tissue infiltration. *The American Journal of Clinical Nutrition* **2009,** *90* (6), 1579–1585. DOI: 10.3945/ajcn.2009.28047.

22. Silva, A. M.; Shen, W.; Heo, M.; Gallagher, D.; Wang, Z.; Sardinha, L. B.; Heymsfield, S. B. Ethnicity-related skeletal muscle differences across the lifespan. *American Journal of Human Biology* **2010,** *22* (1), 76–82. DOI: 10.1002/ajhb.20956.

23. Walowski, C. O.; Herpich, C.; Enderle, J.; Braun, W.; Both, M.; Hasler, M.; Müller, M. J.; Norman, K.; Bosy-Westphal, A. Analysis of the adiponectin paradox in healthy older people. *Journal of Cachexia, Sarcopenia and Muscle* **2022,** *14* (1), 270–278. DOI: 10.1002/jcsm.13127.

24. Sattar, N.; Nelson, S. M. Adiponectin, diabetes, and coronary heart disease in older persons: unraveling the paradox. *The Journal of Clinical Endocrinology and Metabolism* **2008,** *93* (9), 3299–3301. DOI: 10.1210/jc.2008-1435.

25. Baker, J. F.; Newman, A. B.; Kanaya, A.; Leonard, M. B.; Zemel, B.; Miljkovic, I.; Long, J.; Weber, D.; Harris, T. B. The Adiponectin Paradox in the Elderly: Associations With Body Composition, Physical Functioning, and Mortality. *The Journals of Gerontology* **2019,** *74* (2), 247–253. DOI: 10.1093/gerona/gly017.

26. MacKinnon, D. P. (**2008**). *Introduction to Statistical Mediation Analysis.;* New York, NY: Tayler & Francis Group/Lawrence Erlbaum Associates.

27. Lam, K. S.-L.; Xu, A.; Tan, K. C.-B.; Wong, L.-C.; Tiu, S.-C.; Tam, S. Serum adiponectin is reduced in acromegaly and normalized after correction of growth hormone excess. *The Journal of Clinical Endocrinology and Metabolism* **2004,** *89* (11), 5448–5453. DOI: 10.1210/jc.2003-032023.

28. Yamaza, H.; Komatsu, T.; To, K.; Toyama, H.; Chiba, T.; Higami, Y.; Shimokawa, I. Involvement of insulin-like growth factor-1 in the effect of caloric restriction: regulation of plasma adiponectin and leptin. *The Journals of Gerontology* **2007,** *62* (1), 27–33. DOI: 10.1093/gerona/62.1.27.

29. Cruz-Jentoft, A. J.; Baeyens, J. P.; Bauer, J. M.; Boirie, Y.; Cederholm, T.; Landi, F.; Martin, F. C.; Michel, J.-P.; Rolland, Y.; Schneider, S. M. *et al*. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age and Ageing* **2010,** *39* (4), 412–423. DOI: 10.1093/ageing/afq034.

30. Lee, S. Y.; Gallagher, D. Assessment methods in human body composition. *Current Opinion in Clinical Nutrition and Metabolic Care* **2008,** *11* (5), 566–572. DOI: 10.1097/MCO.0b013e32830b5f23.

31. Müller, M. J.; Braun, W.; Pourhassan, M.; Geisler, C.; Bosy-Westphal, A. Application of standards and models in body composition analysis. *The Proceedings of the Nutrition Society* **2016,** *75* (2), 181–187. DOI: 10.1017/S0029665115004206.

32. Schautz, B.; Later, W.; Heller, M.; Peters, A.; Müller, M. J.; Bosy-Westphal, A. Impact of age on leptin and adiponectin independent of adiposity. The *British Journal of Nutrition* **2012,** *108* (2), 363–370. DOI: 10.1017/S0007114511005605.

33. Bosy-Westphal, A.; Jensen, B.; Braun, W.; Pourhassan, M.; Gallagher, D.; Müller, M. J. Quantification of whole-body and segmental skeletal muscle mass using phase-sensitive 8-electrode medical bioelectrical impedance devices. *European Journal of Clinical Nutrition* **2017,** *71* (9), 1061–1067. DOI: 10.1038/ejcn.2017.27.

34. Tylavsky, F.; Lohman, T., Blunt, B.A.; Schoeller, D.A.; Fuerst, T.; Cauley, J.A.; Nevitt, M.C.; Visser, M.; Harris, T.B. QDR 4500 DXA overestimates fat-free mas compared with criterion methods. *Journal of Applied Physiology* **2003,** *94* (3), 959–965. DOI: 10.1152/japplphysiol.00732.2002.

35. Walowski, C. O.; Herpich, C.; Enderle, J.; Braun Wiebke; Both, M.; Hasler, M.; Müller, J. M.; Norman, K.; Bosy-Westphal, A. Determinants of bone mass in healthy older adults derived from the crosstalk with muscle and adipose tissue (*submitted*, ***2023)***.

36. Albala, C.; Yáñez, M.; Devoto, E.; Sostin, C.; Zeballos, L.; Santos, J. L. Obesity as a protective factor for postmenopausal osteoporosis. *International Journal of Obesity and Related Metabolic Disorders* **1996,** *20* (11), 1027–1032.

37. Maïmoun, L.; Mura, T.; Leprieur, E.; Avignon, A.; Mariano-Goulart, D.; Sultan, A. Impact of obesity on bone mass throughout adult life: Influence of gender and severity of obesity. *Bone* **2016,** *90*, 23–30. DOI: 10.1016/j.bone.2015.11.020.

38. Salamat, M. R.; Salamat, A. H.; Janghorbani, M. Association between Obesity and Bone Mineral Density by Gender and Menopausal Status. *Endocrinology and Metabolism* **2016,** *31* (4), 547–558. DOI: 10.3803/EnM.2016.31.4.547.

39. Santos, V. R. D.; Christofaro, D. G. D.; Gomes, I. C.; Júnior, I. F. F.; Gobbo, L. A. Relationship between obesity, sarcopenia, sarcopenic obesity, and bone mineral density in elderly subjects aged 80 years and over. *Revista brasileira de ortopedia* **2018,** *53* (3), 300–305. DOI: 10.1016/j.rboe.2017.09.002.

40. Mendonça, F. M.; Soares, R.; Carvalho, D.; Freitas, P. The impact of obesity on bone health: an overview. *Endokrynologia Polska* **2022,** *73* (6), 954–958. DOI: 10.5603/EP.a2022.0063.

41. Hsu, Y.-H.; Venners, S. A.; Terwedow, H. A.; Feng, Y.; Niu, T.; Li, Z.; Laird, N.; Brain, J. D.; Cummings, S. R.; Bouxsein, M. L. *et al*. Relation of body composition, fat mass, and serum lipids to osteoporotic fractures and bone mineral density in Chinese men and women. *The American Journal of Clinical Nutrition* **2006,** *83* (1), 146–154. DOI: 10.1093/ajcn/83.1.146.

42. Kim, K.-C.; Shin, D.-H.; Lee, S.-Y.; Im, J.-A.; Lee, D.-C. Relation between obesity and bone mineral density and vertebral fractures in Korean postmenopausal women. *Yonsei Medical Journal* **2010,** *51* (6), 857–863. DOI: 10.3349/ymj.2010.51.6.857.

43. Greco, E. A.; Fornari, R.; Rossi, F.; Santiemma, V.; Prossomariti, G.; Annoscia, C.; Aversa, A.; Brama, M.; Marini, M.; Donini, L. M. *et al*. Is obesity protective for osteoporosis? Evaluation of bone mineral density in individuals with high body mass index. *International Journal of Clinical Practice* **2010,** *64* (6), 817–820. DOI: 10.1111/j.1742-1241.2009.02301.x.

44. Etherington, J.; Harris, P. A.; Nandra, D.; Hart, D. J.; Wolman, R. L.; Doyle, D. V.; Spector, T. D. The effect of weight-bearing exercise on bone mineral density: a study of female ex-elite athletes and the general population. *Journal of Bone and Mineral Research* **1996,** *11* (9), 1333–1338. DOI: 10.1002/jbmr.5650110918.

45. Vainionpää, A.; Korpelainen, R.; Leppäluoto, J.; Jämsä, T. Effects of high-impact exercise on bone mineral density: a randomized controlled trial in premenopausal women. *Osteoporosis international* **2005,** *16* (2), 191–197. DOI: 10.1007/s00198-004-1659-5.

46. Rosen, C. J.; Bouxsein, M. L. Mechanisms of disease: is osteoporosis the obesity of bone? *Nature Clinical Practice. Rheumatology* **2006,** *2* (1), 35–43. DOI: 10.1038/ncprheum0070.

47. Tan, S. D.; Vries, T. J. de; Kuijpers-Jagtman, A. M.; Semeins, C. M.; Everts, V.; Klein-Nulend, J. Osteocytes subjected to fluid flow inhibit osteoclast formation and bone resorption. *Bone* **2007,** *41* (5), 745–751. DOI: 10.1016/j.bone.2007.07.019.

48. Vezeridis, P. S.; Semeins, C. M.; Chen, Q.; Klein-Nulend, J. Osteocytes subjected to pulsating fluid flow regulate osteoblast proliferation and differentiation. *Biochemical and Biophysical Research Communications* **2006,** *348* (3), 1082–1088. DOI: 10.1016/j.bbrc.2006.07.146.

49. Aguirre, J. I.; Plotkin, L. I.; Stewart, S. A.; Weinstein, R. S.; Parfitt, A. M.; Manolagas, S. C.; Bellido, T. Osteocyte apoptosis is induced by weightlessness in mice and precedes osteoclast recruitment and bone loss. *Journal of Bone and Mineral Research* **2006,** *21* (4), 605–615. DOI: 10.1359/jbmr.060107.

50. Reid, D. M. (**1993**) *Osteoporosis*; *Baillière's Clinical Rheumatology*. Volume 7, Issue 3. Baillière Tindall London.

51. Liu, Y.; Xu, Y.; Wen, Y.; Guan, K.; Ling, W.; He, L.; Su, Y.; Chen, Y. Association of weight-adjusted body fat and fat distribution with bone mineral density in middle-aged chinese adults: a cross-sectional study. *PLoS ONE* **2013,** *8* (5), e63339. DOI: 10.1371/journal.pone.0063339.

52. Bredella, M. A.; Torriani, M.; Ghomi, R. H.; Thomas, B. J.; Brick, D. J.; Gerweck, A. V.; Harrington, L. M.; Breggia, A.; Rosen, C. J.; Miller, K. K. Determinants of bone mineral density in obese premenopausal women. *Bone* **2011,** *48* (4), 748–754. DOI: 10.1016/j.bone.2010.12.011.

53. Bredella, M. A.; Lin, E.; Gerweck, A. V.; Landa, M. G.; Thomas, B. J.; Torriani, M.; Bouxsein, M. L.; Miller, K. K. Determinants of bone microarchitecture and mechanical properties in obese men. *The Journal of Clinical Endocrinology and Metabolism* **2012,** *97* (11), 4115–4122. DOI: 10.1210/jc.2012-2246.

54. Katzmarzyk, P. T.; Barreira, T. V.; Harrington, D. M.; Staiano, A. E.; Heymsfield, S. B.; Gimble, J. M. Relationship between abdominal fat and bone mineral density in white and African American adults. *Bone* **2012,** *50* (2), 576–579. DOI: 10.1016/j.bone.2011.04.012.

55. Sharma, D. K.; Anderson, P. H.; Morris, H. A.; Clifton, P. M. Visceral Fat Is a Negative Determinant of Bone Health in Obese Postmenopausal Women. *International Journal of Environmental Research and Public Health* **2020,** *17* (11), 3996. DOI: 10.3390/ijerph17113996.

56. Lee, S. W.; Jo, H. H.; Kim, M. R.; You, Y. O.; Kim, J. H. Association between obesity, metabolic risks and serum osteocalcin level in postmenopausal women. *Gynecological Endocrinology* **2012**, *28* (6), 472–477. DOI: 10.3109/09513590.2011.633660.

57. Cohen, A.; Dempster, D. W.; Recker, R. R.; Lappe, J. M.; Zhou, H.; Zwahlen, A.; Müller, R.; Zhao, B.; Guo, X.; Lang, T. *et al*. Abdominal fat is associated with lower bone formation and inferior bone quality in healthy premenopausal women: a transiliac bone biopsy study. *The Journal of Clinical Endocrinology and Metabolism* **2013,** *98* (6), 2562–2572. DOI: 10.1210/jc.2013-1047.

58. Luo, Y.; Ma, X.; Hao, Y.; Xu, Y.; Xiong, Q.; Tang, J.; Pan, X.; Xiao, Y.; Bao, Y.; Jia, W. Association between serum osteocalcin level and visceral obesity in Chinese postmenopausal women. *Clinical Endocrinology* **2015,** *83* (3), 429–434. DOI: 10.1111/cen.12793.

59. Bredella, M. A.; Torriani, M.; Ghomi, R. H.; Thomas, B. J.; Brick, D. J.; Gerweck, A. V.; Rosen, C. J.; Klibanski, A.; Miller, K. K. Vertebral bone marrow fat is positively associated with visceral fat and inversely associated with IGF-1 in obese women. *Obesity* **2011,** *19* (1), 49–53. DOI: 10.1038/oby.2010.106.

60. Crivelli, M.; Chain, A.; da Silva, I. T. F.; Waked, A. M.; Bezerra, F. F. Association of Visceral and Subcutaneous Fat Mass With Bone Density and Vertebral Fractures in Women With Severe Obesity. *Journal of Clinical Densitometry* **2021,** *24* (3), 397–405. DOI: 10.1016/j.jocd.2020.10.005.

61. Dolan, S. E.; Carpenter, S.; Grinspoon, S. Effects of weight, body composition, and testosterone on bone mineral density in HIV-infected women. *Journal of Acquired Immune Deficiency Syndromes* **2007,** *45* (2), 161–167. DOI: 10.1097/QAI.0b013e31804a7f4d.

62. Gilsanz, V.; Chalfant, J.; Mo, A. O.; Lee, D. C.; Dorey, F. J.; Mittelman, S. D. Reciprocal relations of subcutaneous and visceral fat to bone structure and strength. *The Journal of Clinical Endocrinology and Metabolism* **2009,** *94* (9), 3387–3393. DOI: 10.1210/jc.2008-2422.

63. Choi, H. S.; Kim, K. J.; Kim, K. M.; Hur, N. W.; Rhee, Y.; Han, D. S.; Lee, E. J.; Lim, S.-K. Relationship between visceral adiposity and bone mineral density in Korean adults. *Calcif Tissue International* **2010,** *87* (3), 218–225. DOI: 10.1007/s00223-010-9398-4.

64. Coppack, S. W. Pro-inflammatory cytokines and adipose tissue. *The Proceedings of the Nutrition Society* **2001,** *60* (3), 349–356. DOI: 10.1079/pns2001110.

65. Terkawi, M. A.; Matsumae, G.; Shimizu, T.; Takahashi, D.; Kadoya, K.; Iwasaki, N. Interplay between Inflammation and Pathological Bone Resorption: Insights into Recent Mechanisms and Pathways in Related Diseases for Future Perspectives. *International Journal of Molecular Sciences* **2022,** *23* (3), 1786. DOI: 10.3390/ijms23031786.

66. Cnop, M.; Havel, P. J.; Utzschneider, K. M.; Carr, D. B.; Sinha, M. K.; Boyko, E. J.; Retzlaff, B. M.; Knopp, R. H.; Brunzell, J. D.; Kahn, S. E. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia* **2003,** *46* (4), 459–469. DOI: 10.1007/s00125-003-1074-z.

67. Gariballa, S.; Alkaabi, J.; Yasin, J.; Al Essa, A. Total adiponectin in overweight and obese subjects and its response to visceral fat loss. *BMC Endocrine Disorders* **2019,** *19* (1), 55. DOI: 10.1186/s12902-019-0386-z.

68. Richards, J. B.; Valdes, A. M.; Burling, K.; Perks, U. C.; Spector, T. D. Serum adiponectin and bone mineral density in women. *The Journal of Clinical Endocrinology and Metabolism* **2007,** *92* (4), 1517–1523. DOI: 10.1210/jc.2006-2097.

69. Araneta, M. R. G.; Mühlen, D. von; Barrett-Connor, E. Sex differences in the association between adiponectin and BMD, bone loss, and fractures: the Rancho Bernardo study. *Journal of Bone and Mineral Research* **2009,** *24* (12), 2016–2022. DOI: 10.1359/jbmr.090519.

70. Blain, H.; Vuillemin, A.; Guillemin, F.; Durant, R.; Hanesse, B.; Talance, N. de; Doucet, B.; Jeandel, C. Serum leptin level is a predictor of bone mineral density in postmenopausal women. *The Journal of Clinical Endocrinology and Metabolism* **2002,** *87* (3), 1030–1035. DOI: 10.1210/jcem.87.3.8313.

71. Njeh, C. F.; Fuerst, T.; Hans, D.; Blake, G. M.; Genant, H. K. Radiation exposure in bone mineral density assessment. *Applied Radiation and Isotopes* **1999,** *50* (1), 215–236. DOI: 10.1016/s0969-8043(98)00026-8.

72. Blake, G. M.; Fogelman, I. The clinical role of dual energy X-ray absorptiometry. *European Journal of Radiology* **2009,** *71* (3), 406–414. DOI: 10.1016/j.ejrad.2008.04.062.

73. Javed, F.; Yu, W.; Thornton, J.; Colt, E. Effect of fat on measurement of bone mineral density. *International Journal of Body Composition Research* **2009,** *7* (1), 37–40.

74. Madsen, O. R.; Jensen, J. E.; Sørensen, O. H. Validation of a dual energy X-ray absorptiometer: measurement of bone mass and soft tissue composition. *European Journal of Applied Physiology and Occupational Physiology* **1997,** *75* (6), 554–558. DOI: 10.1007/s004210050204.

75. Svendsen, O. L.; Hassager, C.; Skødt, V.; Christiansen, C. Impact of soft tissue on in vivo accuracy of bone mineral measurements in the spine, hip, and forearm: a human cadaver study. *Journal of Bone and Mineral Research* **1995,** *10* (6), 868–873. DOI: 10.1002/jbmr.5650100607.

76. Yu, E. W.; Thomas, B. J.; Brown, J. K.; Finkelstein, J. S. Simulated increases in body fat and errors in bone mineral density measurements by DXA and QCT. *Journal of Bone and Mineral Research* **2012,** *27* (1), 119–124. DOI: 10.1002/jbmr.506.

77. Evans, E. M.; Mojtahedi, M. C.; Kessinger, R. B.; Misic, M. M. Simulated change in body fatness affects Hologic QDR 4500A whole body and central DXA bone measures. *Journal of Clinical Densitometry* **2006,** *9* (3), 315–322. DOI: 10.1016/j.jocd.2006.04.117.

78. Bolotin, H. H.; Sievänen, H.; Grashuis, J. L. Patient-specific DXA bone mineral density inaccuracies: quantitative effects of nonuniform extraosseous fat distributions. *Journal of Bone and Mineral Research* **2003,** *18* (6), 1020–1027. DOI: 10.1359/jbmr.2003.18.6.1020.

79. Tothill, P.; Weir, N.; Loveland, J. Errors in dual-energy X-ray scanning of the hip because of nonuniform fat distribution. *Journal of Clinical Densitometry* **2014,** *17* (1), 91–96. DOI: 10.1016/j.jocd.2013.02.008.

80. Cherif, R.; Vico, L.; Laroche, N.; Sakly, M.; Attia, N.; Lavet, C. Dual-energy X-ray absorptiometry underestimates in vivo lumbar spine bone mineral density in overweight rats. *Journal of Bone and Mineral Metabolism* **2018,** *36* (1), 31–39. DOI: 10.1007/s00774-017-0813-z.

81. Kim, K. M.; Lee, E. Y.; Lim, S.; Jang, H.-C.; Kim, C.-O. Favorable effects of skeletal muscle on bone are distinguished according to gender and skeletal sites. *Osteoporosis and Sarcopenia* **2017,** *3* (1), 32–36. DOI: 10.1016/j.afos.2016.11.001.

82. Ito, K.; Ookawara, S.; Hibino, Y.; Imai, S.; Fueki, M.; Bandai, Y.; Yasuda, M.; Kamimura, T.; Kakuda, H.; Kiryu, S. *et al*. Skeletal Muscle Mass Index Is Positively Associated With Bone Mineral Density in Hemodialysis Patients. *Frontiers in Medicine* **2020,** *7*, 187. DOI: 10.3389/fmed.2020.00187.

83. Qin, H.; Jiao, W. Correlation of muscle mass and bone mineral density in the NHANES US general population, 2017-2018. *Medicine* **2022,** *101* (39), e30735. DOI: 10.1097/MD.0000000000030735.

84. Kang, D.; Liu, Z.; Wang, Y.; Zhang, H.; Feng, X.; Cao, W.; Wang, P. Relationship of body composition with bone mineral density in northern Chinese men by body mass index levels. *Journal of Endocrinological Investigation* **2014,** *37* (4), 359–367. DOI: 10.1007/s40618-013-0037-6.

85. Liu, P.-Y.; Ilich, J. Z.; Brummel-Smith, K.; Ghosh, S. New Insight into Fat, Muscle and Bone Relationship in Women: Determining the Threshold at Which Body Fat Assumes Negative Relationship with Bone Mineral Density. *International Journal of Prevention Medicine* **2014,** *5* (11), 1452–1463.

86. Ilesanmi-Oyelere, B. L.; Coad, J.; Roy, N.; Kruger, M. C. Lean Body Mass in the Prediction of Bone Mineral Density in Postmenopausal Women. *BioResearch Open Access* **2018,** *7* (1), 150–158. DOI: 10.1089/biores.2018.0025.

87. Ho-Pham, L. T.; Nguyen, U. D. T.; Nguyen, T. V. Association between lean mass, fat mass, and bone mineral density: a meta-analysis. *The Journal of Clinical Endocrinology and Metabolism* **2014,** *99* (1), 30–38. DOI: 10.1210/jc.2014-v99i12-30A.

88. Thouverey, C.; Caverzasio, J. Sclerostin inhibits osteoblast differentiation without affecting BMP2/SMAD1/5 or Wnt3a/β-catenin signaling but through activation of platelet-derived growth factor receptor signaling in vitro. *BoneKEy reports* **2015,** *4*, 757. DOI: 10.1038/bonekey.2015.126.

89. Sutherland, M. K.; Geoghegan, J. C.; Yu, C.; Turcott, E.; Skonier, J. E.; Winkler, D. G.; Latham, J. A. Sclerostin promotes the apoptosis of human osteoblastic cells: a novel regulation of bone formation. *Bone* **2004,** *35* (4), 828–835. DOI: 10.1016/j.bone.2004.05.023.

90. Winkler, D. G.; Sutherland, M. K.; Geoghegan, J. C.; Yu, C.; Hayes, T.; Skonier, J. E.; Shpektor, D.; Jonas, M.; Kovacevich, B. R.; Staehling-Hampton, K. *et al*. Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. *The EMBO Journal* **2003,** *22* (23), 6267–6276. DOI: 10.1093/emboj/cdg599.

91. Li, X.; Ominsky, M. S.; Niu, Q.-T.; Sun, N.; Daugherty, B.; D'Agostin, D.; Kurahara, C.; Gao, Y.; Cao, J.; Gong, J. *et al*. Targeted deletion of the sclerostin gene in mice results in increased bone formation and bone strength. *Journal of Bone and Mineral Research* **2008,** *23* (6), 860–869. DOI: 10.1359/jbmr.080216.

92. Li, X.; Ominsky, M. S.; Warmington, K. S.; Morony, S.; Gong, J.; Cao, J.; Gao, Y.; Shalhoub, V.; Tipton, B.; Haldankar, R.*et al*. Sclerostin antibody treatment increases bone formation, bone mass, and bone strength in a rat model of postmenopausal osteoporosis. *Journal of Bone and Mineral Research* **2009,** *24* (4), 578–588. DOI: 10.1359/jbmr.081206.

93. Neve, A.; Corrado, A.; Cantatore, F. P. Osteocalcin: skeletal and extra-skeletal effects. *Journal of Cellular Physiology* **2013,** *228* (6), 1149–1153. DOI: 10.1002/jcp.24278.

94. He, C.; He, W.; Hou, J.; Chen, K.; Huang, M.; Yang, M.; Luo, X.; Li, C. Bone and Muscle Crosstalk in Aging. *Frontiers in Cell and Developmental Biology* **2020,** *8*, 585644. DOI: 10.3389/fcell.2020.585644.

95. Wang, J. S.; Mazur, C. M.; Wein, M. N. Sclerostin and Osteocalcin: Candidate Bone-Produced Hormones. *Frontiers in Endocrinology* **2021,** *12*, 584147. DOI: 10.3389/fendo.2021.584147.

96. Amrein, K.; Amrein, S.; Drexler, C.; Dimai, H. P.; Dobnig, H.; Pfeifer, K.; Tomaschitz, A.; Pieber, T. R.; Fahrleitner-Pammer, A. Sclerostin and its association with physical activity, age, gender, body composition, and bone mineral content in healthy adults. *The Journal of Clinical Endocrinology and Metabolism* **2012,** *97* (1), 148–154. DOI: 10.1210/jc.2011-2152.

97. Urano, T.; Shiraki, M.; Ouchi, Y.; Inoue, S. Association of circulating sclerostin levels with fat mass and metabolic disease--related markers in Japanese postmenopausal women. *The Journal of Clinical Endocrinology and Metabolism* **2012,** *97* (8), E1473-477. DOI: 10.1210/jc.2012-1218.

98. Reppe, S.; Noer, A.; Grimholt, R. M.; Halldórsson, B. V.; Medina-Gomez, C.; Gautvik, V. T.; Olstad, O. K.; Berg, J. P.; Datta, H.; Estrada, K. *et al*. Methylation of bone SOST, its mRNA, and serum sclerostin levels correlate strongly with fracture risk in postmenopausal women. *Journal of Bone and Mineral Research* **2015,** *30* (2), 249–256. DOI: 10.1002/jbmr.2342.

99. Gianicolo, E. A. L.; Eichler, M.; Muensterer, O.; Strauch, K.; Blettner, M. Methods for Evaluating Causality in Observational Studies. *Deutsches Arzteblatt International* **2020,** *116* (7), 101–107. DOI: 10.3238/arztebl.2020.0101.

100. Tönnies, T.; Kahl, S.; Kuss, O. Collider Bias in Observational Studies. *Deutsches Arzteblatt International* **2022,** *119* (7), 107–122. DOI: 10.3238/arztebl.m2022.0076.

101. Meuli, L.; Dick, F. Understanding Confounding in Observational Studies. *European Journal of Vascular and Endovascular Surgery* **2018,** *55* (5), 737. DOI: 10.1016/j.ejvs.2018.02.028.

102. Edelstein, S. L.; Barrett-Connor, E. Relation between body size and bone mineral density in elderly men and women. *American Journal of Epidemiology* **1993,** *138* (3), 160–169. DOI: 10.1093/oxfordjournals.aje.a116842.

103. Guney, E.; Kisakol, G.; Ozgen, G.; Yilmaz, C.; Yilmaz, R.; Kabalak, T. Effect of weight loss on bone metabolism: comparison of vertical banded gastroplasty and medical intervention. *Obesity Surgery* **2003,** *13* (3), 383–388. DOI: 10.1381/096089203765887705.

104. Lach-Trifilieff, E.; Minetti, G. C.; Sheppard, K.; Ibebunjo, C.; Feige, J. N.; Hartmann, S.; Brachat, S.; Rivet, H.; Koelbing, C.; Morvan, F. *et al*. An antibody blocking activin type II receptors induces strong skeletal muscle hypertrophy and protects from atrophy. *Molecular and Cellular Biology* **2014,** *34* (4), 606–618. DOI: 10.1128/MCB.01307-13.

105. Rooks, D.; Praestgaard, J.; Hariry, S.; Laurent, D.; Petricoul, O.; Perry, R. G.; Lach-Trifilieff, E.; Roubenoff, R. Treatment of Sarcopenia with Bimagrumab: Results from a Phase II, Randomized, Controlled, Proof-of-Concept Study. *Journal of the American Geriatrics Society* **2017,** *65* (9), 1988–1995. DOI: 10.1111/jgs.14927.

106. Amato, A. A.; Sivakumar, K.; Goyal, N.; David, W. S.; Salajegheh, M.; Praestgaard, J.; Lach-Trifilieff, E.; Trendelenburg, A.-U.; Laurent, D.; Glass, D. J. *et al*. A. Treatment of sporadic inclusion body myositis with bimagrumab. *Neurology* **2014,** *83* (24), 2239–2246. DOI: 10.1212/WNL.0000000000001070.

107. Garito, T.; Roubenoff, R.; Hompesch, M.; Morrow, L.; Gomez, K.; Rooks, D.; Meyers, C.; Buchsbaum, M. S.; Neelakantham, S.; Swan, T. *et al*. Bimagrumab improves body composition and insulin sensitivity in insulin-resistant individuals. *Diabetes, Obesity & Metabolism* **2018,** *20* (1), 94–102. DOI: 10.1111/dom.13042.

108. Rooks, D.; Petricoul, O.; Praestgaard, J.; Bartlett, M.; Laurent, D.; Roubenoff, R. Safety and pharmacokinetics of bimagrumab in healthy older and obese adults with body composition changes in the older cohort. *Journal of Cachexia, Sarcopenia and Muscle* **2020,** *11* (6), 1525–1534. DOI: 10.1002/jcsm.12639.

109. Heymsfield, S. B.; Coleman, L. A.; Miller, R.; Rooks, D. S.; Laurent, D.; Petricoul, O.; Praestgaard, J.; Swan, T.; Wade, T.; Perry, R. G. *et al*. Effect of Bimagrumab vs Placebo on Body Fat Mass Among Adults With Type 2 Diabetes and Obesity: A Phase 2 Randomized Clinical Trial. *JAMA Network Open* **2021,** *4* (1), e2033457. DOI: 10.1001/jamanetworkopen.2020.33457.

110. Sun, L.; Sun, S.; Zhao, X.; Zhang, J.; Guo, J.; Tang, L.; Ta, D. Inhibition of myostatin signal pathway may be involved in low-intensity pulsed ultrasound promoting bone healing. *Journal of Medical Ultrasonics* **2019,** *46* (4), 377–388. DOI: 10.1007/s10396-019-00962-2.

111. Chen, Y.-S.; Guo, Q.; Guo, L.-J.; Liu, T.; Wu, X.-P.; Lin, Z.-Y.; He, H.-B.; Jiang, T.-J. GDF8 inhibits bone formation and promotes bone resorption in mice. *Clinical and Experimental Pharmacology & Physiology* **2017,** *44* (4), 500–508. DOI: 10.1111/1440-1681.12728.

112. Qin, Y.; Peng, Y.; Zhao, W.; Pan, J.; Ksiezak-Reding, H.; Cardozo, C.; Wu, Y.; Divieti Pajevic, P.; Bonewald, L. F. *et al*. Myostatin inhibits osteoblastic differentiation by suppressing osteocyte-derived exosomal microRNA-218: A novel mechanism in muscle-bone communication. *The Journal of Biological Chemistry* **2017,** *292* (26), 11021–11033. DOI: 10.1074/jbc.M116.770941.

113. Wu, L.-F.; Zhu, D.-C.; Wang, B.-H.; Lu, Y.-H.; He, P.; Zhang, Y.-H.; Gao, H.-Q.; Zhu, X.-W.; Xia, W.; Zhu, H. *et al*. Relative abundance of mature myostatin rather than total myostatin is negatively associated with bone mineral density in Chinese. *Journal of Cellular and Molecular Medicine* **2018,** *22* (2), 1329–1336. DOI: 10.1111/jcmm.13438.

114. Hamrick, M. W. Increased bone mineral density in the femora of GDF8 knockout mice. *The Anatomical Record. Part A, Discoveries in Molecular, Cellular, and Evolutionary Biology* **2003,** *272* (1), 388–391. DOI: 10.1002/ar.a.10044.

115. Hamrick, M. W.; Pennington, C.; Byron, C. D. Bone architecture and disc degeneration in the lumbar spine of mice lacking GDF-8 (myostatin). *Journal of Orthopaedic Research* **2003,** *21* (6), 1025–1032. DOI: 10.1016/S0736-0266(03)00105-0.

116. Clarke, B. L. Anti-sclerostin antibodies: utility in treatment of osteoporosis. *Maturitas* **2014,** *78* (3), 199–204. DOI: 10.1016/j.maturitas.2014.04.016.

117. Fabre, S.; Funck-Brentano, T.; Cohen-Solal, M. Anti-Sclerostin Antibodies in Osteoporosis and Other Bone Diseases. *Journal of Clinical Medicine* **2020,** *9* (11). DOI: 10.3390/jcm9113439.

SUMMARY

Increased demographic pressure due to a rising life expectancy and growing proportion of older adults in the total population results in challenges for the state, society, economy and healthcare sector. Aging-associated changes in body composition and hormone production favor the development of an *obese osteosarcopenic* phenotype that is characterized by sarcopenia (reduced muscle mass (MM) and/or muscle strength), osteoporosis (low bone mineral density) and increased body fat (overweight/obesity). This triad of conditions results in adverse health outcomes including a higher risk of frailty, cognitive impairment and mortality. The present thesis contributes to an improved diagnosis of a low MM that is needed for secondary prevention and therapy. In addition, analysis of the hormonal crosstalk between skeletal muscle, bone and adipose tissue leads to a better understanding of the etiology of the *obese osteosarcopenic* phenotype.

The main results of the thesis are:

(i) Current reference values for skeletal MM depend on the measurement technique, on characteristics of the study population (e.g. sex, age, ethnicity, disease) as well as on normalization for body size and fat mass (FM). The adverse effects of obesity on muscle quality and function may lead to an underestimation of sarcopenia in obesity and therefore normalization of MM for FM is required. Random and systematic errors of measurement techniques which provide proxies for MM like single images or appendicular lean mass limit the assessment of individual cases and the accurate detection of changes in MM. Reference data for total skeletal MM based on the gold standard whole body magnetic resonance imaging (MRI) are scarce due to high costs and cumbersome image-segmentation. Therefore, normal values for skeletal muscle mass index (MM (kg) normalized by height (m^2)) assessed by bioelectrical impedance analysis and validated against MRI were generated based on a young and healthy Caucasian population stratified into underweight, normal weight, overweight and obesity.

(ii) Adiponectin, commonly known for its beneficial metabolic effects, was negatively correlated with muscle and bone mass and positively with muscle fat (so called *adiponectin paradox*). Negative correlations between adiponectin and the anabolic hormone insulin-like growth factor 1 (IGF-1) suggest that higher adiponectin levels are not causally related to lower muscle or bone masses in advanced age. The associations may rather be mediated by an age-related decrease in IGF-1 that results in an increase in adiponectin.

(iii) The anabolic effect of muscle on bone was partly explained by the mechanical loading effect. By contrast, a higher FM exerts adverse effects on bone because bone mass was negatively associated with leptin levels as well as with inflammation markers that were positively related with FM. Based on these findings, nutritional recommendations for the prevention of osteoporosis can be derived. For example, an energy-restricted diet rich in anti-inflammatory foods (e.g. vegetables, fruits, fish, whole grains and nuts) may lead to a reduction in FM and improvement in inflammatory status and therefore to a reduced risk of bone loss and fracture. In conclusion, endocrine determinants of bone mass and density derived from the crosstalk with muscle and adipose tissue could provide targets for preventing and mitigating bone degradation.

ZUSAMMENFASSUNG

Der zunehmende demografische Wandel durch die steigende Lebenserwartung und den kontinuierlich wachsenden Anteil älterer Erwachsener an der Gesamtbevölkerung führt zu Herausforderungen für den Staat, die Gesellschaft, die Wirtschaft und den Gesundheitssektor. Altersbedingte Veränderungen der Körperzusammensetzung und der Hormonproduktion begünstigen die Entwicklung eines *adipösen osteosarkopenen* Phänotyps, der durch Sarkopenie (reduzierte Muskelmasse (MM) und/oder Muskelkraft), Osteoporose (niedrige Knochenmineraldichte) und erhöhtes Körperfett (Übergewicht/Adipositas) gekennzeichnet ist. Diese Triade übt negative gesundheitliche Folgen aus wie ein erhöhtes Risiko für Gebrechlichkeit, kognitive Beeinträchtigungen und Mortalität. Die vorliegende Arbeit leistet einen Beitrag für eine verbesserte Diagnose einer niedrigen MM, die für die Sekundärprävention und Therapie erforderlich ist. Darüber hinaus trägt die Analyse des hormonellen Zusammenspiels zwischen Skelettmuskel, Knochen und Fettgewebe zu einem erweiterten Verständnis der Ätiologie des *adipösen osteosarkopenen* Phänotyps bei.

Die wichtigsten Ergebnisse der Arbeit sind:

(i) Die aktuellen Referenzwerte für die skelettale MM hängen von der Messmethode, den Merkmalen der Studienpopulation (z.B. Geschlecht, Alter, ethnische Zugehörigkeit, Krankheit) sowie von der Normalisierung für die Körperhöhe und Fettmasse (FM) ab. Die nachteiligen Auswirkungen eines hohen Körperfettgehalts auf die Muskelqualität und -funktion können zu einer Unterschätzung der Sarkopenie-Prävalenz bei Adipositas führen. Daher ist eine Normalisierung der MM für die FM zwingend erforderlich. Einzelne Schichtbildaufnahmen mit Computertomographie oder Magnetresonanztomographie (MRT) sowie die mittels Dual-Röntgen-Absorptiometrie oder bioelektrischer Impendanzanalyse (BIA) gemessene appendikuläre Magermasse werden als valide Parameter für die gesamte MM angesehen. Jedoch schränken zufällige und systematische Messfehler die Anwendbarkeit dieser Surrogate bei der Einzelfall-Bewertung ein und limitieren die Erkennung von Veränderungen der MM. Referenzwerte für die gesamte MM, die auf dem Goldstandard der Ganzkörper-MRT beruhen, sind aufgrund der hohen Kosten und der zeitaufwändigen Segmentierung der Bilder rar. Daher wurden mittels BIA, die gegen Ganzkörper-MRT validiert wurde, Normalwerte für den Skelettmuskelmassen-Index (MM (kg) normalisiert für Körperhöhe (m^2)) auf der Grundlage einer jungen und gesunden kaukasischen Population ermittelt, die nach Untergewicht, Normalgewicht, Übergewicht und Adipositas stratifiziert wurde.

(ii) Adiponektin, ein für seine günstigen Effekte auf den Stoffwechsel bekanntes Hormon, korrelierte negativ mit der Muskel- und Knochenmasse und positiv mit der Verfettung des Muskels (sogenanntes *Adiponektin Paradoxon*). Die negative Korrelation zwischen Adiponektin und dem anabolen Hormon *Insulin-like growth factor 1* (IGF-1) lässt vermuten, dass höhere Adiponektin-Spiegel nicht kausal mit einer geringeren Muskel- oder Knochenmasse im fortgeschrittenen Alter zusammenhängen, sondern dass die Assoziationen möglicherweise durch eine altersbedingte Abnahme von IGF-1-Spiegeln vermittelt werden, die zu einem Anstieg von Adiponektin führen.

(iii) Die anabole Wirkung der MM auf den Knochen konnte teilweise durch mechanische Belastung erklärt werden. Im Gegensatz dazu zeigte eine höhere FM ungünstige Wirkungen auf den Knochen, die über die negativen Assoziationen zwischen der Knochenmasse und den Leptin-Spiegeln sowie Entzündungsmarkern, die wiederum positiv mit der FM korrelierten, erklärt werden konnten. Auf Basis dieser Ergebnisse lassen sich Ernährungsempfehlungen zur Prävention von Osteoporose ableiten. Eine energiereduzierte Ernährung, die reich an entzündungshemmenden Lebensmitteln (z.B. Gemüse, Obst, Fisch, Vollkornprodukten und Nüssen) ist, könnte daher zu einer Verringerung der FM und einer Verbesserung des Entzündungsstatus und damit zu einem geringeren Risiko für Knochenschwund und Frakturen führen. Endokrine Determinanten der Knochenmasse und -dichte, die sich aus der Interaktion mit Muskel- und Fettgewebe ergeben, liefern daher Ansatzpunkte zur Reduktion des Knochenabbaus.

APPENDIX

Ethical approvals

Declaration of co-authorship Chapter II

Declaration of co-authorship Chapter III

Declaration of co-authorship Chapter IV

Curriculum vitae

Danksagung

MEDIZINISCHE FAKULTÄT
DER CHRISTIAN-ALBRECHTS-UNIVERSITÄT ZU KIEL

ETHIK-KOMMISSION

A.D. 1665

Universitäts-Kinderklinik · Schwanenweg 20 · 24105 Kiel

Prof. Dr. Dr. Bosy-Westphal
Institut für Humanernährung u.
Lebensmittelkunde der CAU
Düsternbrooker Weg 17
24105 Kiel

Postadresse:
Arnold-Heller-Straße 3 / Haus 9
D-24105 Kiel

Telefon 0431 / 500-14 191
Telefax 0431 / 500-14 195
ethikkomm@email.uni-kiel.de

Datum: 12. Juni 2018

Vorab per Fax: 0431 880 5679

cc: BfArM

AZ.:	**A 106/18** (bitte stets angeben)
Studienplan:	**Generierung von Prädiktionsformeln zur Analyse der Körperzusammensetzung von älteren Erwachsenen mit Hilfe der Bioelektrischen Impedanzanalyse (BIA)**
Formular-Nr.:	**000487755**
EUDAMED-Nr.:	**CIV-18-02-023164**
Antragsnummer:	**00011286**
Studiencode:	**BCA-12**
Sponsor:	**seca GmbH & co. kg, 22089 Hamburg**
Prüfer:	**Prof. Dr. Dr. Bosy-Westphal, Institut für Humanernährung u. Lebensmittelkunde, CAU Kiel**
Erstantrag:	**28. Februar 2018 (Mail des DIMDI)**
Nachreichungen:	**07./ 13./ 15. März 2018, 23. April 2018 (via DIMDI)**
Überarb. Antrag:	**12. Juni 2018 (Mail des DIMDI)**

Votum

Die Ethik-Kommission der Medizinischen Fakultät der Christian-Albrechts-Universität zu Kiel hat als federführende Kommission die oben genannte Monozenterstudie auf mögliche berufsethische und berufsrechtliche Bedenken hin überprüft. Alle Voraussetzungen gemäß § 20 MPG wurden erfüllt. Die Kommission stimmt darin überein, dass gegen die Durchführung der Studie nunmehr keine Bedenken bestehen.

An der Beratung und Beschlussfassung haben die in Anhang 1 aufgeführten Mitglieder der hiesigen Ethik-Kommission teilgenommen. Es wird bestätigt, dass Prüfärzte, die an der oben genannten Studie beteiligt sind, nicht an der Abstimmung teilgenommen haben.

Die Ethik-Kommission erteilt eine zustimmende Bewertung, weil die klinische Prüfung ärztlich vertretbar und ein Nutzen für die Heilkunde ableitbar ist.
Für das Forschungsvorhaben besteht ein zwingendes Bedürfnis im Sinne des § 28b Abs. 1 Nr. 1 RöV, § 24 Abs.1 Nr. 1 StrlSchV, weil die bisherigen Forschungsergebnisse und die medizinischen Erkenntnisse nicht ausreichen.

Die Ethik-Kommission gibt folgende allgemeine Hinweise:

1. Die ärztliche und juristische Verantwortung verbleibt bei den jeweiligen Prüfern.
2. Auf die Einhaltung einschlägiger Gesetze und Rechtsvorschriften insbesondere der Dokumentations- und Mitteilungspflichten wird hingewiesen.
3. Die Ethik-Kommission bestätigt, dass sie auf Grundlage nationaler Gesetze, Vorschriften sowie der GCP-Verordnung arbeitet.
4. Datenschutzrechtliche Aspekte von Forschungsvorhaben werden durch die Ethik-Kommission grundsätzlich nur kursorisch geprüft. Diese Bewertung ersetzt mithin nicht die Konsultation des zuständigen Datenschutzbeauftragten.
5. Eine Kopie dieser Stellungnahme wird der zuständigen Behörde zugeleitet.

Prof. Dr. med. H. M. Mehdorn
Vorsitzender der Ethik-Kommission

Dr. med. Christine Glinicke
Geschäftsführung der Ethik-Kommission

Anhang 1
Nachfolgend sind die Mitglieder der Ethik-Kommission aufgeführt, die diese Studie im Umlaufverfahren und bei der Sitzung am 06. März 2018 beurteilt haben:
Dr. rer. nat. Amke Caliebe (Medizinische Informatik und Statistik)
PD Dr. med. Ulf Lützen (Radiologie)
Prof. Dr. med. Maximilian Mehdorn (Neurochirurgie)
PD Dr. med. Hiltrud Muhle (Neuropädiatrie)
Prof. Dr. med. Heiner Mönig (Innere Medizin)
Prof. Dr. med. Christoph Mundhenke (Gynäkologie und Geburtshilfe)
PD Dr. med. Susanna Nikolaus (Innere Medizin)
Prof. Dr. med. Martin Schrappe (Pädiatrie)
Dr. jur. Kurt Weigle (Medizin- und Arbeitsrecht)

Anhang 2
Das Votum ist gültig für nachfolgend aufgeführte Prüfer und Prüfzentrum:

HP: Prof. Dr. Anja Bosy-Westphal
P: Dr. Wiebke Braun
Christian-Albrecht-Universität zu Kiel, Institut für Humanernährung und Lebensmittel, Abtl. Humanernährung, Düsternbrooker Weg 17, 24105 Kiel

Anhang 3
Folgende Unterlagen wurden per DIMDI am 28. Februar 2018 vorgelegt:

APPENDIX_CIP
#1_BCA-12_clinical investigation plan_1_1_signed
#2_BCA-12_Studiensynopsis

[redacted]

MEDIZINISCHE FAKULTÄT
DER CHRISTIAN-ALBRECHTS-UNIVERSITÄT ZU KIEL

ETHIK-KOMMISSION

A.D. 1665

Universitäts-Kinderklinik · Schwanenweg 20 · 24105 Kiel

Postadresse:
Arnold-Heller-Straße 3 / Haus 9
D-24105 Kiel

Telefon 04 31 / 597-18 09
Telefax 04 31 / 597-53 33
ethikkomm@email.uni-kiel.de

Prof. Dr. med. M. J. Müller
Institut für Humanernährung und Lebensmittelkunde
CAU zu Kiel
Düsternbrooker Weg 17
24105 Kiel

Datum: 18.9.2014

NEUES AZ.:	**A 100/13 – A** (bitte stets angeben)
In Bezug auf AZ.: A 100/13	
Studienplan:	**Zusammenhang zwischen der Beziehung von Muskelmasse und Fettmasse zu Muskelkraft, Mobilität und Ernährung bei gesunden Senioren**
Studienleiter und Antragsteller:	**Prof. Dr. M. J. Müller, Institut für Humanernährung und Lebensmittelkunde, Christian-Albrechts-Universität zu Kiel**
Erstantrag vom:	**11.1.2013 (Eingang 15.1.2013)**
Datum des 1. überarb. Antrages:	**16.10.2013 (Eingang 1.11.2013)**
Datum des 2. überarb. Antrages:	**16.9.2014 (Eingang 17.9.2014)** Antrag mit Studienprotokoll (exclusive DXA-Untersuchung) - Version 1 vom 16.9.2014, CE-Zertifikate, Probandeninformation und Einwilligungserklärung- Version 1 vom 16.9.2014, Aktivitätsfragebogen, Protokoll zu Ernährungsgewohnheiten, Text zur Rekrutierung der Probanden, Schreiben Forschungsförderung Institut Danone vom 5.2.2013

V o t u m

Die Ethik-Kommission der Medizinischen Fakultät der Christian-Albrechts-Universität zu Kiel hat die zu dem oben genannten Antrag gemäß § 15 Berufsordnung (BO) der Ärztekammer Schleswig-Holstein eingereichten Unterlagen auf mögliche berufsethische und berufsrechtliche Bedenken hin überprüft.

Die Kommission stimmt darin überein, dass gegen die Durchführung der Studie nunmehr keine Bedenken bestehen.

Die im Folgenden aufgeführten Hinweise müssen beachtet werden:

1. In der Probandeninformation müssen folgende Änderungen erfolgen:
Zur Blutentnahme muss zusätzlich auf das seltene Risiko der chronischen Nervenschädigung als mögliche seltene Komplikation bei Blutentnahmen hingewiesen werden. Das

1

Blutvolumen muss benannt werden, die „Blutwerte" müssen kurz erklärt werden.
Zur Versicherung muss die Police-Nr. und die Telefonnummer aufgeführt werden.

2. In der Einwilligungserklärung ist der Passus „Terminvereinbarung der ausstehenden DXA-Messung" für die Studienteilnehmer unverständlich und muss laienverständlich erklärt werden.

3. Es wird darauf hingewiesen, dass künftige Änderungen der Studie der Ethik-Kommission anzuzeigen sind und gegebenenfalls eine erneute Beratung erforderlich machen.
Falls in Zukunft eine Erweiterung der Studie mit zusätzlichen DXA-Messungen erfolgen soll, ist diesbezüglich ein erneuter Antrag bei der Ethik-Kommission zu stellen unter Vorlage der Genehmigung des Bundesamt für Strahlenschutz (BfS) und einer dazugehörigen Probandeninformation und Einwilligungserklärung.

4. Die ethische und rechtliche Verantwortung für die Durchführung dieser Studie verbleibt beim Projektleiter und den an der Studie teilnehmenden und Mitarbeitern.

5. Über alle schwerwiegenden oder unerwarteten unerwünschten Ereignisse, die während der Studie auftreten, muss die Kommission umgehend benachrichtigt werden.

6. Die Ethik-Kommission weist darauf hin, dass für eventuell in Zukunft weitere teilnehmende Zentren eine berufsrechtliche Beratung bei der jeweils für sie zuständigen Ethik-Kommission erforderlich ist.

7. Nach Abschluss der Studie erbittet die Kommission einen kurzen Bericht mit einem Hinweis, ob im Laufe der Studie ethische oder juristische Probleme aufgetreten sind.

8. Die Ethik-Kommission benötigt vor Studienbeginn ein Exemplar der Versicherungsbestätigung in Kopie sowie ein Exemplar der entsprechend geänderten Probandeninformation und Einwilligungserklärung, gekennzeichnet mit aktuellem Versionsdatum und Seitenzahlen.

Prof. Dr. med. H. M. Mehdorn
Vorsitzender der Ethik-Kommission

Dr. med. Christine Glinicke
Geschäftsführung der Ethik-Kommission

Nachfolgend sind die Mitglieder der Ethik-Kommission aufgeführt, die diese Studie im Umlaufverfahren beurteilt haben:

Frau Prof. Dr. med. R. Fölster-Holst (Dermatologie)

Prof. Dr. med. N. Frey (Innere Medizin und Kardiologie)

Prof. Dr. med. Dr. jur. H.-J. Kaatsch (Rechtsmedizin)

Prof. Dr. med. H.M. Mehdorn (Neurochirurgie)

Frau PD Dr. med. S. Nikolaus (Innere Medizin)

PD Dr. med. D. Proppe (Innere Medizin und Klinische Pharmakologie)

PD Dr. med. A. Rohr (Neuroradiologie)

Prof. Dr. med. M. Schrappe (Pädiatrie)

Frau Dr. M. Schwinge (Pröpstin i.R.)

2

Declaration of co-authorship Chapter II

C | A | U Christian-Albrechts-Universität zu Kiel	Agrar- und Ernährungs-wissenschaftliche Fakultät

Declaration of co-authorship

If a dissertation is based on already published or submitted co-authored articles, a declaration from each of the authors regarding the part of the work done by the doctoral candidate must be enclosed when submitting the dissertation.

1. Doctoral candidate
Name: Carina Walowski

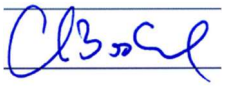
2. This co-author declaration applies to the following article:
Reference Values for Skeletal Muscle Mass - Current Concepts and Methodological Considerations

The extent of the doctoral candidate's contribution to the article is assessed on the following scale:

A. Has contributed to the work (0-33%)
B. Has made a substantial contribution (34-66%)
C. Did the majority of the work independently (67-100%)

3. Declaration on the individual phases of the scientific work (A, B, C)	**Extent**
Concept: Formulation of the basic scientific problem based on theoretical questions which require clarification, including a summary of the general questions which, it is assumed, will be answerable via analyses or concrete experiments/investigations	B
Planning: Planning of experiments/analyses and formulation of investigative methodology, including choice of method and independent methodological development, in such a way that the scientific questions asked can be expected to be answered	B
Execution: Involvement in the analysis or the concrete experiments/investigation	C
Manuscript preparation: Presentation, interpretation and discussion of the results obtained in article form	B

4. Signature of all co-authors		
Date	**Name**	**Signature**
12.12.2022	**Wiebke Braun**	W. Braun
14.12.2022	**Michael J. Maisch**	Dr. med. Michael J. Maisch

12.12.2022	**Björn Jensen**	
20.12.2022	**Sven Peine**	
19.12.2022	**Kristina Norman**	
16.12.2022	**Manfred J. Müller**	
05.01.2023	**Anja Bosy-Westphal**	

5. Signature of doctoral candidate		
Date	**Name**	**Signature**
12.12.2022	**Carina Walowski**	

Declaration of co-authorship Chapter III

C | A | U Christian-Albrechts-Universität zu Kiel	Agrar- und Ernährungs-wissenschaftliche Fakultät

Declaration of co-authorship

If a dissertation is based on already published or submitted co-authored articles, a declaration from each of the authors regarding the part of the work done by the doctoral candidate must be enclosed when submitting the dissertation.

1. Doctoral candidate
Name: Carina Walowski

2. This co-author declaration applies to the following article:
Analysis of the adiponectin paradox in healthy older people

The extent of the doctoral candidate's contribution to the article is assessed on the following scale:

A. Has contributed to the work (0-33%)
B. Has made a substantial contribution (34-66%)
C. Did the majority of the work independently (67-100%)

3. Declaration on the individual phases of the scientific work (A, B, C)	**Extent**
Concept: Formulation of the basic scientific problem based on theoretical questions which require clarification, including a summary of the general questions which, it is assumed, will be answerable via analyses or concrete experiments/investigations	B
Planning: Planning of experiments/analyses and formulation of investigative methodology, including choice of method and independent methodological development, in such a way that the scientific questions asked can be expected to be answered	B
Execution: Involvement in the analysis or the concrete experiments/investigation	C
Manuscript preparation: Presentation, interpretation and discussion of the results obtained in article form	B

4. Signature of all co-authors		
Date	**Name**	**Signature**
19.12.2022	**Catrin Herpich**	C. Herpich
12.12.2022	**Janna Enderle**	Janna Enderle

12.12.2022	**Wiebke Braun**	
15.12.2022	**Marcus Both**	
12.12.2022	**Mario Hasler**	
16.12.2022	**Manfred J. Müller**	
19.12.2022	**Kristina Norman**	
05.01.2023	**Anja Bosy-Westphal**	

5. Signature of doctoral candidate		
Date	**Name**	**Signature**
12.12.2022	**Carina Walowski**	

Declaration of co-authorship Chapter IV

C | A | U Christian-Albrechts-Universität zu Kiel	Agrar- und Ernährungs-wissenschaftliche Fakultät

Declaration of co-authorship

If a dissertation is based on already published or submitted co-authored articles, a declaration from each of the authors regarding the part of the work done by the doctoral candidate must be enclosed when submitting the dissertation.

1. Doctoral candidate
Name: Carina Walowski

2. This co-author declaration applies to the following article:
Determinants of bone mass in healthy older adults derived from the crosstalk with muscle and adipose tissue

The extent of the doctoral candidate's contribution to the article is assessed on the following scale:

A. Has contributed to the work (0-33%)
B. Has made a substantial contribution (34-66%)
C. Did the majority of the work independently (67-100%)

3. Declaration on the individual phases of the scientific work (A, B, C)	**Extent**
Concept: Formulation of the basic scientific problem based on theoretical questions which require clarification, including a summary of the general questions which, it is assumed, will be answerable via analyses or concrete experiments/investigations	B
Planning: Planning of experiments/analyses and formulation of investigative methodology, including choice of method and independent methodological development, in such a way that the scientific questions asked can be expected to be answered	B
Execution: Involvement in the analysis or the concrete experiments/investigation	C
Manuscript preparation: Presentation, interpretation and discussion of the results obtained in article form	B

4. Signature of all co-authors		
Date	**Name**	**Signature**
19.12.2022	**Catrin Herpich**	C. Herpich
12.12.2022	**Janna Enderle**	Janna Enderle

12.12.2022	**Wiebke Braun**	
15.12.2022	**Marcus Both**	
12.12.2022	**Mario Hasler**	
16.12.2022	**Manfred J. Müller**	
19.12.2022	**Kristina Norman**	
05.01.2023	**Anja Bosy-Westphal**	

5. Signature of doctoral candidate		
Date	**Name**	**Signature**
12.12.2022	**Carina Walowski**	

PERSÖNLICHE DATEN

Name:	Carina Ottilie Walowski
Geburtsdatum:	21.06.1988
Geburtsort:	Ravensburg
Staatsangehörigkeit:	deutsch

AKADEMISCHER WERDEGANG

10/2019-02/2023 **Promotion an der Christian-Albrechts-Universität zu Kiel**

- Institut für Humanernährung und Lebensmittelkunde, Abteilung Humanernährung (Prof. Dr. Dr. Anja Bosy-Westphal)
- Titel der Dissertation: „*Association between body composition and the endocrine crosstalk of tissues in older adults*"

02/2017-10/2019 **Studium an der Christian-Albrechts-Universität zu Kiel**

- Studiengang: Ernährungs- und Verbraucherökonomie
- Master of Science (**1,1)**
- Titel der Masterarbeit: *„Beziehung zwischen Leberfett und Schilddrüsenhormon-Spiegel bei euthyreoten Erwachsenen"*

09/2013-01/2017 **Studium an der Semmelweis Universität Budapest**

- Studiengang: Humanmedizin (ohne Abschluss) - auf eigenen Wunsch Studienfach gewechselt
- Notendurchschnitt nach Bayerischer Formel: **1,5**

10/2012-05/2013 **McDaniel College Budapest**

- Vorbereitungsjahr Humanmedizin
- Notendurchschnitt nach Bayerischer Formel: **1,6**

10/2008-09/2012 **Studium an der Hochschule Weihenstephan-Triesdorf**

- Studiengang: Ernährung und Versorgungsmanagement
- Bachelor of Science (**2,0**)
- Titel der Bachelorarbeit: *„Epidemiologische und ernährungsmedizinische Aspekte nach kanzerogen bedingter Gastrektomie"*

08/2000-06/2007 **Ursulaschule Osnabrück**

- Abitur (**2,6**)

PRAKTIKA UND BERUFSERFAHRUNG

07.07.2014-05.08.2014 Praktikantin, *Niels-Stensen-Kliniken*, *Marienhospital Osnabrück*

- Krankenpflegepraktikum: **Kardiologie**

- Mithilfe bei pflegerischen Tätigkeiten an Patient*innen, bei der Mobilisation, am Patient*innenbett
- Kommunikation und Dokumentation: Teilnahme an Visiten sowie an Pflegeberatungsgesprächen mit Patient*innen

17.06.2013-16.07.2013 Praktikantin, *Niels-Stensen-Kliniken*, *Marienhospital Osnabrück*

- Krankenpflegepraktikum: **Kardiologie**

01.06.2012-30.06.2012 Praktikantin, *Niels-Stensen-Kliniken*, *Marienhospital Osnabrück*

- Krankenpflegepraktikum: **Kardiologie**/**Gefäßchirurgie**

01.09.2010-05.01.2011 Praktikantin, *apetito convenience GmbH & Co. KG, Hilter:*

- **Qualitätssicherung**
- Praxissemester im Rahmen des Bachelor-Studiums
- Wareneingangskontrolle, Produktionslabor, chemisches und mikrobiologisches Labor, Endproduktionskontrolle, Dokumentation

02.06.2008-13.06.2008 Praktikantin, *Niels-Stensen-Kliniken*, *Marienhospital Osnabrück*

- **Physiotherapie**

01.01.2008-29.02.2008 Praktikantin, *Niels-Stensen-Kliniken*, *Marienhospital Osnabrück*

- **Diätassistenz**
- Eigenständige Zubereitung von Kostformen für Patient*innen mit speziellen Krankheitsbildern (Diabetes, Adipositas, Herzkrankheiten)

01.11.2007-31.12.2007 Praktikantin, *Niels-Stensen-Kliniken*, *Marienhospital Osnabrück:*

- **Diabetologie**
- Teilnahme an Gruppenschulungen für Diabetiker*innen mit Themen wie Insuline, Folgeerkrankungen, Selbstkontrolle
- Teilnahme an Ernährungsberatungen (Gewichtsreduktion, Intoleranzen)

02.07.2007-31.08.2007 *Niels-Stensen-Kliniken*, *Marienhospital Osnabrück:*

- **Patient*innen-Transport**

ORIGINALARBEITEN

Walowski, C. O. *et al*. Reference Values for skeletal muscle mass - current concepts and methodological considerations. *Nutrients*. 12;12(3):755. doi:10.3390/nu12030755 (2020).

Walowski, C. O. *et al*. Analysis of the adiponectin paradox in healthy older people. *Journal of Cachexia, Sarcopenia and Muscle*. 14(1):270-278. doi:10.1002/jcsm.13127 (2022).

Müller, M.J.; **Walowski, C.O**.; Bosy-Westphal, A. Kommentar zu Körpergewicht oder Fett-freie Masse als Bezugsgröße für die Berechnung des Proteinbedarfs. *Aktuelle Ernährungsmedizin* 2022. Vol. 47 Ausgabe 4: 273-277. doi: 10.1055/a-1848-388 (2022).

KONGRESSBEITRÄGE

Walowski, C.O. *et al*. Reference values for skeletal muscle mass - current concepts and methodological considerations. Conference Paper im Rahmen des Kongresses Ernährung 2020 Medizin fürs Leben in Bremen, 25.-27. Juni 2020. In: Aktuelle Ernährungsmedizin 2020 Vol. 45 Ausgabe 3: 224. doi: 10.1055/s-0040-1710224.

Walowski, C.O. *et al*. Rationale for associations between adiponectin and muscle and bone mass in healthy older people. Postervorstellung im Rahmen des Kongresses Ernährung 2022 Medizin fürs Leben in Bremen, 23.-25. Juni 2022. In: Aktuelle Ernährungsmedizin 2022 Vol. 47 Ausgabe 3: 242-243. doi:10.1055/s-0042-1748248.

DANKSAGUNG

Mein außerordentlicher Dank gilt meiner Doktormutter Frau Prof. Dr. Dr. Anja Bosy-Westphal für das entgegengebrachte Vertrauen einer Promotion in der Abteilung Humanernährung. Du hast mir nicht nur die Möglichkeit eingeräumt, spannende sowie herausfordernde Fragestellungen zu bearbeiten und die Ergebnisse in namenhaften *Journals* zu veröffentlichen, sondern auch einen forschungsnahen Beitrag zur Entwicklung eines Medizinproduktes leisten zu dürfen. Besonders dankbar bin ich Dir, dass Du mir den Raum und die Freiheiten gegeben hast, mich wissenschaftlich zu entfalten und Ideen zu verfolgen. Danke für Deine immer offene Tür, Deine Diskussionsbereitschaft sowie bereichernden und konstruktiven Anmerkungen, die mir stets geholfen haben, Ergebnisse kritisch zu hinterfragen und mich auf das Wesentliche zu konzentrieren, wenn ich mal wieder zu weit in die Materie abgedriftet bin. Danke für Dein unermüdliches Engagement zu jeder Tages- und Nachtzeit und Deine positive Energie, die Du in unseren Arbeitsalltag versprüht hast.

Ein ganz besonderer Dank gilt dem Zweitgutachter.

Für die finanzielle Unterstützung der Studien möchte ich mich bei der *seca gmbh & co.kg*, Hamburg und dem *Danone Institut Ernährung für Gesundheit e.V.*, Deutschland bedanken.

Der Klinik für diagnostische Radiologie des Universitätsklinikums Schleswig-Holstein danke ich sehr für die Möglichkeit der Durchführung der MRT und DXA-Untersuchungen.

Zudem möchte ich Frau Britta Jux für ihren Einsatz bei der Planung und Realisierung der Ganzkörper-MRT-Aufnahmen danken.

Dem Deutschen Institut für Ernährungsforschung, Potsdam-Rehbrücke und der Charité, Universitätsmedizin Berlin, vertreten durch Frau Prof. Dr. Kristina Norman und Frau Dr. Catrin Herpich, danke ich herzlich für die Kooperation und für die arbeitsintensive Analytik der Blutproben.

Herrn Prof. Dr. Mario Hasler danke ich ganz besonders für seine persönliche und engagierte Unterstützung bei der zeitintensiven Beantwortung statistischer Fragestellungen.

Besonders möchte ich all „meinen“ studentischen Hilfskräften für ihre fleißige und zuverlässige Unterstützung bei den Segmentierungen der MRT-*scans* bedanken.

Liebe Kolleginnen. Ich danke Euch für die freundliche Aufnahme in die Abteilung, für die gute Teamarbeit, das motivierende Arbeitsklima, für den fachlichen Austausch, für Euer offenes Ohr und Eure Unterstützung. Wir hatten gemeinsam viel Spaß auf der Arbeit und ich werde die

Erinnerungen an diese schöne Zeit stets bei mir tragen. Ihr habt maßgeblich zu meiner fachlichen und persönlichen Entwicklung beigetragen. Dafür bin ich jedem einzelnen von euch dankbar.

Tief verbunden und dankbar bin ich meinen Eltern für ihren unermüdlichen Beistand, ihre stetigen Ermutigungen sowie ihren zweifelslosen Glauben an mich und meine Fähigkeiten. Egal ob in positiven oder weniger positiven Lebensabschnitten, Ihr habt mir immer ein offenes Ohr geschenkt, mich unterstützt, motiviert und in schwierigen Zeiten wieder auf den richtigen Kurs zurückgebracht.

Von ganzem Herzen möchte ich meinem Bruder für seine uneingeschränkte Unterstützung und sein immerwährendes Vertrauen in mich danken. Seine Gabe, Erlebtes aus einem anderen Blickwinkel zu betrachten, sein Zuspruch und sein Rückhalt haben maßgeblich dazu beigetragen meine bisherigen Ziele zu erreichen.