Doctor of Philosophy

Detrimental Effects of β2-Microglobulin on Muscle Metabolism: Evidence from In Vitro, Animal, and Human Research

The Graduate School
of the University of Ulsan

Department of Medicine
Jang, Il-Young

Detrimental Effects of β2-Microglobulin on Muscle Metabolism: Evidence from In Vitro, Animal, and Human Research

Supervisor : Kim, Beom-Jun

A Dissertation

Submitted to
the Graduate School of the University of Ulsan
In partial Fulfillment of the Requirements
for the Degree of

Doctor of Philosophy

by

Jang, Il-Young

Department of Medicine
University of Ulsan, Korea
February, 2024

Detrimental Effects of β2-Microglobulin on Muscle Metabolism: Evidence from In Vitro, Animal, and Human Research

This certifies that the Doctor of Medicine
of Jang, Il-Young is approved.

Committee Chair Dr. Lee, Eunju

Committee Member Dr. Kim, Beom-jun

Committee Member Dr. Choi, Eun-Ji

Committee Member Dr. Jung, Hee-Won

Committee Member Dr. Lim, Jihye

Department of Medicine

Ulsan, Korea

February, 2024

Acknowledgements

I would like to express my deepest gratitude to Professor Beom-Jun Kim for his invaluable support and guidance throughout this research. His expertise, dedication, and unwavering commitment to excellence have been instrumental in the successful completion of this study. Professor Kim's insightful advice and encouragement have greatly influenced the direction and quality of this research, and I am truly grateful for his mentorship. I would also like to extend my heartfelt appreciation to the professors, nurses, and researchers in the Division of Geriatrics in Asan Medical Center, who have contributed to the advancement of both clinical and experimental research. Despite the challenging circumstances, their support and collaboration have been instrumental in conducting comprehensive geriatric assessments and enhancing the overall quality of this study. Their expertise and dedication have been invaluable in overcoming obstacles and achieving meaningful results. I would like to express my heartfelt gratitude to my parents, spouse, daughter, and son for their unwavering support and patience throughout this research. I am truly thankful to my family for enabling me to dedicate myself to this study and for patiently waiting for me during this time. I consider myself truly blessed to have received their love and support, and I am grateful to be able to share this journey with them.

Abstract

Background: β2-Microglobulin (B2M) has garnered considerable interest as a potential pro-aging factor, leading to speculation about its involvement in muscle metabolism and the development of sarcopenia, a key component of aging phenotypes. To explore this hypothesis, we conducted a comprehensive investigation into the impact of B2M on cellular and animal muscle biology, as well as its clinical implications concerning sarcopenia parameters in older individuals.

Methods: In vitro myogenesis was induced in mouse C2C12 myoblasts with 2% horse serum. For in vivo research, C57BL/6 mice aged 3 months were intraperitoneally given 250 μg of B2M daily, and muscular alterations were assessed one month later. Human blood samples were obtained from 158 participants who underwent assessments of muscle mass and function at an outpatient geriatric clinic affiliated with a teaching hospital. Sarcopenia and associated parameters were assessed using cutoff values specifically tailored for the Asian population. The concentration of serum B2M was quantified through an enzyme-linked immunosorbent assay.

Results: Treatment with recombinant B2M exhibited a dose-dependent inhibition of myogenesis from mouse C2C12 myoblasts and consistently lowered the expression of myogenic differentiation markers. Furthermore, B2M notably elevated intracellular levels of reactive oxygen species (ROS) in myotubes, and administration of N-acetyl cysteine, a potent biological thiol antioxidant, reversed the decrease of myotube area, myotube area per myotube, nucleus number per myotube, and fusion index by B2M through mitigating oxidative stress. Animal experiments showed that mice with systemic B2M treatment exhibited significantly smaller cross-sectional area of tibialis anterior and soleus muscle, weaker grip strength, shorter grid hanging time, and decreased latency time to fall off the rotating rod, compared to untreated controls. In a clinical study, serum B2M levels were inversely associated with grip strength, usual gait speed and short physical performance battery (SPPB) total score after adjustment for age, sex, and body mass index, whereas sarcopenia phenotype score showed a

positive association. Consistently, higher serum B2M levels were associated with higher risk for weak grip strength, slow gait speed, low SPPB total score, and poor physical performance.

Conclusion: These results provide experimental evidence that B2M exerted detrimental effects on muscle metabolism mainly by increasing oxidative stress. Furthermore, we made an effort to translate the results of in vitro and animal research into clinical implication and found that circulating B2M could be one of blood-based biomarkers to assess poor muscle health in older adults.

Keywords: β2-Microglobulin, sarcopenia, aging, biomarker, pro-aging factor

Contents

List of Tables and Figures

Introduction

Aging is an intricate process that not only leads to biologically intrinsic changes within individuals but also imposes a substantial burden on social, medical, and welfare systems (1). As individuals age, they often experience a decline in sensory functions, cognitive abilities, and physical capacities, which may lead to increased dependency and a need for assistance in daily living activities. The personal challenges brought about by aging include a decline in the quality of life, a loss of independence, and the increased prevalence of chronic diseases, which can place a significant emotional and financial burden on the individual and their families (2). From a societal perspective, the aging population results in increased healthcare and welfare demands, with a higher prevalence of age-related illnesses requiring long-term care services and support. The shift in demographics toward an older population also presents challenges in terms of workforce composition, retirement funding, and the sustainability of pension systems. Healthcare systems worldwide are thus compelled to adapt, both structurally and financially, to the growing needs of this demographic (3).

An understanding of the biological mechanisms underlying aging is pivotal in addressing these challenges. Aging is a universal, progressive, and multi-step deterioration at cellular, tissue, and organismal levels, resulting in increased susceptibility to major debilitating diseases and death (4). Aging at a cellular level is characterized by a series of progressive deterioration: genomic instability due to DNA damage, reduced telomerase activity leading to telomere shortening, disruptions in mitochondrial function, and an increase in oxidative stress (4, 5). Additionally, aging is associated with systemic changes such as chronic low-grade inflammation, known as 'inflammaging', hormonal dysregulation, and alterations in tissue homeostasis (6-8). The growing number of individuals in the senior population and advancements in healthcare have sparked interest in finding efficient ways to reverse or slow this time-dependent functional decline. Although a number of factors contribute to aging process (9), this process is uniquely experienced by each individual, manifesting at different rates and affecting physiological systems to varying extents (10). In other words, the heterogeneity of aging is such that chronological age alone is an inadequate measure of an individual’s biological

age or functional status, and biomarkers of aging, encompassing genetic, epigenetic, and physiological factors, are more accurate indicators of an individual's aging process and health span (11, 12).

As a clinical biomarker, frailty from geriatric assessment serves as a critical measure in the evaluation of aging, providing a more clinical perspective on the biological aging processes (13, 14). It is a distinct health state related to the aging process where multiple body systems gradually lose their in-built reserves. The necessity to assess frailty arises from its strong correlation with adverse health outcomes, including falls, hospitalization, disability, and mortality (13). Frailty is commonly evaluated through two primary methods: the frailty phenotype and the frailty index (13, 15, 16). The frailty phenotype assesses five physical components: unintentional weight loss, self-reported exhaustion, weakness (grip strength), slow walking speed, and low physical activity (17). Alternatively, the frailty index is a more comprehensive approach, considering a broader range of deficits across various domains (18). The frailty index has emerged as a highly precise method for assessing the degree of aging and predicting outcomes in the older adults. It quantifies frailty based on a comprehensive account of an individual's health deficits, ranging from comorbidities and cognitive impairments to physical performance and psychosocial risk factors. The index's granularity allows for a detailed depiction of an individual's health status, making it an excellent predictor of mortality, hospitalization, and other adverse health outcomes (13). However, the complexity of conducting a comprehensive geriatric assessment to gather the necessary data for the frailty index means it is not always practical for every patient, particularly in clinical settings where time and resources are limited. This limitation underscores the need for a more selective indicator that can be used to identify high-risk patients efficiently (19). In essence, there is a pressing need for simpler biomarkers, akin to routine blood tests, which can be easily administered and interpreted (14). Such biomarkers would allow clinicians to quickly assess frailty without the exhaustive process currently required, thus streamlining the identification of patients who might benefit most from interventions.

Despite the extensive mechanistic research into aging, the quest to translate this complex knowledge into practical clinical applications, such as the development of new drugs and biomarkers, has been a significant challenge. The development of therapeutic strategies

that can slow down, halt, or reverse the aging process requires a nuanced understanding of aging's multifactorial nature. The pursuit of such pharmacological interventions has led to the identification of potential drug targets and the development of novel biomarkers that can predict the onset and progression of age-related diseases. These advancements hold the promise of personalized medicine, where interventions are tailored to the individual's specific aging profile, potentially revolutionizing the management and treatment of aging and its associated conditions. However, pioneering studies, such as the heterochronic parabiosis models, which involve surgically joining two animals of different ages, have highlighted the importance of circulating pro-aging or pro-youthful factors in age-related physiological changes (20-22). In detail, an exchange with young blood rejuvenated aging phenotypes of old mice (23, 24), while exposing to old blood impaired neurogenesis, cognitive function, muscle strength of young mice (25). These experiments have revealed that factors present in the blood of young animals can actually rejuvenate aged tissues, suggesting that some aspects of aging may be modifiable. This research has opened new avenues for understanding the systemic nature of aging and holds potential for developing therapies that could counteract some of the effects of aging, thus offering a new vision for clinical application in age-related health management. Therefore, researchers around the world have been making tremendous endeavors to uncover specific blood-borne factors present in the systemic milieu for the purpose of further understanding aging process (26).

The process of understanding frailty necessitates the identification of various accelerated aging pathways. Despite this diversity, there is a shared characteristic amongst these pathways: they all contribute to a physiological decline that spans from cellular to organ levels, marking the early phases of the aging process (27, 28). While multiple organs and systems age at different rates and can be individually measured, most diagnoses based on societal consensus are recognized only after a certain extent of progression. However, muscle stands out due to its continuous and vigorous metabolic processes, and highly responsive and often present early symptomatic clues that can be readily observed, making them an ideal metric for detecting physiological decline (29, 30). Moreover, since muscle function directly impacts physical ability, the measurement of the muscle mass and strength can serve as an optimal gauge of the physiologic reservoir. Hence, sarcopenia, characterized by a gradual and general-

ized loss of skeletal muscle mass and function, often considered an early hallmark of frailty, can be provided the significant value in evaluating physical frailty and, by extension, provides a window into the intricate capacity of individuals (31, 32).

Sarcopenia is mainly attributable to imbalance between muscle protein synthesis and breakdown during aging (33). This condition is closely linked to adverse outcomes, including frailty, falls, and functional decline, all of which make it impossible for older people to live independently (34, 35). As such, sarcopenia provides a tangible measure that extends beyond the prognostication of frailty; it serves as a pivotal tool for the early detection of frailty. (29) Sarcopenia is thus a crucial trait of aging and is regarded as a "geriatric giant" due to the explosively increased incidence in super-aged societies (36). Importantly, long thought to be an inevitable byproduct of aging, sarcopenia is now acknowledged as a disease to be overcome due to advances in knowledge about muscle homeostasis (37, 38). Consequently, in order to lead a healthy old life, it is essential to identify various muscle-related contributors and to develop biomarkers and therapeutic targets for sarcopenia based on them.

Beta-2 microglobulin (B2M) constitutes the light chain of major histocompatibility complex class I (MHC-I) molecules, which dissociate from nucleated cells and the cell membrane in response to various stimuli, such as endoplasmic reticulum stress and inflammatory reaction (39, 40). Apart from its traditional immunological function (41), B2M plays a pivotal role in a multitude of physiological processes, encompassing protein homeostasis, cellular adhesion, tissue repair, oxidative stress, and cell signaling (42, 43), and serum B2M is considered an indicator of disease severity in cases of amyloidosis renal injury, infections, and lymphoproliferative disorders (44, 45). Notably, a growing body of evidence suggests the potential involvement of B2M in age-related degeneration as well. When compared to age-matched young isochronic parabionts, plasma from young heterochronic parabionts exhibited increased B2M levels after exposure to aged blood (21, 46), and circulating B2M concentrations demonstrated a consistent rise with chronological age in both humans and mice (46). Furthermore, exogenous B2M injection disrupts cognitive function and neurogenesis in young mice, while the lack of inherent B2M expression or the blockade of B2M function counteract cognitive decline associated with aging and augments neurogenesis in aged mice (46, 47).

These findings have brought great attention to circulating B2M as a systemic factor with implications in the pro-aging process, and have raised the possibility that B2M may also be involved in the pathogenesis of sarcopenia, a key component of aging phenotypes. To clarify the potential role of B2M in muscle metabolism, we investigated the effects of B2M on in vitro and animal muscle biology and its clinical relevance for sarcopenia parameters in older adults.

Materials and Methods

1. Cell culture and reagents

Mouse C2C12 myoblasts were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 15% fetal bovine serum (FBS), 20 mM hydroxyethyl piperazine ethane sulfonic acid (HEPES), 2 mM L-glutamine, 100 U/mL penicillin, and 0.1 mg/mL streptomycin (Life Technologies Corp., Carlsbad, CA, USA). The cells were maintained at 37°C in a humidified atmosphere with 5% CO2. To induce myogenesis, cells were grown to 90% confluence in the maintenance medium and subsequently switched to differentiation medium (DMEM with 2% horse serum) for a duration of 3 to 4 days. Recombinant B2M was procured from Lee Biosolutions (Cat. No. 126-11-10; Maryland Heights, MO).

2. Immunofluorescence

Differentiated C2C12 cells were first fixed with 4% paraformaldehyde (PFA) for 15 min, followed by two washes with phosphate-buffered saline (PBS). Subsequently, they were permeabilized in a solution containing 0.01 M sodium citrate buffer and 0.1% Triton X-100 for 10 min, and then washed twice with PBS. The cells were blocked for 1 h with 2% bovine serum albumin (BSA) in PBS and then incubated overnight at 4°C with an anti-myosin heavy chain (MyHC) antibody (MF20; Developmental Studies Hybridoma Bank, Iowa City, IA). After this primary antibody incubation, the cells were treated with Alexa Fluor 555-conjugated secondary antibodies (diluted 1:1000; Cell Signaling) for 1 h and washed with PBST (PBS containing 0.2% Tween-20). Following that, the cells were stained with 4,6-diamidino-2-phenylindole (DAPI, diluted 1:10,000; Sigma-Aldrich, St. Louis, MO) for 2 min and washed with PBS. The prepared samples were then mounted using Fluoromount G (Southern Biotech, Birmingham, AL), and fluorescence images were captured using a Carl Zeiss fluorescence microscope (Jena, Germany). The area

occupied by MyHC-stained myotubes was quantified using ZEN 2 (blue edition) software (Carl Zeiss). The fusion index (%) was determined using the following formula: 100 × (number of nuclei in $MyHC^+$myotubes) divided by the total number of nuclei in $MyHC^+$ myocytes and myotubes (48).

3. Western blot analysis

Cell lysis was performed utilizing RIPA buffer (composed of 50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 0.1% SDS, 1% sodium deoxycholate, 1 mM Na3VO4, 1 mM NaF, 1 mM PMSF, and a protease inhibitor cocktail). Following a 30-min incubation on ice, the lysates were subjected to centrifugation at 14,000 rpm for 20 min at 4°C. The protein concentration was quantified using a BCA protein assay kit (Pierce Chemical Co., Rockford, IL). Protein samples were separated through sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred onto polyvinylidene fluoride membranes, and subsequently subjected to immunoblotting using the following antibodies: MyHC (MF20) and myogenin (sc-12732; Santa Cruz Biotechnology, Dallas, TX).

4. Quantitative reverse-transcription polymerase chain reaction

Total RNA was extracted following the manufacturer's protocol using TRIzol reagent (Invitrogen, Carlsbad, CA). Subsequently, the first-strand cDNA synthesis was carried out with the Superscript III First-Strand Synthesis System (Invitrogen), employing oligo dT primers. Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was conducted in triplicate using Light Cycler® 480 SYBR Green I Master (Roche, Mannheim, Germany). The primers for myogenin (NM_031189.2) and MyHC (NM_001013397.2) were sourced from Applied Biosystems (Foster City, CA). The threshold cycle (Ct) value for each target gene was normalized to the Ct value of 18S rRNA (NR_003278.3).

5. Migration assay

The chemotaxis assay was conducted utilizing a Boyden chamber system, employing transwells with an 8-μm-pore size polycarbonate membrane (Corning, NY). Cells were seeded into the inner chamber at a density of 8 × 104 cells per transwell, with DMEM containing 0.2% FBS, and subsequently exposed to recombinant B2M in the outer chamber for a duration of 5 h. The cells located on the inner membrane were then thoroughly removed by gently wiping with a cotton swab. Meanwhile, the cells situated on the lower surface of the membrane were fixed using 4% PFA and subsequently stained with crystal violet. To quantify the number of C2C12 cells, images of the stained C2C12 cells were captured using cellSens Standard BX53 software (Olympus, Tokyo, Japan) and analyzed using Image J software (NIH, Bethesda, MD).

6. Viability assay

Cell viability was assessed using the Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan) as per the manufacturer's instructions. In brief, 10 μL of WST-8 dye [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] was introduced into each well of a 96-well plate (49). The mixture was incubated for 1 h, and absorbance readings were subsequently taken at 450 nm with a reference wavelength of 650 nm using a microplate reader (SPECTRAmax 340PC; Molecular Devices, Palo Alto, CA).

7. Measurement of intracellular reactive oxygen species levels

Intracellular ROS levels were quantified by employing the chloromethyl derivative of 2′,7′-dichlorofluorescein diacetate (CM-H2DCFDA, C6827; Invitrogen). Initially, cells were rinsed with serum-free DMEM and then incubated with 10 μM CM-H2DCFDA at 37°C for 30 min, shielded from light, in a 5% CO2 environment. Following this incubation, cells were washed with PBS and visualized under a fluorescent microscope (Carl Zeiss, Jena, Germany). Fluorescence intensity was also measured

using a microplate reader (Infinite 200PRO; Tecan Life Sciences, Zürich, Switzerland) at an excitation wavelength of 490 nm and an emission wavelength of 520 nm.

8. Animals

Animal experimental procedures were approved by the Institutional Animal Care and Use Committee of the Asan Institute for Life Sciences (No. 2019-12-143). Three-month-old male C57BL/6 mice (Orientbio, Seongnam, South Korea) were used in this study. Recombinant B2M (250 μg/ 100 μL) or PBS (100 μL) was intraperitoneally injected with a 31-gage needle 5 times per week for 4 weeks (6 mice per group). Mice were sacrificed at 16 weeks of age following treatment by cardiac puncture. All treatment groups were weight matched and randomized to treatment at the initiation of an experiment. The researcher conducting the treatment was not blinded to the experimental groups, but the researcher assessing muscle parameters was blinded to the analyses.

9. Immunofluorescence and muscle fiber size measurement

Muscle tissues that had been frozen in an optimal cutting temperature compound were sectioned to a thickness of 10 μm using a cryostat microtome (Leica Microsystems, Wetzlar, Germany) and were stained with laminin-DAPI to measure muscle fiber size. Specifically, the frozen muscle tissues were blocked for 1 h and then incubated with primary antibodies (laminin, 1:1000, Sigma-Aldrich) overnight at 4°C. After washing with PBS, the sectioned tissue slides were incubated with secondary antibodies (Alexa Fluor 647 goat anti-rabbit IgG, 1:1000, Invitrogen) for 1 h at room temperature. Subsequently, they were mounted using a mounting medium with DAPI aqueous fluoroshield. The cross-sectional area (CSA) of muscle fibers was determined by manually drawing contours around individual fibers using ZEN 2 (blue edition) software (Carl Zeiss).

10. Skeletal muscle function exploration in mice

The grip strength test was conducted by securing all four limbs of the mouse on a metal grid and pulling them backward five times. From these trials, the three values were averaged after excluding the maximum and minimum values, and this average was used as the measured value, expressed in Newtons (N). Another method for assessing muscle strength was the Kondziella's inverted screen test (50). In this test, a weight equivalent to 8-10% of the mouse's body weight was attached to its tail, and the mouse was placed on a wire cage, which was then slowly inverted. The muscle strength of the mice was determined based on the duration they were able to hang onto the wire mesh. The rota-rod performance test involved placing the mouse on a rotating rod and measuring both the distance the mouse traveled before falling and the time it took for the mouse to fall (Jeungdo Bio & Plant Co., Seoul, South Korea). To ensure accurate measurements, the mice underwent acclimatization exercises the day before the actual testing. Each measurement was taken twice with a break in between, rather than continuously, for increased accuracy.

11. Study participants for clinical research

This cross-sectional study was conducted among a cohort of older adults aged 65 years or older residing in the community in South Korea. These individuals underwent a comprehensive functional assessment at the outpatient geriatric clinic of Asan Medical Center, a teaching hospital in Seoul, between May 2020 and November 2020. Their visits to the clinic were primarily for the management of chronic conditions such as osteoporosis, osteoarthritis, hyperlipidemia, and hypertension, or for the evaluation of common nonspecific symptoms associated with aging, such as fatigue and loss of appetite. None of the participants were residing in hospitals or nursing homes; all of them had the capability to move around independently, either with or without walking aids. Individuals with a life expectancy of less than one year due to malignancy, advanced heart failure, or end-stage renal disease were not included in the study. All 158 eligible participants provided written

informed consent and agreed to have their blood samples collected for enrollment in the research. The study received approval from the Institutional Review Board (IRB no. 2020-0259) and adhered to the ethical guidelines for human experimentation outlined in the Declaration of Helsinki.“

12. Evaluation of sarcopenia in older adults

Experienced nurses conducted interviews and analyzed medical records to gather demographic information and medical histories. Body composition, including muscle mass, were assessed using bioelectrical impedance analysis (InBody S10; InBody, Seoul, South Korea) at frequencies of 1, 5, 50, 250, 500, and 1000 kHz. Appendicular skeletal muscle mass (ASM), representing the combined muscle mass of the upper and lower extremities, was calculated. Additionally, the skeletal muscle mass index (SMI) was computed by dividing ASM by the square of the individual's height (kg/m2). Handgrip strength of the dominant arm was measured with a hand dynamometer (Patterson Medical, Warrenville, IL, USA) (51). Participants were instructed to sit comfortably, bend their elbows at a 90-degree angle, and grasp the dynamometer as firmly as possible. Two measurements of grip strength were taken, with at least one minute between measurements, and the maximum value was recorded. Gait speed was determined over a 4-meter distance, and the time needed to complete five chair stands was documented (52). The short physical performance battery (SPPB) was administered, encompassing assessments of repeated chair stands, standing balance, and gait speed (53).

The 2019 Consensus Guidelines from the Asian Working Group for Sarcopenia were employed to establish the definition of sarcopenia (54). Sarcopenia was diagnosed when individuals presented with low muscle mass combined with weak muscle strength and/or poor physical performance. Low muscle mass was defined as having a SMI of < 7.0 kg/m^2 for men and < 5.7 kg/m^2 for women. Weak muscle strength was determined by handgrip strength measurements of less than 28 kg for men and less than 18 kg for women. Poor physical performance was identified by

slow gait speed (less than 1.0 m/s), prolonged completion time for the five-time chair stand test (12 seconds or more), or a low SPPB total score (9 points or less).

The sarcopenia phenotype score (SPS) for a continuous measure incorporating multiple features of sarcopenia, which ranged from 0 (best) to 3 (worst), was calculated as the numbers of abnormal parameters among the following three items: low muscle mass, weak muscle strength, and slow gait speed (55).

13. Measurement of serum B2M

Blood samples were collected from the antecubital veins of all participants following an overnight fast. Subsequently, the samples underwent centrifugation at 3000 rpm for 5 min at 4°C, resulting in the careful separation of the supernatant to eliminate cellular components. Samples displaying indications of hemolysis or clotting were excluded from the analysis. The obtained serum samples were then stored at -80°C until the quantification of serum B2M concentrations was conducted using the Parameter immunoassay kit (Cat. No. KGE019; R&D Systems, Minneapolis, MN, USA) as per the manufacturer's guidelines. The ELISA kit possessed a lower limit of detection of 0.132 μg/mL, with both intra-assay and inter-assay coefficients of variation measuring below 7.5% and 18.4%, respectively.

14. Statistical analysis

The in vitro data are presented as the mean ± standard error of the mean (SEM) derived from a minimum of three independent experiments, each with triplicate measurements, unless stated otherwise. We assessed the significance of variations among three or more groups using analysis of variance (ANOVA) with subsequent *post hoc* analysis using Tukey's honest significance test, and we evaluated the differences between two groups using the Mann-Whitney *U* test."

The clinical data were presented as means ± standard deviation (SD), along with numbers and percentages. To compare the baseline characteristics of participants

with and without sarcopenia, we used the Student's *t*-test for continuous variables and the chi-square test for categorical variables. We assessed the association between age and serum B2M levels through Pearson correlation analyses. Analysis of covariance (ANCOVA) was employed to compare the estimated mean serum B2M levels with respect to sarcopenia status and related parameters, while adjusting for sex, age, and body mass index (BMI). Linear regression analysis was conducted to evaluate the relationship between serum B2M levels and specific muscle parameters relevant to sarcopenia, both before and after controlling for sex, age, and BMI. Logistic regression analysis was performed to calculate odds ratios (ORs) for the risk of sarcopenia and adverse muscle outcomes based on serum B2M increments. All statistical analyses were carried out using SPSS version 18.0 (SPSS Inc., Chicago, IL). A significance level of $P < 0.05$ was considered statistically significant.

Results

1. Effects of recombinant B2M on in vitro myogenesis

Myoblasts from the C2C12 cell line were differentiated into mature myotube with and without recombinant B2M in order to examine the effects of B2M on *in vitro* muscle metabolism. Myotube number, myotube area, myotube area per myotube, nuclei number per myotube, and fusion index were all significantly reduced following recombinant B2M treatment in a dose-dependent manner (**Fig. 1A**). Furthermore, recombinant B2M treatment resulted in a decrease in the proportion of large myotube area and an increase in the proportion of small myotube area (**Fig. 1A**). Consistently, western blot and quantitative RT-PCR analyses revealed that recombinant B2M significantly reduced the protein and mRNA expression levels of myogenic differentiation markers, including myogenin and MyHC were markedly decreased by recombinant B2M (**Fig. 1B** and **1C**, respectively). When we looked into the potential impacts of B2M on other muscle biology, recombinant B2M significantly suppressed the migration of C2C12 myoblasts (**Fig. 1D**) without affecting the viability (**Fig. 1E**). These findings provide *in vitro* proof that B2M plays a detrimental role in muscle metabolism primarily by inhibiting muscle differentiation.

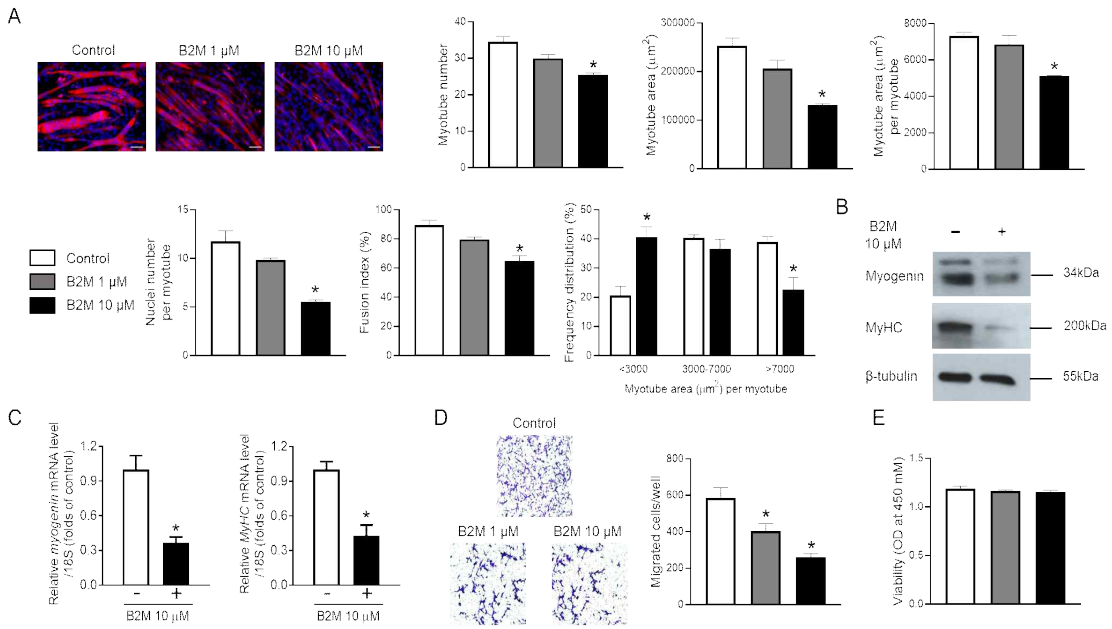


Figure 1. Recombinant B2M suppresses in vitro muscle differentiation.

(A) Mouse C2C12 myoblasts were differentiated into myotubes with 2% horse serum after exposure to the indicated concentrations of recombinant B2M for 3 days. Myotubes were stained with anti-myosin heavy chain (MyHC) antibody while nuclei were counterstained with 4,6-diamidino-2-phenyindole. Quantitative results per field are presented (n = 4). (B) Westen blot and (C) quantitative reverse-transcription polymerase chain reaction analyses of myogenin and MyHC in C2C12 cells with 2% horse serum in the presence or absence of 10 μM B2M for 3 days (n = 3). (D) The directional migration and (E) viability of mouse C2C12 myoblasts were assessed by a Boyden chamber system and cell counting kit-8 assay after exposure to the indicated concentrations of recombinant B2M for 6 and 24 h, respectively (n = 5). Scale bars: 100 μm (A) and 50 μm (D). B2M, β2-Microglobulin; OD, optical density. Data are expressed as mean ± standard error of the mean. $^{*}P < 0.05$ vs. untreated control.

2. Increased intracellular reactive oxygen species (ROS) production underlies the inhibitory effects of B2M on myogenesis

To ascertain the extent of intracellular ROS production, we assessed CM-H2DCFDA intensity in the presence or absence of recombinant B2M treatment during myogenesis. The intracellular ROS levels in myotubes were enhanced by B2M in a dose-dependent manner with a 1.9-fold rise in CM-H2DCFDA content at 10 μM B2M (**Fig. 2A**). It was interesting to note that pretreatment with NAC, a strong biological thiol antioxidant, dramatically decreased the stimulation of intracellular ROS production caused by B2M in myotubes (**Fig. 2B**). NAC also significantly attenuated the effects of B2M on myotube area, myotube area per myotube, nucleus number per myotube, and fusion index (**Fig. 2C**). Consistently, decreased mRNA and protein expressions of myogenin and/or MyHC by B2M were markedly reversed by NAC pretreatment during myogenesis (**Fig. 2D** and **2E**, respectively). These data demonstrated the significance of oxidative stress in the suppression of muscle differentiation brought on by treatment with recombinant B2M.

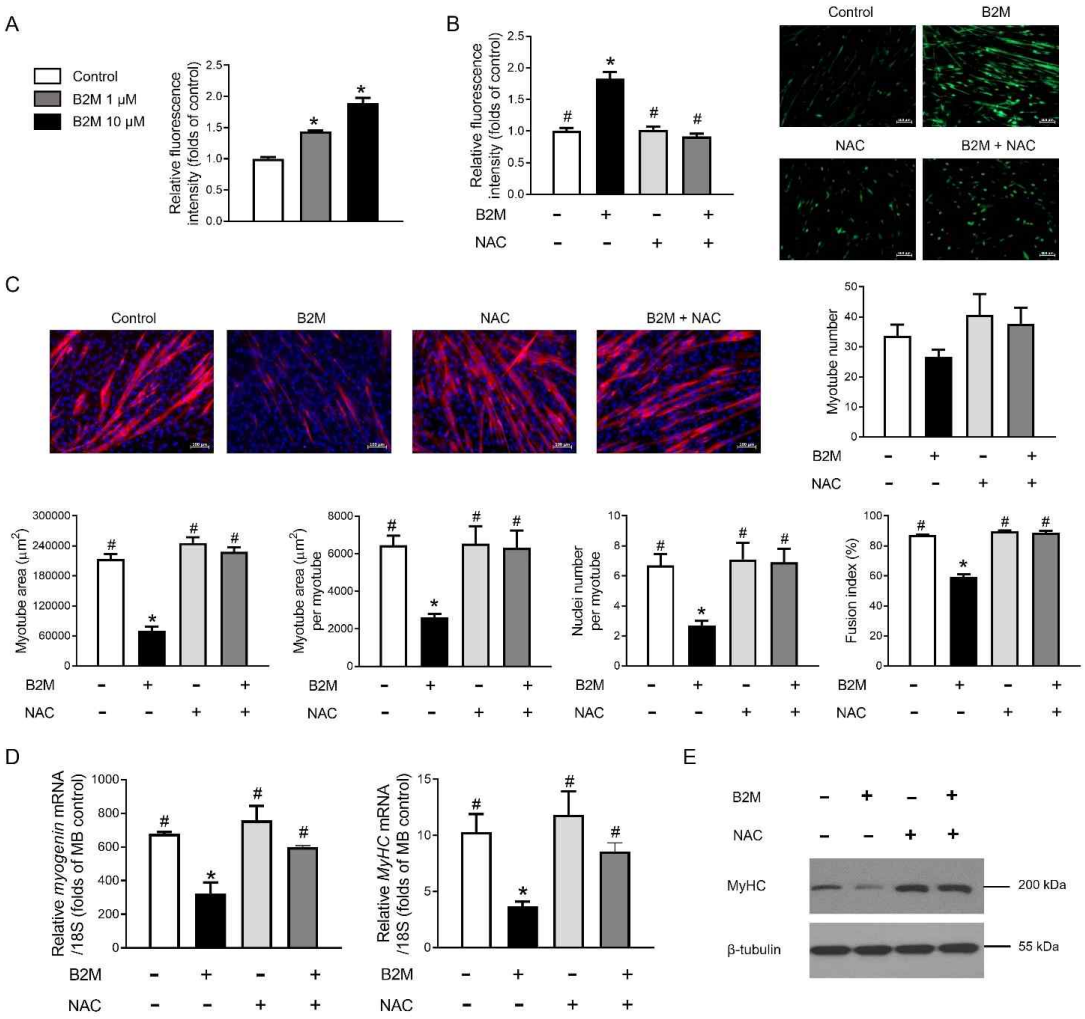


Figure 2. The inhibitory effects of B2M on myogenesis are mediated by increased intracellular reactive oxygen species (ROS) generation.

(A) Mouse C2C12 myoblasts were differentiated into myotubes with 2% horse serum after exposure to the indicated concentrations of recombinant B2M for 3 days. Intracellular ROS levels were measured using a fluorescent probe, chloromethyl derivative of 2′,7′-dichlorofluorescein diacetate (CM-H_2DCFDA;n = 5). (B and C) Mouse C2C12 myoblasts were differentiated into myotubes with 2% horse serum in the presence or absence of 10 μM B2M and/or 1 mM N-acetyl cysteine (NAC) for 3 days. (B) Intracellular ROS levels were measured using H_2DCFDA(n = 3). (C) Myotubes were stained with anti-myosin heavy chain (MyHC) antibody while nuclei

were counterstained with 4,6-diamidino-2-phenyindole. Quantitative results per field are presented (n = 3). (D) Quantitative reverse-transcription polymerase chain reaction and (E) westen blot analyses of MyHC and/or myogenin in C2C12 cells with 2% horse serum in the presence or absence of 10 μM B2M and/or 1 mM NAC for 3 days (n = 3). Scale bars: 100 μm (B) and 100 μm (C). B2M, β2-Microglobulin. $^{*}P < 0.05$ vs. untreated control; $^{\#}P < 0.05$ vs. 10 μM B2M.

3. Decrease muscle size, muscle strength, and physical performance by systemic B2M treatment in mice

To investigate the in vivo effects of B2M on muscle phenotypes, 3-month-old male mice were intraperitoneally injected with 250 μg of B2M for 4 weeks. Untreated controls and B2M-injected mice were matched for body weight at baseline, and there was no difference in body weight after 4 weeks of treatment as well (**Fig. 3A**). Compared to the controls, B2M treatment reduced the cross-sectional area (CSA) of TA and soleus muscles by 28.5% and 16.1%, respectively (**Fig. 3B** and **Fig. 3C**, respectively), and increased the number of small fibers while decreasing the number of large fibers in both muscle types. Furthermore, the grip strength, grid hanging time, and latency time to fall from the rotating rod in mice treated with B2M decreased by 21.2%, 45.4%, and 41.5%, respectively, compared to untreated controls (**Fig. 3D**, **Fig. 3E**, and **Fig. 3F**, respectively). Next, changes in muscle functions were evaluated before and after 4 weeks of treatment in each of the control mice and B2M-injected mice. In both groups, body weight significantly increased to a similar extent after 4 weeks. However, grip strength, grid hanging time, and latency time to fall from the rotating rod decreased by 29.9%, 51.9%, and 40.7%, respectively, after 4 weeks of B2M treatment, whereas there was no change in the untreated controls (**Fig. 3H**, **Fig. 3I**, and **Fig. 3J**, respectively). Consistent with these results, western blot analyses of TA and soleus muscles showed that B2M treatment markedly inhibited the expression of MyHC, a myogenesis marker (**Fig. 3K**).

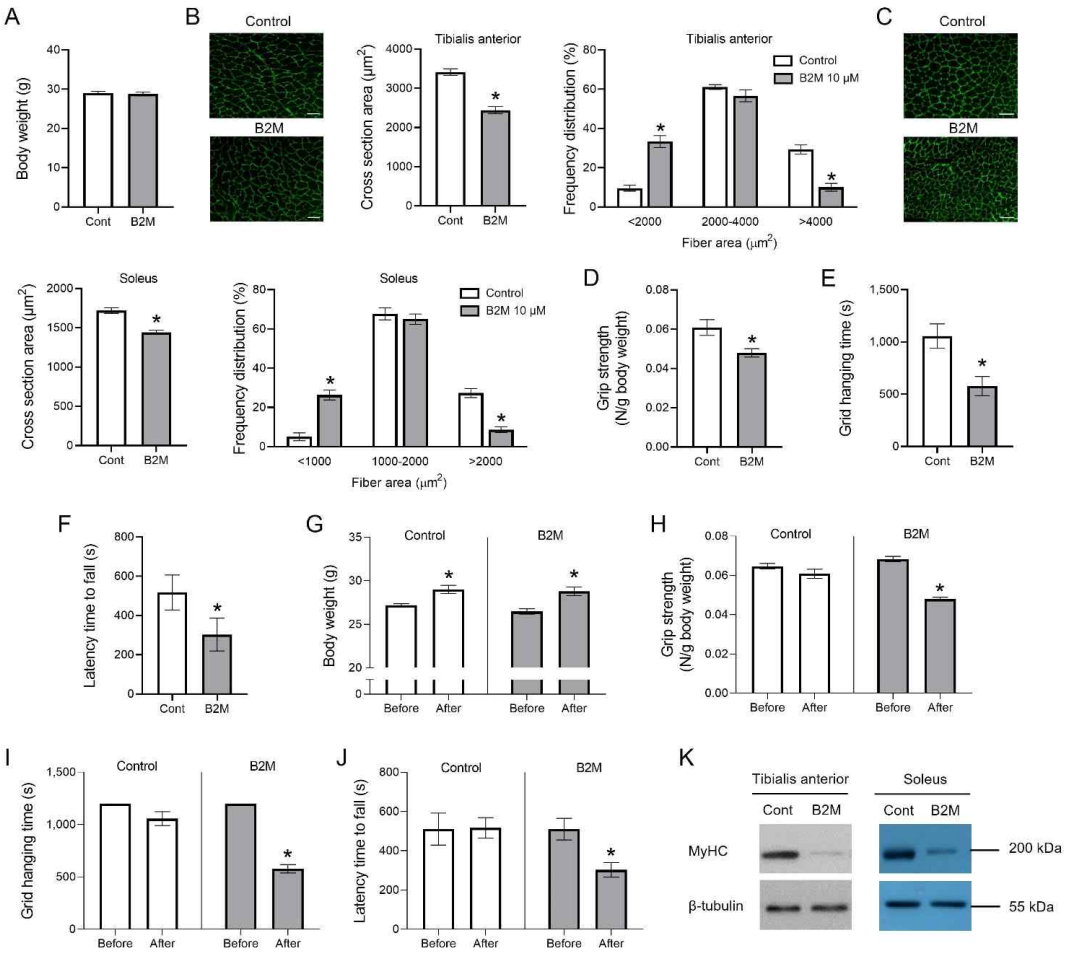


Figure 3. Systemic B2M treatment reduces muscle strength as well as the size of muscle fiber in mice.

Three-month-old male mice were intraperitoneally injected with PBS (100 μL) or recombinant B2M (250 μg/ 100 μL) for 4 weeks (n = 6 per group). (A–F) Muscle phenotypes were compared between untreated controls and B2M-injected mice after 4 weeks. (A) Body weight, (B) tibialis anterior size, (C) soleus size, (D) grip strength, (E) grid hanging time, and (F) latency time to fall from the rotating rod. (G–J) Muscle phenotypes were compared before and after 4 weeks of treatment in each of the control mice and B2M-injected mice. (G) Body weight, (H) grip strength, (I) grid hanging time, and (J) latency time to fall from the rotating rod. (K) Westen blot analyses in tibialis anterior and soleus muscles after 4 weeks of treatment. Scale bars: 100 μm (B) and 100 μm (C). B2M, β2-Microglobulin; PBS, phosphate-buffered

saline. $^{*}P < 0.05$ vs. untreated control or before treatment.

4. Differences in serum B2M level according to the status of sarcopenia and muscle phenotypes in older adults

To ascertain the clinical relevance of B2M's effects on muscle homeostasis in *in vitro* and animal experiments, circulating B2M concentrations were measured in 158 older adults aged 65 and older and their baseline characteristics are presented in **Table 1**. Among the 118 controls without sarcopenia and 40 cases with sarcopenia, 97 (82.2%) and 29 (72.5%) were women, respectively. The mean age of controls was 75.3 ± 5.2 years, whereas that of cases was 79.7 ± 4.6 years (P <0.001). Compared to the control group, participants with sarcopenia exhibited lower weight, BMI, ASM, SMI, grip strength, gait speed, and SPPB total score (P = 0.023 to <0.001). On the other hand, their chair stand test time and SPS score were significantly higher (P = 0.003 and <0.001, respectively).

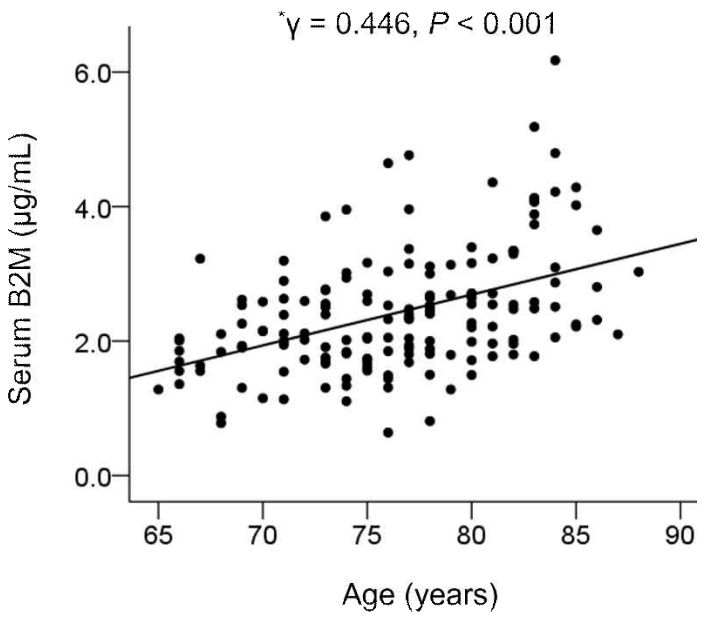
Table 1. Basic clinical characteristics of the study participants

	No sarcopenia (n = 118)	Sarcopenia (n = 40)	*p*
Age, y	**75.3 ± 5.2**	**79.7 ± 4.6**	**<0.001**
Female	97 (82.2)	29 (72.5)	0.254
Body weight, kg	**59.4 ± 9.6**	**53.6 ± 5.5**	**<0.001**
Height, cm	155.0 ± 6.5	153.2 ± 6.9	0.157
Body mass index, kg/m^2	**25.4 ± 5.5**	**22.9 ± 2.5**	**0.007**
Diabetes mellitus	46 (39.0)	13 (32.5)	0.571

Polypharmacy	59 (50.0)	24 (60.0)	0.360
Fall in previous year	21 (17.8)	8 (20.0)	0.814
Appendicular skeletal muscle mass, kg	**15.2 ± 2.9**	**13.2 ± 2.3**	**<0.001**
Skeletal muscle mass index, kg/m^2	**6.30 ± 0.79**	**5.56 ± 0.52**	**<0.001**
Grip strength, kg	**25.9 ± 6.3**	**19.9 ± 5.1**	**<0.001**
Usual gait speed, m/s	**1.04 ± 0.24**	**0.77 ± 0.26**	**<0.001**
Chair stand test time, s	**10.4 ± 5.5**	**17.3 ± 13.5**	**0.003**
SPPB total score (ranges, 0-12)	**10.9 ± 1.6**	**8.7 ± 3.1**	**<0.001**
SPS score (range, 0-3)	**0.73 ± 0.57**	**2.40 ± 0.50**	**<0.001**
Serum B2M, μg/mL	**2.26 ± 0.87**	**2.62 ± 0.86**	**0.023**

Data are presented as mean ± standard deviation or n (%). Differences between the two groups were assessed using Student's *t*-tests for continuous variables and χ^2 test for categorical variables. B2M, β2-microglobulin; SPS, sarcopenia phenotype score; SPPB, short physical performance battery.

Pearson correlation analyses with scatter plots revealed that serum B2M concentrations increased with age, which in consistent with previous reports (γ = 0.446, P <0.001; **Supplemental Fig. 1**) (46).



Supplemental Figure 1. Pearson correlation coefficient with scatter plots for the association of age with serum B2M level.

* indicates a statistically significant value. B2M, β2-microglobulin.

Differences in serum B2M levels according to status of sarcopenia and related specific component were evaluated using ANCOVA before and after adjustment for confounding factors. In a crude analysis, serum B2M levels were higher in participants with sarcopenia, weak grip strength, slow gait speed, prolonged chair stand test time, low SPPB total score, and poor physical performance (P = 0.023 to <0.001), although there was no significant difference of serum B2M between those with and without low muscle mass (**Fig. 4A**). After adjustment for sex, age, and BMI, the statistical significance of serum B2M levels in relation to the status of weak grip strength, slow gait speed, low SPPB total score, and poor physical performance persisted (P = 0.006 to 0.048; **Fig. 4B**). However, in this multivariable adjustment model, serum B2M concentrations were no longer different between those with and without sarcopenia or prolonged chair stand test time.

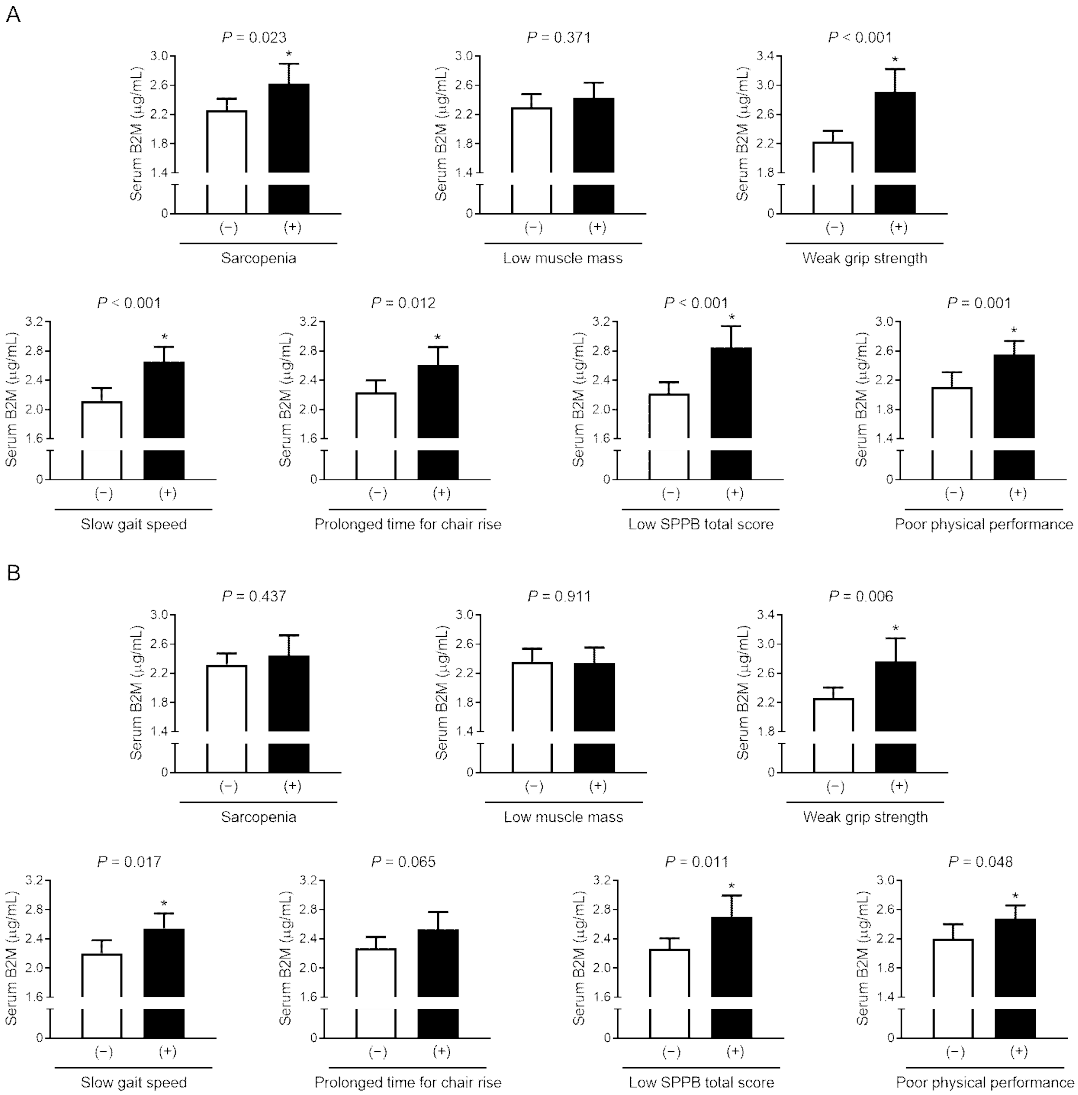


Figure 4. Serum B2M level by sarcopenia and muscle-related parameters before (A) and after (B) adjusting for age, sex, and body mass index.

The estimated mean values with 95% confidence intervals were calculated and compared using analysis of covariance. * indicates a statistically significant difference from control. B2M, β2-microglobulin.

5. Association between serum B2M level and sarcopenia-related parameters in older adults

Linear regression analyses were conducted to explore the relationship of serum B2M levels with specific muscle parameters relevant to sarcopenia (**Table 2**). Regardless of adjustment models, circulating B2M levels were positively associated with SPS and inversely associated with grip strength, gait speed, and SPPB total score (P <0.001 to 0.024), but not with SMI and chair stand test time.

Table 2. Associations of serum B2M level with muscle-related parameters by linear regression analysis

Dependent variable	Unadjusted		Age, sex, and BMI adjusted	
	B	*P*	B	*P*
Sarcopenia phenotype score	**0.300**	**<0.001**	**0.178**	**0.024**
Skeletal muscle mass index	−0.013	0.868	0.053	0.396
Grip strength	**−0.291**	**<0.001**	**−0.216**	**0.001**
Usual gait speed	**−0.364**	**<0.001**	**−0.249**	**0.002**
Chair rise test time	0.154	0.054	0.060	0.472
SPPB total score	**−0.316**	**<0.001**	**−0.212**	**0.008**

The Enter method is applied to this model with serum B2M level as an independent variable. Bold numbers indicate statistically significant values. B, standardized regression coefficient; B2M, β2-microglobulin; SPPB, short physical performance battery.

The risk of sarcopenia and poor muscle outcomes according to serum B2M levels in older adults was assessed by logistic regression analyses (**Table 3**). Prior to

adjustment for confounding variables, the ORs for weak grip strength, slow gait speed, low SPPB total score, and poor physical performance per 1 μg/mL increase in serum B2M level were 2.23, 2.18, 2.16, and 1.95, respectively (P <0.001 to 0.002), and increased risk of these conditions with elevated serum B2M levels was still significant even after accounting for age, sex, and BMI (P = 0.019 to 0.046). However, higher ORs for sarcopenia and prolonged chair stand test time by serum B2M increase were only evident in the univariate model (P = 0.027 and 0.015, respectively), with statistical significance diminishing upon adjustment for potential confounding factors. Furthermore, the risk of low muscle mass per 1 μg/mL increase in serum B2M level was insignificant in either unadjusted or adjusted analyses.

Table 3. Association of presence of sarcopenia or abnormalities in sarcopenia parameters with serum B2M level by logistic regression analysis

Dependent variable	Unadjusted		Age, sex, and BMI adjusted	
	OR (95% CI) per serum B2M increment	*P*	OR (95% CI) per serum B2M increment	*P*
Sarcopenia	**1.57 (1.05–2.35)**	**0.027**	1.22 (0.78–1.93)	0.382
Low muscle mass	1.18 (0.82–1.69)	0.370	1.03 (0.66–1.16)	0.898
Weak grip strength	**2.23 (1.40–3.55)**	**0.001**	**1.85 (1.11–3.10)**	**0.019**
Slow gait speed	**2.18 (1.42–3.36)**	**<0.001**	**1.72 (1.08–2.72)**	**0.021**
Prolonged time for chair rise	**1.62 (1.10–2.40)**	**0.015**	1.47 (0.96–2.23)	0.075
Low SPPB total score	**2.16 (1.38–3.37)**	**0.001**	**1.74 (1.06–2.85)**	**0.029**
Poor physical performance	**1.95 (1.27–2.99)**	**0.002**	**1.60 (1.01–2.52)**	**0.046**

Bold numbers indicate statistically significant values. B2M, β2-microglobulin; OR, odds ration; CI, confidence interval; SPPB, short physical performance battery.

Discussion

Given the backgrounds implicating B2M in various age-related diseases, we conducted in vitro and animal research, which demonstrated that recombinant B2M treatment suppresses myogenesis by triggering the generation of intracellular ROS. Moreover, our subsequent investigation to understand its clinical implications revealed that higher serum B2M levels were associated with a higher risk of weak grip strength, slow gait speed, a low SPPB total score, and poor physical performance in older adults. These results provide experimental evidence that B2M, which exerts detrimental effects on muscle metabolism, could be a potential therapeutic target for sarcopenia. Additionally, they suggest that circulating B2M could serve as one of the blood-based biomarkers for assessing poor muscle health in humans.

Blood, often referred to as the lifeline of our body, plays a pivotal role in transporting oxygen, nutrients, hormones, inflammatory markers, various signaling molecules, and even genetic material such as microRNAs to all organs and tissues. Consequently, it holds a wealth of information about an individual's comprehensive pathophysiological condition. One prominent feature of aging phenotypes is the simultaneous deterioration of multiple organs. For instance, older adults with cognitive impairment are known to be more susceptible to cardiovascular diseases, osteoporosis, and sarcopenia (56-58). Therefore, to elucidate these concurrent functional declines associated with aging, researchers have increasingly focused on age-related changes in the blood, which courses through every corner of our body (12, 59-61). The significance of employing the circulatory system in aging research is strongly underscored by the results from parabiosis, an experimental technique where two animals, typically a young and an old individual, are surgically connected, allowing their circulatory systems to intermingle. The key findings in this model are that exposure to young blood rejuvenates the physiological status of the heart, pancreas, brain, skeletal muscle, and bone in old mice (20, 21, 23, 62, 63), whereas exchange with old blood markedly reduces the functional and cognitive reserve of young mice (25). Subsequently, through the efforts of many researchers, specific circulating

factors that either exacerbate or ameliorate the aging phenotypes have been reported (26). In more detail, apelin, cadrerin13, extracellular nicotinamide phosphoribosyltransferase (eNAMPT), and oxytocin have been proposed as systemic rejuvenating factors that extend murine lifespan, reverse muscle wasting, inhibit cellular senescence, and prevent bone loss (64-68). On the contrary, C-C motif chemokine ligand 11 (CCL11) and growth differentiation factor 11 (GDF11) have been suggested as pro-aging factors in blood, impairing cognitive function, liver regeneration, and muscle regeneration (21, 69). However, since aging process is not as simple as can be explained by one key molecule, continuous efforts to discover various circulating factors that regulate aging and to elucidate their interrelationships are necessary to extend the healthy lifespan in old age.

Sarcopenia, characterized by the loss of muscle mass and function, is becoming increasingly important from a public health perspective in the era of extreme aging, as it is associated with various adverse outcomes in the elderly population (34, 37). Despite its growing significance, there is currently no approved treatment for this condition. This underscores the fact that sarcopenia remains a hot topic in aging research, highlighting the urgent need for innovative approaches to address this complex syndrome and improve the quality of life for older individuals. As part of our efforts to uncover potential factors contributing to the development of sarcopenia, we have been particularly focused on exploring the role of B2M in muscle metabolism. B2M is a nonglycosylated protein consisting of 119 amino acid residues, with a secreted form comprising 99 amino acids and a molecular weight of 12 kDa (70). The circulating B2M concentration consistently showed elevation not only in young partners within heterochronic parabiosis experiments and aged mice but also in older humans (21, 46), and our clinical study, which included older adults, confirmed a positive correlation between age and serum B2M level (Supplemental Figure 1). Importantly, the administration of recombinant B2M resulted in impaired neurogenesis, synaptic dysfunction, and memory deficits in mice, whereas the use of B2M antagonists or genetic ablation of B2M effectively mitigated these detrimental phenotypes in aged mice (46, 47). These discoveries have garnered significant interest

in circulating B2M as a systemic factor with relevance to the aging process. Given that sarcopenia is a key phenotype of aging, we conducted current experiments and found that recombinant B2M inhibited in vitro myogenesis in a dose-dependent manner and decreased muscle size, muscle strength, and physical performance in mice. Collectively, manipulations mitigating the effects of various pro-aging factors have been proposed as potentially effective approaches for slowing or reversing the aging process, and B2M may be one of these therapeutic targets for age-related diseases, including sarcopenia and dementia.

What sets this study apart from previous research is that we conduced translation research to elucidate the role of B2M in human muscle health by expanding upon the findings from an experimental study that confirmed its detrimental impact on muscle metabolism. Due to the impossibility of conducting interventional trials in humans without prior safety verification from preclinical data, the roles of candidate factors in humans must be extrapolated from observational results, which has led us to undertake current clinical study. Consequently, in line with results from in vitro and animal experiments, high serum B2M concentrations were associated with poor muscle phenotypes in older adults. A particularly intriguing finding in our clinical study is that serum B2M levels were primarily associated with muscle strength and physical performance, showing limited correlation with muscle mass, among the diagnostic criteria for sarcopenia. Indeed, muscle mass, muscle strength, and physical performance each offer unique insights into different aspects of muscle health. Muscle mass primarily reflects the quantity or size of muscle tissue in the body, signifying its structural composition (71). Muscle strength represents how much force a muscle or group of muscles can generate during contraction (72), while physical performance encompasses a range of abilities such as coordination, endurance, and power, reflecting the integrated efficiency of muscle groups during activities (73). Importantly, various factors, such as neural control, coordination, and the quality of muscle tissue, can impact muscle strength and physical performance independently (56, 74) of muscle mass (75, 76). Therefore, we speculate that B2M may directly affect the functional aspects of muscles, such as their contractile properties,

neuromuscular efficiency, or energy utilization, rather than causing a significant change in muscle size in humans. All these findings emphasize the complexity and multifaceted nature of muscular physiology.

Since Dr. Rosenberg first described sarcopenia as the age-associated loss of skeletal muscle mass in 1989 (77), there have been updates and refinements to this definition over the years as our understanding of sarcopenia has grown. One significant change was the introduction of the concept that muscle mass alone does not fully capture the functional consequences of sarcopenia and that muscle strength and physical performance are important aspects when assessing muscle health (37, 78). Despite this continued emphasis on muscle function, most existing guidelines still consider muscle mass as a crucial parameter for defining sarcopenia (34, 54, 79, 80). To maintain consistency with our previous study, this research adopts the AWGS guideline (54), which necessarily includes the criterion of low muscle mass, among the various operational definitions of sarcopenia proposed by different working groups. As a result, we could not find a significant association between circulating B2M levels and the risk of sarcopenia before and after adjustment for confounders. However, recent evidence-based analyses conducted by the Sarcopenia Definition and Outcomes Consortium (SDOC) have highlighted that muscle weakness and slow gait speed independently predict falls, self-reported mobility limitations, hip fractures, and mortality among community-dwelling older adults, whereas appendicular lean mass was not associated with these adverse health-related outcomes, regardless of adjustments for body size (72). Consequently, SDOC opted not to include muscle mass as part of their sarcopenia definition. Furthermore, the Korean Working Group on Sarcopenia (KWGS) recognized the clinical significance of weak muscle strength combined with poor physical performance and coined the term 'functional sarcopenia' to describe this condition, even in the absence of low muscle mass (81). In this context, if this study applies a definition of sarcopenia that prioritizes muscle function over one based on muscle mass, there is a 1.5-fold increase in the risk of sarcopenia for every 1 μg/mL increase in serum B2M concentration. The fact that research results can be interpreted differently depending on which guidelines are used

underscores the necessity for establishing an international consensus on the definition and diagnosis of sarcopenia (32, 80).

In order to explore the potential mechanisms that underlie the adverse impact of B2M on muscle metabolism, our research has centered on examining alterations in redox signaling. Within a well-functioning skeletal muscle system, the generation of oxidative species is carefully balanced by their elimination through both exogenous and endogenous antioxidant molecules (82, 83). Nonetheless, an imbalance between these processes under specific pathological conditions disrupts the usual redox equilibrium, a phenomenon commonly described as "oxidative stress" (84). Several research findings have consistently suggested that oxidative stress can result in a range of detrimental effects within skeletal muscle. These effects encompass a triggered inflammatory response, excitation-contraction uncoupling, an increase in myonuclear apoptosis, dysregulated autophagy, heightened ubiquitin proteasome activity, disruptions in mineral homeostasis, and mitochondrial dysfunction (82, 85, 86). These multifaceted processes collectively contribute to muscle fatigue, impaired recovery after exercise or injury, and muscle atrophy due to increased protein breakdown, ultimately compromising the strength, endurance, and overall performance of skeletal muscle. Notably, experimental research has revealed that B2M induces cellular damage by augmenting the ROS production (87). Moreover, a correlation between B2M and oxidative stress has been observed in the elderly population (88), suggesting that oxidative stress could potentially mediate the role of B2M as a pro-aging factor. Building upon this background, our study delved into the investigation of whether recombinant B2M leads to an increase in intracellular ROS production within muscle cells and whether antioxidants reverse the detrimental impact of B2M on myogenesis. As a result, our findings underscore the pivotal role of oxidative stress as a key regulator in mediating the influence of B2M on muscle metabolism.

This study presents a significant advantage as it incorporates comprehensive investigations at the cellular, animal, and clinical levels, collectively providing robust

evidence to elucidate the influence of B2M on muscle metabolism. The other major strength is our clinical assessment of all re-quired parameters to define sarcopenia, including muscle mass, handgrip strength, gait speed, SPPB score, and 5-time chair-stand test, thereby enhancing the reliability of our findings (34, 54). Furthermore, we utilized Asian-specific cutoff values for the diagnosis of sarcopenia in older adults, recognizing that muscle phenotypes can exhibit variations based on lifestyles, ethnicities, cultural backgrounds, and body sizes (54).

Despite these strengths, it is essential to acknowledge several limitations that should be taken into account when interpreting the data. Most importantly, due to the cross-sectional design of our clinical study, we can ascertain the association between serum B2M concentration and muscle phenotypes in older adults, but we cannot confirm whether the current circulating B2M level can predict the future occurrence of sarcopenia. Secondly, the inclusion of participants from a single medical center may limit the generalizability of our findings. Thirdly, the use of bioelectrical impedance analysis represents an indirect approach to assess muscle mass, which could potentially affect measurement accuracy (89). In fact, recent studies have indicated that newer methods, like the D3 creatinine dilution method, offer superior reliability in assessing muscle mass when compared to other techniques, such as dual-energy X-ray absorptiometry (90). Future studies adopting these advanced methods may yield results that differ from those of the present study. Lastly, while we made efforts to consider various confounding factors in our analyses, we cannot rule out the possibility that the observed association may have been influenced by uncontrolled factors affecting B2M and/or muscle variables.

Conclusion

In conclusion, recombinant B2M dose-dependently inhibited in vitro myogenesis by increasing oxidative stress and reduced muscle size, muscle strength, and physical performance in mice. Furthermore, higher serum B2M levels, which increased with age, were significantly associated with poor muscle phenotypes in older adults. These consistent experimental and clinical findings support the detrimental impact of B2M on muscle homeostasis, suggesting that B2M may be a therapeutic target for sarcopenia and raising the possibility that measuring serum B2M levels could offer additional insights for evaluating muscle health in old age. Further meticulously designed large-scale longitudinal studies are imperative to confirm the role of circulating B2M as a blood-based biomarker for predicting the risk of sarcopenia.

References

1. Dall TM, Gallo PD, Chakrabarti R, West T, Semilla AP, Storm MV. An aging population and growing disease burden will require a large and specialized health care workforce by 2025. Health Aff (Millwood). 2013;32(11):2013-20.
2. Atella V, Piano Mortari A, Kopinska J, Belotti F, Lapi F, Cricelli C, et al. Trends in age-related disease burden and healthcare utilization. Aging Cell. 2019;18(1):e12861.
3. Tang B, Li Z, Hu S, Xiong J. Economic Implications of Health Care Burden for Elderly Population. Inquiry. 2022;59:469580221121511.
4. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013;153(6):1194-217.
5. Mylonas A, O'Loghlen A. Cellular Senescence and Ageing: Mechanisms and Interventions. Front Aging. 2022;3:866718.
6. Ferrucci L, Fabbri E. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. Nat Rev Cardiol. 2018;15(9):505-22.
7. van den Beld AW, Kaufman JM, Zillikens MC, Lamberts SWJ, Egan JM, van der Lely AJ. The physiology of endocrine systems with ageing. Lancet Diabetes Endocrinol. 2018;6(8):647-58.
8. Pataky MW, Young WF, Nair KS. Hormonal and Metabolic Changes of Aging and the Influence of Lifestyle Modifications. Mayo Clin Proc. 2021;96(3):788-814.
9. Barja G. Towards a unified mechanistic theory of aging. Exp Gerontol. 2019;124:110627.
10. Sharkey BJ. Functional vs chronologic age. Med Sci Sports Exerc. 1987;19(2):174-8.
11. Peretz L, Rappoport N. Deviation of Physiological from Chronological Age Is Associated with Health. Stud Health Technol Inform. 2022;294:224-8.
12. Menni C, Kastenmüller G, Petersen AK, Bell JT, Psatha M, Tsai PC, et al. Metabolomic markers reveal novel pathways of ageing and early development in human populations. Int J Epidemiol. 2013;42(4):1111-9.
13. Clegg A, Young J, Iliffe S, Rikkert MO, Rockwood K. Frailty in elderly people. Lancet. 2013;381(9868):752-62.

14. Diebel LWM, Rockwood K. Determination of Biological Age: Geriatric Assessment vs Biological Biomarkers. Curr Oncol Rep. 2021;23(9):104.
15. Abellan van Kan G, Rolland Y, Houles M, Gillette-Guyonnet S, Soto M, Vellas B. The assessment of frailty in older adults. Clin Geriatr Med. 2010;26(2):275-86.
16. Cesari M, Gambassi G, van Kan GA, Vellas B. The frailty phenotype and the frailty index: different instruments for different purposes. Age Ageing. 2014;43(1):10-2.
17. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, et al. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci. 2001;56(3):M146-56.
18. Rockwood K, Mitnitski A. Frailty in relation to the accumulation of deficits. J Gerontol A Biol Sci Med Sci. 2007;62(7):722-7.
19. Soo WK, King MT, Pope A, Parente P, Dārziņš P, Davis ID. Integrated Geriatric Assessment and Treatment Effectiveness (INTEGERATE) in older people with cancer starting systemic anticancer treatment in Australia: a multicentre, open-label, randomised controlled trial. Lancet Healthy Longev. 2022;3(9):e617-e27.
20. Katsimpardi L, Litterman NK, Schein PA, Miller CM, Loffredo FS, Wojtkiewicz GR, et al. Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. Science. 2014;344(6184):630-4.
21. Villeda SA, Luo J, Mosher KI, Zou B, Britschgi M, Bieri G, et al. The ageing systemic milieu negatively regulates neurogenesis and cognitive function. Nature. 2011;477(7362):90-4.
22. Laviano A. Young blood. N Engl J Med. 2014;371(6):573-5.
23. Villeda SA, Plambeck KE, Middeldorp J, Castellano JM, Mosher KI, Luo J, et al. Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. Nat Med. 2014;20(6):659-63.
24. Loffredo FS, Steinhauser ML, Jay SM, Gannon J, Pancoast JR, Yalamanchi P, et al. Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. Cell. 2013;153(4):828-39.

25. Rebo J, Mehdipour M, Gathwala R, Causey K, Liu Y, Conboy MJ, et al. A single heterochronic blood exchange reveals rapid inhibition of multiple tissues by old blood. Nat Commun. 2016;7:13363.
26. Kang JS, Yang YR. Circulating plasma factors involved in rejuvenation. Aging (Albany NY). 2020;12(22):23394-408.
27. Taylor JA, Greenhaff PL, Bartlett DB, Jackson TA, Duggal NA, Lord JM. Multisystem physiological perspective of human frailty and its modulation by physical activity. Physiol Rev. 2023;103(2):1137-91.
28. Romero-Ortuño R, Martínez-Velilla N, Sutton R, Ungar A, Fedorowski A, Galvin R, et al. Network Physiology in Aging and Frailty: The Grand Challenge of Physiological Reserve in Older Adults. Front Netw Physiol. 2021;1:712430.
29. Cruz-Jentoft AJ, Michel JP. Sarcopenia: A useful paradigm for physical frailty. European Geriatric Medicine. 2013;4(2):102-5.
30. Dent E, Morley JE, Cruz-Jentoft AJ, Woodhouse L, Rodríguez-Mañas L, Fried LP, et al. Physical Frailty: ICFSR International Clinical Practice Guidelines for Identification and Management. J Nutr Health Aging. 2019;23(9):771-87.
31. Zhu L, Zong X, Shi X, Ouyang X. Association between Intrinsic Capacity and Sarcopenia in Hospitalized Older Patients. J Nutr Health Aging. 2023;27(7):542-9.
32. Sayer AA, Cruz-Jentoft A. Sarcopenia definition, diagnosis and treatment: consensus is growing. Age Ageing. 2022;51(10).
33. Dao T, Green AE, Kim YA, Bae SJ, Ha KT, Gariani K, et al. Sarcopenia and Muscle Aging: A Brief Overview. Endocrinol Metab (Seoul). 2020;35(4):716-32.
34. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing. 2019;48(1):16-31.
35. Phillips A, Strobl R, Vogt S, Ladwig KH, Thorand B, Grill E. Sarcopenia is associated with disability status-results from the KORA-Age study. Osteoporos Int. 2017;28(7):2069-79.
36. Lee SY. Sarcopenia: A Geriatric Giant Facing a Huge Transition. Ann Geriatr Med Res. 2021;25(1):1-3.
37. Cruz-Jentoft AJ, Sayer AA. Sarcopenia. Lancet. 2019;393(10191):2636-46.

38. Yoon JH, Kwon K-S. Receptor-Mediated Muscle Homeostasis as a Target for Sarcopenia Therapeutics. Endocrinol Metab. 2021;36(3):478-90.
39. Pérarnau B, Siegrist CA, Gillet A, Vincent C, Kimura S, Lemonnier FA. Beta 2-microglobulin restriction of antigen presentation. Nature. 1990;346(6286):751-4.
40. Dirscherl C, Löchte S, Hein Z, Kopicki J-D, Harders AR, Linden N, et al. Dissociation of β2m from MHC class I triggers formation of noncovalent transient heavy chain dimers. Journal of Cell Science. 2022;135(9).
41. Zijlstra M, Bix M, Simister NE, Loring JM, Raulet DH, Jaenisch R. Beta 2-microglobulin deficient mice lack CD4-8+ cytolytic T cells. Nature. 1990;344(6268):742-6.
42. Nomura T, Huang WC, Zhau HE, Josson S, Mimata H, Chung LW. β 2-Microglobulin-mediated signaling as a target for cancer therapy. Anticancer Agents Med Chem. 2014;14(3):343-52.
43. Wang H, Liu B, Wei J. Beta2-microglobulin(B2M) in cancer immunotherapies: Biological function, resistance and remedy. Cancer Letters. 2021;517:96-104.
44. Dogan A. Amyloidosis: Insights from Proteomics. Annu Rev Pathol. 2017;12:277-304.
45. Barton KT, Kakajiwala A, Dietzen DJ, Goss CW, Gu H, Dharnidharka VR. Using the newer Kidney Disease: Improving Global Outcomes criteria, beta-2-microglobulin levels associate with severity of acute kidney injury. Clin Kidney J. 2018;11(6):797-802.
46. Smith LK, He Y, Park JS, Bieri G, Snethlage CE, Lin K, et al. β2-microglobulin is a systemic pro-aging factor that impairs cognitive function and neurogenesis. Nat Med. 2015;21(8):932-7.
47. Gao Y, Hong Y, Huang L, Zheng S, Zhang H, Wang S, et al. β2-microglobulin functions as an endogenous NMDAR antagonist to impair synaptic function. Cell. 2023;186(5):1026-38.e20.
48. Lee JY, Kim DA, Choi E, Lee YS, Park SJ, Kim BJ. Aldosterone Inhibits In Vitro Myogenesis by Increasing Intracellular Oxidative Stress via Mineralocorticoid Receptor. Endocrinol Metab (Seoul). 2021;36(4):865-74.
49. Lee JY, Park SJ, Kim DA, Lee SH, Koh JM, Kim BJ. Muscle-Derived Lumican Stimulates Bone Formation via Integrin α2β1 and the Downstream ERK Signal. Front Cell Dev Biol. 2020;8:565826.

50. Deacon RM. Measuring the strength of mice. J Vis Exp. 2013(76).
51. Roberts HC, Denison HJ, Martin HJ, Patel HP, Syddall H, Cooper C, et al. A review of the measurement of grip strength in clinical and epidemiological studies: towards a standardised approach. Age Ageing. 2011;40(4):423-9.
52. Peel NM, Kuys SS, Klein K. Gait speed as a measure in geriatric assessment in clinical settings: a systematic review. J Gerontol A Biol Sci Med Sci. 2013;68(1):39-46.
53. Jung HW, Roh H, Cho Y, Jeong J, Shin YS, Lim JY, et al. Validation of a Multi-Sensor-Based Kiosk for Short Physical Performance Battery. J Am Geriatr Soc. 2019;67(12):2605-9.
54. Chen LK, Woo J, Assantachai P, Auyeung TW, Chou MY, Iijima K, et al. Asian Working Group for Sarcopenia: 2019 Consensus Update on Sarcopenia Diagnosis and Treatment. J Am Med Dir Assoc. 2020;21(3):300-7.e2.
55. Ahn SH, Jung HW, Lee E, Baek JY, Jang IY, Park SJ, et al. Decreased Serum Level of Sclerostin in Older Adults with Sarcopenia. Endocrinol Metab (Seoul). 2022;37(3):487-96.
56. Boyle PA, Buchman AS, Wilson RS, Leurgans SE, Bennett DA. Association of muscle strength with the risk of Alzheimer disease and the rate of cognitive decline in community-dwelling older persons. Arch Neurol. 2009;66(11):1339-44.
57. Gray SL, Anderson ML, Hubbard RA, LaCroix A, Crane PK, McCormick W, et al. Frailty and incident dementia. J Gerontol A Biol Sci Med Sci. 2013;68(9):1083-90.
58. Loskutova N, Honea RA, Vidoni ED, Brooks WM, Burns JM. Bone density and brain atrophy in early Alzheimer's disease. J Alzheimers Dis. 2009;18(4):777-85.
59. Lehallier B, Gate D, Schaum N, Nanasi T, Lee SE, Yousef H, et al. Undulating changes in human plasma proteome profiles across the lifespan. Nature Medicine. 2019;25(12):1843-50.
60. Tanaka T, Biancotto A, Moaddel R, Moore AZ, Gonzalez-Freire M, Aon MA, et al. Plasma proteomic signature of age in healthy humans. Aging Cell. 2018;17(5):e12799.
61. Chaleckis R, Murakami I, Takada J, Kondoh H, Yanagida M. Individual variability in human blood metabolites identifies age-related differences. Proc Natl Acad Sci U S A. 2016;113(16):4252-9.

62. Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature. 2005;433(7027):760-4.

63. Baht GS, Silkstone D, Vi L, Nadesan P, Amani Y, Whetstone H, et al. Exposure to a youthful circulaton rejuvenates bone repair through modulation of β-catenin. Nat Commun. 2015;6:7131.

64. Vinel C, Lukjanenko L, Batut A, Deleruyelle S, Pradère J-P, Le Gonidec S, et al. The exerkine apelin reverses age-associated sarcopenia. Nature Medicine. 2018;24(9):1360-71.

65. Rai R, Ghosh AK, Eren M, Mackie AR, Levine DC, Kim S-Y, et al. Downregulation of the Apelinergic Axis Accelerates Aging, whereas Its Systemic Restoration Improves the Mammalian Healthspan. Cell Reports. 2017;21(6):1471-80.

66. Yang YR, Kabir MH, Park JH, Park JI, Kang JS, Ju S, et al. Plasma proteomic profiling of young and old mice reveals cadherin-13 prevents age-related bone loss. Aging (Albany NY). 2020;12(9):8652-68.

67. Yoshida M, Satoh A, Lin JB, Mills KF, Sasaki Y, Rensing N, et al. Extracellular Vesicle-Contained eNAMPT Delays Aging and Extends Lifespan in Mice. Cell Metabolism. 2019;30(2):329-42.e5.

68. Elabd C, Cousin W, Upadhyayula P, Chen RY, Chooljian MS, Li J, et al. Oxytocin is an age-specific circulating hormone that is necessary for muscle maintenance and regeneration. Nature Communications. 2014;5(1):4082.

69. Egerman Marc A, Cadena Samuel M, Gilbert Jason A, Meyer A, Nelson Hallie N, Swalley Susanne E, et al. GDF11 Increases with Age and Inhibits Skeletal Muscle Regeneration. Cell Metabolism. 2015;22(1):164-74.

70. Güssow D, Rein R, Ginjaar I, Hochstenbach F, Seemann G, Kottman A, et al. The human beta 2-microglobulin gene. Primary structure and definition of the transcriptional unit. J Immunol. 1987;139(9):3132-8.

71. Tosato M, Marzetti E, Cesari M, Savera G, Miller RR, Bernabei R, et al. Measurement of muscle mass in sarcopenia: from imaging to biochemical markers. Aging Clin Exp Res. 2017;29(1):19-27.

72. Bhasin S, Travison TG, Manini TM, Patel S, Pencina KM, Fielding RA, et al. Sarcopenia Definition: The Position Statements of the Sarcopenia Definition and Outcomes Consortium. J Am Geriatr Soc. 2020;68(7):1410-8.
73. Meza-Valderrama D, Marco E, Dávalos-Yerovi V, Muns MD, Tejero-Sánchez M, Duarte E, et al. Sarcopenia, Malnutrition, and Cachexia: Adapting Definitions and Terminology of Nutritional Disorders in Older People with Cancer. Nutrients. 2021;13(3).
74. Kuschel LB, Sonnenburg D, Engel T. Factors of Muscle Quality and Determinants of Muscle Strength: A Systematic Literature Review. Healthcare (Basel). 2022;10(10).
75. Sartori R, Romanello V, Sandri M. Mechanisms of muscle atrophy and hypertrophy: implications in health and disease. Nat Commun. 2021;12(1):330.
76. Cisterna BA, Cardozo C, Sáez JC. Neuronal involvement in muscular atrophy. Front Cell Neurosci. 2014;8:405.
77. Rosenberg IH. Sarcopenia: origins and clinical relevance. J Nutr. 1997;127(5 Suppl):990s-1s.
78. Sanchez-Rodriguez D, Marco E, Cruz-Jentoft AJ. Defining sarcopenia: some caveats and challenges. Curr Opin Clin Nutr Metab Care. 2020;23(2):127-32.
79. Studenski SA, Peters KW, Alley DE, Cawthon PM, McLean RR, Harris TB, et al. The FNIH sarcopenia project: rationale, study description, conference recommendations, and final estimates. J Gerontol A Biol Sci Med Sci. 2014;69(5):547-58.
80. Coletta G, Phillips SM. An elusive consensus definition of sarcopenia impedes research and clinical treatment: A narrative review. Ageing Research Reviews. 2023;86:101883.
81. Baek JY, Jung HW, Kim KM, Kim M, Park CY, Lee KP, et al. Korean Working Group on Sarcopenia Guideline: Expert Consensus on Sarcopenia Screening and Diagnosis by the Korean Society of Sarcopenia, the Korean Society for Bone and Mineral Research, and the Korean Geriatrics Society. Ann Geriatr Med Res. 2023;27(1):9-21.
82. Szentesi P, Csernoch L, Dux L, Keller-Pintér A. Changes in Redox Signaling in the Skeletal Muscle with Aging. Oxid Med Cell Longev. 2019;2019:4617801.

83. Powers SK, Morton AB, Ahn B, Smuder AJ. Redox control of skeletal muscle atrophy. Free Radic Biol Med. 2016;98:208-17.

84. Dröge W. Free radicals in the physiological control of cell function. Physiol Rev. 2002;82(1):47-95.

85. Ábrigo J, Elorza AA, Riedel CA, Vilos C, Simon F, Cabrera D, et al. Role of Oxidative Stress as Key Regulator of Muscle Wasting during Cachexia. Oxid Med Cell Longev. 2018;2018:2063179.

86. Mecocci P, Fanó G, Fulle S, MacGarvey U, Shinobu L, Polidori MC, et al. Age-dependent increases in oxidative damage to DNA, lipids, and proteins in human skeletal muscle. Free Radic Biol Med. 1999;26(3-4):303-8.

87. Gordon J, Wu CH, Rastegar M, Safa AR. Beta2-microglobulin induces caspase-dependent apoptosis in the CCRF-HSB-2 human leukemia cell line independently of the caspase-3, -8 and -9 pathways but through increased reactive oxygen species. Int J Cancer. 2003;103(3):316-27.

88. Althubiti M, Elzubier M, Alotaibi GS, Althubaiti MA, Alsadi HH, Alhazmi ZA, et al. Beta 2 microglobulin correlates with oxidative stress in elderly. Exp Gerontol. 2021;150:111359.

89. Aleixo GFP, Shachar SS, Nyrop KA, Muss HB, Battaglini CL, Williams GR. Bioelectrical Impedance Analysis for the Assessment of Sarcopenia in Patients with Cancer: A Systematic Review. Oncologist. 2020;25(2):170-82.

90. Evans WJ, Hellerstein M, Orwoll E, Cummings S, Cawthon PM. D(3)-Creatine dilution and the importance of accuracy in the assessment of skeletal muscle mass. J Cachexia Sarcopenia Muscle. 2019;10(1):14-21.

Korean Abstract

근육 대사에서 β2-Microglobulin 의 작용기전 및 효과 검증에 관한 연구

Detrimental Effects of β2-Microglobulin on Muscle Metabolism: Evidence from In Vitro, Animal, and Human Research

장 일 영

울산대학교 대학원

의 학 과

1. 연구 배경 및 목적

사람의 세포들은 나이가 들면서 일련의 노화의 과정들을 겪으며 근감소증으로 진행하게 된다. 임상적으로 근감소증은 근육의 양과 기능의 저하로 정의하지만, 노쇠, 삶의 질, 조기 사망 등 다양한 부정적 예후들과 밀접히 연관되어 있다. 노화를 이해하고 제어하기 위해 근육 대사의 바이오마커를 발굴하고 검증하는 것은 사람에게 근감소증의 가능성 및 예후 예측뿐만 아니라 신약 후보물질 개발에도 적용될 수 있어 그 가치가 크다. 혈액에서 노화를 제어하는 circulating factor 가 존재한다는 점은 선행연구에서 잘 알려진바, 아직까지 근감소증의 확실한 바이오마커가 없는 현시점에서 근육 대사에 관여하는 강력한 circulating factor 를 in vitro 부터 clinical evidence 까지 확보하는 것은 그 가치가 매우 클 것이다.

본 연구에서는 pro-aging factor 로 알려지기 시작한 β2-Microglobulin (B2M) 에 대하여 muscle metabolism 에 대한 영향을 확인하고 검증하는 것을 목표로 한다.

2. 연구대상자 및 연구 방법

In vitro setting 에서 muscle metabolism 에 대한 B2M 의 역할과 기전을 이해하고, animal muscle 에서 B2M 의 근육에 대한 영향을 검증하며, clinical human aging cohort 에서

sarcopenia phenotype 및 parameter 와 비교하여 muscle 에 대한 detrimental effect 를 검증한다.

(1) In vitro 에서 B2M 의 myogenesis 영향 확인

Mouse 의 myoblasts 에서 myogenesis 를 유도한 후 B2M 을 처리하여 dose 별 myogenesis 의 suppression 여부 및 수준을 확인한다.

(2) Anti-myogenesis 의 기전 확인

In vitro myoblasts 에 B2M 처리 후 dose 별 intracellular reactive oxygen species (ROS) 생성의 증가를 확인하며, 알려진 antioxidant 인 N-acetyl cysteine 을 전처리한 cell 과 그 효과를 함께 비교하여 myoblast 에 대한 효과 (myotube area, nucleus number, fusion index 감소 등)을 확인하고 muscle 에 대한 B2M 의 inhibitory mechanism 을 확인한다.

(3) In vivo 에서 muscle phenotype 에 대한 B2M 의 영향 확인

3 개월령 mice 복강에 B2M 을 4 주간 매일 주입하고, 대조군과 주요 횡문근(skeletal muscle)들에 대한 단면적, 근력 및 근 기능을 비교한다.

(4) Human aging cohort 에서 circulating B2M level 과 근감소증 phenotype 및 parameter 의 비교

1) 노인으로 구성된 human cohort 에서 근감소증 진단 기준에 의거 근감소증 유무를 구분하고, serum B2M level 을 근감소증 유무에 따라 비교한다.
2) 근육량, 근력, 근 기능 등 근감소증 주요 parameter 들에 대해서도 serum B2M level 을 비교하여 muscle function 에 대한 negative effect 를 확인한다.
3) Serum B2M level 과 muscle 관련 parameter 들에 대한 회귀분석을 통해 human muscle 에 대한 B2M 의 inhibitory effect 를 확인한다.

3. 연구 결과 및 고찰

Mouse C2C12 myoblast 에 recombinant B2M 처리 시 myogenesis 가 용량 의존적으로 억제됨을 확인하였고 근육 분화(생성) 표지자들의 발현도 일관되게 감소하였다. B2M 은 myotube 내부에서 활성 산소(ROS) 생성을 상당히 증가시켰으며, 반대로 강력한 항산화

제인 N-아세틸시스테인을 투여했을 경우 B2M 에 의해 감소한 근세포 면적, 근 세포당 면적, 근 세포당 핵수, 융합 지수는 역행함을 확인하였다.

동물 실험에서도, B2M 을 투여받은 mouse 는 주요 골격근의 면적 및 근육량뿐만 아니라 근력, 활동력 등이 유의하게 감소하였다.

사람을 대상으로 한 임상 코호트 연구에서는, 혈청 B2M 수준이 근감소증 주요 지표들인 근력(grip strength), 보행속도(gait speed) 및 신체 기능(Short Physical Performance Battery, SPPB)과 reverse correlation 을 보였으며 근감소증 주요 지표들을 합산한 sarcopenia phenotype score 와는 positive correlation 을 보였다. 이 결과는 나이, 성별 및 체질량 지수를 보정하여도 일관된 결과를 보였으며, 특히 사람에서 혈청 B2M 수준이 높은 경우 근감소증의 주요 지표들(근력, 보행속도, 신체 기능)은 일관되게 낮아 근감소증 위험이 높음을 확인할 수 있었다.

이 연구는 B2M 이 주로 산화 스트레스를 증가시킴으로써 근육 대사에 해로운 영향을 미친다는 실험적 증거를 제공한다. 특히 우리 연구는 그 방법론적으로서 B2M 의 근육에 대한 효과를 in vitro 및 animal 연구에서부터 확인한 후, 사람(노인)의 혈액과 임상 정보들을 통해 실제 검증함으로써, B2M 이 노인의 근육 건강을 평가하는 혈액 바이오마커가 될 수 있고, 잠재적으로 pro-aging marker 가 될 수 있다는 중요한 근거를 제공한다.