

Official Title:

Nutrition Ameliorates the Muscle Loss of Pre-sarcopenia in Elderly

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1. Background and Research Objectives

Population aging has become a global concern. Taiwan is projected to enter a “super-aged society” by 2025, with individuals aged 65 and above comprising over 20% of the total population (approximately 4.81 million people). Older adults often suffer from degenerative diseases, leading to progressive disability and diminished quality of life and dignity. Among the physiological declines associated with aging, loss of muscle mass is particularly prominent. When accompanied by reduced muscle strength or physical function, this condition is defined as sarcopenia. Sarcopenia has recently been recognized as a geriatric syndrome and is associated with adverse clinical outcomes such as disability, falls, injuries, hospitalization, and poor recovery from illness. It is considered a major contributor to loss of independence in older adults. Currently, there is no definitive clinical biomarker for sarcopenia. Functional assessments often reflect fatigue and frailty but do not necessarily indicate sarcopenia itself.

This subproject will recruit participants from the main integrated aging study and identify individuals with **pre-sarcopenia** for nutritional intervention. We plan to collect blood and urine samples from 120 participants at two time points: mid-intervention (6 weeks) and post-intervention (12 weeks). Functional assessments related to sarcopenia will be conducted after the intervention. Using metabolomics approaches, we aim to evaluate the effectiveness of nutritional supplementation, identify potential biomarkers for risk sarcopenia, and assess their prognostic value. The findings will contribute to understanding the physiological mechanisms of sarcopenia and support future therapeutic monitoring.

2. Experimental design and methods

2.1 Study Period: April 1, 2021 – May 31, 2023

2.2 Participant Recruitment: Participants will be recruited from annual health check-ups conducted in Songshan District, Taipei City, and at the Chang Gung Health and Wellness Village. Those enrolled in the main integrated aging study and meeting inclusion criteria will be invited to participate.

2.3 Inclusion Criteria:

- 2.3.1 Willingness to sign informed consent
- 2.3.2 Participation in the designated health check-up programs or prior pilot studies
- 2.3.3 Age \geq 60 years (male or female)
- 2.3.4 Able to ambulate independently.
- 2.3.5 Meeting one of the following pre-sarcopenia criteria
 - Grip strength: <28 kg (men), <18 kg (women)
 - Walking speed: <1.0 m/s
 - Muscle mass: <7.0 kg/m² (men), <5.7 kg/m² (women)

2.4 Exclusion Criteria:

- 2.4.1 Impaired renal function.
- 2.4.2 Allergy or intolerance to protein supplements.
- 2.4.3 Currently undergoing cancer treatment.
- 2.4.4 Autoimmune diseases.
- 2.4.5 Assessed by a physician as unsuitable to participate.

2.5 Sample Size: 120 participants

2.6 Measurements, Duration, and Risks Study Procedures:

- 2.6.1 Participants will undergo nutritional assessment via questionnaire and receive a daily supplement containing 8.5 g whey protein and 1.5 g leucine for 12 weeks.
- 2.6.2 At week 6, participants will return for blood (5 mL) and urine (10 mL) collection and report supplement adherence.
- 2.6.3 At week 12, participants will return for a second round of blood and urine collection, supplement adherence assessment, and sarcopenia-related functional tests (body composition, grip strength, walking speed, and calf circumference).

2.7 Potential Risks:

- 2.7.1 Blood sampling: Two draws of 5 mL each; minimal risk anticipated.
- 2.7.2 Supplementation: Participants with known allergies to supplement ingredients will be excluded. Any adverse reactions will be reported to the principal investigator immediately.

2.8 Nutritional supplementation

Participants received sachets containing the nutritional supplement and were instructed to consume two sachets daily over a 12-week intervention period. Each 8 g serving provided 35 kcal, comprising 4.1 g of protein, 1 g of fat, and 2.4 g of carbohydrates. Follow-up assessments were conducted throughout the intervention to monitor adherence. Participants were asked to return both used and unused sachets at each visit, and compliance was defined as consumption of at least 80% of the provided sachets.

2.9 Sample collect for NMR spectroscopy analysis and LC-MS spectrometry analysis

Blood and urine samples were collected from participants following an overnight fast, both before and after the supplementation period. Plasma was separated within 4 hours of collection by centrifugation at $1,500 \times g$ for 10 minutes. The resulting supernatant was aliquoted into microtubes and stored at -80°C until analysis.

2.10 Statistical Analysis

Metabolomic data will be processed using Topspin and MassLynx for spectral alignment and peak extraction, followed by multivariate analysis with SIMCA-P, including principal component analysis (PCA). Univariate statistical comparisons will be conducted using t-tests and ANOVA for group-level differences. Paired t-tests will be applied to assess within-subject changes before and after nutritional supplementation. Changes in metabolite concentrations in plasma and urine will be linked to individual health indicators to identify biomarkers associated with healthy aging.