しており，細胞では上皮細胞，血管内皮細胞，線維芽細胞，樹状細胞やマクロファージに発現する[3]。IL-33受容体は膜結合型ST2（IL-33Rα鎖）とIL-1RAcP（IL-1 receptor accessory protein）（IL-33Rβ鎖）のヘテロ二量体から構成される。IL-33のST2への結合に伴いIL-1RAcPからシグナルが伝達され，細胞質内領域のTIR（Toll/IL-1R）ドメインにMyD88およびIRAKが会合し，TRAF6を介したシグナルによりNF-κBおよびMAP kinase経路が活性化される。

2. IL-33の免疫学的役割

ST2はヘルパーT細胞のうちTh2サブセットに選択的に発現するほか，肥満細胞，好塩基球，好酸球，CD8⁺T細胞および調節性T細胞に発現が認められ，IL-33に応答してTh2サイトカインが生産されることが示されている[6]。IL-33は組織損傷の初期段階に検出されることがいくつかの実験モデルにおいて示されている。鞭虫*Trichuris muris*を野生型マウスに感染させると，感染3日後をピークとして腸管組織におけるIL-33 mRNA発現が亢進される[7]。感染防御にTh2応答が必要とされる*T. muris*感染モデルにおいてIL-33は感染防御に働く。同感染におけるIL-33生産は一過性であり，感染初期に生産されるIL-33の刺激によりTSLPを始めとする他のTh2サイトカイン誘導因子が生産されることから，IL-33はTh2応答の増幅および持続に作用すると捉えられる。また，IL-33は炎症反応の初期段階において肥満細胞や好酸球など自然免疫系の誘導に係わる多様な細胞を活性化させる。炎症性疾患においてIL-33はアレルギー性疾患を増悪させる因子と考えられており，気管支喘息，炎症性腸疾患や慢性関節リウマチなどさまざまな疾患の発症に係わることが報告されている。近年，腸間膜の脂肪組織に存在する新たなリンパ球集積にIL-33やIL-25に反応し高いTh2サイトカイン生産能を持つinnate lymphoid cell［ナチュラルヘルパー細胞，nuocyte，multipotent progenitor type 2（$MPP^{type 2}$）細胞，innate helper type 2（Ih2）細胞など］が局在することが相次いで発見され，IL-33が炎症反応の増幅および慢性化に係わる可能性が注目されている[8]。他方，アルツハイマー病の発症は脳に蓄積されるアミロイドβ（Aβ）による神経細胞の機能傷害が一因とされるが，IL-33はAβの発現を減少させることが示されておりIL-33がアルツハイマー病の進行を抑制させる可能性が注目されている。

3. *P. gingivalis*ジンジパインによる歯肉上皮細胞のIL-33発現誘導

成人性歯周炎は歯周炎関連細菌*Porphyromonas gingivalis*による細菌感染症であり，同菌により引き起こされる炎症反応による歯周組織の破壊は歯を喪失する最大の要因である。最近われわれは，*P. gingivalis*により歯肉上皮細胞からIL-33が誘導される可能性について検討を行った。ヒト歯肉上皮細胞株Ca9-22細胞を凍結乾燥*P. gingivalis*全菌体で刺激すると，48時間後にIL-33 mRNA発現が約30倍以上亢進されることを見出した（図1）。同様に，*P. gingivalis*全菌体の刺激によりIL-25ならびにTSLP mRNA発現も著明に亢進された。そこで*P. gingivalis*により誘導されたIL-33蛋白の局在を免疫染色法ならびにウェスタンブロット法にて検討した結果，IL-33蛋白は刺激4日後をピークに細胞質に局在していた。それでは，歯肉上皮細胞におけるIL-33誘導活性を担う*P. gingivalis*の菌体成分はどのようなものであろうか。われわれは*P. gingivalis*由来のfimbriae（線毛），リポペプチドないしリポポリサッカライドをそれぞれ歯肉上皮細胞に刺激した結果，いずれの菌体成分もIL-33誘導活性を示さなかった。これらの実験結果から，歯肉上皮細胞におけるIL-33誘導活性はToll-like receptorアゴニストではなく他の成分により担われる可能性が推測された。これまでの報告から，肺上皮細胞を真菌*Alternaria alternata*由来抽出液で刺激するとTSLPが生産され，同誘導作用はシステインプロテアーゼ阻害剤により抑制されることが示されている[9]。*P. gingivalis*はジンジパインというシステインプロテアーゼを分泌する。ジンジパインは同菌が生産する総プロテアーゼ活性の約85%を占めており，多彩な病原性を発揮することで歯周炎の増悪に深く関与することが明らかにされて

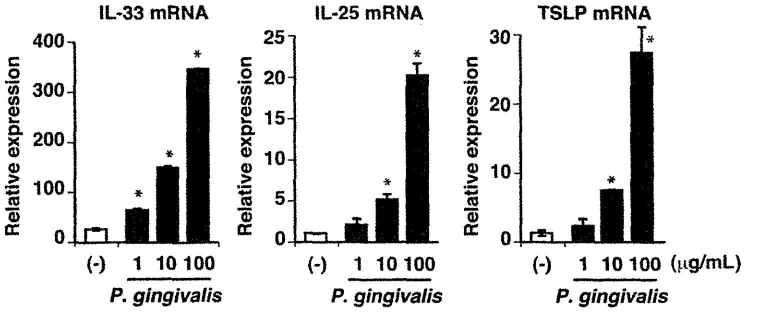

図 1 *P. gingivalis* によるヒト歯肉上皮細胞からの IL-33, IL-25 ならびに TSLP mRNA 発現誘導

ヒト歯肉上皮細胞株 Ca9-22 を *P. gingivalis* W83 株全菌体で 48 時間刺激した際の IL-33, IL-25 ならびに TSLP mRNA 発現をリアルタイム PCR 法により測定した。

*：ANOVA 検定にて無刺激群と刺激群間に有意差（$p<0.01$）が認められた。

いる[10]。ジンジパインはアルギニン残基を切断するジンジパイン-R（Rgp）とリジン残基を切断するジンジパイン-K（Kgp）にそれぞれ分類される。そこで *P. gingivalis* 全菌体を Rgp ないし Kgp の特異的阻害剤でそれぞれ前処理した後に Ca9-22 細胞に刺激した結果，IL-33 発現誘導は完全に抑制された。また，すべてのジンジパインを変異する KDP136 株（長崎大学口腔病原微生物，中山浩次博士より分与）では IL-33 誘導活性はみられなかった。これらの事実から，歯肉上皮細胞における IL-33 誘導活性は *P. gingivalis* が発現するジンジパインにより担われることが示された。

それでは，ジンジパインによる IL-33 誘導活性はどのようなシグナル伝達機構により担われるのであろうか。Rgp はヒト好中球に発現する G 蛋白共役 7 回膜貫通型受容体 protease-activated receptor-2（PAR-2）を活性化させることが明らかにされている[11]。Ca9-22 細胞は mRNA レベルおよび蛋白レベルにおいて構成的に PAR-2 を発現していることから，RNA 干渉法により PAR-2 mRNA 発現を抑制させた同細胞に *P. gingivalis* 全菌体を刺激した結果，コントロール siRNA 導入細胞に比べて PAR-2 siRNA 導入細胞では IL-33 mRNA 発現誘導は有意に減少した。PAR-2 を介したシグナルにより MAP キナーゼ経路ならびに NF-κB 経路が活性化されることから，*P. gingivalis* による MAP キナーゼ ERK，JNK ならびに p38 の活性化について検討した。全菌体を刺激すると 18 時間後に p38 の著明なリン酸化が検出され，IL-33 誘導活性は p38 阻害剤の処理により著明に抑制された。次に *P. gingivalis* による NF-κB 経路の活性化について，NF-κB プロモーターをトランスフェクションした Ca9-22 細胞を *P. gingivalis* 全菌体で刺激した際のルシフェラーゼ活性を測定した結果，刺激 3 時間後から NF-κB の活性化がみられ IL-33 誘導活性は NF-κB 阻害剤により抑制された。以上の結果から，ジンジパインによる歯肉上皮細胞からの IL-33 発現は，PAR-2，p38 および NF-κB を介した活性化により誘導されることが明らかになった（図 2）。今後，*P. gingivalis* により歯肉上皮細胞に誘導された IL-33 の放出メカニズムならびに IL-33 が歯肉炎へおよぼす影響について検討する必要がある。

おわりに

IL-33 は IL-25 や TSLP とともに皮膚や粘膜組織を構成する上皮細胞を中心に発現しており，Th2 応答の誘導を調節する。IL-33 はアレルギー性炎症の誘導を促進させることにより，気管支喘

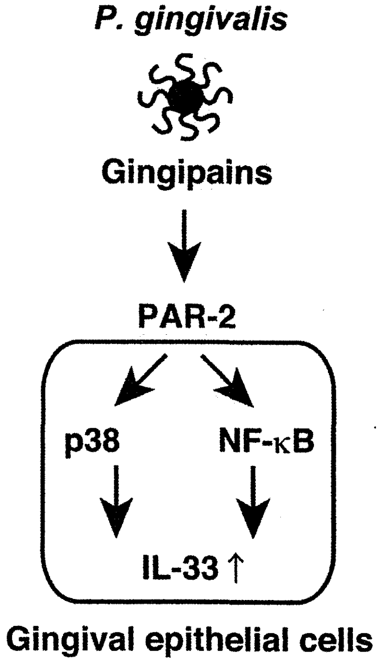

図 2　*P. gingivalis* によるヒト歯肉上皮細胞からの IL-33 誘導機構

息，炎症性腸疾患や慢性関節リウマチなどさまざまな疾患を増悪させる知見が報告されているが，IL-33 の発現および生産機構は十分に解明されていない。われわれは *P. gingivalis* 由来プロテアーゼであるジンジパインにより歯肉上皮細胞においてIL-33 発現が亢進されることを示した。今後，歯周炎の病態形成においてIL-33 が炎症の増悪因子となる可能性が想定され，今後の検討課題といえる。

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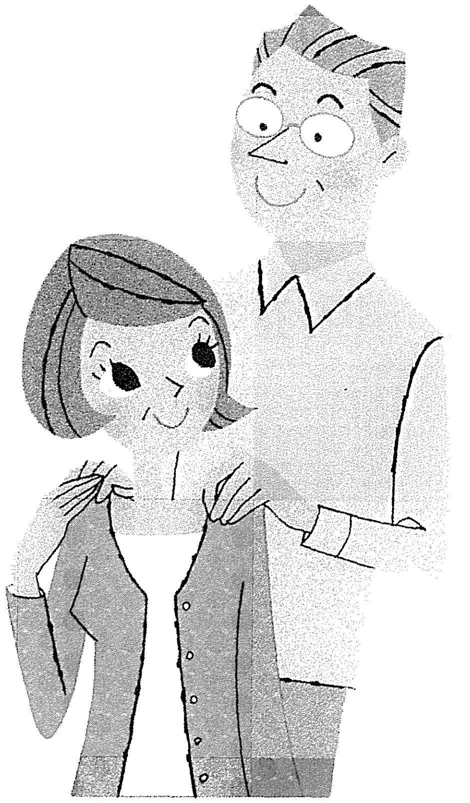
世界口腔保健学術大会記念

第18回口腔保健シンポジウム

健康寿命の鍵は、口の健康！

歯周病と全身の密接な関係



最近の研究によって、お口の病気である歯周病が、
糖尿病などのさまざまな全身疾患と関係していることが判明しつつあります。
また21世紀における国民の健康運動の取り組み「第二次健康日本21」が
本年から始まる予定で、歯と口腔の健康もクローズアップされています。
すべての人たちが健康寿命を延ばし、毎日を楽しく過ごせますよう、
歯周病の正しい知識と予防はもちろん、正しいお口のケアを身につけましょう。

監修●松下 健二 先生（独立行政法人 国立長寿医療研究センター 口腔疾患研究部 部長）／髙柴 正悟 先生（岡山大学大学院 医歯薬学総合研究科 教授）

健康寿命と口腔の密接な関係

口腔は、全身の健康を保つためにとても重要です。特に健康寿命と口腔には密接な関係があります。

健康寿命とは、日常で介護を必要とせずに自立した生活のできる期間を指します。厚生労働省は今年、初めてその数値を発表しました。2010年の平均は男性70.42歳、女性は73.62歳でした。一方、同じ2010年の平均寿命は男性79.55歳、女性86.30歳。両者の間に男性約9年、女性約13年のギャップがあります。これは、介護などを必要とする期間にあたります。

厚労省は、運動や食習慣などを改善することで、健康寿命を1.6年以上延ばすことを提案していますが、高齢になるとどうしても認知症や寝たきりといった問題が生まれます。

この問題に口腔、つまり口や歯の健康が深く関わっているのです。また脳卒中や心臓病、糖尿病などの発症と悪化にも口腔が深く関わっていることも分かっています。自分の歯がたくさん残っていると、全身疾患のリスクが低く、長生きになるというデータが、さまざまな国の研究から報告されています。

具体的には、歯がある人と無い人を比べると、残りの寿命が全然違います。歯を失った人のその後を観察すると、急に身体にいろいろな症状が出たり、生活習慣病の発症率が高くなったりしているのです。この他にも歯が無くなることは、がんの発症や死亡に関連があるというのです。歯が無いということは、特に消化器系のがんの発症と関連が高いと言われています。

第18回口腔保健シンポジウムが開催されました。

1994年に東京でおこなわれた世界口腔保健学術大会を記念し、口腔保健に関するさまざまな話題を取り上げて、理解を深める、第18回口腔保健シンポジウム（主催：社団法人日本歯科医師会、協賛：サンスター株式会社）が「健康寿命の鍵は、口の健康！～歯周病と全身の密接な関係～」をテーマに、2012年7月7日・津田ホール（東京）にて開催されました。松下健二先生からは「口腔と全身の密接な関係」と題して、健康長寿と口腔ケアについてお話いただきました。続いて高柴正悟先生からは同じテーマに関し、健康長寿と歯周病についてご説明いただきました。

また大林素子さんのミニトーク「マイドリーム～あきらめないで頑張れる、大林流・健康管理～」では、元バレーボール全日本代表の経験を活かした健康管理術やお口の健康について、楽しくお話しいただきました。

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国立長寿医療研究センター
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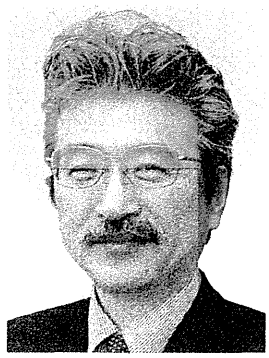

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健康寿命を延ばすために

今後、日本でますます課題になると思われるのは認知症です。この認知症についても、歯との関係についていろいろな報告があります。健康な歯が多いこと、むし歯があってもちゃんと治療していること、もしくは歯が残っていることは、認知機能と関係があるというのです。

また高齢の方にとって肺炎は深刻な疾患です。日本人全体の死因に占める肺炎の割合は約10%ですが、そのうち96%までが65歳以上の高齢者です。高齢になると飲み込む機能が低下するため、誤嚥性肺炎が原因で亡くなる人が多いからです。

そうしたことから口のなかをきれいにする口腔ケアをおこなうと、肺炎の予防になると言われています。うがいや歯みがきはいつもしていると思いますが、顔の表情をうまく作ったり、口のまわりの筋肉を鍛えたり、舌の運動をしたり、唾液腺のマッサージで唾液の分泌をスムーズにすることも大切です。

歯の健康とともに口腔の機能を維持すると、おいしく食べられます。これは健康寿命を延ばすことにつながるのです。

OnePoint

知っていますか、「唾液の大切な役割」

唾液には重要な役割がたくさんあります。
何らかの原因で唾液が減ってしまうと
歯周病やむし歯になりやすくなります。
もし下記の症状に心当たりがあるならば、
歯医者さんにご相談ください。

唾液の主な役割

- **抗菌作用**：むし歯、歯周病から肺炎まで感染症から身体を守る
- **再石灰化作用**：脱灰で失われたミネラルの回復、歯質の保護
- **浄化作用**：唾液の水分と嚥下により口のなかがきれいになる
- **緩衝作用**：口のなかの酸性化を和らげる
- **粘膜修復作用**：傷を治し、老化を防止する神経成長因子を全身に届ける
- **円滑作用・作用**：食物や粘膜を湿らせて咀嚼・嚥下・発音などの運動を円滑にする

こんな症状があれば要注意！

口が乾く	のどが渇く	味がおかしい
口のなかがネバネバする	目が乾く	パンなど乾いたものが食べにくい

歯科医院での定期的なケアを

まず口のなかのばい菌が感染して炎症が起こることから始まります。その結果、歯ぐきが腫れたり、骨がやせたりして、歯のまわりの組織が壊れます。やがて歯が抜けて、機能を失います。これが歯周病で、いま話題になっている健康寿命と密接に関わっています。

歯周病になると、歯肉の上の皮が欠落して壊れ、ばい菌がどんどん身体のなかに入ります。その結果、動脈硬化などの病気が進行することもあります。

歯周病は生活習慣病や呼吸器系などの病気にも関わっています。早産や低体重児の出産、糖尿病にも関係があります。末期の腎疾患に悪い影響を与えるとも言われています。

歯は、特に治療を必要とする場合でなくても、定期的に専門的なクリーニングを受けていると、非常によい状態を保てます。歯周病対策の基本は「プラーク（歯垢）を除去する」「炎症を抑える」「形態を改善する」「リハビリ（歯を削ってかぶせたり、つないだりする補てつ治療）をする」というものです。

歯周病の治療としては歯みがきのほか、外科治療もあります。再生治療などを用い、歯周組織を再び感染が起きにくい形態へと改善する治療です。ただ、歯周病治療のいちばんの基本は「プラークの除去」。それがうまくできれば、高価な治療を受けなくても済むようになります。

GUEST SPEAKER

マイドリーム
～あきらめないで頑張れる、大林流健康管理～

イタリアのプロリーグでプレーしていたとき、私は1年の1/3が海外遠征でした。1年に10カ国ほど回っていたのです。その遠征中、むし歯になったこともありました。

日本だと、どんな地方でも歯科医院はありますよね。でも海外だと、無い地域も多くて、結構困りました。

あるときのこと、むし歯治療中なのに遠征で1ヶ月、家に戻ることができず、とても痛むので抜くことにしました。アジアのある国で病院に行ったら麻酔を打てる医師がおらず、結局断られてしまいました。そのときは海外に身を置いてしまったことで、当たり前のことをしっかりとやっている日本のすごさを感じました。それをしっかりやり続けることが、とても大切なのですね。

歯が痛むとプレーに集中できません。試合中であっても痛いものは痛いですから。そうしたことを避けるために、日々の歯みがきに気を付けるようになりました。

スポーツキャスター
大林 素子 氏

中学1年からバレーボールを始め、中学3年の時に東京都中学選抜に選出される。その後、高校バレーボール界の名門八王子実践高校に進む。86年日立入社、88年ソウル五輪、92年バルセロナ五輪に出場する。95年にイタリアセリエA・アンコーナに所属、日本人初のプロ選手となる。帰国後、東洋紡オーキスに所属、96年アトランタ五輪出場後、97年に引退する。現在、日本スポーツマスターズ委員会シンボルメンバー、JOC環境アンバサダー、福島県・しゃくなげ大使、環境省チャレンジ25キャンペーン応援団、JVA（日本バレーボール協会）広報委員、2012オリンピックデーラン・アンバサダー、観光庁2012「スポーツ観光マイスター」。

Effects of Exercise and Amino Acid Supplementation on Body Composition and Physical Function in Community-Dwelling Elderly Japanese Sarcopenic Women: A Randomized Controlled Trial

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OBJECTIVES: To evaluate the effectiveness of exercise and amino acid supplementation in enhancing muscle mass and strength in community-dwelling elderly sarcopenic women.

DESIGN: Randomized controlled trial.

SETTING: Urban community in Tokyo, Japan.

PARTICIPANTS: One hundred fifty-five women aged 75 and older were defined as sarcopenic and randomly assigned to one of four groups: exercise and amino acid supplementation (exercise + AAS; n = 38), exercise (n = 39), amino acid supplementation (AAS; n = 39), or health education (HE; n = 39).

INTERVENTION: The exercise group attended a 60-minute comprehensive training program twice a week, and the AAS group ingested 3 g of a leucine-rich essential amino acid mixture twice a day for 3 months.

MEASUREMENTS: Body composition was determined using bioelectrical impedance analysis. Data from interviews and functional fitness parameters such as muscle strength and walking ability were collected at baseline and after the 3-month intervention.

RESULTS: A significant group × time interaction was seen in leg muscle mass ($P = .007$), usual walking speed ($P = .007$), and knee extension strength ($P = .017$). The within-group analysis showed that walking speed significantly increased in all three intervention groups, leg muscle mass in the exercise + AAS and exercise groups, and knee extension strength only in the exercise + AAS group (9.3% increase, $P = .01$). The odds ratio for leg muscle mass and knee extension strength improvement was more than four times as great in the exercise + AAS group (odds ratio = 4.89, 95% confidence interval = 1.89–11.27) as in the HE group.

CONCLUSION: The data suggest that exercise and AAS together may be effective in enhancing not only muscle strength, but also combined variables of muscle mass and walking speed and of muscle mass and strength in sarcopenic women. **J Am Geriatr Soc 60:16–23, 2012.**

Key words: sarcopenic women; exercise; amino acid supplementation; muscle mass; muscle strength

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Sarcopenia, defined as age-related involuntary loss of skeletal muscle mass and strength,[1,2] has been associated with physical disability, functional decline, falls, impaired mobility, and mortality in elderly people.[3,4] Therefore, treating or reversing sarcopenia is important in the maintenance of health and life expectancy in the elderly population. Although many factors, such as chronic disease, physical inactivity, and decreased muscle protein synthesis, may contribute to loss of muscle mass,[5–7] it has been suggested that only skeletal muscle disuse and undernutrition are potentially preventable or reversible with targeted interventions.[8]

Many studies have shown a strong relationship between resistance exercise and strength improvement, through which the efficacy of resistance exercise for the prevention and treatment of sarcopenia has been confirmed.[9] The previous studies have also shown that ingestion of essential amino acids can induce muscle protein anabolism in elderly adults.[10,11] One study showed that the combination of resistance exercise and essential amino acid supplementation (AAS) augmented muscle protein

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synthesis, suggesting it as a strategy to reverse sarcopenia[12] but in a small sample size. There are few randomized controlled trials (RCTs) on the effects of exercise and AAS on body composition and functional capacity.

The purpose of this study was to investigate the effects of exercise and AAS on muscle mass, strength, and walking ability in sarcopenic women.

METHODS

Subjects

A letter outlining the comprehensive geriatric health examination survey, describing its objective and the way that the personal data would be used, was mailed to the women randomly selected from the Basic Resident Register of 5,932 people aged 75 and older residing in the Itabashi ward of metropolitan Tokyo inviting them to participate in the study. Two thousand eighteen people responded to the mailed letters of invitation to participate in the study, with 1,670 people agreeing and 348 people declining to participate. The baseline assessment was conducted at the Tokyo Metropolitan Institute of Gerontology (TMIG) from October 12 to November 3, 2008. One thousand three hundred eighty-three women aged 75 and older were screened; 287 who originally agreed to participation were absent. Written informed consent was obtained for baseline screening; six people did not sign the informed consent form and were not included in this study.

Three hundred four of 1,377 women (22.1%) were operationally defined as sarcopenic (Figure 1), with selection based on categorization into one or more of the following inclusion criteria groups: appendicular skeletal muscle mass/height^2 less than 6.42 kg/m^2 and knee extension strength less than 1.01 Nm/kg[13,14] (n = 68), appendicular skeletal muscle mass/height^2 less than 6.42 kg/m^2 and usual walking speed less than 1.22 m/s (n = 65),[14] body mass index (BMI) less than 22.0 kg/m^2[14] and knee

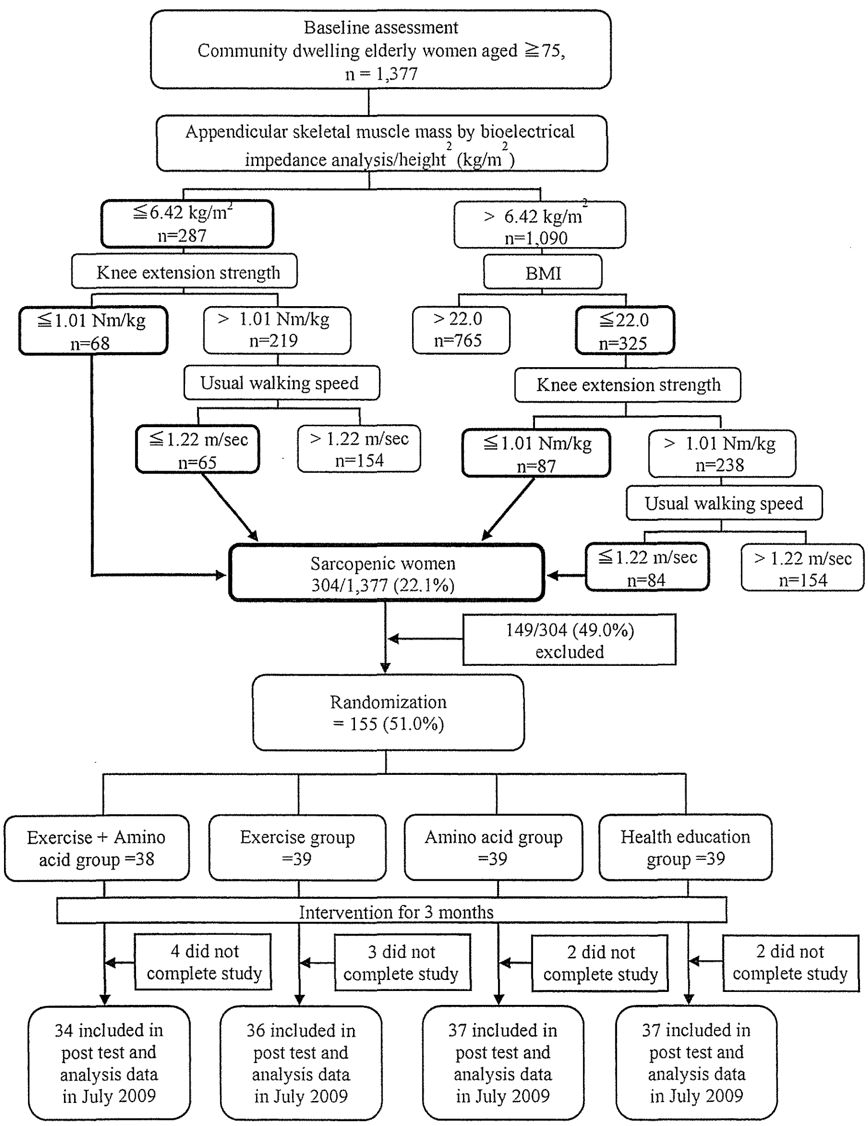

Figure 1. Algorithm for the selection of women who were operationally defined as sarcopenic and flowchart of participants in the randomized controlled trial of exercise and amino acid supplementation.

extension strength less than 1.01 Nm/kg (n = 87), and BMI less than 22.0 kg/m^2 and usual walking speed less than 1.22 m/s (n = 84). Exclusion criteria were severe knee or back pain; severely impaired mobility; impaired cognition (Mini-Mental State Examination (MMSE) score < 24);[16] missing baseline data; and unstable cardiac conditions such as ventricular dysrhythmias, pulmonary edema, or other musculoskeletal conditions. One hundred forty-nine (49.0%) of the potential sarcopenic participants were excluded because they were classified into one or more of the exclusion criteria or declined participation. The Clinical Research Ethics Committee of TMIG approved the study protocol. The intervention procedures were fully explained to all participants, and written informed consent was obtained (Figure 1).

Randomization

Randomization was performed after the baseline assessment; any variable that identified personal information was not included in the randomization process. Computer-generated random numbers were assigned to 155 participants who were then sorted and divided into four equal groups. The groups were randomly assigned to one of the four interventions groups: exercise + AAS (n = 38), exercise (n = 39), AAS (n = 39), or health education (HE; n = 39). All participants agreed to the group allocations that were mailed to them. There was no attempt to equalize the size of the groups based on their characteristics or to recruit subjects with specific characteristics. The co-investigators were blind to the randomization procedure and group allocations, separate physical therapy staff members who were also blind to the allocation of treatments collected data.

Outcome Measures

Outcome measures were evaluated according to data collected from interviews, body composition assessments using bioelectrical impedance analysis (BIA), and physical fitness tests at baseline and after the 3-month intervention.

Interview Survey

Face-to-face interviews were conducted to assess the individual's history of fractures and falls over the previous year, number of falls, cause of falls, urinary incontinence, exercise habits, smoking status, and MMSE score.

Body Composition Assessment

Measurements of height and weight were used to calculate BMI (kg/m^2). Body composition was measured using a segmental multifrequency BIA instrument that operated at frequencies of 5, 50, 250, and 550 kHz (Well-Scan 500, Elk Corp., Tokyo, Japan). Participants removed their socks, stood on two metallic electrodes on the floor scale barefoot, and held metallic grip electrodes placed in the palm of the hand with the fingers wrapped around the handrails. Using segmental body composition and muscle mass values of both legs, both arms, and the trunk, appendicular skeletal muscle mass and leg muscle mass values were obtained and used for analysis by summing the appropriate segmental muscle mass values.[13,17,18] Reliability of body composition measurements in all 155 participants in this study was not analyzed, although for the AAS group (n = 39), measurements were taken for a second time 1 week after baseline testing, and reliability was examined; the intraclass correlation coefficients (ICC) were: 0.98 for the right arm, 0.97 for the left arm, 0.97 for the right leg, 0.96 for the left leg, and 0.93 for the trunk.

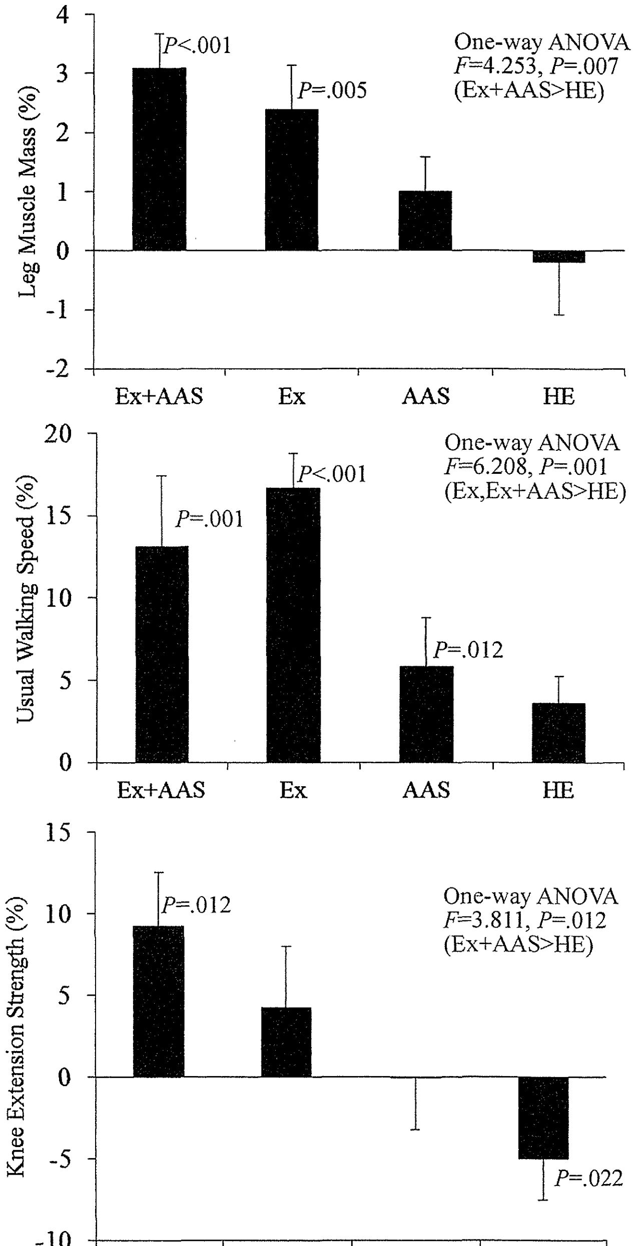

Figure 2. Mean percentage changes (standard errors) in leg muscle mass, usual walking speed, and knee extension strength after exercise (Ex), amino acid supplementation (AAS), both (Ex + AAS), or health education (HE). Bars indicate average changes from baseline to after the 3-month intervention. ANOVA = analysis of variance.

Functional Fitness Test

Calf girth and functional fitness variables including usual and maximum walking speeds and knee extension strength were measured. In measures of walking speed, participants were allowed to use assistive walking devices only if they expressed strong concerns about walking without a device or if there was any danger of falling. The knee extension strength measurement was taken twice, and the higher value divided by body weight (Nm/kg) were analyzed. The procedures for the functional fitness tests have been described in detail in previous reports.[19,20]

Intervention

Exercise

A comprehensive physical fitness and muscle mass enhancement training program of moderate intensity was provided for the participants in the exercise groups. The exercise intervention consisted of 60-minute exercise sessions held at the TMIG twice per week for 3 months. Each exercise intervention group was divided into two subgroups, with participants exercising together within their assigned group in one of four exercise sessions offered per day.

Each exercise session consisted of a 5-minute warm-up, 30 minutes of strengthening exercise, 20 minutes of balance and gait training, and 5 minutes of cool down. The strengthening exercises were performed in a progressive sequence from seated to standing positions. For each type of exercise, participants were instructed to complete up to eight repetitions of the movements. When the exercises were properly executed without significant fatigue or loss of proper execution, the resistance was increased. The progressive resistance was provided through the use of resistance bands or ankle weights. Intensity was maintained at approximately 12 to 14 on the Borg Rate of Perceived Exertion scale.[21] The principal investigator, along with the exercise instructor and assistant trainers, assessed each individual's ability to increase intensity.

Chair exercise: The chair-seated exercises were used in the early stages of the program because the participants were frail older adults and it provided a secure and stable position. Repetitions of toe raises, heel raises, knee lifts, knee extensions, and others were performed while seated on a chair. Hip flexions, lateral leg raises, and repetitions of other exercises were performed standing upright behind the chair and holding the back of the chair for stability.

Ankle-weight exercise: To strengthen lower extremities, a fixed weight was placed on the ankle while participants performed strengthening exercises. Weights of 0.50, 0.75, 1.00, and 1.50 kg were prepared and used in accordance with each participant's strength level as the resistance progressively increased. The exercises performed using these ankle weights included seated knee flexion and extension and standing knee flexion and extensions.

Exercises using a resistance band: Resistance bands were used to strengthen the upper and lower body. Lower body exercises included leg extension and hip flexion. Upper body exercises included double-arm pull downs and biceps curls.

Balance and gait training: The balance training was focused on improvement of static, dynamic, and lateral balancing ability. Exercises included standing on one leg, multidirectional weight shifts, tandem stand, and tandem walk. Participants practiced proper gait mechanics that focused on the maintenance of stability during walking and increasing stride length, toe elevation of the forward limb, heel elevation of the rear limb, frequency of stepping, and heel–floor angle. Exercises included raising the toes (dorsiflexion) during the forward swing of the leg, kicking off the floor with the ball of the foot, walking with directional changes, and gait pattern variations.

Amino Acid Supplementation

Essential AAS was provided for the participants in the AAS groups every 2 weeks. Packets of powdered amino acid supplements (42.0% leucine, 14.0% lysine, 10.5% valine, 10.5% isoleucine, 10.5% threonine, 7.0% phenylalanine, and 5.5% other) were provided for the participants to be taken with water or milk, and they were instructed to take the 3-g supplement two times a day (6 g daily) every day for 3 months.[22] To monitor their amino acid intake accurately, participants were given record sheets that were collected every 2 weeks on which they recorded what time of day they took the supplement and the amount of amino acid taken every day.

Health Education

Participants in the HE group took a class once a month for 3 months, a total of three times. The classes focused on cognitive function, osteoporosis, and oral hygiene. Participants were asked to continue their regular lifestyle habits, and no specific instructions on diet or physical activity were given.

Data Analysis

Sample size calculations using univariate one-factor repeated-measures analysis of variance (ANOVA) to examine significant differences in means at baseline and after the 3-month intervention ($\alpha = 0.05$, power = 0.80) with an effect size of 0.15 required a sample size of 28 participants. Esimating a potential attrition rate of 25%, 38 subjects per group were required.[23] One-way ANOVA was used to test any differences in baseline measures and percentage changes between groups, and chi-square tests were performed on categorical variables. Percentage changes in muscle mass and functional fitness after the intervention were calculated using the following formula: % change = ((postintervention value–baseline value)/(baseline value) × 100). Two-way repeated-measures ANOVA was used to evaluate the differences in the effect of the intervention on the outcome measures between groups, and a post hoc test was done on variables showing significant differences to determine which groups were different. Multiple logistic regressions were performed to compare the effects of the four intervention groups on each outcome variable after 3 months of intervention. All analyses were performed using SPSS version 15.0 of Windows (SPSS, Inc., Tokyo, Japan).

RESULTS

The baseline demographic, fitness, and interview variables of the participants in the four groups are summarized in Table 1. All of the baseline characteristics were similar between the groups.

The mean attendance rates during the 3-month intervention were 70.3% in the exercise + AAS group, 80.5% in the exercise group, 72.2% in the AAS group, and 71.8% in the HE group. Eleven participants (exercise + AAS = 4, exercise = 3, AAS = 2, HE = 2) were unable to complete the study after randomization because of spouse care (n = 3), admission to nursing home (n = 2), lack of motivation (n = 2), severe knee or back pain (n = 1), death (n = 1), falls and hip fracture (n = 1), and hospitalization (n = 1; Figure 2).

In comparing the pre- and postintervention changes in body composition and functional fitness of the groups (Table 2), there was a significant group × time interaction for leg muscle mass ($F = 4.253$, $P < .007$; exercise + AAS > HE), usual and maximum walking speeds (exercise and exercise + AAS > HE), and knee extension strength ($F = 3.558$, $P = .02$; exercise + AAS > HE).

The within-group analysis showed significant changes in leg muscle mass in the exercise + AAS ($P < .001$) and exercise ($P = .005$) groups and changes in usual walking speed in the exercise + AAS ($P = .001$), exercise ($P < .001$), and AAS groups ($P = .01$). Knee extension strength improved significantly only in the exercise + AAS group ($P = .01$), no improvement was seen in exercise or AAS, and a statistically significant decrease was observed in the HE group ($P = .02$; Figure 1).

Table 3 shows the effects of the type of intervention on changes in combined variables of muscle mass and physical function. Significant increases in leg muscle mass and knee extension strength (odds ratio (OR) = 4.89, 95% confidence interval (CI) = 1.89–11.27) and leg muscle mass and usual walking speed (OR = 4.11, 95% CI = 1.33–13.68) were observed in only the exercise + AAS group.

DISCUSSION

Although many definitions of sarcopenia have been reported,[1–3,24] there has recently been a focus not only on the loss of appendicular skeletal muscle mass, but also on functional decline.[25] In this study, sarcopenic women were operationally defined based on declines in muscle strength or walking ability that accompany the loss of skeletal muscle mass or low BMI. Because defining sarcopenia was beyond the scope of this study, the focus of the discussion will be on the effects of the intervention. To evaluate the intervention effects, the changes observed in the single variables as well as the combined variables will be discussed.

Many studies have focused on exercise or nutrition as interventions to reverse sarcopenia, but the results of these studies have not always been consistent.[8,9,12,26]

This study demonstrated that appendicular muscle mass and walking speed increased with the combination of exercise and essential amino acid ingestion, as well as with the separate exercise and amino acid interventions, but muscle strength improved only with the combination of exercise and amino acid ingestion.

A recently published meta-analysis[9] and a Cochrane review article also confirmed that resistance training two to three times a week can improve physical function and functional limitations and can reduce disability and muscle weakness in older people.[27] Previous studies have demonstrated that resistance training in elderly people produces

Table 1. Selected Variable Characteristics of Participants at Baseline According to Study Group

Characteristic	Exercise + AAS (n = 38)	Exercise (n = 39)	AAS (n = 39)	Health Education (n = 39)	*F*-Value*	*P*-Value*
Age, mean ± SD	79.5 ± 2.9	79.0 ± 2.9	79.2 ± 2.8	78.7 ± 2.8	0.577	.63
Height, cm, mean ± SD	147.1 ± 6.7	147.7 ± 4.4	145.8 ± 4.5	146.5 ± 4.9	0.960	.41
Body weight, kg, mean ± SD	39.5 ± 5.5	41.1 ± 4.7	40.1 ± 3.2	40.4 ± 3.9	0.874	.46
Body mass index, kg/m^2, mean ± SD	18.3 ± 2.5	18.9 ± 2.0	18.9 ± 1.6	18.8 ± 1.7	0.745	.53
Calf girth, cm, mean ± SD	18.3 ± 2.5	18.9 ± 2.0	18.9 ± 1.6	18.8 ± 1.7	0.745	.53
Lean body mass, kg, mean ± SD	29.1 ± 3.4	30.0 ± 2.6	28.8 ± 2.0	29.3 ± 2.4	1.505	.22
Muscle mass, kg, mean ± SD	26.9 ± 3.1	27.7 ± 2.3	26.5 ± 1.8	27.0 ± 2.2	1.538	.21
Appendicular muscle mass, kg, mean ± SD	13.3 ± 1.6	13.7 ± 1.3	13.1 ± 1.0	13.3 ± 1.2	1.502	.22
Legs muscle mass, kg, mean ± SD	9.8 ± 1.2	10.1 ± 1.0	9.7 ± 0.7	9.9 ± 0.9	1.570	.20
Usual walking speed, m/s, mean ± SD	1.26 ± 0.27	1.29 ± 0.28	1.29 ± 0.20	1.18 ± 0.22	1.701	.17
Maximal walking speed, m/s, mean ± SD	1.62 ± 0.37	1.67 ± 0.31	1.67 ± 0.27	1.55 ± 0.32	1.150	.33
Knee extension strength, Nm, mean ± SD	45.9 ± 11.3	46.6 ± 11.1	46.7 ± 7.8	47.4 ± 10.5	0.139	.94
Falls, %	21.1	17.9	15.4	20.5	0.519	.91
Exercise habit, %	26.3	25.6	38.5	33.3	2.029	.57
Urinary incontinence, %	44.7	38.5	41.0	25.6	3.414	.33
Osteoporosis history, %	36.8	43.6	48.7	30.8	2.987	.39
Heart disease history, %	10.5	15.4	12.8	17.9	0.977	.81
Diabetes mellitus history, %	7.9	5.1	5.1	12.8	2.156	.54

* One-way analysis of variance for continuous variables and chi-square test for categorical variables.

AAS = amino acid supplementation; SD = standard deviation.

Table 2. Comparison of Muscle Mass and Functional Fitness Variables Between Groups After 3-Month Intervention

Variable	Group	Mean ± Standard Deviation: Baseline	Mean ± Standard Deviation: After 3-Month Intervention	Analysis of Variance (Group × Time), *P*-Value	Post Hoc Analysis*
Muscle mass, kg	Exercise + AAS	26.76 ± 2.77	27.26 ± 3.04	*F* = 1.076, .36	
	Exercise	28.09 ± 1.90	28.51 ± 2.39		
	AAS	26.25 ± 1.81	26.53 ± 2.10		
	HE	27.48 ± 2.04	27.66 ± 2.23		
Appendicular muscle mass, kg	Exercise + AAS	13.25 ± 1.35	13.59 ± 1.53	*F* = 1.354, .26	
	Exercise	13.90 ± 1.06	14.19 ± 1.33		
	AAS	12.86 ± 0.99	13.03 ± 1.10		
	HE	13.57 ± 1.16	13.67 ± 1.05		
Legs muscle mass, kg	Exercise + AAS	9.76 ± 1.01	10.07 ± 1.13	*F* = 4.253, .007	Exercise + AAS > HE
	Exercise	10.28 ± 0.81	10.53 ± 1.05		
	AAS	9.55 ± 0.73	9.65 ± 0.83		
	HE	10.14 ± 0.87	10.11 ± 0.81		
BMI, kg/m^2	Exercise + AAS	18.30 ± 2.64	18.14 ± 2.68	*F* = 0.606, .61	
	Exercise	18.80 ± 1.30	18.50 ± 1.41		
	AAS	18.84 ± 1.43	18.56 ± 1.62		
	HE	18.83 ± 1.75	18.77 ± 1.67		
Usual walking speed, m/s	Exercise + AAS	1.27 ± 0.25	1.43 ± 0.29	*F* = 4.213, .007	Exercise and Exercise + AAS > HE
	Exercise	1.31 ± 0.24	1.50 ± 0.23		
	AAS	1.30 ± 0.18	1.36 ± 0.18		
	HE	1.19 ± 0.21	1.22 ± 0.23		
Maximum walking speed, m/s	Exercise + AAS	1.64 ± 0.34	1.92 ± 0.37	*F* = 9.374, <.001	Exercise and Exercise + AAS > HE
	Exercise	1.72 ± 0.27	2.04 ± 0.27		
	AAS	1.71 ± 0.28	1.92 ± 0.27		
	HE	1.57 ± 0.31	1.64 ± 0.31		
Knee extension strength, Nm/kg	Exercise + AAS	1.15 ± 0.27	1.23 ± 0.29	*F* = 3.558, .02	Exercise + AAS > HE
	Exercise	1.12 ± 0.30	1.14 ± 0.26		
	AAS	1.15 ± 0.25	1.14 ± 0.25		
	HE	1.14 ± 0.26	1.00 ± 0.26		

* A post hoc analysis was performed using the Scheffe method.
AAS = amino acid supplementation; HE = health education; BMI = body mass index.

Table 3. Change in Leg Muscle Mass and Functional Fitness After Intervention According to Study Group

Dependent Variable*	Adjusted Odds Ratio (95% Confidence Interval): AAS	Exercise	Exercise + AAS
Change in leg muscle mass and knee extension strength	1.99 (0.72–5.65)	2.61 (0.88–8.05)	4.89 (1.89–11.27)
Change in leg muscle mass and usual walking speed	1.35 (0.45–4.08)	2.41 (0.79–7.58)	4.11 (1.33–13.68)

Reference: health education.
* 1 = improve, 0 = no change or decrease.
AAS = amino acid supplementation.

9% to 15% increases in strength and approximately 5% in thigh muscle volume.[28,29] Also, many studies have shown that resistance training in elderly people must be conducted at high intensities and volumes to see improvements.[9,27] In contrast, less-intense resistance exercise programs have produced little or no strength gains.

The data in this study show improvements of 2.4% in leg muscle mass, 2.0% in appendicular muscle mass, and 4.3% in leg strength in the exercise group. The moderate-intensity exercise provided in this trial produced strength gains that were smaller than those seen in previous studies, but the combination of moderate intensity exercise and AAS increased muscle mass 3.1% and muscle strength 9.3%, gains that are comparable with those observed in previous studies of high-intensity exercise.[28]

The results of the current study showed that total muscle mass, appendicular muscle mass, and walking speed significantly increased in the exercise group, suggesting that exercise is effective in the improvement of muscle mass and functional fitness, but increases in muscle strength were not observed. These results indicate that exercise alone is insufficient for recovery in sarcopenic elderly women.

Previous studies have indicated that declines in muscle mass are related to declines in muscle protein synthesis rates in older adults and that leucine-enriched essential amino acid mixtures are primarily responsible for the amino acid-induced muscle protein anabolism in elderly people.[11,22] These studies investigated the effects of different amino acid dosages (from 6.7 to 20.0 g/d) on protein synthesis, and the 6.0-g/d dosage provided in this study is lower than in previous studies, but the mean weights of the subjects in such studies were from 71.0 to 81.3 kg, making the dosage of amino acid between 0.090 and 0.246 g/kg of body weight. The amino acid dosage in the current study was 0.151 g/kg, which is comparable with the amounts found in the literature.[11,22,26] The results of the current study showed that muscle mass, appendicular muscle mass, and leg muscle mass significantly increased in the AAS group, which is consistent with previous findings.

Many studies have demonstrated an increase in muscle mass from nutritional supplementation, but an increase in muscle strength does not always accompany an increase in muscle mass. A recent study concluded that essential AAS alone was not sufficient to increase muscle strength.[26] Similarly, although the results of the current study showed that AAS alone increased muscle mass, improvement in muscle strength was not observed. The results of the present study showed that muscle mass increased significantly with exercise or essential AAS, although muscle strength, measured according to knee extension strength, improved significantly only in the exercise + AAS group.

Next, the discussion will focus on the changes in the combined variables. One study that investigated the effects of resistance exercise and nutritional supplementation on muscle mass and strength in older adults concluded that high-intensity resistance exercise was beneficial in increasing muscle mass and muscle strength, but the nutritional supplementation, which contained only a small percentage of a soy-based protein within a mixture of mainly carbohydrates, did not contribute to those gains.[8] As illustrated in Figure 2, exercise alone was effective in enhancing single variables such as leg muscle mass or usual walking speed. Similarly, the AAS group improved usual walking speed, but rationally, to treat sarcopenia, improvements in single variables are not sufficient. Improvements observed in the combined variables would presumably lead to the most-efficient reversal of sarcopenia. Significant improvements in the combinations of leg muscle mass, knee extension strength, and walking speed were seen only in the exercise + AAS group. Although whether exercise + AAS was better than either intervention alone remains inconclusive, these results suggest that exercise + AAS may be necessary for benefits in muscle mass and strength.

This study has several limitations. First is the measurement of body composition estimated using BIA. Although magnetic resonance imaging (MRI), computed tomography, and dual-energy X-ray absorptiometry are common, accurate clinical methods of measuring muscle mass,[30,31] they are cost ineffective and are not always appropriate for field studies. BIA is simple, noninvasive, and inexpensive and has been widely used in field studies. The comparison of MRI and BIA measurements has revealed a strong correlation between the two, confirming the validity of the BIA method for muscle mass measurement in older adults.[13,17,18] Therefore, the validity of the data collected using BIA has little influence on the interpretation of the results of this study. Second, it has been reported that AAS enhances muscle protein synthesis,[11,22,32] but the mechanism of the increase in muscle mass from AAS was not explored in the current investigation. Therefore, the results of this study were interpreted based on the assumption that muscle protein synthesis had been enhanced. Third, the effects of the exercise + AAS should have been determined with the use of placebos, but placebo treatments were not provided in this study, so future research should include placebos to observe the effects of exercise and AAS on physical function and muscle strength. Fourth, the total number of dropouts in this study was 11 people, and they were not included in the data analysis. Many studies have used intention-to-treat (ITT) analyses to determine the effects of RCTs, and the use of ITT analyses are increasing, although one previous study found that only approximately 35% of 274 RCTs used ITT analyses.[33] The current study was not an ITT analysis because it confirmed that there were no significant differences between the dropouts and the participants who completed the study, and the exclusion of the 11 dropouts from the analysis did not affect the integrity of the baseline randomization. Finally, previous research has shown that milk contains essential amino acids.[34,35] Because some of the participants took the AAS with milk, the exact essential amino acid dosage in this study could not be determined, and the effect of drinking milk on the results of this study was not confirmed. Future research should avoid the intake of milk with amino acids when investigating the effects of amino acids on muscle strength and mass and physical function.

This study demonstrated that exercise and nutrition may be necessary for the basic treatment of increasing muscle mass and strength to reverse the effects of sarcopenia in community-dwelling sarcopenic women. Exercise and AAS together have significant effects on enhancing not only muscle strength, but also the combined variables of muscle mass and walking speed and of muscle mass and strength in this study population, but further follow-up studies on larger populations are required to confirm these results.

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Author Contributions: H. Kim developed the study concept and design, recruited subjects, developed the intervention program, analyzed and interpreted the data, and prepared the manuscript. S. Takao interpreted the data and reviewed the manuscript for accuracy. K. Saito assisted in AAS and supervised the interview survey. Y. Hideyo assisted in subject recruitment, supervised the interviewers, and interpreted the data. M. Kobayashi assisted in AAS and subject recruitment and interpreted the data. H. Kato assisted in assisted AAS and body composition assessment. M. Katayama assisted in AAS and interview survey.

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Relationship Between Serum Isoflavone Levels and Disability-Free Survival Among Community-Dwelling Elderly Individuals: Nested Case–Control Study of the Tsurugaya Project

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Background. The longer healthy life expectancy observed in Japan may be partly attributed to the Japanese diet. The researchers sought to examine whether serum isoflavone levels are associated with disability and death.

Methods. The researchers used a nested case–control study to compare serum isoflavones (daidzein, genistein, glycitein, and equol) levels between 165 participants that died or were certificated as disabled (cases) and 177 controls. Disability was defined by certification of long-term care insurance. Conditional logistic regression models were used to calculate the risk of isoflavones for the composite outcome.

Results. The proportion of cases was lower in the group with the highest levels of equol (34/91, 37%) compared with equol nonproducers (84/161, 52%). The risk of disability or death among equol producers remained reduced after adjusting for age and sex (odds ratio: 0.55, 95% confidence interval: 0.33–0.93). In a multivariate model, this *risk was also unchanged* (odds ratio: 0.51, 95% confidence interval: 0.27–0.96). There were no significant associations between daidzein, genistein, and glycitein with the composite endpoint.

Conclusions. Higher serum equol levels, but not any other isoflavones, were inversely associated with the composite endpoint of disability and death. Although it cannot be concluded that equol per se has preventive effects on disability or death, *higher equol levels appear associated with better health.*

Key Words: Isoflavone—Disability—Mortality—Nested case–control study.

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ACCORDING to the Health Report published in 2004 by the World Health Organization, both healthy life expectancy at age 0 and 60 years were the longest in Japan compared with all other countries in the world (1). Therefore, it might be important to explore the determinants of the prolonged healthy life expectancy among Japanese. For instance, this longer healthy life expectancy may be, in part, attributed to the Japanese diet, which is high in foods such as fish, green tea, and soybean. In this study, the researchers focused on the relationship between soy isoflavones and disability-free survival.

A questionnaire survey is frequently used to estimate food consumption. However, in Japan, soybean is frequently used as a raw material in seasonings, such as miso paste and soy sauce. Therefore, it might be difficult to estimate the amount of soybean consumed from food frequency questionnaires. Indeed, the researchers previously found that there was a poor correlation between the assessment of soybean consumption by dietary record and food frequency questionnaire (2). Furthermore, studies on equol, a metabolite of daidzein that is produced by intestinal bacteria in some, but not all, adults have shown that those individuals who possessed equol-producing intestinal bacteria were more likely to benefit from soyfood consumption than those who did not (3,4). Importantly, equol production can only be assessed from blood or urine samples. Therefore, in the present study, the researchers decided to assess serum isoflavone as markers of soy intake.

Isoflavones, including genistein, daidzein, and glycitein, are found in soy and soy products (3,4). Isoflavones are known to have estrogenic effects, and consequently, may possess an ability to lower cholesterol and inhibit bone loss (3,4). Furthermore, emerging evidence suggests that isoflavones may be associated with lower risk of various cancers, including lung (5–7), prostate (8,9), and breast (10). Therefore, isoflavone levels may be associated with a lower risk of incident disability and mortality.

In the present study, the researchers used a nested case–control study design to investigate the relationship between serum isoflavone levels and risk of composite outcome of disability and death; a good indicator of healthy life expectancy.

Methods

Study Participants

As implemented in 2002 and 2003, the Tsurugaya Project was a comprehensive geriatric assessment of medical status, as well as physical and cognitive functions (11–17). The present study is based on data collected in 2002, as blood samples from that time period were available (16,17).

Of the 2,730 inhabitants aged 70 and older living in the Tsurugaya area of Sendai, Japan, 1,177 provided written informed consent to participate in the study. Because the researchers did not obtain agreement to review information regarding long-term care insurance (LTCI) in 2002, they requested agreement from the participants who underwent a comprehensive geriatric assessment in 2003. Of the 1,177 participants who underwent a comprehensive geriatric assessment in 2002, 671 underwent another comprehensive geriatric assessment in 2003, of which 657 agreed to a review of their LTCI information. The researchers excluded data from participants who were identified as having a disability on their LTCI certificate in 2003 ($n = 55$), participants who did not agree to their blood samples being analyzed or stored ($n = 6$), and participants who moved prior to being certified as disabled ($n = 6$). Of the 590 remaining participants, 208 developed a disability or died by June 30, 2009. The eligible participants were divided into eight strata according to sex and age (every 5 years; Table 1). Specifically, a select 178 cases (ie, participants that developed a disability or died) and 178 controls (ie, participants who lived without disability until June 30, 2009) were stratified. Because 14 serum samples (1 control and 13 cases) did not have sufficient serum to measure isoflavone levels (<1 mL of serum), the researchers assessed a total of 342 participants in the present study (Table 1). The Ethics Committee of the Tohoku University Graduate School of Medicine approved the study protocol.

Table 1. Age and Sex Distribution of the Eligible Participants from the Tsurugaya Project (2002–2009)

		Condition in June 2009	
Age in 2002 (y)	Sex	No Disability and Alive	Disabled or Deceased
70–74	Men	35	32
	Women	36	33
75–79	Men	27	25
	Women	50	47
80–84	Men	9	9
	Women	16	15
85–89	Men	1	1
	Women	3	3

Serum Isoflavone Measurements

Blood samples collected under non-fasting conditions were immediately cooled at 4°C, centrifuged within 4 hours at 3,000*g* at 4°C for 10 minutes, and stored at −80°C. Concentrations of serum isoflavones, namely genistein, daidzein, glycitein, and equol, were measured using triple quadrupole tandem liquid chromatography–mass spectrometry (18). These measurements were determined at a clinical testing laboratory (SRL, Tokyo, Japan). Serum albumin, total cholesterol (TC), and casual glucose levels were also measured.

Other Measurements

Information regarding smoking status, drinking status, food intake, physical activity (PA), and history of disease was surveyed via a questionnaire, and drug information was confirmed by an experienced pharmacist. The participants were instructed to fill out a brief self-administered diet history questionnaire that included 75 food items with specified serving sizes described by natural portions or standard weight and volume measures of the servings commonly consumed in the study population. The mean daily intake of nutrients was calculated by using an ad hoc computer program developed to analyze the questionnaire (14). Participants indicated the mean frequency of consumption of green tea over the previous 1 month in terms of the specified serving size by selecting one of the eight frequency categories: almost never, less than 1 cup/wk, 1 cup/wk, 2–3 cups/wk, 4–6 cups/wk, 1 cup/d, 2–3 cups/d, and greater than or equal to 4 cups/d. Subsequently, the researchers summarized this information into three groups as follows: greater than or equal to 4 cups/d, 2–3 cups/d, and less than 2 cups/d (14). In terms of meat consumption, participants indicated the mean frequency of consuming a specified serving size of (1) chicken, (2) pork or beef, (3) ham, sausage, or bacon, and (4) liver over the previous 1 month by selecting one of the frequency categories: 2 times/d, 1 time/d, 4–6 times/wk, 2–3 times/wk, 1 time/wk, less than 1 time/wk, and none. These four frequencies were summed and a total meat consumption frequency was calculated. According to the distribution, the researchers classified participants into four groups of meat consumers: greater than or equal to 6.5 times/wk, 4.5–6.4 times/wk, 2.5–4.4 times/wk, and less than 2.5 times/wk. PA was first assessed by a self-reported single question on whether the participant had any PA in the past year. If "yes," further questions were asked about the frequency and duration of walking, brisk walking, and sports. Each PA was classified into three categories on the basis of the frequency and duration of participation: (i) "high" PA (≥3–4 times/wk for ≥30 minutes each time), (ii) "low" PA (some PA in the past year, but not enough), and (iii) "none" (no PA). In this study, the researchers used three categories according to the distribution; participants who did any level of sports or high frequency of brisk walking, participants who did low frequency of brisk walking or any level of walking, and participants who did not have PA. Symptoms of depression were assessed via the Japanese version of the 30-item geriatric depression scale (12,14). The anthropometric variables (height and body weight) were recorded according to standard protocol. Body mass index was calculated as weight in kilograms divided by height in meters squared. Functional reach, which measures how far an individual can reach forward beyond their arm's length while standing without losing balance, was measured and used as an indicator of physical function (13). The researchers used average stiffness of the right and left calcaneus as an indicator of bone mineral density. To assess stiffness, the researchers determined quantitative ultrasound parameters, such as the speed of sound (m/s), broadband ultrasound attenuation (dB/MHz), and the stiffness index (Stiffness), which was derived from speed of sound and broadband ultrasound attenuation. These parameters were measured in the right and left calcaneus using an Achilles Ultrasound Bone Densitometer (A-1000, GE-Lunar Corporation, Madison, WI) (19). Participants self-measured blood pressure at home using an automated device (HEM747IC: Omron Life Science Co. Ltd., Tokyo, Japan) (12). Participants were classified into groups based on the following categories: home hypertension, home borderline hypertension, and home normotension, according to the guidelines for home blood pressure (20). Participants prescribed antihypertensive medication were classified into the home hypertension group. The presence of diabetes was defined as a non-fasting blood glucose greater than or equal to 200 mg/dL (11.1 mmol/L) or use of antidiabetic drugs. Impaired blood glucose was classified as non-fasting blood glucose between 140–199 mg/dL (7.7–11.0 mmol/L). Participants were categorized into four TC groups: TC greater than or equal to 240 mg/dL or use of cholesterol lowering drug, TC between 200–239 mg/dL, TC between 160–199 mg/dL, and TC less than 160 mg/dL.

LTCI Certification

The researchers defined incident disability based on the LTCI certification system, which was launched as a national insurance scheme in April 2000 (21–23), and followed up those with certified incident disability until June 30, 2009.

Individuals aged 40–64 years and living in Japan, who were diagnosed with aging-related diseases (eg, Alzheimer's disease and stroke), and those aged 65 and older, who were certified as requiring care, are eligible for benefits under the LTCI certification (24). To receive LTCI services, elderly individuals or their caregivers (family or professional) must contact the municipal government to have their care requirements officially certified (22). A trained local government official visits their home to evaluate nursing care needs via a questionnaire that assesses their current physical and mental status, and use of medical services (21). Standardized scores for physical and mental functioning, as well as the estimated amount of time required for care under nine categories (ie, grooming and/or bathing, eating, using the toilet, transferring, eating, assistance with instrumental activities of daily living, behavioral problems, rehabilitation, and medical services), are then calculated using software. Based on the national average values, it is decided whether applicants should be certified to receive LTCI services, and then, the system assigns a care needs level, which is determined by a certification board comprising physicians, nurses, and other experts in health and social services, who were appointed by the local mayor. The minimum standard for LTCI was care support level 1, which requires 25 minutes of total care/d (21,25).

Care needs are assessed according to seven levels, which closely correlate with the Barthel Index (Spearman's coefficient: −0.86) and the Mini-Mental State Examination (Spearman's coefficient: −0.42) (24,26). The outcome represents a comprehensive measure of disability among elderly individuals (26).

The Sendai City Municipal Authority provided annual information regarding LTCI certification, including the care level, date of certification, relocation, and death, between June 30, 2003 and June 30, 2009. The researchers defined incident disability as the certification of an individual by the LTCI to any level of care, and the date of disability as the first date of certification. Six participants were removed from the study due to relocation during the follow-up. The researchers used a composite outcome of disability and death, which can also be considered as an indicator of disability-free survival.

Statistical Analysis

The researchers classified participants into four groups based on quartiles of isoflavone levels. However, with respect to equol, almost half of participants were nonproducers of equol (ie, equol level < 1.0 ng/mL), and consequently, the researchers used three categories, specifically, the nonproducers, lower half of equol producers (ie, equol level ≥ 1.0 ng/mL), and upper half of equol producers.

The characteristics of cases and controls were compared using the χ^2 test or t test, as appropriate. Characteristics with respect to isoflavone levels were compared using the χ^2 test for categorical variables or ANOVA for continuous variables, as appropriate. A multiple logistic regression analysis was used to determine factors that predict equol production. This model included the following factors: smoking, alcohol drinking, blood pressure (ie, home hypertension, home borderline hypertension, and home normotension), blood glucose (diabetes, impaired blood glucose, and normal range), TC group, albumin, sex-specific quartile of functional reach, depression (geriatric depression scale ≥ 11), body mass index, sex-specific quartile of stiffness of calcaneus, history of cardiovascular disease, history of cancer, sex-specific quartile of total energy intake, green tea consumption, meat consumption, PA group, and serum daidzein concentration. The researchers also determined the factors that predict higher equol values in equol producers via a linear regression model with log-transformed equol and the above-mentioned factors.

A conditional logistic regression model on the age and sex strata was used to calculate the odds ratios (ORs) and 95% confidence intervals of isoflavones and risk of disability or death. The researchers used both crude and multiple adjusted models. In the multivariate model, the researchers adjusted for the potential confounders associated with isoflavone levels or incident disability or death mentioned above excluding serum daidzein level.

The researchers also calculated the risk of disability only (case = 142) or death only (case = 40). Furthermore, the researchers calculated the relationship of daidzein, genistein, and glycitein with composite outcome of disability and death among equol producers. The level of statistical significance was set at $p < .05$. All statistical analyses were performed using SAS software, version 9.1 (SAS Institute, Cary, NC).

Results

The baseline characteristics of control (ie, those that live without disability) and case (ie, those that developed a disability or died by the end of the follow-up period) groups are presented in Table 2. Due to age and sex matching, there were no differences observed in age and proportion of sex. TC levels, functional reach, stiffness, and PA were statistically lower among cases than controls ($p < .05$).

When the researchers compared the baseline characteristics of the participants according to each isoflavone type and their respective levels, there were no apparent age differences across all isoflavone groups. However, the proportions of women were generally lower in the higher ranges of all isoflavone levels. TC levels were also generally lower in the higher ranges of all serum isoflavone levels. The proportion of bone mineral density was significantly different in the glycitein group, but not in the daidzein, genistein, or equol groups. Although the proportion of women was lower at higher serum equol levels, the proportion of current smokers was also lower at the higher serum equol levels. When a multiple logistic regression analysis was performed to determine the predictors of equol producers, male gender was revealed as a significant predictor of higher equol production. Of all equol producers, men, nonsmokers, and participants with diabetes had higher log-transformed equol values. A higher concentration of daidzein also predicted higher log-transformed equol values ($\beta = 0.003$, $p = .002$).

The relationship between levels of different isoflavones and the composite endpoint of disability or death are presented in Table 3. There were no significant associations between daidzein, genistein, and glycitein with the composite outcome of disability or death after adjusting for age and sex. However, in the equol group, the risk of composite endpoint was lower with higher levels of equol, after control for age and sex (OR = 0.55; 95% confidence interval = 0.33–0.93). These associations remained unchanged when additional potential confounders were added to the model (OR = 0.51; 95% confidence interval = 0.27–0.96). Similarly, the relationship was unchanged when the researchers excluded participants who died without disability. Although participants with the highest isoflavone quartiles consistently showed lower risk of death (OR ≤ 0.46), this observation did not reach statistical significant due to the small number of deaths. The relationships between daidzein, genistein, and glycitein levels with the composite endpoint were also assessed among equol producers only (Table 4). The highest quartiles of the daidzein, genistein, and glycitein groups showed a nonsignificant trend for a lower risk of the composite endpoint (OR ≤ 0.86).

Discussion

The present nested case–control study is the first to show that higher levels of equol are associated with lower risk of disability or mortality. This inverse relation was also observed when the researchers compared participants with disability with controls. However, whether equol per se plays a causal role in increasing healthy life expectancy remains to be determined in future research.

There are several advantages of the present study. First, this study assessed comprehensive geriatric parameters, including physical function and depressive symptoms, which have been previously associated with incident disability or mortality. Second, the researchers used a nested case–control design, in which the measurement precedes the onset of the outcome, thus establishing the temporal relationship between the putative cause and the hypothesized effect. Third, the researchers used LTCI certification to assess disability, which is based on strictly established and uniform rules throughout Japan. This methodology enabled us to achieve higher follow-up rates, and eliminated potential selection bias in both the case and control groups. Nevertheless, this system is not perfect, as

Table 2. Comparison of Baseline Characteristics Between Control and Case Groups, the Tsurugaya Project 2002–2009.

		Condition at June 2009		
		Control	Case	
		Alive without Disability	Disability or Death	*p* Value
Numbers of participants		177	165	
Age	y, mean (*SD*)	75.9 (3.8)	76.5 (4.2)	.14
Sex	Women	59%	59%	
Smoking	Current	10%	13%	
	Past	27%	29%	
	Never	63%	55%	
Drinking	≥46 g of alcohol/d	8%	8%	.15
	23–45.9 g of alcohol/d	8%	3%	
	0–22.9 g of alcohol/d	15%	21%	
	0 g	69%	68%	
Blood pressure	Normotension	19%	16%	.76
	Borderline hypertension	12%	9%	
	Hypertension	65%	70%	
	Home BP not measured	5%	5%	
Blood glucose	Diabetes	7%	8%	.09
	Impaired blood glucose	4%	10%	
	Normoal blood glucose	89%	82%	
Cholesterol	TC ≥ 240 mg/dL or cholesterol lowering drug	34%	26%	.32
	TC 200–239 mg/dL	36%	36%	
	TC 160–199 mg/dL	24%	30%	
	TC < 160 mg/dL	6%	8%	
Albumin	g/dL, mean (*SD*)	4.4 (0.3)	4.3 (0.3)	.32
Functional reach	Could not measure	1%	1%	<.01
	Men 0–28.8 cm, women 0–25.6 cm	15%	35%	
	Men 28.9–32.1 cm, women 25.7–28.6 cm	21%	28%	
	Men 32.2–36.3 cm, women 28.7–32.2 cm	31%	18%	
	Men 36.4 cm–, women 32.3 cm–	33%	19%	
Depression	GDS ≥ 11 point	25%	35%	.0497
Body mass index	kg/m^2 (SD)	23.7 (3.0)	23.6 (3.6)	.76
Stiffness of calcaneus	Men 0%–60.4%, women 0%–49.4%	19%	30%	<.01
	Men 60.5%–71.4%, women 49.5%–56.9%	23%	25%	
	Men 71.5%–81.9%, women 58.5%–65.9%	31%	20%	
	Men 82.0%–, women 66.0%–	28%	25%	
History of CVD	Present	11%	18%	.10
History of cancer	Present	5%	10%	.06
Total energy intake	kcal/d (SD)	1616 (398)	1654 (468)	.41
Green tea consumption	≥4 cups/d	50%	45%	.43
Meat consumption	6.5 times/wk	29%	28%	.71
Physical activity	Sports or higher amount of brisk walking	27%	16%	.02
	Lower amount of brisk walking or any amount of walking	42%	56%	

Notes: CVD = cardiovascular diseases; diabetes = casual blood glucose ≥ 200 mg/dL or taking antidiabetic drugs; GDS = geriatric depression scale; home hypertensive = home systolic BP ≥ 135 mmHg and/or home diastolic BP ≥ 85 mmHg and/or user of antihypertensive medication; home borderline hypertensive = not satisfied with home hypertensive critetria and home systolic BP ≥ 125 mmHg and/or home diastolic BP ≥ 80 mmHg; home normotensive = home systolic BP < 125 mmHg and home diastolic BP < 80 mmHg without antihypertensive medication; impaired blood glucose = casual blood glucose ≥ 140 mg/dL and not taking antidiabetic drugs; *SD* = standard deviation; TC = total cholesterol.

elderly individuals or their caregivers must initiate contact with the municipal government to receive LTCI services, and thus, some elderly individuals with disability may not be certified. However, this confounder would attenuate the relationship between equol levels and the composite outcome of disability and death. Therefore, the researchers' conclusion that higher serum equol was associated with lower risk of composite endpoint of incident disability and death, should remain true. Another limitation of this study was that blood samples were collected in non-fasting conditions, potentially affecting serum isoflavone levels. Similarly, because equol production has been found to vary over time within individuals (27), the misclassification of equol status is a possibility. However, these limitations would only attenuate the relationship between equol levels and the composite outcome of disability and death.

In the present study, half of the participants were classified as equol producers, which corroborate the findings of previous reports from Asia (4,17). In the researchers' attempt to determine the predictors of equol production, they were able to only identify sex. Interestingly, among all equol producers, men, nonsmokers, and participants with diabetes had

Table 3. Serum Isoflavone Levels in the Control and Case Groups from the Tsurugaya Project (2002–2009)

		All Samples				Control vs Disabled			Control vs Death		
		Control	Case	OR1 (95% CI)	OR2 (95% CI)	Control	Case	OR2 (95% CI)	Control	Case	OR2 (95% CI)
Isoflavones	ng/mL	No Disability and Alive	Disabled or Deceased	Age and Sex Only	Multiple Adjusted	No Disability and Alive	Disabled*	Multiple Adjusted	No Disability and Alive	Deceased	Multiple Adjusted
Daidzein	–36	48	38	1	1	48	36	1	48	6	1
	36.1–76.6	43	41	1.21 (0.66–2.21)	1.42 (0.68–2.97)	43	31	1.22 (0.55–2.67)	43	15	9.31 (1.50–57.94)
	76.7–141.0	42	44	1.32 (0.73–2.41)	1.64 (0.77–3.50)	42	35	1.63 (0.73–3.66)	42	16	9.53 (1.28–71.20)
	141.1–	44	42	1.21 (0.66–2.21)	1.52 (0.73–3.19)	44	40	1.56 (0.72–3.36)	44	3	0.24 (0.02–2.95)
Genistein	–63.5	47	39	1	1	47	37	1	47	7	1
	63.6–145.2	41	44	1.30 (0.71–2.38)	1.42 (0.68–2.95)	41	35	1.21 (0.55–2.66)	41	13	4.89 (0.90–26.52)
	145.3–269.1	41	44	1.30 (0.71–2.37)	1.31 (0.63–2.71)	41	33	1.09 (0.50–2.39)	41	15	2.04 (0.43–9.71)
	269.2–	48	38	0.96 (0.52–1.75)	0.99 (0.47–2.07)	48	37	1.05 (0.49–2.28)	48	5	0.37 (0.05–2.49)
Glycitein	–1.9	43	37	1	1	43	31	1	43	10	1
	2.0–4.6	46	43	1.09 (0.60–2.01)	1.06 (0.51–2.22)	46	37	1.09 (0.49–2.43)	46	10	1.26 (0.31–5.08)
	4.7–9.8	46	41	1.04 (0.57–1.91)	1.12 (0.53–2.35)	46	36	1.27 (0.57–2.84)	46	11	0.98 (0.25–3.82)
	9.9–	42	44	1.22 (0.66–2.27)	1.31 (0.62–2.74)	42	38	1.52 (0.68–3.37)	42	9	0.46 (0.10–2.08)
Equol	–0.9	77	84	1	1	77	75	1	77	16	1
	1.0–23.5	43	47	1.00 (0.60–1.68)	1.10 (0.59–2.05)	43	38	1.01 (0.52–1.98)	43	14	1.90 (0.55–6.62)
	23.6–	57	34	0.55 (0.33–0.93)	0.51 (0.27–0.96)	57	29	0.52 (0.27–1.02)	57	10	0.45 (0.12–1.68)

Notes: 95% CI = 95% confidence interval; diabetes = casual blood glucose ≥ 200 mg/dL or taking antidiabetic drugs; OR = odds ratio; OR1 = stratified for age and sex; OR2 = further adjusted for smoking status, drinking status, blood pressure category (home hypertensive, home borderline hypertensive, home normotensive), casual blood glucose (normal glucose, impaired blood glucose, diabetes), total cholesterol (total cholesterol ≥ 240 mg/dL or user of cholesterol lowering drugs, total cholesterol between 200–239 mg/dL, total cholesterol between 160–199 mg/dL, total cholesterol < 160 mg/dL), serum albumin, sex-specific quartile of functional reach, body mass index, depressive symptom (geriatric depression scale ≥ 11 or user of antidepressants), sex-specific quartile of stiffness of calcaneus, history of cardiovascular diseases, history of cancer, and sex-specific quartile of total energy intake, green tea consumption, meat consumption, and physical activity; home hypertensive = home systolic BP ≥ 135 mmHg and/or home diastolic BP ≥ 85 mmHg and/or user of antihypertensive medication; home borderline hypertensive = does not satisfy the home hypertensive critetria, and home systolic BP ≥ 125 mmHg and/or home diastolic BP ≥ 80 mmHg; home normotensive = home systolic BP < 125 mmHg and home diastolic BP < 80 mmHg without antihypertensive medication; impaired blood glucose = casual blood glucose ≥ 140 mg/dL and not taking antidiabetic drugs.

*Participants died without incident disability was not included in this analysis.