

1 **Title page**

2 **Extracellular-to-intracellular water ratios are associated with functional disability levels in patients**
3 **with knee osteoarthritis: Results from the Nagahama Study**

4

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26

27 **Abstract**

28 **Introduction/objectives:** To test the hypothesis that greater extracellular-to-intracellular water

29 (ECW/ICW) ratios in lower-limb muscles are associated with worsened functional abilities in patients

30 with knee osteoarthritis (OA).

31 **Methods:** We analyzed data from 787 participants (82.2% female; mean age, 69.6 ± 5.3 years) from the

32 Nagahama Prospective Cohort who were ≥ 60 years old and had radiographically confirmed bilateral knee

33 OA. The Knee Scoring System (KSS) was used to assess functional abilities. Lower-limb ECW/ICW

34 ratios and skeletal mass index values were determined with multi-frequency bioelectrical impedance

35 analysis (BIA). Multiple linear regression analysis was used to test for associations between ECW/ICW

36 ratios and functional abilities. Subgroup analyses based on OA severities and symptomatology were also

37 conducted.

38 **Results:** Increased ECW/ICW ratios were associated with a 4.38-point decrease in the KSS function
39 scores (95% confidence interval [CI], 3.15–5.62 points) after adjusting for covariates. This association
40 varied according to the degree of knee symptoms, especially in individuals with radiologically mild OA.
41 ECW/ICW ratios in individuals with asymptomatic mild OA were associated with a 2.14-point decrease
42 in the KSS function score (95% CI, 0.32–3.96 points), whereas those in individuals with severe
43 symptomatic mild OA were associated with a 6.16-point decrease (95% CI, 2.13–10.19 points).

44 **Conclusions:** Our findings indicate that higher ECW/ICW ratios are associated with greater functional
45 disability in patients with knee OA. Therefore, ECW/ICW ratio measurements with multi-frequency BIA
46 can serve as valuable indicators for functional disability in patients with knee OA.

47

48 **Key Points**

- 49 ● Higher extracellular-to-intracellular water (ECW/ICW) ratios are associated with greater functional
50 disability levels in patients with knee osteoarthritis (OA).
- 51 ● ECW/ICW ratios are useful clinical signs as a biomarker for poor functional abilities in patients with
52 knee OA.

53

54 **Keywords**

55 Knee osteoarthritis, functional disability, muscle quality, extracellular-to-intracellular water ratio,
56 bioelectrical impedance analysis

57

58

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76

77 **Conflicts of Interest:**

78 On behalf of all authors, the corresponding author states that there is no conflict of interest.

79

80 **Ethics approval:**

81 All study procedures were approved by the Ethics Committee of the Kyoto University Graduate School
82 of Medicine and the Nagahama Municipal Review Board (G278) and were conducted in accordance with
83 the principles of the Declaration of Helsinki.

84

85 **Consent to participate and consent for publication:**

86 Written informed consent for the use of data was obtained from all participants in the Nagahama Study.

87

88 **Availability of data and material:**

89 Data not available due to ethical restrictions.

90

91 **Code availability:**

92 Not applicable

93

94 **Authors' contributions:**

95 All authors have made substantial contributions to (1) the conception and design of the study; (2) revising

96 it critically for important intellectual content; and (3) final approval of the version to be submitted. The

97 specific contributions of each author are as follows:

98 (1) Analysis and interpretation of the data: MT, TI, TK, and NI.

99 (2) Drafting of the article: MT, TI, TK, and NI.

100 (3) Statistical expertise: MT and TK.

101

102 **Acknowledgments**

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106

107 **Text**

108 **Introduction**

109 Dysfunction in thigh muscles is an established risk factor for incident knee osteoarthritis (OA) and the
110 loss of functional abilities [1-3]. However, decreased thigh muscle mass cannot fully explain the muscle
111 weakness observed in patients with knee OA, so researchers have suggested that high levels of muscular
112 fat infiltration, which indicate poor muscle quality, accompany muscle dysfunction in such patients [4,5].
113 Therefore, examining local measures of muscle composition may elucidate the causes of functional
114 disability in patients with knee OA.

115 A recent meta-analysis [6] reported that thigh muscle fat infiltration levels are higher in
116 patients with knee OA than in healthy controls. Furthermore, models with adjustments for body mass
117 index (BMI) values show that patients with knee OA have elevated levels of skeletal adipose tissue
118 within the quadriceps muscle [7]. Interestingly, lower physical functionality in patients with knee OA
119 are associated with higher intramuscular fat fractions but not with muscle size [7]. Additionally,
120 previous studies [8,9] have reported that greater fat infiltration levels are associated with OA progression
121 and knee pain. Therefore, greater fat infiltration levels may worsen mobility and the ability to perform
122 activities of daily living, and knee OA severities may influence the association between knee function
123 and muscle quality.

124 Multi-frequency bioelectrical impedance analysis (BIA) is a convenient, affordable, and

125 noninvasive method for measuring skeletal muscle mass and adipose tissue levels within localized
126 regions. Skeletal muscle tissue contains abundant water, and multi-frequency BIA can separately
127 evaluate intracellular water (ICW) and extracellular water (ECW) [10,11]. ICW generally reflects
128 muscle cell mass, and ECW reflects adipose tissue and interstitial fluid in the extracellular space [12].
129 Higher ECW/ICW ratios are indicative of greater levels of noncontractile tissue relative to skeletal
130 muscle and are therefore biomarkers for loss of muscle quality [13]. Higher ECW/ICW ratios in lower-
131 extremity muscles are associated with physical impairments independently of muscle mass, age, sex,
132 and BMI [13]. However, no previous study has investigated the association between ECW/ICW ratios
133 and functional abilities in patients with knee OA. It is also unknown whether this potential association
134 is affected by the radiological grade and/or degree of knee pain.

135 The purpose of this study was to examine the associations between ECW/ICW ratios and
136 functional abilities in patients with knee OA. We hypothesized that greater ECW/ICW ratios would be
137 associated with worsened functional abilities in patients with knee OA. We also hypothesized that such
138 associations would be particularly strong in patients with severe OA and those experiencing symptoms
139 of knee pain or stiffness.

140

141

142 **Materials and Methods**

143 *Study participants and selection*

144 This cross-sectional study was conducted with participants from the Nagahama Prospective Cohort for
145 Comprehensive Human Bioscience (herein referred to as the Nagahama Study). The Nagahama Study's
146 participants were recruited between 2013 and 2016 from the general population of Nagahama City, a
147 city with 125,000 inhabitants located in a predominantly rural area of the Shiga Prefecture of central
148 Japan. In total, 9,850 individuals aged 35–81 years who lived independently in the community were
149 enrolled. Of these individuals, 4,990 were aged ≥ 60 years, underwent body composition analysis, and
150 completed a questionnaire about their habits in daily life, and 3,270 of those individuals underwent
151 optional knee X-rays. We further restricted our sample to 850 participants with radiographically
152 confirmed bilateral knee OA and then excluded 63 individuals with any of the following comorbidities:
153 rheumatoid arthritis, central or peripheral nervous system impairments, chronic obstructive pulmonary
154 disease, or chronic kidney disease necessitating dialysis. Ultimately, 787 individuals were included in
155 our analyses (Figure 1).

156 All study procedures were approved by the Ethics Committee of the Kyoto University
157 Graduate School of Medicine and the Nagahama Municipal Review Board (G278) and were conducted
158 in accordance with the principles of the Declaration of Helsinki. Written informed consent for the use
159 of data was obtained from all participants in the Nagahama Study.

160

161 *Assessments of physical characteristics, clinical features, and exercise habits*

162 Each individual's height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively, and
163 BMI values (in kg/m²) were calculated as weight divided by height squared. The presence of diabetes
164 or osteoporosis was detected by reviewing the cohort data. The participants reported their exercise
165 behaviors and whether they experienced back pain on a questionnaire concerning activities of daily
166 living. An exercise habit was defined as engaging in moderate-intensity physical activity for >30
167 minutes twice a week for at least a year.

168

169 *Definition of radiographically confirmed knee OA*

170 For bilateral X-ray knee assessments, anteroposterior weight-bearing views were obtained while
171 participants kept both knees fully extended. Two experienced orthopedists who were blinded to clinical
172 data evaluated the radiographic images according to the Kellgren-Lawrence (KL) grading system, with
173 radiographically confirmed knee OA being defined as KL grades ≥ 2 for both knees [14]. For the present
174 study, we defined mild knee OA as the presence of KL grades of 2 in both knees and moderate to severe
175 knee OA (greater OA severities) as the presence of KL grade ≥ 3 in one or both knees.

176

177 *Quantification of knee function and knee impairments*

178 The Knee Society's Knee Scoring System (KSS), a standard measure of knee function and knee

179 impairments, was used in the present study. The KSS is a self-administered assessment tool that reflects
180 physical function and radiographically determines knee OA grades in the general Japanese population
181 [15]. For the present analyses, we focused on two KSS categories: the functional activities category and
182 the symptoms category.

183 The functional activities category of the KSS was chosen to measure the degree of disability
184 in performing daily activities. This category is divided into four components: walking and standing (30
185 points), standard activities (30 points), advanced activities (25 points), and discretionary activities (15
186 points). The maximum possible functional activities score is 100 points, and higher scores represent
187 better physical function levels.

188 The symptom category of the KSS is based on three components: the degree of knee pain
189 during walking, the degree of knee pain while traveling up and down stairs, and knee stiffness. The
190 scores range from 25 (i.e., no pain or stiffness) to 0 (i.e., the worst possible pain and stiffness). For this
191 study, we categorized patients into groups based on three quantiles of the KSS symptom score as
192 follows: asymptomatic: KSS symptom score ≥ 23 , moderate: KSS symptom score ≥ 18 , and severe: KSS
193 symptom score < 18 .

194

195 *Quantification of lower-limb ECW/ICW ratios and skeletal muscle mass index values*

196 ECW/ICW ratios and skeletal muscle mass index (SMI) values in the lower limbs were assessed with a

multi-frequency BIA device (InBody 430; InBody Co., Seoul, Republic of Korea) that featured an eight-polar tactile-electrode impedance meter. Bioelectrical impedances were obtained in each leg at frequencies of 5 and 250 kHz and an alternating current of 250 A. The impedance measurements at 5 kHz (Z_5) mainly reflected ECW, and the impedance measurements at 250 kHz (Z_{250}) reflected ICW. In accordance with the protocols of previous studies [16,17], the impedance for the ECW (in cm^2/Ω) was calculated as $(\text{body height})^2/Z_5$. The impedance of the ICW compartment (Z_{250-5}) was calculated as $1/[(1/Z_{250}) - (1/Z_5)]$, and the impedance for the ICW (in cm^2/Ω) was calculated as $(\text{body height})^2/(Z_{250-5})$. For each individual, the ECW/ICW ratio in each leg was calculated as Z_5/Z_{250-5} , and the average ratio for both legs was then calculated. The summed muscle mass of both legs was divided by the square of the individual's height to yield a lower-limb SMI value (in kg/m^2) [18].

207

208 *Statistical analysis*

For descriptive analyses, continuous variables are expressed as means $\pm$ standard deviations (SDs), and categorical variables are expressed as counts and percentages.

For our primary analysis of whether ECW/ICW ratios were associated with functional abilities, we performed a multiple linear regression analysis with ECW/ICW ratios as the independent variable and KSS function scores as the dependent variable. A multiple linear regression analysis was conducted with adjustments for lower-limb SMI values, age, sex, BMI values, radiographically

215 measured OA severities, symptomaticity, and the presence or absence of diabetes, osteoporosis, exercise
216 habit, and back pain.

217 We also performed several secondary analyses. First, we repeated the primary analysis in
218 each subgroup, separated by radiographically determined OA severity (i.e., mild or greater OA
219 severities) and by three quantiles of the KSS symptom score (i.e., asymptomatic, moderate, or severe),
220 with the resulting scheme including six subgroups. Second, we performed a multiple linear regression
221 analysis with ECW/ICW ratios as the dependent variable to identify the variables associated with
222 ECW/ICW ratios. All statistical analyses were performed with SPSS software version 25.0 (SPSS Japan
223 Inc., Tokyo, Japan). The statistical significance threshold was set at $p < 0.05$.

224

225

226 **Results**

227 Of the 787 individuals in our sample, 82.2% were female. The mean age was 69.6 ± 5.3 years, and the
228 mean BMI value was 23.4 ± 3.2 kg/m². The mean KSS function and symptoms scores were 82.3 ± 17.4
229 points and 19.0 ± 6.0 points, respectively. Table 1 shows the baseline characteristics of the individuals
230 in our sample.

231 In the primary analysis, an increased ECW/ICW ratio was associated with a 4.38-point
232 decrease in the KSS function score (95% confidence interval [CI], 3.15–5.62 points) after adjustments

for covariates (Table 2). In subgroup analyses, the association varied according to the degree of knee symptoms, especially in individuals with mild OA (Table 3). For example, an increase in ECW/ICW ratio was associated with a 2.14-point decrease in the KSS function score (95% CI, 0.32–3.96 points) in individuals with asymptomatic mild OA but with a 6.16-point decrease (95% CI, 2.13–10.19 points) in those with severe symptomatic mild OA.

In exploratory analyses, greater lower-limb SMI values, higher BMI, and the presence of an exercise habit were associated with lower ECW/ICW ratios, and severe OA, worse KSS symptom scores, older, female sex, and the presence of osteoporosis were associated with greater ECW/ICW ratios (Table 4).

Discussion

The present study is the first to show that higher ECW/ICW ratios, which reflect greater noncontractile tissue masses within the skeletal muscles, are associated with worse KSS function scores in patients with knee OA. This association was particularly strong in individuals who were symptomatic and had greater OA severities. These results are consistent with our hypotheses.

Recently, Misra et al. [19] reported that body composition-based obesity and sarcopenic obesity, but not sarcopenia, are associated with knee OA, and an earlier study [20] reported that higher

251 ratios of fat mass to muscle mass, as measured with BIA, are associated with symptomatic knee OA.
252 These previous findings suggest that greater adipose tissue levels are associated with knee OA.
253 Adiposity enhances the metabolic effect of adipose tissue products such as cytokines and adipokines,
254 which regulate chondrocyte anabolism and thus play key roles in joint cartilage pathophysiology [21].
255 Furthermore, an association between adiposity and knee OA suggests that obesity increases mechanical
256 stress across the knee joint and thus leads to cartilage damage [22]. However, there is no consensus
257 regarding the association between high BMI values and functional disability [23-25]. This disagreement
258 may arise from the fact that the BMI formula does not distinguish between fat mass and lean body mass.
259 Our results indicate that higher ECW/ICW ratios, which indicate relative increase of adipose tissue to
260 muscle mass, are associated with low KSS function scores in patients with knee OA.

261 The results of the primary analysis indicated that not only higher ECW/ICW ratios but also
262 OA severities and symptomaticity were associated with worse KSS function scores. Furthermore, our
263 findings in subgroup analyses suggested that the association between an increase in ECW/ICW ratio
264 and functional ability was strong in individuals with more severe symptoms who had radiologically
265 mild knee OA. Muscle inflammation and increased adipose tissue levels within the quadriceps muscle
266 often accompany symptomatic knee OA and may contribute to physical dysfunction in patients [26,27].
267 A previous study [7] that used magnetic resonance imaging (MRI) to evaluate quadriceps muscle mass
268 and muscular fat fractions in patients with knee OA reported that muscular fat fractions, but not muscle

269 mass, are associated with pain and self-reported functional disability. This is consistent with our
270 observation that lower muscle quality, as measured with multi-frequency BIA, is associated with self-
271 reported disability. Thus, our results and those of other studies suggest that muscle quality is an
272 important factor related to physical dysfunction, especially in patients with symptomatic severe knee
273 OA.

274 The ECW/ICW ratio is a recognized biomarker for muscle quality because it is associated
275 with muscle strength and physical function independent of age, BMI values, and SMI values in
276 community-dwelling older adults [28]. Our results indicate that increased ECW/ICW ratios in patients
277 with knee OA are associated with decreased lower-limb SMI values, worsened symptoms, and greater
278 OA severities. Therefore, increased ECW/ICW ratios may be useful biomarkers for symptomaticity and
279 poor functional abilities in patients with knee OA. Moreover, the results of exploratory analyses showed
280 that advanced age, female sex, and the presence of osteoporosis were associated with greater ECW/ICW
281 ratios. As the amount of physical activity is reduced in postmenopausal females with osteoporosis [29],
282 the increased ECW/ICW ratios could be caused by inactivity. In fact, our results indicated that the
283 presence of an exercise habit was associated with lower ECW/ICW ratios. Approaches to factors that
284 are associated with ECW/ICW ratios may also be important for improving functional abilities in patients
285 with knee OA.

286 Although multi-frequency BIA lacks the ability of MRI and computed tomography (CT) to

287 differentiate individual thigh muscles, it is free of the major limitations of quantitative methods such as
288 MRI, CT, and dual-energy X-ray absorptiometry (DXA), which include inconvenience, high costs, and
289 radiation exposure. The lower-limb lean tissue mass measurements, obtained with the device used in
290 the present study, correlate strongly with lower-limb muscle mass measurements obtained with DXA
291 [30]. Multi-frequency BIA therefore shows great potential as a tool for future research into markers of
292 muscle quality in patients with knee OA.

293 This study has some limitations. First, its cross-sectional design means that it could not
294 determine whether increased ECW/ICW ratios cause dysfunction in patients with knee OA.
295 Intramuscular fat content is a predictor of OA progression [8], so future studies should examine whether
296 increased ECW/ICW ratios worsen functional disability in patients with knee OA. Second, our focus on
297 Nagahama Study participants who opted for X-ray examinations is a possible source of selection bias.
298 Indeed, when we compared the characteristics of our study population with those of Nagahama Study
299 participants who did not opt for X-ray examinations, we found that the latter group had lower KSS
300 function scores and higher ECW/ICW ratios (Supplementary Table). If many of the participants who
301 did not undergo X-ray examinations had bilateral knee OA, then our analyses could have underestimated
302 the strength of the association between functional disability and muscle quality. Finally, multi-frequency
303 BIA with an eight-polar tactile electrode cannot differentiate the thigh from the shank. However,
304 segmental-bioelectrical impedance spectroscopy (S-BIS) has recently been used for regionally specific

305 muscle quality assessments [31-33]. Future studies should use longitudinal S-BIS assessments of
306 muscle quality in the thigh to clarify how poor muscle quality influences functional disability in patients
307 with knee OA.

308 In conclusion, higher ECW/ICW ratios are associated with greater functional disability levels
309 in patients with knee OA, and the association is stronger in the patients with symptomatic OA and
310 greater OA severities. Muscle quality assessments based on multi-frequency BIA measurements of
311 ECW/ICW ratios are therefore more useful than muscle quantity assessments as a biomarker for poor
312 functional abilities in patients with knee OA.

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315 **Conflicts of Interest:**

316 On behalf of all authors, the corresponding author states that there is no conflict of interest.

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323

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437

438 **Table 1.** Characteristics of the individuals with knee OA

Characteristic	Mean $\pm$ SD or n (%)
ECW/ICW ratio	5.2 $\pm$ 0.8
Lower-limb SMI, kg/m ²	2.4 $\pm$ 0.3
OA severity	
Mild; KL grades = 2 for both knees	539 (68.5%)
Moderate-severe; KL grade $\geq$ 3 in one or both knees	248 (31.5%)
Symptom severity	
Less: KSS symptom score $\geq$ 23	275 (34.9%)
Mild: KSS symptom score $\geq$ 18	264 (33.6%)
Severe: KSS symptom score <18	248 (31.5%)
Diabetes	79 (10.0%)
Osteoporosis	143 (18.2%)
Exercise Habit: >2 days/wk	328 (41.7%)
Back Pain	434 (55.1%)

439 Symptom severities were categorized into patient subgroups based on three quantiles of the KSS
 440 symptom score.

441 Abbreviations: ECW/ICW, extracellular-to-intracellular water; KSS, Knee Scoring System; OA,
 442 osteoarthritis; SD, standard deviation; SMI, skeletal muscle mass index

443

444 **Table 2.** Associations between study variables and KSS function scores

Variable	Association with KSS function scores		
	Regression coefficient	95% CI	<i>P</i> value
ECW/ICW ratio	-4.38	-5.62 to -3.15	<0.001
Lower-limb SMI, kg/m ²	2.29	-0.76 to 2.40	0.340
Greater OA severities	-2.14	-4.09 to -0.20	0.031
Symptom severities: Less	ref.		
Mild	-5.75	-7.85 to -3.65	<0.001
Severe	-19.90	-22.2 to -17.6	<0.001
Age, y	-0.40	-0.58 to -0.22	<0.001
Female sex	0.49	-2.66 to 3.64	0.760
BMI, kg/m ²	-1.17	-1.50 to -0.84	<0.001
Diabetes	-3.29	-5.98 to 0.43	0.022
Osteoporosis	-1.09	-3.40 to 1.22	0.355
Exercise habit	3.07	1.31 to 4.83	0.001
Back pain	-4.25	-6.07 to -2.43	<0.001

445 A multiple linear regression analysis was conducted with ECW/ICW ratios as the independent variable
446 and KSS function scores as the dependent variable, with adjustments for lower-limb SMI values, age,
447 sex, BMI values, OA severities (reference, mild OA), symptomatology, and the presence or absence of
448 diabetes, osteoporosis, an exercise habit, and back pain.

449 Abbreviations: BMI, body mass index; CI, confidence interval; ECW/ICW, extracellular-to-intracellular
450 water; KSS, Knee Scoring System; OA, osteoarthritis; Ref, reference; SMI, skeletal muscle mass index

Table 3. Associations between ECW/ICW ratios and KSS function scores for subgroups defined by OA severities and symptomaticity

Category		n	Change in KSS function scores with ECW/ICW ratios		
OA severity	Symptom severity		Regression coefficient (B)	95% CI	P value
Mild OA	Asymptomatic	233	-2.14	-3.96 to -0.32	0.022
	Moderate	187	-3.86	-6.43 to -1.29	0.003
	Severe	119	-6.16	-10.19 to -2.13	0.003
Greater OA severities	Asymptomatic	42	-6.10	-9.94 to -2.26	0.003
	Moderate	77	-4.36	-9.04 to 0.32	0.067
	Severe	129	-5.36	-8.67 to -2.06	0.002

The secondary analysis was conducted in six subgroups, separated by radiographically determined OA severity (i.e., mild or greater OA severities) and by three quantiles of the KSS symptom score (i.e., asymptomatic, moderate, or severe). A multiple linear regression analysis was conducted with adjustments for lower-limb SMI values, age, sex, BMI values, radiographically measured OA severities, symptomaticity, and the presence or absence of diabetes, osteoporosis, an exercise habit, and back pain.

Regression coefficient represents changes in KSS function score (points) in each group.

Abbreviations: CI, confidence interval; ECW/ICW, extracellular-to-intracellular water; KSS, Knee Scoring System; OA, osteoarthritis

461 **Table 4.** Factors associated with ECW/ICW ratios

Variable	Association with ECW/ICW ratios		
	Regression coefficient	95% CI	<i>P</i> value
Lower-limb SMI (kg/m ²)	-0.42	-0.68 to -0.15	0.002
Greater OA severities	0.18	0.07 to 0.29	0.002
Symptom severities; asymptomatic	ref		
moderate	0.11	-0.10 to 0.23	0.072
severe	0.23	0.10 to 0.36	0.001
Age, y	0.04	0.03 to 0.05	<0.001
Female sex	0.37	0.19 to 0.55	<0.001
BMI, kg/m ²	-0.03	-0.05 to -0.01	0.006
Diabetes	0.12	-0.04 to 0.28	0.143
Osteoporosis	0.27	0.14 to 0.40	<0.001
Exercise habit	-0.16	-0.26 to -0.06	0.002
Back pain	0.02	-0.09 to 0.12	0.720

462 A multiple linear regression analysis was conducted with ECW/ICW ratios as the dependent variable to
463 identify the variables associated with ECW/ICW ratios.

464 Abbreviations: BMI, body mass index; CI, confidence interval; ECW/ICW, extracellular-to-intracellular
465 water; KSS, Knee Scoring System; OA, osteoarthritis; Ref, reference; SMI, skeletal muscle mass index

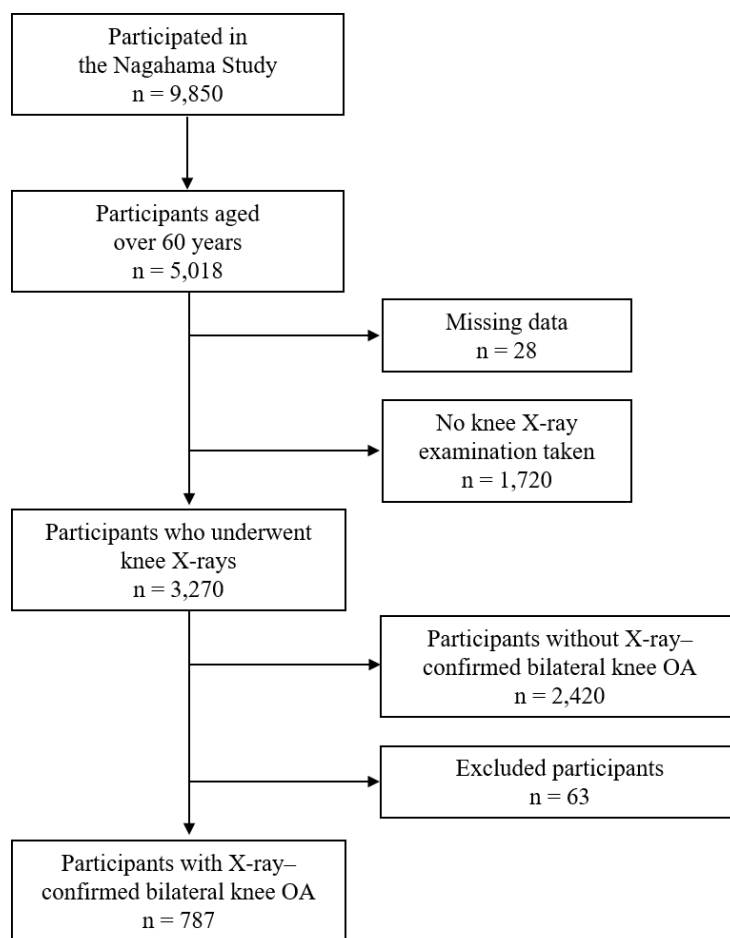


Fig. 1 Flowchart for selection of participants from the Nagahama Study

Abbreviation: OA, osteoarthritis