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Skeletal Muscle Functions as prevention of needing long-term care: the Bunkyo Health Study, a prospective cohort study of urban elderly Japanese

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Cohort profiles

Skeletal Muscle Functions as prevention of needing long-term care: the Bunkyo Health Study, a prospective cohort study of urban elderly Japanese

Yuki Someya1,2,10, Yoshifumi Tamura1,2, Hideyoshi Kaga2, Shuko Nojiri3, Kazunori Shimada1,4, Hiroyuki Daida1,4, Muneaki Ishijima1,5, Kazuo Kaneko1,5, Shigeki Aoki1,6, Takashi Miida1,7, Satoshi Hirayama1,7, Seiki Konishi1,7, Nobutaka Hattori1,8, Yumiko Motoi1,9, Hisashi Naito1,10, Ryuzo Kawamori1,2, Hirotaka Watada1,2

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Abstract

Purpose: The proportion of elderly individuals in Japan reached 27.7% in 2017, the highest in the world. A serious social problem in such a super-aged society is the rise in the number of elderly people who need long-term care, which is mainly due to cerebrovascular disease (17.2%), dementia (16.4%), age-related frailty (13.9%), and fall, fracture and joint disease (12.2%). We hypothesized that decreases in muscle mass, muscle strength, or insulin sensitivity play a central role in the need of long-term care. We developed a cross-sectional and prospective longitudinal cohort study of elderly subjects in an urban community to test this hypothesis.

Participants: Participants were 1,629 elderly people aged 65–84 years living in 13 communities in an urban area (Bunkyo-ku, Tokyo, Japan).

Findings to date: We mainly evaluated cognitive function, physical fitness, cerebral small vessel disease, body composition, bone mineral density, arteriosclerosis, knee osteoarthritis, and glucose tolerance. Population distribution of this cohort was similar to that of Bunkyo-ku or Tokyo, respectively. Average age was 73.1 ± 5.4 years old (male: 73.0 ± 5.3, female: 73.2 ± 5.4) and gender ratio was 0.73:1 (male/female; male (n=687, 42.2%); female (n=942, 57.8%)), the latter was also similar to that of Bunkyo-ku or Tokyo. Most of characteristics of study subjects were similar to previous Japanese cohort.

Future plans: We are planning to re-evaluate cognitive function and cerebrovascular events 5 and 10 years after the baseline evaluation. In these cross-sectional and prospective longitudinal cohort studies, we will evaluate the association between muscle
mass, muscle strength, or insulin sensitivity and main causes and risk factors for needing long-term care.
**Strengths and limitations of this study**

- Prospective cohort study over 10 years to identify risk factors for needing long-term care.

- Relationships between muscle mass, muscle strength, or insulin sensitivity and multiple diseases necessitating long-term care will be evaluated simultaneously.

- A relatively large cohort was assembled for evaluating muscle mass, muscle strength, or insulin sensitivity, whole brain magnetic resonance imaging (MRI), cognitive function, glucose tolerance, vascular function, physical fitness, and knee osteoarthritis, simultaneously.

- Study results may suggest novel strategies to prevent the need for long-term care.

- This study has several limitations: (1) including only participants living in an urban part of Japan, (2) challenges in confirming causal relationships, and (3) a relatively small number of participants in the prospective cohort.
Introduction

The number of elderly people has increased worldwide. For example, World Health Organization defined the aging rate as proportion of persons aged 65 years or older. The aging rate has been increasing in both developed and developing countries. In 2015, the worldwide aging rate was 8.3% and it is estimated to increase to 17.8% by 2055. While the aging rate in developed countries is predicted to reach 27.8% in 2060, the aging rate in Japan reached 27.7% in 2017; it is the highest in the world. Japan is now categorized as a super-aged society, defined as a country with an aging rate greater than 21%. The rate will reach 30% in 2025 and 40% in 2060. A serious social problem in such a super-aged society is the increase in the number of elderly people who need long-term care. Thus, the Japanese government had launched a policy to extend the healthy life span without long-term care; however, evidence-based strategies to prevent the need for long-term care have not been established yet.

In Japan, the main causes of requiring long-term care are cerebrovascular disease (17.2%), dementia (16.4%), age-related frailty (13.9%), and fall, fracture, and joint disease (12.2%). Thus, multiple approaches to prevent these diseases will be useful for preventing the need for long-term care. However, if there are common risk factors for the onset of these diseases, it would be helpful to find an efficient strategy to prevent the need for long-term care. Several previous studies have demonstrated that a decrease in muscle mass or strength and insulin sensitivity in muscle may be common risk factors for diseases related to needing long-term care. Indeed, aging-related decreases in muscle strength and muscle mass, defined as sarcopenia, are associated with an increased risk for metabolic syndrome, cognitive decline, cerebrovascular disease, and all-cause mortality. In addition, sarcopenia is also closely associated with not only
decreased mobility function, falls, and fractures\textsuperscript{13, 14}, but decreased psychosocial well-being\textsuperscript{15} and quality of life,\textsuperscript{16} which are associated with senile depression. On the other hand, insulin resistance is associated with cardiovascular disease\textsuperscript{17, 18} and lacunar infarction\textsuperscript{19, 20} as well as microstructural white matter changes\textsuperscript{21} and decreased cognitive performance\textsuperscript{22, 23}. Therefore, parameters of muscle function such as muscle mass, muscle strength, or insulin sensitivity might be common risk factors for diseases related to needing long-term care. However, there is insufficient clinical evidence to conclude that such associations exist.

In this context, we developed a cross-sectional study and a prospective longitudinal cohort of elderly subjects in an urban community to investigate the association between muscle mass, muscle strength, or insulin sensitivity and main causes and risk factors for needing long-term care.
Cohort description

Study design
This study contains a cross-sectional study and a prospective study for over 10 years. We recruited elderly subjects aged 65–84 years old living in Bunkyo-ku, an urban area in Tokyo, Japan (Figure 1). Bunkyo-ku was selected because the age distribution in Bunkyo-ku is similar to that of Tokyo overall and there is high accessibility to the research center. Among 68 communities in Bunkyo-ku, we selected 13 communities based on probability proportionate to size sampling. We obtained the name and address of all residents aged 65–84 years in the selected communities from residential registries. We mailed invitations to a group briefing session at our institution (Sportology Center, Juntendo University). After the briefing session, subjects who provided written informed consent were included as research participants.

All subjects participated in examinations over two visits to the Sportology Center. During the first visit, subjects participated in cognitive function and physical fitness testing. Magnetic resonance imaging (MRI) of the knee of the dominant leg or leg with pain and whole brain was performed. We also interviewed subjects about their medical history, family history, and current pain. During the second visit, we evaluated body composition and bone mineral density using dual-energy X-ray absorptiometry (DXA), cardio-ankle vascular index (CAVI), and abdominal fat distribution with MRI after an overnight fast. Next, we carried out a 75 g oral glucose tolerance test (OGTT). All data were collected and stored at the Sportology Center. After the baseline evaluation (15/Oct/2015 - 1/Oct/2018), we will ask participants about their health status every year by mail. At 5 (2020 - 2023) and 10 (2025 - 2028) years after the baseline examination,
we are planning to re-evaluate cognitive function and cerebrovascular events at the institution.

**Sample size calculations**

In the current study, we set primary endpoints as the onset of cerebral small vessel disease/cerebrovascular disease and cognitive decline, respectively. Cognitive decline was defined as the presence of dementia or mild cognitive impairment (MCI). Since the prevalence of cognitive decline is lower than the prevalence of cerebral small vessel disease/cerebrovascular disease \(^{24-26}\), the sample size was calculated to allow for evaluation of the association between cognitive decline and muscle functions. Since this study includes both cross-sectional and prospective components, we calculated two sample sizes for each study. In the cross-sectional study, we estimated that rates of dementia and MCI in the group with higher muscle functions (muscle mass, muscle strength, or insulin sensitivity) was 12%, compared with 24% in the group with lower muscle functions \(^{25,26}\). Therefore, a sample of 1,584 subjects was estimated to be necessary based on statistical power of 80% at a significance level of 5%. In the prospective cohort study, the assumed prevalence of cognitive decline per year was 1–2% and the assumed cumulative incident rate over 10 years was 15% in the population. Therefore, a sample of 1,291 subjects was estimated to be necessary. Thus, the estimated required number of study subjects in the cross-sectional study (1,584) was sufficient for the prospective study, if we estimate a dropout rate of 15% in 10 years. Thus, we set the overall sample size as 1,600 in the present study.

**Participants**
For the baseline examination (15/Oct/2015 - 1/Oct/2018), we mailed invitations to attend the group briefing session to all 8,629 elderly subjects living in 13 communities of Bunkyo-ku in Tokyo, Japan. Then, 1,984 (23.0%) subjects participated in group briefing session at our institution (Sportology Center, Juntendo University). After the briefing session, 1,830 subjects provided written informed consent. However, 198 subjects withdrew the consent during the study, and 3 subjects were excluded by over/under age of criteria. Therefore, the study subjects were 1,629 elderly people (18.9% of all elderly individuals in selected 13 communities of Bunkyo-ku) (Figure 2).

**Muscle Functions (Muscle mass, muscle strength, or insulin sensitivity)**

Muscle mass was measured using DXA (Discovery DXA System, Hologic, Inc., Tokyo, Japan) \(^{27}\). Skeletal muscle mass index (SMI) was calculated by dividing appendicular muscle mass (kg) by height squared (m\(^2\)) \(^{28}\). Lower limb isokinetic muscle strength (angular velocity of 60 degrees/second) was measured using a dynamometer (BIODEX system 3 or 4: Biodex Medical Systems, Upton, NY, USA) \(^{29}\). Insulin sensitivity in muscle was estimated with the Matsuda index using data on glucose and insulin during the 75 g OGTT \(^{30}\).

**Cognitive function**

In this study, cognitive function was primarily evaluated using the Montreal Cognitive Assessment [MoCA] \(^{31,32}\). In addition, the Mini-Mental State Examination [MMSE] \(^{33,34}\), Neurobehavioral Cognitive Examination [COGNISTAT] \(^{35,36}\), Wechsler Memory Scale-Revised [WMS-R Part II] \(^{37,38}\), and Trail Making Test [TMT] \(^{39,40}\) were also used for sub-evaluation of cognitive function. We also used the Geriatric Depression Scale short
version [GDS-S] to assess depression.

**Whole brain MRI**

All participants in this study were scanned with a 0.3-T clinical MR scanner (AIRIS Vento, Hitachi, Tokyo, Japan). Sequences included axial three-dimensional (3D) time-of-flight magnetic resonance angiography (repetition time (TR), 35 ms; echo time (TE), 7.1 ms; and slice thickness, 1.2 mm), T2*-weighted gradient echo imaging (TR, 1000 ms; TE, 45 ms; flip angle, 20°; and slice thickness, 5 mm), fluid-attenuated inversion recovery imaging (TR, 11,000 ms; TE, 100 ms; inversion time (TI), 2000 ms; and slice thickness, 5 mm). We also obtained 3D-volumetric T1-weighted imaging using a gradient echo with inversion recovery (GEIR) sequence with these following parameters: TR, 25 ms; TE, 5.8 ms; TI, 600 ms; flip angle, 12°; number of excitations (NEX), 1; field of view (FOV), 200 × 250 × 250 mm^3; resolution, 0.98 × 0.98 × 2 mm^3; slice orientation, sagittal; total scan time, 10.1 min.

For advanced MRI analysis, brain MRI was also performed in some participants on a 3-T clinical MR scanner (MAGNETOM Prisma, Siemens Healthcare, Erlangen, Germany) with a 64-channel head coil. The protocol includes 3D T1-weighted images and multi-shell diffusion weighted images (DWI). Three-dimensional T1-weighted images were obtained using magnetization-prepared 180 degrees radio-frequency pulses and rapid gradient-echo (MP-RAGE) with these following parameters: TR, 2300 ms; TE, 2.32 ms; TI: 900 ms; FOV, 240 × 240 mm; matrix size, 256 × 256; resolution, 0.9 × 0.9 mm; slice thickness, 0.9 mm; and acquisition time, 5.21 min. For DWI, echo planar imaging (EPI) consisting of two b values (1000, and 2000 s/mm^2) along 64 isotropic diffusion gradients was acquired in the anterior-posterior phase-encoding direction with
the following parameters: TR, 3,300 ms; TE, 70 ms; FOV, 229 × 229 mm; matrix size, 130 × 130; resolution 1.8 × 1.8 mm; slice thickness, 1.8 mm; and acquisition time, 7.29 min. Each DWI acquisition was completed with a gradient-free image (b = 0). We also acquired standard and reverse phase encoded blipped images with no diffusion weighting (Blip Up and Blip Down) to correct for magnetic susceptibility-induced distortions related to EPI acquisitions.

Evaluation of brain MRI

Primary brain MRI evaluation was conducted by an experienced neuroradiologist based on axial T2*-WI and FLAIR images obtained on a 0.3-T MR scanner. The evaluation included cerebral small vessel disease such as lacunar infarcts, cerebral microhemorrhages, periventricular hyperintensities (PVHs), and deep and subcortical white matter hyperintensities (DSWMHs). PVH and DSWMH were categorized using the Fazekas scale. In this study, level III was defined as a cerebral small vessel disease. In addition, whole brain volume, regional brain volume, regional cortical thickness, and white and grey matter integrity were also included on subsequent evaluations. Furthermore, 0.3-T and 3-T 3D T1-weighted images were used for the quantification of whole brain volume as well as regional cortical volume and thickness; multi-shell DWI was used for the quantification of white and grey matter integrity.

Lifestyle and physical activity level

We investigated lifestyle and physical activity level using questionnaires: sleeping status, Pittsburgh Sleep Quality Index [PSQI-J]; exercise habits and physical activity level, International Physical Activity Questionnaire [IPAQ]; nutritional status, brief-type
self-administered diet history questionnaire [BDHQ]; \(^{48,49}\) and mental status, 12-Item Short Form Health Survey [SF-12v2; Standard Japanese, Quality Metric Inc., Lincoln RI, USA] \(^{50}\). In addition, the Instrumental Activities of Daily Living [IADL] scale was used to estimate the ability to live independently \(^{51}\).

**Physical fitness and function**

We evaluated physical fitness and function based on muscle strength (hand grip dynamometer; T. K. K. 5401, Takei Scientific Instruments Co., Ltd., Niigata, Japan) \(^{52}\), balance (one-leg standing test) \(^{53}\), flexibility (sit-and-reach test) \(^{54}\), gait speed (10-meter walking test) \(^{55}\), and the Timed Up and Go test \(^{56}\). In addition, locomotive functions were evaluated using the stand-up test, two-step test, and a 25-question risk assessment on physical condition and lifestyle (Locomotive Challenge! Council, Japanese Orthopaedic Association, Tokyo, Japan).

**Blood and urine analysis**

Blood and urine samples were collected in the morning after an overnight fast. Subsequently, a standard 75 g OGTT was performed. Blood samples were obtained before and 30, 60, 90, and 120 minutes after ingesting 75 g of glucose to determine plasma glucose and serum insulin levels. Serum and plasma samples were sent to a commercial clinical laboratory (SRL Inc., Tokyo, Japan). Biochemical analysis for each parameter in Table 1 was performed using standard methods. Urine samples were used for albumin and qualitative tests. Other exploratory measurements will be planned for each study.

**DNA extraction and genome-wide association study**
Genomic DNA will be extracted from peripheral leukocytes and used for genetic analyses to explore genes associated with clinical characteristics.

**Bone mineral density**

Bone mineral density (BMD) of the lumbar spine and femoral neck were measured using DXA (Discovery DXA System, Hologic, Inc., Tokyo, Japan). A quality assurance of the longitudinal adjustment was performed by calibrating the machine with standardized phantoms. The co-efficient of variation (CV) for the *in vivo* LS-BMD measurements was <1% \(^{57}\). The diagnosis of osteoporosis was based on the criteria proposed by the Japanese Society of Bone and Mineral Metabolism 2012 \(^{58}\), in which postmenopausal women with either ≥1 prevalent non-traumatic radiographic vertebral fractures according to clinical radiological criteria or hip fractures, and a those with low BMD (≤70% of the young adult mean value) or a BMD ≤80% of the young adult mean and ≥1 prevalent non-traumatic fragility fractures other than vertebral or hip fractures were diagnosed with osteoporosis.

**Arteriosclerosis**

Arteriosclerosis was estimated using CAVI and the ankle brachial index (ABI), which were measured using an automatic waveform analyzer (Vascular Screening System VaSera VS-1500, Fukuda Denshi Co., Ltd., Tokyo, Japan) \(^{59}\). Recording was performed in the supine position after 10 minutes of rest. Occlusion and monitoring cuffs were placed snugly upper and lower extremities. Pressure waveforms were then recorded simultaneously from the brachial arteries using the oscillometric method. All scans were automatically conducted by well-trained investigators. A resting ABI ≤ 0.90 was considered to reflect the presence of peripheral artery disease.
Abdominal Fat Area

Intra-abdominal fat area was measured with 0.3-T MRI (AIRIS Vento, Hitachi, Tokyo, Japan). Subjects were in the prone position in the magnet with their arms placed straight overhead. Using the intervertebral space between the fourth and fifth lumbar vertebrae (L4–L5) as the point of origin, transverse images of 10-mm slice thickness were obtained every 100 mm from head to foot, resulting in a total of 10 images for each subject. All MRI data were transferred to a computer workstation for analysis using specially designed image analysis software (AZE Virtual Place, AZE, Tokyo, Japan) to measure visceral and subcutaneous fat area.

Anthropometric Measurement

Height was measured within 0.1 cm using a stadiometer in the upright position in the morning. Body weight was measured within 0.1 kg using an electronic scale (InBody770; Biospace Co., Ltd., Seoul, Korea). Circumference of the waist, thigh and calf of the dominant leg were measured within 0.5 cm with a plastic tape measure.

Evaluation of osteoarthritis (OA) of the knee joint and knee pain

The radiographic OA severity using the Kellgren-Lawrence [K/L] grade was evaluated based on the weight-bearing antero-posterior radiographs of the femoro-tibial joint for both knees using the bilateral standing extended view and based on the weight-bearing postero-anterior radiographs of the femoro-tibial joint with the knee in 45° of flexion.

The knee joints of the participants were also examined by MRI. Imaging sequences included coronal and sagittal proton density-weighted images with/without fat-
suppression and T2 mapping. The morphological changes of knee OA were semi-quantitatively evaluated according to the Whole Organ Magnetic Resonance Imaging Score (WORMS) system. A medial meniscus extrusion (MME) was also evaluated, as we previously described.

Clinical manifestation was evaluated using a visual analog scale (VAS scale; 0–100mm) for pain and the Japanese Knee Osteoarthritis Measure (JKOM) score. JKOM is a self-reported score that includes four subcategories: pain and stiffness (0–32 points), activities of daily living (0–40 points), social activities (0–20 points), and general health condition (0–8 points), with 100 points being the maximum score.

Findings to date
Population distribution of this cohort was similar to that of Bunkyo-ku or Tokyo, respectively (Figure 3). Average age was 73.1 ± 5.4 years old (male: 73.0 ± 5.3, female: 73.2 ± 5.4) and gender ratio was 0.73:1 (male/female; male (n=687, 42.2%); female (n=942, 57.8%)), the latter was also similar to that of Bunkyo-ku or Tokyo. Other cohort characteristics are shown in Table 2. Most of characteristics of study subjects were similar to previous Japanese cohort.

Strength and limitation
Strengths of the present study were as follows; (1) prospective cohort study over 10 years to identify risk factors for needing long-term care, (2) random sampling of elderly individual in urban city was performed, (3) relatively large cohort was assembled for evaluating muscle mass, muscle strength, or insulin sensitivity, whole brain magnetic resonance imaging (MRI), cognitive function, glucose tolerance, vascular function,
physical fitness, and knee osteoarthritis, simultaneously. Therefore, this study can evaluate the relationships between muscle mass, muscle strength, or insulin sensitivity and multiple diseases necessitating long-term care and may suggest novel strategies to prevent the need for long-term care.

On the other hand, this study has several limitations: (1) including only participants living in an urban part of Japan, (2) a relatively healthy participants, (3) challenges in confirming causal relationships, and (4) a relatively small number of participants in the prospective cohort.

**Ethics and dissemination**

This study protocol was approved by the ethics committee of Juntendo University in November 2015 (No. 2015078, 2016138, 2016131, and 2017121). This study is being carried out in accordance with the principles outlined in the Declaration of Helsinki. All participants gave written informed consent at the orientation meeting. Participants were told that they have the right to withdraw from the trial at any time. Collected data are coded with non-identifying numbers and stored securely in password-protected files. Accessibility to the files is limited to principal investigators. The findings of the study will be presented in local and international conferences and disseminated in peer-reviewed journals.
Authors’ contribution

YS, YT, HK, HW, and RK conceived the study and obtained grant funding. YS, YT, and HK drafted the protocol. SN advised on the statistical analysis. SK, HD, MI, KK, TM, SH, SK, SA, NH, YM, and HN reviewed and revised the protocol.

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Competing interests statement

The authors have nothing to disclose.
Figure legend

Figure 1. Geographical location of Bunkyo-ku in Japan

Left: Japan is divided into 47 prefectures. The location of Tokyo is indicated by a circle.
Center: Tokyo is a prefecture that includes 23 special wards, 26 cities, 5 towns, and 8 villages. Bunkyo is one of the special wards (Bunkyo-ku in Japanese). It located in east Tokyo, as indicated by the circle. Right: Bunkyo-ku includes 68 communities and has 227,902 residents.

Figure 2. Flowchart of participant recruitment process

Figure 3. Distribution of elderly population in this cohort, Bunkyo-ku, and Tokyo

Population rate of each age among elderly individuals (65-84 years old) in this cohort, Bunkyo-ku, and Tokyo are presented.
References


62. Hada S, Kaneko H, Sadatsuki R, et al. The degeneration and destruction of femoral articular cartilage shows a greater degree of deterioration than that of the tibial and


Table 1. Parameters for blood and urine analyses.

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Table 2 Characteristics of study subjects

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<td>13.9±2.5</td>
<td>14.9±2.5</td>
<td>13.2±2.2</td>
</tr>
<tr>
<td>Worker / Volunteer (n: %)</td>
<td>563 (34.6%)</td>
<td>288 (41.9%)</td>
<td>275 (29.2%)</td>
</tr>
<tr>
<td>Needing long-term care (n: %)</td>
<td>27 (1.7%)</td>
<td>14 (2.0%)</td>
<td>13 (1.4%)</td>
</tr>
<tr>
<td>Solitude (n: %)</td>
<td>342 (21.0%)</td>
<td>84 (12.2%)</td>
<td>258 (27.4%)</td>
</tr>
<tr>
<td>Hypertension (n: %)</td>
<td>748 (45.9%)</td>
<td>368 (53.6%)</td>
<td>380 (40.3%)</td>
</tr>
<tr>
<td>Diabetes (n: %)</td>
<td>187 (11.5%)</td>
<td>113 (16.4%)</td>
<td>74 (7.9%)</td>
</tr>
<tr>
<td>Hyperlipidemia (n: %)</td>
<td>639 (39.2%)</td>
<td>231 (33.6%)</td>
<td>408 (43.3%)</td>
</tr>
<tr>
<td>Cerebrovascular disease (n: %)</td>
<td>68 (4.2%)</td>
<td>34 (4.9%)</td>
<td>34 (3.6%)</td>
</tr>
<tr>
<td>Cardiovascular disease (n: %)</td>
<td>75 (4.6%)</td>
<td>50 (7.3%)</td>
<td>25 (2.7%)</td>
</tr>
<tr>
<td>Cancer (n: %)</td>
<td>221 (13.6%)</td>
<td>110 (16.0%)</td>
<td>111 (11.8%)</td>
</tr>
<tr>
<td>Skeletal muscle mass (kg/m²)</td>
<td>7.1±1.1</td>
<td>7.9±0.9</td>
<td>6.5±0.8</td>
</tr>
<tr>
<td>Muscle strength (Nm/kg)</td>
<td>133.2±37.5</td>
<td>148.4±38.8</td>
<td>122.2±32.3</td>
</tr>
<tr>
<td>Matsuda index</td>
<td>7.3±4.1</td>
<td>7.4±4.6</td>
<td>7.2±3.7</td>
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<tr>
<td>MoCA-J (point)</td>
<td>25.1±3.0</td>
<td>24.7±3.0</td>
<td>25.5±3.0</td>
</tr>
<tr>
<td>MMSE (point)</td>
<td>27.7±1.9</td>
<td>27.5±2.0</td>
<td>27.9±1.9</td>
</tr>
<tr>
<td>Small vessel disease (n: %)</td>
<td>402 (24.8%)</td>
<td>178 (26.1%)</td>
<td>224 (23.9%)</td>
</tr>
</tbody>
</table>

MoCA-J: Montreal Cognitive Assessment Japanese version, MMSE: Mini Mental State Examination
Figure 1

Japan is divided into 47 prefectures.

Tokyo is a prefecture that includes 23 special wards, 26 cities, 5 towns, and 8 villages.

Bunkyo-ku is one of the special wards and includes 68 communities and 227,902 people.
Elderly subjects living in 13 communities of Bunkyo-ku (n = 8,629)

Excluded (n = 6,646)
- Unknown address: 242
- Declined to participate: 292
- Could not participate: 22
- Did not reply: 6,090

Participating group briefing (n = 1,983)

Excluded (n = 153)
- Declined consent: 153

Informed consent obtained (n = 1,830)

Excluded (n = 201)
- Withdrew consent: 198
- Did not meet age criteria: 3

This cohort subjects: 1,629
Figure 3

Population rate of each age in elderly

(B years old)

Tokyo Participants (Male) Participants (Female) Bunkyo-ku

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<thead>
<tr>
<th>Section/Topic</th>
<th>Item #</th>
<th>Recommendation</th>
<th>Reported on page #</th>
</tr>
</thead>
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<tr>
<td><strong>Title and abstract</strong></td>
<td>1</td>
<td>(a) Indicate the study’s design with a commonly used term in the title or the abstract</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Provide in the abstract an informative and balanced summary of what was done and what was found</td>
<td>3</td>
</tr>
<tr>
<td><strong>Introduction</strong></td>
<td></td>
<td></td>
<td>6-7</td>
</tr>
<tr>
<td>Background/rationale</td>
<td>2</td>
<td>Explain the scientific background and rationale for the investigation being reported</td>
<td></td>
</tr>
<tr>
<td><strong>Objectives</strong></td>
<td>3</td>
<td>State specific objectives, including any pre-specified hypotheses</td>
<td>6</td>
</tr>
<tr>
<td><strong>Methods</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study design</td>
<td>4</td>
<td>Present key elements of study design early in the paper</td>
<td>8</td>
</tr>
<tr>
<td>Setting</td>
<td>5</td>
<td>Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection</td>
<td>8</td>
</tr>
<tr>
<td><strong>Participants</strong></td>
<td>6</td>
<td>(a) <strong>Cohort study</strong>—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Case-control study</strong>—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Cross-sectional study</strong>—Give the eligibility criteria, and the sources and methods of selection of participants</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>(b) <strong>Cohort study</strong>—For matched studies, give matching criteria and number of exposed and unexposed</td>
<td>8</td>
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<tr>
<td></td>
<td></td>
<td><strong>Case-control study</strong>—For matched studies, give matching criteria and the number of controls per case</td>
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</tr>
<tr>
<td><strong>Variables</strong></td>
<td>7</td>
<td>Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable</td>
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</tr>
<tr>
<td>Data sources/ measurement</td>
<td>8*</td>
<td>For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group</td>
<td>8</td>
</tr>
<tr>
<td><strong>Bias</strong></td>
<td>9</td>
<td>Describe any efforts to address potential sources of bias</td>
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</tr>
<tr>
<td><strong>Study size</strong></td>
<td>10</td>
<td>Explain how the study size was arrived at</td>
<td>9</td>
</tr>
<tr>
<td>Quantitative variables</td>
<td>11</td>
<td>Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why</td>
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</tr>
<tr>
<td><strong>Statistical methods</strong></td>
<td>12</td>
<td>(a) Describe all statistical methods, including those used to control for confounding</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Describe any methods used to examine subgroups and interactions</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
<td>(c) Explain how missing data were addressed</td>
<td>-</td>
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<td></td>
<td>(d) <strong>Cohort study</strong>—If applicable, explain how loss to follow-up was addressed</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
<td><strong>Case-control study</strong>—If applicable, explain how matching of cases and controls was addressed</td>
<td>-</td>
</tr>
<tr>
<td>Cross-sectional study</td>
<td>Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy. (e) Describe any sensitivity analyses.</td>
<td></td>
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<td>----------------------------------------------------------------------------------------------------------</td>
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</table>

**Results**

<table>
<thead>
<tr>
<th>Participants</th>
<th>13*</th>
<th>(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(b) Give reasons for non-participation at each stage.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Consider use of a flow diagram. Figure 2.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Descriptive data</th>
<th>14*</th>
<th>(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(b) Indicate number of participants with missing data for each variable of interest.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Cohort study—Summarise follow-up time (eg, average and total amount).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcome data</th>
<th>15*</th>
<th><strong>Cohort study</strong>—Report numbers of outcome events or summary measures over time.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Case-control study</strong>—Report numbers in each exposure category, or summary measures of exposure.</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Cross-sectional study</strong>—Report numbers of outcome events or summary measures.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Main results</th>
<th>16</th>
<th>(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(b) Report category boundaries when continuous variables were categorized.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period.</td>
</tr>
</tbody>
</table>

| Other analyses   | 17  | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses. |

**Discussion**

<table>
<thead>
<tr>
<th>Key results</th>
<th>18</th>
<th>Summarise key results with reference to study objectives.</th>
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<tbody>
<tr>
<td>Limitations</td>
<td>19</td>
<td>Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.</td>
</tr>
<tr>
<td>Interpretation</td>
<td>20</td>
<td>Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.</td>
</tr>
<tr>
<td>Generalisability</td>
<td>21</td>
<td>Discuss the generalisability (external validity) of the study results.</td>
</tr>
</tbody>
</table>

**Other information**

| Funding          | 22  | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based. |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.
# Cohort profile: skeletal muscle function and need for long-term care in a prospective cohort study of urban elderly people in Japan (the Bunkyo Health Study)

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<td>Date Submitted by the Author:</td>
<td>02-Aug-2019</td>
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| Complete List of Authors: | Someya, Yuki; Juntendo University Graduate School of Medicine, Sportology Center; Juntendo University Graduate School of Medicine, Metabolism & Endocrinology  
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Kawamori, Ryuzo; Juntendo University Graduate School of Medicine, Sportology Center  
Watada, Hirotaka; Juntendo University Graduate School of Medicine, Metabolism & Endocrinology |
| <b>Primary Subject Heading</b>: | Epidemiology |

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<th>Epidemiology, Research methods</th>
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<tr>
<td>Keywords:</td>
<td>muscle mass, muscle strength, insulin sensitivity, long-term care, community based study</td>
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Cohort profile: skeletal muscle function and need for long-term care in a prospective cohort study of urban elderly people in Japan (the Bunkyo Health Study)

Yuki Someya1,2,10, Yoshifumi Tamura1,2, Hideyoshi Kaga2, Shuko Nojiri3, Kazunori Shimada1,4, Hiroyuki Daida1,4, Muneaki Ishijima1,5, Kazuo Kaneko1,5, Shigeki Aoki1,6, Takashi Miida1,7, Satoshi Hirayama1,7, Seiki Konishi1,7, Nobutaka Hattori1,8, Yumiko Motoi1,9, Hisashi Naito1,10, Ryuzo Kawamori1,2, Hirotaka Watada1,2

1: Sportology Center, 2: Department of Metabolism & Endocrinology, 3: Clinical Research Support Center, 4: Department of Cardiovascular Medicine, 5: Department of Medicine for Orthopaedics and Motor Organ, 6: Department of Radiology 7: Department of Clinical Laboratory Medicine, 8: Department of Neurology, 9: Department of Diagnosis, Prevention and Treatment of Dementia, Juntendo University Graduate School of Medicine, Tokyo, Japan, and 10: Juntendo University Graduate School of Health and Sports Science, Chiba, Japan.

Word count:

Abstract: 300 words    Text: 4,680 words

Number of figures and tables: 6

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Abstract

Purpose: The proportion of elderly individuals (age ≥ 65 years) in Japan reached 27.7% in 2017, the highest in the world. A serious social problem in a super-aged society is the rise in the number of elderly people who need long-term care (LTC), which is mainly due to cerebrovascular disease, dementia, age-related frailty, falls and fractures, and joint disease. We hypothesized that decreased muscle mass, muscle strength, and insulin sensitivity are common risk factors for these diseases related to needing LTC. We developed a prospective cohort study of elderly subjects in an urban community to test this hypothesis. The primary objective is to prospectively investigate associations between muscle mass, muscle strength, and insulin sensitivity and incidence of main disease and risk factors of needing LTC. The primary outcomes are incidence of cerebrovascular disease and cognitive decline.

Participants: Participants were 1,629 people aged 65–84 years living in 13 communities in an urban area (Bunkyo-ku, Tokyo, Japan). Average age was 73.1 ± 5.4 years.

Findings to date: We obtained baseline data on cognitive function, cerebral small vessel disease (SVD) determined by brain magnetic resonance imaging (MRI), body composition, bone mineral density, arteriosclerosis, physical function, muscle mass, muscle strength, and insulin sensitivity. Mild cognitive impairment and dementia were observed in 26.0% and 3.3% of participants, respectively. The prevalence of cerebral SVD was 24.8%. These characteristics are similar to those previously reported in elderly Japanese subjects.
**Future plans:** We will ask participants about their health status, including incidence of cerebrovascular disease, falls, fractures, and other diseases every year by mail. We plan to re-evaluate cognitive function, brain MRI parameters and other parameters at 5 and 10 years after the baseline evaluation. We will evaluate whether low muscle function (muscle mass, muscle strength, or insulin sensitivity) is a risk factor for cognitive decline or cerebrovascular disease.
Introduction

The number of elderly people (aged ≥65 years) has increased worldwide. For example, World Health Organization defined the aging rate as proportion of persons aged 65 years or older. The aging rate has been increasing in both developed and developing countries. In 2015, the worldwide aging rate was 8.3% and it is estimated to increase to 17.8% by 2055. While the aging rate in developed countries is predicted to reach 27.8% in 2060, the aging rate in Japan reached 27.7% in 2017; it is the highest in the world. Japan is now categorized as a super-aged society, defined as a country with an aging rate greater than 21%. The rate will reach 30% in 2025 and 40% in 2060. A serious social problem in such a super-aged society is the increase in the number of elderly people who need long-term care. Need for long-term care is defined as a condition where an elderly individual needs care to perform activities of daily living due to age-related diseases such as cerebrovascular disease, dementia, and musculoskeletal disease. The number of individuals who need long-term care in Japan increased from 2.18 million in 2000 to 6.06 million in 2015. Social security expenses for long-term care increased from 3.6 trillion JPY in 2000 to 10.1 trillion JPY in 2015. These figures are predicted to increase and cause increased social and economic burden in Japan. Thus, the Japanese government has launched a policy to extend healthy life expectancy without long-term care; however, evidence-based strategies to prevent the need for long-term care have not been established yet.

In Japan, the main causes of needing long-term care are cerebrovascular disease (17.2%), dementia (16.4%), age-related frailty (13.9%), falls and fractures (12.2%), and joint disease (11.0%). Thus, multiple approaches to prevent these diseases will be useful for preventing the need for long-term care. However, if there are common risk factors for
the onset of these diseases, it would be helpful to find an efficient strategy to prevent the need for long-term care. Several previous studies have demonstrated that a decrease in muscle mass or muscle strength\(^8\) could be common risk factors for diseases related to needing long-term care. Indeed, aging-related decreases in muscle strength or muscle mass\(^8\) are associated with an increased risk for metabolic syndrome\(^{10-12} 26\), cognitive decline\(^{12, 14}\), cerebrovascular disease\(^{27, 28}\), and all-cause mortality\(^{13} 29\). In addition, decreased muscle mass or muscle strength are also closely associated with decreased mobility, falls, and fractures\(^{15, 16}\) as well as decreased psychosocial well-being\(^{17}\) and quality of life,\(^{18}\) which are associated with geriatric depression. On the other hand, impaired insulin sensitivity is associated with cardiovascular disease\(^{19, 20}\) and lacunar infarcts\(^{21, 22}\) as well as microstructural white matter changes\(^{23}\) and decreased cognitive performance\(^{24, 25}\). Given that skeletal muscle is the main organ that determines whole-body insulin sensitivity\(^{30, 31}\), we hypothesized that parameters related to muscle function such as muscle mass, muscle strength, and insulin sensitivity might be common risk factors for diseases related to needing long-term care. However, there is insufficient clinical evidence to support this hypothesis.

On the other hand, several other risk factors for diseases related to needing long-term care have been reported. For example, arteriosclerosis determined by cardio-ankle vascular index (CAVI) predicts cognitive decline in elderly Japanese individuals\(^{32}\). A recent meta-analysis showed a modest association between CAVI and incident cardiovascular disease risk\(^{33}\). In addition, cerebral small vessel disease (SVD), defined as lacunar infarcts, cerebral microhemorrhages, periventricular hyperintensities (PVHs), or deep and subcortical white matter hyperintensities (DSWMHs) detected on magnetic resonance imaging (MRI), is strongly associated with incident ischemic and hemorrhagic...
stroke, all-cause dementia and depression, and all-cause mortality $^{34}$. Furthermore, a meta-analysis of data from 12 cohort studies showed that low hip bone mineral density (BMD) is an important predictor of fracture risk $^{35}$. We hypothesized that muscle mass, muscle strength, and insulin sensitivity are also common risk factors for arteriosclerosis, cerebral SVD, and lower BMD; however, this hypothesis has not been tested yet.

In this context, we developed a prospective cohort study of elderly individuals in an urban community. The primary objective of the present study is to prospectively investigate associations between muscle mass, muscle strength, or insulin sensitivity and main causes and risk factors for needing long-term care. The primary outcomes of the present study are incidence of cerebrovascular disease and cognitive decline. Secondary outcomes are incidence of age-related frailty, falls and fractures, and joint disease, respectively.
Cohort description

Study design
The Bunkyo Health Study is a prospective cohort study of over 10 years. We recruited elderly subjects aged 65–84 years living in Bunkyo-ku, an urban area in Tokyo, Japan (Figure 1). Bunkyo-ku was selected because the age distribution in Bunkyo-ku is similar to that of Tokyo overall and there is high accessibility to the research center. Among 68 communities in Bunkyo-ku, we selected 13 communities based on probability proportionate to size sampling. We obtained the name and address of all residents aged 65–84 years in the selected communities from residential registries. We mailed invitations to a group briefing session at our institution (Sportology Center, Juntendo University). The inclusion criterion of the present study was age of 65–84 years. The exclusion criteria were pacemaker or defibrillator placement and diabetes requiring insulin therapy. After the briefing session, subjects who provided written informed consent were included as research participants. The primary outcomes of the present study are incidence of cerebrovascular disease and cognitive decline. The secondary outcomes are age-related frailty, falls and fractures, and joint disease. The exploratory outcomes are arteriosclerosis, cerebral SVD, and osteoporosis as well as cognitive function, brain volume and structure, depression, physical function and all-cause mortality.

The study protocol is outlined in Figure 2. All subjects participated in examinations over two visits to the Sportology Center. During the first visit, subjects participated in cognitive function and physical fitness testing. MRI of the whole brain and the knee of the dominant leg or the leg with pain was performed. We also interviewed subjects about their medical history, family history, and current pain. During
the second visit, we evaluated body composition and bone mineral density using dual-energy X-ray absorptiometry (DXA) \textsuperscript{43}, CAVI \textsuperscript{44}, and abdominal fat distribution with MRI after an overnight fast \textsuperscript{45}. Next, we carried out a 75 g oral glucose tolerance test (OGTT) \textsuperscript{46}. All data were collected and stored at the Sportology Center.

After the baseline evaluation (October 15, 2015 to October 1, 2018), we will ask participants about their health status every year by mail. For example, we will ask about incidence of cerebrovascular disease, falls, fractures, and other diseases every year. We also will send the Kihon Check List questionnaire \textsuperscript{47} to participants every year. This questionnaire was developed to identify elderly individuals with frailty or at high risk for needing long-term care. It consists of 25 questions regarding instrumental and social activities of daily living, physical function, nutritional status, oral function, cognitive function, and depressed mood \textsuperscript{47,48}. At 5 years (2020–2023) and 10 years (2025–2028) after the baseline examination, we are planning to re-evaluate cognitive function, brain MRI parameters and other parameters measured at baseline except OGTT.

**Sample size calculations**

Since the primary outcomes are incidence of cerebrovascular disease and cognitive decline and the incidence of cognitive decline is lower than the incidence of cerebrovascular disease \textsuperscript{49-51}, the sample size was calculated to allow for evaluation of associations between cognitive decline and muscle mass, muscle strength, and insulin sensitivity, respectively. Cognitive decline was defined as the presence of dementia or mild cognitive impairment (MCI) estimated using the Montreal Cognitive Assessment (MoCA), Japanese version \textsuperscript{36,52} or the Mini-Mental State Examination (MMSE) \textsuperscript{37,53}, respectively. In the prospective analysis, we plan to categorize subjects in two groups for
muscle function (e.g. low and high muscle strength groups). The assumed incidence of
cognitive decline per year is 1–2% and the assumed cumulative incidence over 10 years
in the population is 15%. The low muscle function group was assumed to have a two-fold
higher risk of cognitive decline. Therefore, a sample of 1,519 subjects was estimated to
be necessary, assuming a dropout rate of 15% over 10 years.

Since we also plan to perform a cross-sectional analysis using baseline data, we
performed a preliminary sample size calculation. Although muscle mass, muscle strength,
and insulin sensitivity reflect different aspects of muscle function, the combined
associations of these parameters with diseases or risk factors related to needing long-term
care remain unclear. Thus, we plan to categorize subjects in nine categories of muscle
function (e.g. low, middle, and high muscle strength × low, middle, and high insulin
sensitivity) and focus on associations between these categories of muscle function and
cognitive decline. We also plan to evaluate other outcomes such as arteriosclerosis,
cerebral SVD, osteoporosis, knee joint condition, cognitive function, brain volume and
structure, depression, and physical function in the cross-sectional analysis. We estimated
that rates of cognitive decline in the group with higher muscle function to be 12%,
compared with 24% in the group with lower muscle function \(^{50,51}\). A sample of 1,584
subjects was estimated to be necessary based on statistical power of 80% at a significance
level of 5%. Thus, the estimated required number of subjects in the cross-sectional
analysis (1,584) is sufficient for the prospective analysis. We set the overall sample size
to be 1,600 in the present study.

Participants

For the baseline examination (October 15, 2015 to October 1, 2018), we mailed
invitations to attend a group briefing session to all 8,629 elderly individuals living in 13 communities in Bunkyo-ku, Tokyo, Japan, of whom 1,984 (23.0%) subjects participated in a group briefing session at our institution (Sportology Center, Juntendo University). After the briefing session, 1,830 subjects provided written informed consent for study participation. However, 190 subjects withdrew consent and 11 subjects were excluded based on age, pacemaker status, and insulin therapy status. Ultimately, the study subjects comprised of 1,629 elderly people, which corresponds to 18.9% of all elderly individuals in 13 selected communities of Bunkyo-ku) (Figure 3).

Measurements

Muscle mass and strength

Muscle mass was measured using DXA (Discovery DXA System, Hologic, Inc., Tokyo, Japan)\(^{54}\). Skeletal muscle mass index (SMI) was calculated by dividing appendicular muscle mass (kg) by height squared (m\(^2\))\(^{55}\). Lower limb isokinetic muscle strength (angular velocity of 60 degrees/second) was measured using a dynamometer (BIODEX system 3 or 4: Biodex Medical Systems, Upton, NY, USA)\(^{56}\).

Insulin sensitivity

Insulin sensitivity was estimated with the Matsuda index using data on glucose and insulin during the 75 g OGTT\(^{46}\). The Matsuda index was calculated using the following equation:

\[
\frac{10,000}{\sqrt{\text{fasting glucose \times fasting insulin} \times \text{mean glucose \times mean insulin during OGTT}}} ^{57}\]

This formula can estimate whole-body glucose disposal during the euglycemic insulin clamp. Skeletal muscle is the main organ absorbing glucose during
the clamp. Thus, the Matsuda index is a surrogate marker of insulin sensitivity, mainly reflecting insulin sensitivity in muscle. Our previous study using the two-step hyperinsulinemic euglycemic clamp technique demonstrated that the Matsuda index is significantly correlated with muscle insulin sensitivity, not hepatic insulin sensitivity.

Cognitive function

Cognitive function was primarily evaluated using the MoCA and the MMSE. The MoCA and MMSE contain 9 and 11 items, respectively. Possible scores range from 0 to 30 points. In this study, we used MoCA score ≤22 as the cutoff for MCI and MMSE score ≤23 as the cutoff for dementia. The Neurobehavioral Cognitive Status Examination (COGNISTAT), which evaluates orientation, attention, language, memory, calculation, construction, and reasoning; the Trail Making Test, which evaluates visual-conceptual and visual-motor tracking; and the Wechsler Memory Scale-Revised (part II) were also used for evaluation of cognitive function. We also used the short version of the Geriatric Depression Scale to assess depression, defined as a score ≥10 points.

Evaluation of whole brain MRI

We performed whole brain MR scanning with a 0.3-T clinical MR scanner (AIRIS Vento, Hitachi, Tokyo, Japan) in all participants. In addition, brain MR scanning was also performed in some participants on a 3-T clinical MR scanner (MAGNETOM Prisma, Siemens Healthcare, Erlangen, Germany) with a 64-channel head coil for advanced brain MRI analysis and validation of brain analysis with the 0.3-T clinical MR scanner. The following sequences were obtained with the 0.3-T clinical MR scanner: axial
three-dimensional (3D) time-of-flight magnetic resonance angiography (repetition time (TR), 35 ms; echo time (TE), 7.1 ms; and slice thickness, 1.2 mm), T2*-weighted gradient echo imaging (TR, 1000 ms; TE, 45 ms; flip angle, 20°; and slice thickness, 5 mm), and fluid-attenuated inversion recovery imaging (TR, 11,000 ms; TE, 100 ms; inversion time (TI), 2000 ms; and slice thickness, 5 mm). We also obtained 3D-volumetric T1-weighted imaging using a gradient echo with inversion recovery (GEIR) sequence with these following parameters: TR, 25 ms; TE, 5.8 ms; TI, 600 ms; flip angle, 12°; number of excitations (NEX), 1; field of view (FOV), 200 × 250 × 250 mm$^3$; resolution, 0.98 × 0.98 × 2 mm$^3$; slice orientation, sagittal; total scan time, 10.1 min.

The protocol with the 3-T clinical MR scanner included 3D T1-weighted imaging and multi-shell diffusion weighted imaging (DWI). Three-dimensional T1-weighted images were obtained using magnetization-prepared 180 degrees radio-frequency pulses and rapid gradient-echo (MP-RAGE) with these following parameters: TR, 2300 ms; TE, 2.32 ms; TI: 900 ms; FOV, 240 × 240 mm; matrix size, 256 × 256; resolution, 0.9 × 0.9 mm; slice thickness, 0.9 mm; and acquisition time, 5.21 min. For DWI, echo planar imaging (EPI) consisting of two b values (1000, and 2000 s/mm$^2$) along 64 isotropic diffusion gradients was acquired in the anterior-posterior phase-encoding direction with the following parameters: TR, 3,300 ms; TE, 70 ms; FOV, 229 × 229 mm; matrix size, 130 × 130; resolution 1.8 × 1.8 mm; slice thickness, 1.8 mm; and acquisition time, 7.29 min. Each DWI acquisition was completed with a gradient-free image (b = 0). We also acquired standard and reverse phase encoded blipped images with no diffusion weighting (Blip Up and Blip Down) to correct for magnetic susceptibility-induced distortions related to EPI acquisitions.

Primary brain MRI evaluation was conducted by an experienced
neuroradiologist based on axial T2*-WI and FLAIR images obtained on a 0.3-T MR scanner. The evaluation included cerebral SVD, characterized by lacunar infarcts, cerebral microhemorrhages, PVHs, and DSWMHs. PVHs and DSWMHs were categorized using the Fazekas scale. In this study, level III was defined as cerebral SVD. In addition, whole brain volume, regional brain volume, regional cortical thickness, and white and grey matter integrity were also included on subsequent evaluations. Furthermore, 0.3-T and 3-T 3D T1-weighted images were used for the quantification of whole brain volume as well as regional cortical volume and thickness; multi-shell DWI was used for the quantification of white and grey matter integrity.

Lifestyle and physical activity levels
We investigated lifestyle and physical activity levels using questionnaires. To evaluate sleeping status, we used the Pittsburgh Sleep Quality Index, which has seven components. We evaluated exercise habits and physical activity levels with the International Physical Activity Questionnaire, which assesses different types of physical activity such as walking, moderate-intensity activities, and vigorous-intensity activities. We evaluated nutritional status using a brief-type self-administered diet history questionnaire, which contains 58 items about fixed portions and food types. We evaluated mental status using the 12-Item Short Form Health Survey (SF-12v2; Standard Japanese, Quality Metric Inc., Lincoln, RI, USA). In addition, the Instrumental Activities of Daily Living scale was used to estimate the ability to live independently.

Physical fitness and function
We evaluated physical fitness and function based on muscle strength with a hand grip.
dynamometer (T. K. K. 5401, Takei Scientific Instruments Co., Ltd., Niigata, Japan) \(^{38}\); balance using the one-leg standing test, which measures the duration of one-leg standing with eyes open \(^{39}\); flexibility using the sit-and-reach test (T.K.K.5112, Takei Scientific Instruments Co., Ltd.) \(^{40}\); gait speed using the 10-meter walking test \(^{41}\); and combined motor function using the Timed Up and Go test, which measures the time it takes a participant to rise from an arm chair, walk 3 meters, turn, walk back, and sit down again \(^{42}\). In addition, locomotive functions were evaluated using the stand-up test, two-step test, and a risk assessment on physical condition and lifestyle with 25 questions (Locomotive Challenge! Council, Japanese Orthopaedic Association, Tokyo, Japan).

**Blood and urine analysis**

Blood and urine samples were collected in the morning after an overnight fast. Subsequently, a standard 75 g OGTT was performed. Blood samples were obtained before and 30, 60, 90, and 120 minutes after ingesting 75 g of glucose to determine plasma glucose and serum insulin levels. Serum and plasma samples were sent to a commercial clinical laboratory (SRL Inc., Tokyo, Japan). Biochemical analysis for each parameter in Table 1 was performed using standard methods. Urine samples were used for albumin and qualitative tests. Other exploratory measurements are being planned for each study.

<table>
<thead>
<tr>
<th>Table 1. Parameters for blood and urine analyses.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete Blood Count</td>
</tr>
<tr>
<td>Glucose</td>
</tr>
<tr>
<td>Insulin</td>
</tr>
<tr>
<td>C-peptide</td>
</tr>
<tr>
<td>HbA1C</td>
</tr>
<tr>
<td>Triglyceride</td>
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<td></td>
</tr>
</tbody>
</table>
DNA extraction and genome-wide association study (GWAS)

Genomic DNA will be extracted from peripheral leukocytes. We plan to evaluate single nucleotide polymorphisms (SNPs) using a SNP array optimized for the Japanese or Asian population. GWAS will be performed to identify genes associated with clinical characteristics, mainly selected from the primary, secondary, and exploratory outcomes.

Bone mineral density

BMD of the lumbar spine and femoral neck were measured using DXA (Discovery DXA System, Hologic, Inc., Tokyo, Japan). Quality assurance for longitudinal evaluation was performed by calibrating the machine with standardized phantoms. The coefficient of variation (CV) for the in vivo lumbar spine-BMD measurements was <1%. The diagnosis of osteoporosis was based on the criteria proposed by the Japanese Society of Bone and Mineral Metabolism in 2012. Postmenopausal women were diagnosed with osteoporosis if they met the following criteria: ≥1 prevalent non-traumatic radiographic vertebral fractures based on clinical radiological criteria; hip fracture; low BMD (≤70% of the young adult mean value); and BMD ≤80% of the young adult mean value and ≥1 prevalent non-traumatic fragility fractures that are not vertebral or hip fractures.
Arteriosclerosis

Arteriosclerosis was estimated using CAVI and the ankle brachial index (ABI), which were measured using an automatic waveform analyzer (Vascular Screening System VaSera VS-1500, Fukuda Denshi Co., Ltd., Tokyo, Japan). Recording was performed in the supine position after 10 minutes of rest. Occlusion and monitoring cuffs were placed snugly on the upper and lower extremities. Pressure waveforms were then recorded simultaneously from the brachial arteries using the oscillometric method. All scans were automatically conducted by well-trained investigators. A resting ABI ≤ 0.90 was considered to reflect the presence of peripheral artery disease.

Abdominal fat area

Intra-abdominal fat area was measured with a 0.3-T MR scanner (AIRIS Vento, Hitachi). Subjects were in the prone position in the magnet with their arms placed straight overhead. Using the intervertebral space between the fourth and fifth lumbar vertebrae (L4–L5) as the point of origin, transverse images of 10-mm slice thickness were obtained every 100 mm from head to foot, resulting in a total of 10 images for each subject. All MRI data were transferred to a computer workstation for analysis using specialized image analysis software (AZE Virtual Place, AZE, Tokyo, Japan) to measure visceral and subcutaneous fat area.

Anthropometric measurement

Height was measured within 0.1 cm using a stadiometer in the upright position in the morning. Body weight was measured within 0.1 kg using an electronic scale (InBody770;
Biospace Co., Ltd., Seoul, Korea). Circumference of the waist as well as the thigh and calf of the dominant leg were measured within 0.5 cm with a plastic tape measure.

**Evaluation of osteoarthritis (OA) of the knee joint and knee pain**

Radiographic OA severity \(^{79}\) was evaluated using the Kellgren-Lawrence grading system based on weight-bearing anteroposterior radiographs of the femorotibial joint for both knees in the bilateral standing extended view as well as weight-bearing posteroanterior radiographs of the femorotibial joint with the knee in 45° of flexion.

Knee joints were also examined with MRI. Imaging sequences included coronal and sagittal proton density-weighted images with or without fat suppression and T2 mapping. Morphological changes in knee OA were semi-quantitatively evaluated according to the Whole Organ Magnetic Resonance Imaging Score (WORMS) \(^{80}\). Medial meniscus extrusion was also evaluated, we previously described \(^{81-84}\).

Clinical manifestations of knee pain were evaluated using a visual analog scale (VAS scale; 0–100 mm) and the Japanese Knee Osteoarthritis Measure (JKOM) score \(^{85}\). JKOM is a self-reported score that includes four subcategories: pain and stiffness (0–32 points), activities of daily living (0–40 points), social activities (0–20 points), and general health condition (0–8 points), with 100 points being the maximum score.

**Patient and public involvement**

No participants were involved in development of the research questions, design of the study or the recruitment.

**Statistical analysis**
Statistical analyses are planned to evaluate associations between muscle function and clinical outcomes. We use logistic regression with adjustment for age, sex, and other potential factors. Sex will be stratified as male and female. Age brackets will be 65–69, 70–74, 75–79, and 80–84 years. The follow-up period is defined as the time from the baseline examination to the occurrence of each event during follow-up examinations. In addition, exploratory analyses will be performed with several exposures and outcomes. Statistical analysis plans will be appropriate to each study.

Findings to date

The cohort was similar to the population of Bunkyo-ku and Tokyo, respectively (Figure 4). This cohort characteristics was shown on Table 2. Average age was 73.1 ± 5.4 years (males: 73.0 ± 5.3, female: 73.2 ± 5.4). The male: female ratio was 0.73:1 (males: n=687, 42.2%; females: n=942, 57.8%), which was also similar to that of Bunkyo-ku and Tokyo, respectively. The MoCA and MMSE scores for cognitive function were 25.1 ± 3.0 and 27.7 ± 1.9, respectively. Subjects with MCI (MoCA score ≤22) and dementia (MMSE score ≤23) represented 26.0% and 3.3% of the study cohort, respectively. The prevalence of MCI, dementia, hypertension, and diabetes was comparable to the prevalence for those conditions in a similar elderly Japanese cohort. There were 402 participants (24.8%) with cerebral SVD, which was also similar to the results of a previous study.

In terms of muscle function, SMI in males and females were 7.9 ± 0.9 kg/m² and 6.5 ± 0.8 kg/m², respectively. Muscle isokinetic strength was 148.4 ± 38.8 Nm/kg in males and 122.2 ± 32.3 Nm/kg in females. These findings are comparable to values in elderly Japanese subjects. Insulin sensitivity estimated using the Matsuda index was 7.3 ± 4.1, which is similar to the value in non-obese, non-diabetic, middle-aged men.
<table>
<thead>
<tr>
<th></th>
<th>All participants</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1629</td>
<td>687</td>
<td>942</td>
</tr>
<tr>
<td>Age (years old)</td>
<td>73.1±5.4</td>
<td>73.0±5.3</td>
<td>73.2±5.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>158.0±8.8</td>
<td>165.8±5.9</td>
<td>152.4±5.6</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>58.2±10.4</td>
<td>65.7±8.6</td>
<td>52.7±7.7</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.2±3.1</td>
<td>23.9±2.8</td>
<td>22.7±3.2</td>
</tr>
<tr>
<td>Education (year)</td>
<td>13.9±2.5</td>
<td>14.9±2.5</td>
<td>13.2±2.2</td>
</tr>
<tr>
<td>Worker / Volunteer (n: %)</td>
<td>563 (34.6%)</td>
<td>288 (41.9%)</td>
<td>275 (29.2%)</td>
</tr>
<tr>
<td>Needing long-term care (n: %)</td>
<td>27 (1.7%)</td>
<td>14 (2.0%)</td>
<td>13 (1.4%)</td>
</tr>
<tr>
<td>Solitude (n: %)</td>
<td>342 (21.0%)</td>
<td>84 (12.2%)</td>
<td>258 (27.4%)</td>
</tr>
<tr>
<td>Hypertension (n: %)</td>
<td>748 (45.9%)</td>
<td>368 (53.6%)</td>
<td>380 (40.3%)</td>
</tr>
<tr>
<td>Diabetes (n: %)</td>
<td>187 (11.5%)</td>
<td>113 (16.4%)</td>
<td>74 (7.9%)</td>
</tr>
<tr>
<td>Dyslipidemia (n: %)</td>
<td>639 (39.2%)</td>
<td>231 (33.6%)</td>
<td>408 (43.3%)</td>
</tr>
<tr>
<td>Cerebrovascular disease (n: %)</td>
<td>68 (4.2%)</td>
<td>34 (4.9%)</td>
<td>34 (3.6%)</td>
</tr>
<tr>
<td>Cardiovascular disease (n: %)</td>
<td>75 (4.6%)</td>
<td>50 (7.3%)</td>
<td>25 (2.7%)</td>
</tr>
<tr>
<td>Cancer (n: %)</td>
<td>40 (2.5%)</td>
<td>25 (3.6%)</td>
<td>15 (1.6%)</td>
</tr>
<tr>
<td>Cerebral small vessel disease (n: %)</td>
<td>402 (24.8%)</td>
<td>178 (26.1%)</td>
<td>224 (23.9%)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>136.6±17.1</td>
<td>136.8±16.4</td>
<td>136.5±17.6</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>84.3±9.8</td>
<td>86.4±9.7</td>
<td>82.8±9.5</td>
</tr>
<tr>
<td>Cardio Ankle Vascular Index: Right</td>
<td>9.0±1.1</td>
<td>9.2±1.1</td>
<td>8.8±1.1</td>
</tr>
<tr>
<td>Cardio Ankle Vascular Index: Left</td>
<td>8.9±1.1</td>
<td>9.1±1.0</td>
<td>8.7±1.0</td>
</tr>
<tr>
<td>Skeletal muscle mass (kg/m²)</td>
<td>7.1±1.1</td>
<td>7.9±0.9</td>
<td>6.5±0.8</td>
</tr>
<tr>
<td>Muscle isokinetic strength (Nm/kg)</td>
<td>133.2±37.5</td>
<td>148.4±38.8</td>
<td>122.2±32.3</td>
</tr>
<tr>
<td>Hang-grip strength (kg)</td>
<td>25.9±7.1</td>
<td>32.3±5.7</td>
<td>21.2±3.5</td>
</tr>
<tr>
<td>Gait speed (m/sec)</td>
<td>1.9±0.4</td>
<td>2.0±0.4</td>
<td>1.8±0.3</td>
</tr>
<tr>
<td>Timed up and Go test (sec)</td>
<td>6.7±1.6</td>
<td>6.5±1.6</td>
<td>6.8±1.5</td>
</tr>
<tr>
<td>Instrumental Activities of Daily Living (point)</td>
<td>6.7±1.5</td>
<td>5.0±0.3</td>
<td>8.0±0.2</td>
</tr>
<tr>
<td>Depression: GDS≥10point (n: %)</td>
<td>31 (1.9%)</td>
<td>15 (2.2%)</td>
<td>16 (1.7%)</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dL)</td>
<td>100.5±16.7</td>
<td>104.5±18.2</td>
<td>97.6±14.8</td>
</tr>
<tr>
<td>Fasting plasma insulin (μU/mL)</td>
<td>4.9±3.3</td>
<td>5.0±3.4</td>
<td>4.8±3.2</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.8±0.6</td>
<td>5.9±0.6</td>
<td>5.8±0.5</td>
</tr>
</tbody>
</table>
Triglycerides (mg/dL) | 98.5±54.2 | 104.5±60.6 | 94.1±48.6
HDL cholesterol (mg/dL) | 64.3±16.5 | 58.7±15.7 | 68.4±16.0
LDL cholesterol (mg/dL) | 121.3±30.8 | 113.8±30.7 | 126.8±29.7
Aspartate aminotransferase (IU/L) | 23.5±9.5 | 23.6±9.4 | 23.4±9.6
Alanine aminotransferase (IU/L) | 19.3±11.1 | 20.4±12.4 | 18.5±10.1
γ-glutamyl transferase (IU/L) | 30.8±35.8 | 40.3±49.5 | 23.8±17.8
Serum albumin (g/dL) | 4.3±0.4 | 4.3±0.4 | 4.3±0.4
Creatinine (mg/dL) | 0.8±0.3 | 0.9±0.4 | 0.7±0.2
Matsuda index | 7.3±4.1 | 7.4±4.6 | 7.2±3.7
Montreal Cognitive Assessment (MoCA) (point) | 25.1±3.0 | 24.7±3.0 | 25.5±3.0
Mild Cognitive Impairment: MoCA≤22 point (n; %) | 292 (17.9%) | 149 (21.7%) | 143 (15.2%)
Mini-Mental State Examination (MMSE) (point) | 27.7±1.9 | 27.5±2.0 | 27.9±1.9
Dementia: MMSE≤23 point (n; %) | 53 (3.3%) | 23 (3.3%) | 30 (3.2%)
Bone mineral density of the femoral neck (g/cm²) | 0.6±0.1 | 0.7±0.1 | 0.6±0.1
Bone mineral density of the lumbar spine (g/cm²) | 0.9±0.2 | 1.1±0.2 | 0.8±0.2

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**Strengths and limitations**

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Strengths of the present study include: (1) long-term prospective cohort study design to identify risk factors for needing long-term care, (2) random sampling of elderly individuals in an urban area, (3) assembly of a relatively large cohort for evaluating muscle mass, muscle strength, and insulin sensitivity, whole brain MRI, cognitive function, vascular function, physical fitness, and knee OA simultaneously. Therefore, this study can evaluate relationships between muscle mass, muscle strength, and insulin sensitivity and multiple diseases that lead to needing long-term care. This study may suggest novel strategies to prevent the need for long-term care.

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This study has several limitations such as (1) including only participants living...
in an urban part of Japan, (2) challenges in confirming causal relationships, and (3) a relatively low participation rate (18.9%), thus, we cannot deny the possibility of selection bias and we should be careful about the interpretation of the results. Before subject recruitment, we recognized that the participation rate in a similar study conducted in Japan was low (18.4%) \(^8\). Thus, we sent invitation letters twice after no initial reply and called the subjects before the briefing session to increase the participation rate. Other plans may be considered to further increase the participation rate in future studies of community dwelling.

**Collaborators**

For more information or potential collaboration, please contact Yoshifumi Tamura (ys-tamur@juntendo.ac.jp) and Yuki Someya (yksomeya@juntendo.ac.jp).

**Ethics and dissemination**

This study protocol was approved by the ethics committee of Juntendo University in November 2015 (No. 2015078, 2016138, 2016131, and 2017121). This study is being carried out in accordance with the principles outlined in the Declaration of Helsinki. All participants gave written informed consent at the orientation meeting. Participants were told that they have the right to withdraw from the trial at any time. Data are coded and stored securely in password-protected files. Only the principal investigators have access to the files. The findings of the study will be presented in local and international conferences and disseminated in peer-reviewed journals.
Authors’ contribution
YS, YT, HK, HW, and RK conceived the study and obtained grant funding. YS, YT, and
HK drafted the protocol. SN advised on the statistical analysis. SK, HD, MI, KK, TM,
SH, SK, SA, NH, YM, and HN reviewed and revised the protocol.

Funding statement
This work is supported by the Strategic Research Foundation at Private Universities
(S1411006) and KAKENHI (18H03184) from the Ministry of Education, Culture, Sports,
Science and Technology of Japan, the Mizuno Sports Promotion Foundation, and the
Mitsui Life Social Welfare Foundation.

Competing interests statement
The authors have nothing to disclose.

Data availability statement
Currently the data are not available since the cohort is ongoing.
Figure legend

Figure 1. Geographical location of Bunkyo-ku in Japan
Left: Japan is divided into 47 prefectures. The location of Tokyo is indicated by a circle.
Center: Tokyo is a prefecture that includes 23 special wards, 26 cities, 5 towns, and 8 villages. Bunkyo is one of the special wards (Bunkyo-ku in Japanese). It located in east Tokyo, as indicated by the circle. Right: Bunkyo-ku includes 68 communities and has 227,902 residents. (Source of map; http://www.start-point.net)

Figure 2. Experimental protocol

Figure 3. Flowchart of the participant recruitment process

Figure 4. Distribution of the elderly population in this cohort, Bunkyo-ku, and Tokyo
The proportion of elderly individuals in each age group (age 65–84 years) in this cohort, Bunkyo-ku, and Tokyo are presented.
References


41. Peters DM, Fritz SL, Krotish DE. Assessing the reliability and validity of a shorter walk


57. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999;22(9):1462-70.


Figure 1

Japan is divided into 47 prefectures.

Tokyo is a prefecture that includes 23 special wards, 26 cities, 5 towns, and 8 villages.

Bunkyo-ku is one of the special wards and includes 68 communities and 227,902 people.
Cognitive function, muscle function, brain magnetic resonance imaging (MRI) parameters, and other variables were evaluated.

Evaluation for incidences of cerebrovascular disease, falls, fractures, and other diseases every year by mail.

Re-evaluation for cognitive function, brain MRI parameters and other parameters measured at baseline except oral glucose tolerance test (OGTT).

Evaluation for incidences of cerebrovascular disease, falls, fractures, and other diseases every year by mail.

Re-evaluation for cognitive function, brain MRI parameters and other parameters measured at baseline except OGTT.
Figure 3

Elderly subjects living in 13 communities of Bunkyo-ku (n = 8,629)

Excluded (n = 6,646)
- Unknown address: 242
- Declined to participate: 292
- Could not participate: 22
- Did not reply: 6,090

Participating group briefing (n = 1,983)

Excluded (n = 153)
- Declined consent: 153

Informed consent obtained (n = 1,830)

Excluded (n = 201)
- Withdrew consent: 190
- Insulin treatment: 4
- Pacemaker implantation: 4
- Did not meet age criteria: 3

This cohort subjects: 1,629
Figure 4

Population rate of each age in elderly

Tokyo
Participants (Male)
Participants (Female)
Bunkyo-ku

(years old)
### STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

<table>
<thead>
<tr>
<th>Section/Topic</th>
<th>Item #</th>
<th>Recommendation</th>
<th>Reported on page #</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Title and abstract</strong></td>
<td>1</td>
<td><em>(a)</em> Indicate the study’s design with a commonly used term in the title or the abstract  &lt;br&gt;    <em>(b)</em> Provide in the abstract an informative and balanced summary of what was done and what was found</td>
<td>1, 3</td>
</tr>
<tr>
<td><strong>Introduction</strong></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td><strong>Background/rationale</strong></td>
<td>2</td>
<td>Explain the scientific background and rationale for the investigation being reported</td>
<td>5-7</td>
</tr>
<tr>
<td><strong>Objectives</strong></td>
<td>3</td>
<td>State specific objectives, including any pre-specified hypotheses</td>
<td>7</td>
</tr>
<tr>
<td><strong>Methods</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Study design</strong></td>
<td>4</td>
<td>Present key elements of study design early in the paper</td>
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<td><strong>Setting</strong></td>
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<td>Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection</td>
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<td><strong>Participants</strong></td>
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<td><em>(a) Cohort study</em>—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up  &lt;br&gt;    <em>(Case-control study)</em>—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls  &lt;br&gt;    <em>(Cross-sectional study)</em>—Give the eligibility criteria, and the sources and methods of selection of participants</td>
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<td><strong>Variables</strong></td>
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<td>Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable  &lt;br&gt;    For matched studies, give matching criteria and number of exposed and unexposed  &lt;br&gt;    For matched studies, give matching criteria and the number of controls per case</td>
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<td>For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group</td>
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<td><strong>Bias</strong></td>
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<td>Describe any efforts to address potential sources of bias</td>
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<td>Explain how the study size was arrived at</td>
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<td><strong>Quantitative variables</strong></td>
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<td>Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why  &lt;br&gt;    <em>(Cohort study)</em>—If applicable, explain how loss to follow-up was addressed  &lt;br&gt;    <em>(Case-control study)</em>—If applicable, explain how matching of cases and controls was addressed</td>
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<td><strong>Statistical methods</strong></td>
<td>12</td>
<td>*(a) Describe all statistical methods, including those used to control for confounding  &lt;br&gt;    *(b) Describe any methods used to examine subgroups and interactions  &lt;br&gt;    *(c) Explain how missing data were addressed  &lt;br&gt;    <em>(d) Cohort study</em>—If applicable, explain how loss to follow-up was addressed  &lt;br&gt;    <em>(Case-control study)</em>—If applicable, explain how matching of cases and controls was addressed</td>
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|   | **Cross-sectional study**—If applicable, describe analytical methods taking account of sampling strategy  
(e) Describe any sensitivity analyses |
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**Participants** | 13*  
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**Descriptive data** | 14*  
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(b) Indicate number of participants with missing data for each variable of interest  
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**Outcome data** | 15*  
**Cohort study**—Report numbers of outcome events or summary measures over time  
**Case-control study**—Report numbers in each exposure category, or summary measures of exposure  
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*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.  
**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.
Cohort profile: skeletal muscle function and need for long-term care in a prospective cohort study of urban elderly people in Japan (the Bunkyo Health Study)

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Cohort profile: skeletal muscle function and need for long-term care in a prospective cohort study of urban elderly people in Japan (the Bunkyo Health Study)

Yuki Someya\textsuperscript{1,2,10}, Yoshifumi Tamura\textsuperscript{1,2}, Hideyoshi Kaga\textsuperscript{2}, Shuko Nojiri\textsuperscript{3}, Kazunori Shimada\textsuperscript{1,4}, Hiroyuki Daida\textsuperscript{1,4}, Muneaki Ishijima\textsuperscript{1,5}, Kazuo Kaneko\textsuperscript{1,5}, Shigeki Aoki\textsuperscript{1,6}, Takashi Miida\textsuperscript{1,7}, Satoshi Hirayama\textsuperscript{1,7}, Seiki Konishi\textsuperscript{1,7}, Nobutaka Hattori\textsuperscript{1,8}, Yumiko Motoi\textsuperscript{1,9}, Hisashi Naito\textsuperscript{1,10}, Ryuzo Kawamori\textsuperscript{1,2}, Hirotaka Watada\textsuperscript{1,2}

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Abstract

Purpose: The proportion of elderly individuals (age ≥ 65 years) in Japan reached 27.7% in 2017, the highest in the world. A serious social problem in a super-aged society is the rise in the number of elderly people who need long-term care (LTC), which is mainly due to cerebrovascular disease, dementia, age-related frailty, falls and fractures, and joint disease. We hypothesized that decreased muscle mass, muscle strength, and insulin sensitivity are common risk factors for these diseases related to needing LTC. We developed a prospective cohort study of elderly subjects in an urban community to test this hypothesis. The primary objective is to prospectively investigate associations between muscle mass, muscle strength, and insulin sensitivity and incidence of main disease and risk factors of needing LTC. The primary outcomes are incidence of cerebrovascular disease and cognitive decline.

Participants: Participants were 1,629 people aged 65–84 years living in 13 communities in an urban area (Bunkyo-ku, Tokyo, Japan). Average age was 73.1 ± 5.4 years.

Findings to date: We obtained baseline data on cognitive function, cerebral small vessel disease (SVD) determined by brain magnetic resonance imaging (MRI), body composition, bone mineral density, arteriosclerosis, physical function, muscle mass, muscle strength, and insulin sensitivity. Mild cognitive impairment and dementia were observed in 18.1% and 3.3% of participants, respectively. The prevalence of cerebral SVD was 24.8%. These characteristics are similar to those previously reported in elderly Japanese subjects.
Future plans: We will ask participants about their health status, including incidence of cerebrovascular disease, falls, fractures, and other diseases every year by mail. We plan to re-evaluate cognitive function, brain MRI parameters and other parameters at 5 and 10 years after the baseline evaluation. We will evaluate whether low muscle function (muscle mass, muscle strength, or insulin sensitivity) is a risk factor for cognitive decline or cerebrovascular disease.

Strengths and limitations

- Prospective cohort study over 10 years to identify risk factors for needing long-term care.
- Relationships between muscle mass, muscle strength, or insulin sensitivity and multiple diseases necessitating long-term care will be evaluated simultaneously.
- A relatively large cohort was assembled for evaluating muscle mass, muscle strength, or insulin sensitivity, whole brain magnetic resonance imaging (MRI), cognitive function, glucose tolerance, vascular function, physical fitness, and knee osteoarthritis, simultaneously.
- Study results may suggest novel strategies to prevent the need for long-term care.
- This study has several limitations: (1) including only participants living in an urban part of Japan, (2) challenges in confirming causal relationships, and (3) a relatively small number of participants in the prospective cohort.
Introduction

The number of elderly people (aged $\geq 65$ years) has increased worldwide. For example, World Health Organization defined the aging rate as proportion of persons aged 65 years or older. The aging rate has been increasing in both developed and developing countries. In 2015, the worldwide aging rate was 8.3% and it is estimated to increase to 17.8% by 2055. While the aging rate in developed countries is predicted to reach 27.8% in 2060 \(^1\), the aging rate in Japan reached 27.7% in 2017 \(^2\); it is the highest in the world. Japan is now categorized as a super-aged society, defined as a country with an aging rate greater than 21\% \(^3\). The rate will reach 30\% in 2025 and 40\% in 2060. A serious social problem in such a super-aged society is the increase in the number of elderly people who need long-term care. Need for long-term care is defined as a condition where an elderly individual needs care to perform activities of daily living due to age-related diseases such as cerebrovascular disease, dementia, and musculoskeletal disease. The number of individuals who need long-term care in Japan increased from 2.18 million in 2000 to 6.06 million in 2015. Social security expenses for long-term care increased from 3.6 trillion JPY in 2000 to 10.1 trillion JPY in 2015 \(^5\) \(^6\). These figures are predicted to increase and cause increased social and economic burden in Japan. Thus, the Japanese government has launched a policy to extend healthy life expectancy without long-term care \(^7\); however, evidence-based strategies to prevent the need for long-term care have not been established yet.

In Japan, the main causes of needing long-term care are cerebrovascular disease (17.2\%), dementia (16.4\%), age-related frailty (13.9\%), falls and fractures (12.2\%), and joint disease (11.0\%) \(^2\). Thus, multiple approaches to prevent these diseases will be useful for preventing the need for long-term care. However, if there are common risk factors for
the onset of these diseases, it would be helpful to find an efficient strategy to prevent the need for long-term care. Several previous studies have demonstrated that a decrease in muscle mass or muscle strength\textsuperscript{8} 9-14 15 16 17 18 or insulin sensitivity\textsuperscript{19} 20 21 22 23 24 25 may be common risk factors for diseases related to needing long-term care. Indeed, aging-related decreases in muscle strength or muscle mass\textsuperscript{8} are associated with an increased risk for metabolic syndrome\textsuperscript{10-12} 26, cognitive decline\textsuperscript{12} 14, cerebrovascular disease\textsuperscript{27,28}, and all-cause mortality\textsuperscript{13} 29. In addition, decreased muscle mass or muscle strength are also closely associated with decreased mobility, falls, and fractures\textsuperscript{15,16} as well as decreased psychosocial well-being\textsuperscript{17} and quality of life,\textsuperscript{18} which are associated with geriatric depression. On the other hand, impaired insulin sensitivity is associated with cardiovascular disease\textsuperscript{19,20} and lacunar infarcts\textsuperscript{21,22} as well as microstructural white matter changes\textsuperscript{23} and decreased cognitive performance\textsuperscript{24,25}. Given that skeletal muscle is the main organ that determines whole-body insulin sensitivity\textsuperscript{30,31}, we hypothesized that parameters related to muscle function such as muscle mass, muscle strength, and insulin sensitivity might be common risk factors for diseases related to needing long-term care. However, there is insufficient clinical evidence to support this hypothesis.

On the other hand, several other risk factors for diseases related to needing long-term care have been reported. For example, arteriosclerosis determined by cardio-ankle vascular index (CAVI) predicts cognitive decline in elderly Japanese individuals\textsuperscript{32}. A recent meta-analysis showed a modest association between CAVI and incident cardiovascular disease risk\textsuperscript{33}. In addition, cerebral small vessel disease (SVD), defined as lacunar infarcts, cerebral microhemorrhages, periventricular hyperintensities (PVHs), or deep and subcortical white matter hyperintensities (DSWMHs) detected on magnetic resonance imaging (MRI), is strongly associated with incident ischemic and hemorrhagic
stroke, all-cause dementia and depression, and all-cause mortality $^{34}$. Furthermore, a
meta-analysis of data from 12 cohort studies showed that low hip bone mineral density
(BMD) is an important predictor of fracture risk $^{35}$. We hypothesized that muscle mass,
muscle strength, and insulin sensitivity are also common risk factors for arteriosclerosis,
cerebral SVD, and lower BMD; however, this hypothesis has not been tested yet.

In this context, we developed a prospective cohort study of elderly individuals in an urban community. The primary objective of the present study is to prospectively investigate associations between muscle mass, muscle strength, or insulin sensitivity and main causes and risk factors for needing long-term care. The primary outcomes of the present study are incidence of cerebrovascular disease and cognitive decline. Secondary outcomes are incidence of age-related frailty, falls and fractures, and joint disease, respectively.
Cohort description

Study design

The Bunkyo Health Study is a prospective cohort study of over 10 years. We recruited elderly subjects aged 65–84 years living in Bunkyo-ku, an urban area in Tokyo, Japan (Figure 1). Bunkyo-ku was selected because the age distribution in Bunkyo-ku is similar to that of Tokyo overall and there is high accessibility to the research center. Among 68 communities in Bunkyo-ku, we selected 13 communities based on probability proportionate to size sampling. We obtained the name and address of all residents aged 65–84 years in the selected communities from residential registries. We mailed invitations to a group briefing session at our institution (Sportology Center, Juntendo University).

The inclusion criterion of the present study was age of 65–84 years. The exclusion criteria were pacemaker or defibrillator placement and diabetes requiring insulin therapy. After the briefing session, subjects who provided written informed consent were included as research participants. The primary outcomes of the present study are incidence of cerebrovascular disease and cognitive decline. The secondary outcomes are age-related frailty, falls and fractures, and joint disease. The exploratory outcomes are arteriosclerosis, cerebral SVD, and osteoporosis as well as cognitive function, brain volume and structure, depression, physical function and all-cause mortality.

The study protocol is outlined in Figure 2. All subjects participated in examinations over two visits to the Sportology Center. During the first visit, subjects participated in cognitive function and physical fitness testing. MRI of the whole brain and the knee of the dominant leg or the leg with pain was performed. We also interviewed subjects about their medical history, family history, and current pain. During
the second visit, we evaluated body composition and bone mineral density using dual-energy X-ray absorptiometry (DXA) \(^43\), CAVI \(^44\), and abdominal fat distribution with MRI after an overnight fast \(^45\). Next, we carried out a 75 g oral glucose tolerance test (OGTT) \(^46\). All data were collected and stored at the Sportology Center.

After the baseline evaluation (October 15, 2015 to October 1, 2018), we will ask participants about their health status every year by mail. For example, we will ask about incidence of cerebrovascular disease, falls, fractures, and other diseases every year. We also will send the Kihon Check List questionnaire \(^47\) to participants every year. This questionnaire was developed to identify elderly individuals with frailty or at high risk for needing long-term care. It consists of 25 questions regarding instrumental and social activities of daily living, physical function, nutritional status, oral function, cognitive function, and depressed mood \(^47\) \(^48\). At 5 years (2020–2023) and 10 years (2025–2028) after the baseline examination, we are planning to re-evaluate cognitive function, brain MRI parameters and other parameters measured at baseline except OGTT.

### Sample size calculations

Since the primary outcomes are incidence of cerebrovascular disease and cognitive decline and the incidence of cognitive decline is lower than the incidence of cerebrovascular disease \(^49\) \(^51\), the sample size was calculated to allow for evaluation of associations between cognitive decline and muscle mass, muscle strength, and insulin sensitivity, respectively. Cognitive decline was defined as the presence of dementia or mild cognitive impairment (MCI) estimated using the Montreal Cognitive Assessment (MoCA), Japanese version \(^36\) \(^52\) or the Mini-Mental State Examination (MMSE) \(^37\) \(^53\), respectively. In the prospective analysis, we plan to categorize subjects in two groups for
muscle function (e.g. low and high muscle strength groups). The assumed incidence of
cognitive decline per year is 1–2% and the assumed cumulative incidence over 10 years
in the population is 15% \(^{54,55}\). The low muscle function group was assumed to have a two-
fold higher risk of cognitive decline. Therefore, a sample of 1,519 subjects was estimated
to be necessary, assuming a dropout rate of 15% over 10 years.

Since we also plan to perform a cross-sectional analysis using baseline data, we
performed a preliminary sample size calculation. Although muscle mass, muscle strength,
and insulin sensitivity reflect different aspects of muscle function, the combined
associations of these parameters with diseases or risk factors related to needing long-term
care remain unclear. Thus, we plan to categorize subjects in nine categories of muscle
function (e.g. low, middle, and high muscle strength × low, middle, and high insulin
sensitivity) and focus on associations between these categories of muscle function and
cognitive decline. We also plan to evaluate other outcomes such as arteriosclerosis,
cerebral SVD, osteoporosis, knee joint condition, cognitive function, brain volume and
structure, depression, and physical function in the cross-sectional analysis. We estimated
that rates of cognitive decline in the group with higher muscle function to be 12%,
compared with 24% in the group with lower muscle function \(^{50,51}\). A sample of 1,584
subjects was estimated to be necessary based on statistical power of 80% at a significance
level of 5%. Thus, the estimated required number of subjects in the cross-sectional
analysis (1,584) is sufficient for the prospective analysis. We set the overall sample size
to be 1,600 in the present study.

Participants

For the baseline examination (October 15, 2015 to October 1, 2018), we mailed
invitations to attend a group briefing session to all 8,629 elderly individuals living in 13 communities in Bunkyo-ku, Tokyo, Japan, of whom 1,984 (23.0%) subjects participated in a group briefing session at our institution (Sportology Center, Juntendo University). After the briefing session, 1,830 subjects provided written informed consent for study participation. However, 190 subjects withdrew consent and 11 subjects were excluded based on age, pacemaker status, and insulin therapy status. Ultimately, the study subjects comprised of 1,629 elderly people, which corresponds to 18.9% of all elderly individuals in 13 selected communities of Bunkyo-ku) (Figure 3).

**Measurements**

**Muscle mass and strength**

Muscle mass was measured using DXA (Discovery DXA System, Hologic, Inc., Tokyo, Japan)\(^{56}\). Skeletal muscle mass index (SMI) was calculated by dividing appendicular muscle mass (kg) by height squared (m\(^2\))\(^{57}\). Lower limb isokinetic muscle strength (angular velocity of 60 degrees/second) was measured using a dynamometer (BIODEX system 3 or 4: Biodex Medical Systems, Upton, NY, USA)\(^{58}\).

**Insulin sensitivity**

Insulin sensitivity was estimated with the Matsuda index using data on glucose and insulin during the 75 g OGTT\(^{46}\). The Matsuda index was calculated using the following equation:

\[
\text{Matsuda index} = \frac{10,000}{\sqrt{(\text{fasting glucose} \times \text{fasting insulin}) \times (\text{mean glucose} \times \text{mean insulin during OGTT})}}
\]

This formula can estimate whole-body glucose disposal during the euglycemic insulin clamp. Skeletal muscle is the main organ absorbing glucose during
the clamp\textsuperscript{30}. Thus, the Matsuda index is a surrogate marker of insulin sensitivity, mainly reflecting insulin sensitivity in muscle. Our previous study using the two-step hyperinsulinemic euglycemic clamp technique demonstrated that the Matsuda index is significantly correlated with muscle insulin sensitivity, not hepatic insulin sensitivity\textsuperscript{31}.

Cognitive function

Cognitive function was primarily evaluated using the MoCA\textsuperscript{36,52} and the MMSE\textsuperscript{37,53}. The MoCA and MMSE contain 9 and 11 items, respectively. Possible scores range from 0 to 30 points. In this study, we used MoCA score $\leq 22$ as the cutoff for MCI\textsuperscript{60,61} and MMSE score $\leq 23$ as the cutoff for dementia\textsuperscript{37,53}. The Neurobehavioral Cognitive Status Examination (COGNISTAT), which evaluates orientation, attention, language, memory, calculation, construction, and reasoning\textsuperscript{62,63}; the Trail Making Test, which evaluates visual-conceptual and visual-motor tracking\textsuperscript{64,65}; and the Wechsler Memory Scale-Revised (part II)\textsuperscript{66,67} were also used for evaluation of cognitive function. We also used the short version of the Geriatric Depression Scale\textsuperscript{68,69} to assess depression, defined as a score $\geq 10$ points.

Evaluation of whole brain MRI

We performed whole brain MR scanning with a 0.3-T clinical MR scanner (AIRIS Vento, Hitachi, Tokyo, Japan) in all participants. In addition, brain MR scanning was also performed in some participants on a 3-T clinical MR scanner (MAGNETOM Prisma, Siemens Healthcare, Erlangen, Germany) with a 64-channel head coil for advanced brain MRI analysis and validation of brain analysis with the 0.3-T clinical MR scanner. The following sequences were obtained with the 0.3-T clinical MR scanner: axial
three-dimensional (3D) time-of-flight magnetic resonance angiography (repetition time (TR), 35 ms; echo time (TE), 7.1 ms; and slice thickness, 1.2 mm), T2*-weighted gradient echo imaging (TR, 1000 ms; TE, 45 ms; flip angle, 20°; and slice thickness, 5 mm), and fluid-attenuated inversion recovery imaging (TR, 11,000 ms; TE, 100 ms; inversion time (TI), 2000 ms; and slice thickness, 5 mm). We also obtained 3D-volumetric T1-weighted imaging using a gradient echo with inversion recovery (GEIR) sequence with these following parameters: TR, 25 ms; TE, 5.8 ms; TI, 600 ms; flip angle, 12°; number of excitations (NEX), 1; field of view (FOV), 200 × 250 × 250 mm^3; resolution, 0.98 × 0.98 × 2 mm^3; slice orientation, sagittal; total scan time, 10.1 min.

The protocol with the 3-T clinical MR scanner included 3D T1-weighted imaging and multi-shell diffusion weighted imaging (DWI). Three-dimensional T1-weighted images were obtained using magnetization-prepared 180 degrees radio-frequency pulses and rapid gradient-echo (MP-RAGE) with these following parameters: TR, 2300 ms; TE, 2.32 ms; TI: 900 ms; FOV, 240 × 240 mm; matrix size, 256 × 256; resolution, 0.9 × 0.9 mm; slice thickness, 0.9 mm; and acquisition time, 5.21 min. For DWI, echo planar imaging (EPI) consisting of two b values (1000, and 2000 s/mm^2) along 64 isotropic diffusion gradients was acquired in the anterior-posterior phase-encoding direction with the following parameters: TR, 3,300 ms; TE, 70 ms; FOV, 229 × 229 mm; matrix size, 130 × 130; resolution 1.8 × 1.8 mm; slice thickness, 1.8 mm; and acquisition time, 7.29 min. Each DWI acquisition was completed with a gradient-free image (b = 0).

We also acquired standard and reverse phase encoded blipped images with no diffusion weighting (Blip Up and Blip Down) to correct for magnetic susceptibility-induced distortions related to EPI acquisitions.

Primary brain MRI evaluation was conducted by an experienced
neuroradiologist based on axial T2*-WI and FLAIR images obtained on a 0.3-T MR scanner. The evaluation included cerebral SVD, characterized by lacunar infarcts, cerebral microhemorrhages, PVHs, and DSWMHs. PVHs and DSWMHs were categorized using the Fazekas scale. In this study, level III was defined as cerebral SVD. In addition, whole brain volume, regional brain volume, regional cortical thickness, and white and grey matter integrity were also included on subsequent evaluations. Furthermore, 0.3-T and 3-T 3D T1-weighted images were used for the quantification of whole brain volume as well as regional cortical volume and thickness; multi-shell DWI was used for the quantification of white and grey matter integrity.

**Lifestyle and physical activity levels**

We investigated lifestyle and physical activity levels using questionnaires. To evaluate sleeping status, we used the Pittsburgh Sleep Quality Index, which has seven components. We evaluated exercise habits and physical activity levels with the International Physical Activity Questionnaire, which assesses different types of physical activity such as walking, moderate-intensity activities, and vigorous-intensity activities. We evaluated nutritional status using a brief-type self-administered diet history questionnaire, which contains 58 items about fixed portions and food types. We evaluated mental status using the 12-Item Short Form Health Survey (SF-12v2; Standard Japanese, Quality Metric Inc., Lincoln, RI, USA). In addition, the Instrumental Activities of Daily Living scale was used to estimate the ability to live independently.

**Physical fitness and function**

We evaluated physical fitness and function based on muscle strength with a hand grip.
dynamometer (T. K. K. 5401, Takei Scientific Instruments Co., Ltd., Niigata, Japan) \(^{38}\), balance using the one-leg standing test, which measures the duration of one-leg standing with eyes open \(^{39}\); flexibility using the sit-and-reach test (T.K.K.5112, Takei Scientific Instruments Co., Ltd.) \(^{40}\); gait speed using the 10-meter walking test \(^{41}\); and combined motor function using the Timed Up and Go test, which measures the time it takes a participant to rise from an arm chair, walk 3 meters, turn, walk back, and sit down again \(^{42}\). In addition, locomotive functions were evaluated using the stand-up test, two-step test, and a risk assessment on physical condition and lifestyle with 25 questions (Locomotive Challenge! Council, Japanese Orthopaedic Association, Tokyo, Japan).

### Blood and urine analysis

Blood and urine samples were collected in the morning after an overnight fast. Subsequently, a standard 75 g OGTT was performed. Blood samples were obtained before and 30, 60, 90, and 120 minutes after ingesting 75 g of glucose to determine plasma glucose and serum insulin levels. Serum and plasma samples were sent to a commercial clinical laboratory (SRL Inc., Tokyo, Japan). Biochemical analysis for each parameter in Table 1 was performed using standard methods. Urine samples were used for albumin and qualitative tests. Other exploratory measurements are being planned for each study.

| Table 1. Parameters for blood and urine analyses. |
|-----------------------------------------------|-----------------------------------------------|
| Complete Blood Count  | Phosphate                                    |
| Glucose              | Homocysteine                                 |
| Insulin              | Adiponectin                                   |
| C-peptide            | C-reactive protein                            |
| HbA1c                | Intact parathyroid hormone                    |
| Triglyceride         | 25-hydroxy vitamin D                          |
|                     | Bone Specific Alkaline Phosphatase            |
DNA extraction and genome-wide association study (GWAS)

Genomic DNA will be extracted from peripheral leukocytes. We plan to evaluate single nucleotide polymorphisms (SNPs) using a SNP array optimized for the Japanese or Asian population. GWAS will be performed to identify genes associated with clinical characteristics, mainly selected from the primary, secondary, and exploratory outcomes.

Bone mineral density

BMD of the lumbar spine and femoral neck were measured using DXA (Discovery DXA System, Hologic, Inc., Tokyo, Japan). Quality assurance for longitudinal evaluation was performed by calibrating the machine with standardized phantoms. The coefficient of variation (CV) for the in vivo lumbar spine-BMD measurements was <1% . The diagnosis of osteoporosis was based on the criteria proposed by the Japanese Society of Bone and Mineral Metabolism in 2012. Postmenopausal women were diagnosed with osteoporosis if they met the following criteria: ≥1 prevalent non-traumatic radiographic vertebral fractures based on clinical radiological criteria; hip fracture; low BMD (≤70% of the young adult mean value); and BMD ≤80% of the young adult mean value and ≥1 prevalent non-traumatic fragility fractures that are not vertebral or hip fractures.
Arteriosclerosis

Arteriosclerosis was estimated using CAVI and the ankle brachial index (ABI), which were measured using an automatic waveform analyzer (Vascular Screening System VaSera VS-1500, Fukuda Denshi Co., Ltd., Tokyo, Japan). Recording was performed in the supine position after 10 minutes of rest. Occlusion and monitoring cuffs were placed snugly on the upper and lower extremities. Pressure waveforms were then recorded simultaneously from the brachial arteries using the oscillometric method. All scans were automatically conducted by well-trained investigators. A resting ABI ≤ 0.90 was considered to reflect the presence of peripheral artery disease.

Abdominal fat area

Intra-abdominal fat area was measured with a 0.3-T MR scanner (AIRIS Vento, Hitachi). Subjects were in the prone position in the magnet with their arms placed straight overhead. Using the intervertebral space between the fourth and fifth lumbar vertebrae (L4–L5) as the point of origin, transverse images of 10-mm slice thickness were obtained every 100 mm from head to foot, resulting in a total of 10 images for each subject. All MRI data were transferred to a computer workstation for analysis using specialized image analysis software (AZE Virtual Place, AZE, Tokyo, Japan) to measure visceral and subcutaneous fat area.

Anthropometric measurement

Height was measured within 0.1 cm using a stadiometer in the upright position in the morning. Body weight was measured within 0.1 kg using an electronic scale (InBody770;
 Biospace Co., Ltd., Seoul, Korea). Circumference of the waist as well as the thigh and calf of the dominant leg were measured within 0.5 cm with a plastic tape measure.

Evaluation of osteoarthritis (OA) of the knee joint and knee pain

Radiographic OA severity was evaluated using the Kellgren-Lawrence grading system based on weight-bearing anteroposterior radiographs of the femorotibial joint for both knees in the bilateral standing extended view as well as weight-bearing posteroanterior radiographs of the femorotibial joint with the knee in 45° of flexion.

Knee joints were also examined with MRI. Imaging sequences included coronal and sagittal proton density-weighted images with or without fat suppression and T2 mapping. Morphological changes in knee OA were semi-quantitatively evaluated according to the Whole Organ Magnetic Resonance Imaging Score (WORMS). Medial meniscus extrusion was also evaluated, we previously described

Clinical manifestations of knee pain were evaluated using a visual analog scale (VAS scale; 0–100 mm) and the Japanese Knee Osteoarthritis Measure (JKOM) score. JKOM is a self-reported score that includes four subcategories: pain and stiffness (0–32 points), activities of daily living (0–40 points), social activities (0–20 points), and general health condition (0–8 points), with 100 points being the maximum score.

Patient and public involvement

No participants were involved in development of the research questions, design of the study or the recruitment.

Statistical analysis
Statistical analyses are planned to evaluate associations between muscle function and clinical outcomes. We use regression analysis with adjustment for age, sex, and other potential factors, appropriately. Sex will be stratified as male and female. Age brackets will be 65–69, 70–74, 75–79, and 80–84 years. The follow-up period is defined as the time from the baseline examination to the occurrence of each event during follow-up examinations. In addition, exploratory analyses will be performed with several exposures and outcomes. Statistical analysis plans will be appropriate to each study.

Findings to date

The cohort was similar to the population of Bunkyo-ku and Tokyo, respectively (Figure 4). This cohort characteristics was shown on Table 2. Average age was 73.1 ± 5.4 years (males: 73.0 ± 5.3, female: 73.2 ± 5.4). The male: female ratio was 0.73:1 (males: n=687, 42.2%; females: n=942, 57.8%), which was also similar to that of Bunkyo-ku and Tokyo, respectively. The MoCA and MMSE scores for cognitive function were 25.1 ± 3.0 and 27.7 ± 1.9, respectively. Subjects with MCI (MoCA score ≤22) and dementia (MMSE score ≤23) represented 18.1% and 3.3% of the study cohort, respectively. The prevalence of MCI, dementia, hypertension, and diabetes was comparable to the prevalence for those conditions in a similar elderly Japanese cohort. There were 402 participants (24.8%) with cerebral SVD, which was also similar to the results of a previous study.

In terms of muscle function, SMI in males and females were 7.9 ± 0.9 kg/m² and 6.5 ± 0.8 kg/m², respectively. Muscle isokinetic strength was 148.4 ± 38.8 Nm/kg in males and 122.2 ± 32.3 Nm/kg in females. These findings are comparable to values in elderly Japanese subjects. Insulin sensitivity estimated using the Matsuda index was 7.3 ± 4.1, which is similar to the value in non-obese, non-diabetic, middle-aged men.
### Table 2 Characteristics of study subjects

<table>
<thead>
<tr>
<th></th>
<th>All participants</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1629</td>
<td>687</td>
<td>942</td>
</tr>
<tr>
<td>Age (years old)</td>
<td>73.1±5.4</td>
<td>73.0±5.3</td>
<td>73.2±5.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>158.0±8.8</td>
<td>165.8±5.9</td>
<td>152.4±5.6</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>58.2±10.4</td>
<td>65.7±8.6</td>
<td>52.7±7.7</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.2±3.1</td>
<td>23.9±2.8</td>
<td>22.7±3.2</td>
</tr>
<tr>
<td>Education (year)</td>
<td>13.9±2.5</td>
<td>14.9±2.5</td>
<td>13.2±2.2</td>
</tr>
<tr>
<td>Worker / Volunteer (n; %)</td>
<td>563 (34.6%)</td>
<td>288 (41.9%)</td>
<td>275 (29.2%)</td>
</tr>
<tr>
<td>Needing long-term care (n; %)</td>
<td>27 (1.7%)</td>
<td>14 (2.0%)</td>
<td>13 (1.4%)</td>
</tr>
<tr>
<td>Solitude (n; %)</td>
<td>342 (21.0%)</td>
<td>84 (12.2%)</td>
<td>258 (27.4%)</td>
</tr>
<tr>
<td>Hypertension (n; %)</td>
<td>748 (45.9%)</td>
<td>368 (53.6%)</td>
<td>380 (40.3%)</td>
</tr>
<tr>
<td>Diabetes (n; %)</td>
<td>187 (11.5%)</td>
<td>113 (16.4%)</td>
<td>74 (7.9%)</td>
</tr>
<tr>
<td>Dyslipidemia (n; %)</td>
<td>639 (39.2%)</td>
<td>231 (33.6%)</td>
<td>408 (43.3%)</td>
</tr>
<tr>
<td>Cerebrovascular disease (n; %)</td>
<td>68 (4.2%)</td>
<td>34 (4.9%)</td>
<td>34 (3.6%)</td>
</tr>
<tr>
<td>Cardiovascular disease (n; %)</td>
<td>75 (4.6%)</td>
<td>50 (7.3%)</td>
<td>25 (2.7%)</td>
</tr>
<tr>
<td>Cancer (n; %)</td>
<td>40 (2.5%)</td>
<td>25 (3.6%)</td>
<td>15 (1.6%)</td>
</tr>
<tr>
<td>Cerebral small vessel disease (n; %)</td>
<td>402 (24.8%)</td>
<td>178 (26.1%)</td>
<td>224 (23.9%)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>136.6±17.1</td>
<td>136.8±16.4</td>
<td>136.5±17.6</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>84.3±9.8</td>
<td>86.4±9.7</td>
<td>82.8±9.5</td>
</tr>
<tr>
<td>Cardio Ankle Vascular Index: Right</td>
<td>9.0±1.1</td>
<td>9.2±1.1</td>
<td>8.8±1.1</td>
</tr>
<tr>
<td>Cardio Ankle Vascular Index: Left</td>
<td>8.9±1.1</td>
<td>9.1±1.0</td>
<td>8.7±1.0</td>
</tr>
<tr>
<td>Skeletal muscle mass (kg/m²)</td>
<td>7.1±1.1</td>
<td>7.9±0.9</td>
<td>6.5±0.8</td>
</tr>
<tr>
<td>Muscle isokinetic strength (Nm/kg)</td>
<td>133.2±37.5</td>
<td>148.4±38.8</td>
<td>122.2±32.3</td>
</tr>
<tr>
<td>Hang-grip strength (kg)</td>
<td>25.9±7.1</td>
<td>32.3±5.7</td>
<td>21.2±3.5</td>
</tr>
<tr>
<td>Gait speed (m/sec)</td>
<td>1.9±0.4</td>
<td>2.0±0.4</td>
<td>1.8±0.3</td>
</tr>
<tr>
<td>Timed up and Go test (sec)</td>
<td>6.7±1.6</td>
<td>6.5±1.6</td>
<td>6.8±1.5</td>
</tr>
<tr>
<td>Instrumental Activities of Daily Living (point)</td>
<td>6.7±1.5</td>
<td>5.0±0.3</td>
<td>8.0±0.2</td>
</tr>
<tr>
<td>Depression: GDS≥10point (n; %)</td>
<td>31 (1.9%)</td>
<td>15 (2.2%)</td>
<td>16 (1.7%)</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dL)</td>
<td>100.5±16.7</td>
<td>104.5±18.2</td>
<td>97.6±14.8</td>
</tr>
<tr>
<td>Fasting plasma insulin (μU/mL)</td>
<td>4.9±3.3</td>
<td>5.0±3.4</td>
<td>4.8±3.2</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.8±0.6</td>
<td>5.9±0.6</td>
<td>5.8±0.5</td>
</tr>
</tbody>
</table>
For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml
in an urban part of Japan, (2) challenges in confirming causal relationships, and (3) a relatively low participation rate (18.9%), thus, we cannot deny the possibility of selection bias and we should be careful about the interpretation of the results. Before subject recruitment, we recognized that the participation rate in a similar study conducted in Japan was low (18.4%)\(^9\). Thus, we sent invitation letters twice after no initial reply and called the subjects before the briefing session to increase the participation rate. Other plans may be considered to further increase the participation rate in future studies of community dwelling.

**Collaborators**

For more information or potential collaboration, please contact Yoshifumi Tamura (ys-tamura@juntendo.ac.jp) and Yuki Someya (yksomeya@juntendo.ac.jp).

**Ethics and dissemination**

This study protocol was approved by the ethics committee of Juntendo University in November 2015 (No. 2015078, 2016138, 2016131, and 2017121). This study is being carried out in accordance with the principles outlined in the Declaration of Helsinki. All participants gave written informed consent at the orientation meeting. Participants were told that they have the right to withdraw from the trial at any time. Data are coded and stored securely in password-protected files. Only the principal investigators have access to the files. The findings of the study will be presented in local and international conferences and disseminated in peer-reviewed journals.
Authors’ contribution

YS, YT, HK, HW, and RK conceived the study and obtained grant funding. YS, YT, and HK drafted the protocol. SN advised on the statistical analysis. SK, HD, MI, KK, TM, SH, SK, SA, NH, YM, and HN reviewed and revised the protocol.

Funding statement

This work is supported by the Strategic Research Foundation at Private Universities (S1411006) and KAKENHI (18H03184) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, the Mizuno Sports Promotion Foundation, and the Mitsui Life Social Welfare Foundation.

Competing interests statement

The authors have nothing to disclose.

Data availability statement

Currently the data are not available since the cohort is ongoing.
Figure legend

Figure 1. Geographical location of Bunkyo-ku in Japan

Left: Japan is divided into 47 prefectures. The location of Tokyo is indicated by a circle.
Center: Tokyo is a prefecture that includes 23 special wards, 26 cities, 5 towns, and 8 villages. Bunkyo is one of the special wards (Bunkyo-ku in Japanese). It located in east Tokyo, as indicated by the circle. Right: Bunkyo-ku includes 68 communities and has 227,902 residents. (Source of map; http://www.start-point.net)

Figure 2. Experimental protocol

Figure 3. Flowchart of the participant recruitment process

Figure 4. Distribution of the elderly population in this cohort, Bunkyo-ku, and Tokyo

The proportion of elderly individuals in each age group (age 65–84 years) in this cohort, Bunkyo-ku, and Tokyo are presented.
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731 articular cartilage shows a greater degree of deterioration than that of the tibial and
732 patellar articular cartilage in early stage knee osteoarthritis: a cross-sectional study.
734 [published Online First: 2014/10/04]
736 medial tibial osteophyte distance detected by T2 mapping MRI in patients with early-


Tokyo is a prefecture that includes 23 special wards, 26 cities, 5 towns, and 8 villages.

Bunkyo-ku is one of the special wards and includes 68 communities and 227,902 people.
Cognitive function, muscle function, brain magnetic resonance imaging (MRI) parameters, and other variables were evaluated.

Evaluation for incidences of cerebrovascular disease, falls, fractures, and other diseases every year by mail.

Re-evaluation for cognitive function, brain MRI parameters and other parameters measured at baseline except oral glucose tolerance test (OGTT).

Evaluation for incidences of cerebrovascular disease, falls, fractures, and other diseases every year by mail.

Re-evaluation for cognitive function, brain MRI parameters and other parameters measured at baseline except OGTT.
Figure 3

Elderly subjects living in 13 communities of Bunkyo-ku (n = 8,629)

Excluded (n = 6,646)
- Unknown address: 242
- Declined to participate: 292
- Could not participate: 22
- Did not reply: 6,090

Participating group briefing (n = 1,983)

Excluded (n = 153)
- Declined consent: 153

Informed consent obtained (n = 1,830)

Excluded (n = 201)
- Withdrew consent: 190
- Insulin treatment: 4
- Pacemaker implantation: 4
- Did not meet age criteria: 3

This cohort subjects: 1,629
Figure 4

Population rate of each age in elderly population in Tokyo and Bunkyo-ku.

- Tokyo
- Participants (Male)
- Participants (Female)
- Bunkyo-ku
<table>
<thead>
<tr>
<th>Section/Topic</th>
<th>Item #</th>
<th>Recommendation</th>
<th>Reported on page #</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Title and abstract</strong></td>
<td>1</td>
<td><em>(a)</em> Indicate the study’s design with a commonly used term in the title or the abstract</td>
<td>1, 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>(b)</em> Provide in the abstract an informative and balanced summary of what was done and what was found</td>
<td>3</td>
</tr>
<tr>
<td><strong>Introduction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Background/rationale</td>
<td>2</td>
<td>Explain the scientific background and rationale for the investigation being reported</td>
<td>5-7</td>
</tr>
<tr>
<td>Objectives</td>
<td>3</td>
<td>State specific objectives, including any pre-specified hypotheses</td>
<td>7</td>
</tr>
<tr>
<td><strong>Methods</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study design</td>
<td>4</td>
<td>Present key elements of study design early in the paper</td>
<td>8-9</td>
</tr>
<tr>
<td>Setting</td>
<td>5</td>
<td>Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection</td>
<td>8-9</td>
</tr>
<tr>
<td>Participants</td>
<td>6</td>
<td><em>(a)</em> <strong>Cohort study</strong>—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up</td>
<td>8, 10-11</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Case-control study</strong>—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Cross-sectional study</strong>—Give the eligibility criteria, and the sources and methods of selection of participants</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>(b)</em></td>
<td><strong>Cohort study</strong>—For matched studies, give matching criteria and number of exposed and unexposed cases</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Case-control study</strong>—For matched studies, give matching criteria and the number of controls per case</td>
<td></td>
</tr>
<tr>
<td>Variables</td>
<td>7</td>
<td>Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable</td>
<td>8</td>
</tr>
<tr>
<td>Data sources/ measurement</td>
<td>8*</td>
<td>For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group</td>
<td>-</td>
</tr>
<tr>
<td>Bias</td>
<td>9</td>
<td>Describe any efforts to address potential sources of bias</td>
<td>-</td>
</tr>
<tr>
<td>Study size</td>
<td>10</td>
<td>Explain how the study size was arrived at</td>
<td>9-10</td>
</tr>
<tr>
<td>Quantitative variables</td>
<td>11</td>
<td>Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why</td>
<td>-</td>
</tr>
<tr>
<td>Statistical methods</td>
<td>12</td>
<td><em>(d)</em> <strong>Cohort study</strong>—If applicable, explain how loss to follow-up was addressed</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Case-control study</strong>—If applicable, explain how matching of cases and controls was addressed</td>
<td>-</td>
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<tr>
<td><strong>Cross-sectional study</strong>—If applicable, describe analytical methods taking account of sampling strategy</td>
<td>(e) Describe any sensitivity analyses</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Results

<table>
<thead>
<tr>
<th>Participants</th>
<th>13*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed</td>
<td>10-11</td>
</tr>
<tr>
<td>(b) Give reasons for non-participation at each stage</td>
<td>10-11, Figure 3</td>
</tr>
<tr>
<td>(c) Consider use of a flow diagram</td>
<td>Figure 3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Descriptive data</th>
<th>14*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders</td>
<td>19-21</td>
</tr>
<tr>
<td>(b) Indicate number of participants with missing data for each variable of interest</td>
<td>-</td>
</tr>
<tr>
<td>(c) <strong>Cohort study</strong>—Summarise follow-up time (eg, average and total amount)</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcome data</th>
<th>15*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cohort study</strong>—Report numbers of outcome events or summary measures over time</td>
<td>-</td>
</tr>
<tr>
<td><strong>Case-control study</strong>—Report numbers in each exposure category, or summary measures of exposure</td>
<td>-</td>
</tr>
<tr>
<td><strong>Cross-sectional study</strong>—Report numbers of outcome events or summary measures</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Main results</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included</td>
<td>-</td>
</tr>
<tr>
<td>(b) Report category boundaries when continuous variables were categorized</td>
<td>-</td>
</tr>
<tr>
<td>(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period</td>
<td>-</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Other analyses</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses</td>
<td>-</td>
</tr>
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</table>

### Discussion

<table>
<thead>
<tr>
<th>Key results</th>
<th>18</th>
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<tbody>
<tr>
<td>Summarise key results with reference to study objectives</td>
<td>-</td>
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</table>

<table>
<thead>
<tr>
<th>Limitations</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias</td>
<td>-</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence</td>
<td>-</td>
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<thead>
<tr>
<th>Generalisability</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discuss the generalisability (external validity) of the study results</td>
<td>-</td>
</tr>
</tbody>
</table>

### Other information

<table>
<thead>
<tr>
<th>Funding</th>
<th>22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based</td>
<td>23</td>
</tr>
</tbody>
</table>

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.