

1 **Serum 25-hydroxy vitamin D and the risk of low muscle mass in young and middle-aged**
2 **Korean adults**

3 **Short title:** Serum 25(OH)D and low muscle mass

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36

ABSTRACT

37 **Objective:** Despite known benefit of vitamin D in reducing sarcopenia risk in older adults, its
38 effect against muscle loss in young population is unknown. We aimed to examine the
39 association of serum 25-hydroxy vitamin D [25(OH)D] level and its changes over time with
40 the risk of incident low muscle mass (LMM) in young and middle-aged adults.

41 **Design:** A cohort study

42 **Methods:** The study included Korean adults (median age, 36.9 years) without LMM at baseline
43 followed up for a median of 3.9 years (maximum, 7.3 years). LMM was defined as the
44 appendicular skeletal muscle (ASM) mass by body weight (ASM/weight) of one standard
45 deviation below the sex-specific mean for young reference group. Cox-proportional hazard
46 models were used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs).

47 **Results:** Among 192,908 individuals without LMM at baseline, 19,526 developed LMM. After
48 adjusting for potential confounders, the multivariable-adjusted HRs (95% CIs) for incident
49 LMM comparing 25(OH)D levels of 25–<50, 50–<75, and ≥ 75 nmol/L to 25(OH)D <25
50 nmol/L were 0.93 (0.90-0.97), 0.85 (0.81-0.89), and 0.77 (0.71-0.83), respectively. The inverse
51 association of 25(OH)D with incident LMM was consistently observed in young (aged <40
52 years) and older individuals (aged ≥ 40 years). Individuals with increased 25(OH)D levels (<50
53 to ≥ 50 nmol/L) or persistently adequate 25(OH)D levels (≥ 50 nmol/L) between baseline and
54 follow-up visit had lower risk of incident LMM than those with persistently low 25(OH)D
55 levels.

56 **Conclusions:** Maintaining sufficient serum 25(OH)D could prevent unfavourable changes in
57 muscle mass in both young and middle-aged Korean adults.

58

INTRODUCTION

Sarcopenia is characterized by a progressive decline in skeletal muscle mass and muscle strength¹ and represents a major public health concern in older adults. Sarcopenia can lead to serious health consequences that impair the quality of life and poses a considerable burden on healthcare systems^{1,2}. Although sarcopenia is more commonly associated with older ages, there is growing recognition that sarcopenia also occurs early in life, partly due to increased sedentary lifestyle and physical inactivity in modern young population^{3,4}. However, risk or protective factors associated with sarcopenia or muscle loss in younger individuals have not been adequately addressed and remain largely unknown.

Beyond its widely recognized effects on bone health, vitamin D is known to affect skeletal muscle via vitamin D receptors (VDRs)⁵. The link between low serum 25-hydroxyvitamin D [25(OH)D] levels, a reliable marker of vitamin D status, and the risk of sarcopenia in older individuals is well established⁶. Several cross-sectional studies explored the relationship between vitamin D deficiency and muscle mass, but the results were conflicting^{7,8}. Also, findings from small randomized-controlled trials (RCTs) showed no benefit of vitamin D supplementation on muscle mass gain in young individuals^{9,10}. With the lack of large and high-quality studies, it remains unclear whether vitamin D has any protective effect against low muscle mass (LMM) development in young people. In addition, no studies have yet to evaluate the effect of changes in serum 25(OH)D levels over time on the risk of developing LMM.

Thus, we examined the association of serum 25(OH)D level and its changes over time with the risk of incident LMM in young and middle-aged adults without LMM at baseline.

MATERIALS AND METHODS

83

84 *Study Participants*

85 The Kangbuk Samsung Health Study is a cohort study of Korean men and women aged
86 ≥ 18 years who participated in comprehensive health examinations every 1-2 years at Kangbuk
87 Samsung Hospital Total Healthcare Center in Seoul and Suwon, South Korea, as previously
88 described¹¹. The present cohort study included participants who underwent comprehensive
89 health examinations between January 2012 and December 2018. From 2012, data on
90 appendicular skeletal muscle mass and serum 25(OH)D levels were available, and all
91 participants had at least one follow-up visit between recruitment and December 31, 2020
92 (n=208,026 participants). A total of 39,656 participants were excluded in a two-step selection
93 process (See **Supplementary Material** for detailed exclusion criteria)

94 This study was approved by the Institutional Review Board of Kangbuk Samsung
95 Hospital (IRB No. KBSMC 2021-09-032), which waived the requirement for informed consent
96 because de-identified retrospective data routinely collected during health screenings were used.

97

98 *Measurements*

99 At baseline and follow-up visits, information on demographic factors, lifestyle factors
100 such as physical activity, medical history, and medication use was obtained using standardized,
101 self-administered questionnaires¹¹.

102 Blood pressure, height, weight, and body composition measurements were performed by
103 trained nurses. A multi-frequency bio- impedance analyzer (BIA) with eight-point tactile
104 electrodes (InBody 720; Biospace Inc., Seoul, Korea) was used to measure body composition
105 including lean body mass of individuals' limbs, appendicular skeletal muscle mass (ASM) and
106 fat mass. Skeletal muscle mass index (SMI) was calculated using BIA as SMI

107 (%)=appendicular skeletal muscle mass (kg)/body weight (kg)×100, according to the methods
108 by Janssen et al¹². Class I LMM was defined as an SMI within minus one to minus two standard
109 deviations below the mean values of young adults, and class II LMM was defined as SMI below
110 minus two standard deviations below the mean values of young adults¹². Because early
111 detection of muscle mass loss in young adults is important, incident LMM was defined
112 according to class I LMM development. (See **Supplementary Material** for definition of SMI)

113 Blood specimens were collected after a fasting period of at least 10 hours and fasting
114 blood tests evaluated glycemic parameters, lipid profiles, liver enzyme levels, high-sensitivity
115 C-reactive protein (hsCRP) levels and 25(OH)D levels (See **Supplementary Material**). Serum
116 25(OH)D levels were categorized as <10, 10-<20, 20-<30, and ≥30 ng/mL (For conversion to
117 SI units: ng/mL×2.5=nmol/L; e.g., <25, 25-<50, 50-<75, and ≥75 nmol/L)¹³. Despite some
118 controversy, serum 25(OH)D level >20 ng/mL (>50 nmol/L) is considered sufficient for
119 skeletal health in the healthy general population^{14, 15}. Therefore, the change in 25(OH)D status
120 from baseline to the second visit was analysed in the following four groups based on the
121 presence/absence of insufficient serum 25(OH)D (defined as serum 25(OH)D level <20 ng/mL
122 [50 nmol/l]): a) insufficient 25(OH)D level at baseline and follow-up (persistently low); b)
123 insufficient 25(OH)D level at baseline but no insufficiency at follow-up (increased); c) no
124 insufficiency at baseline but insufficiency at follow-up (decreased); and d) no 25(OH)D
125 insufficiency at baseline and follow-up (persistently adequate).

126

127 ***Statistical Analyses***

128 The primary outcome was the development of incident LMM. Each participant was
129 followed from the baseline visit until either the occurrence of incident LMM or the last health
130 examination conducted through the end of 2020, whichever occurred first. The incidence rates

131 were calculated as the number of incident cases divided by person-years of follow-up. Cox
132 proportional hazard models were used to estimate the hazard ratios (HRs) with 95% confidence
133 intervals (CIs) for incident LMM in each 25(OH)D category compared with the reference
134 category, while using three models with progressive adjustment to control for potential
135 confounders (See **Supplementary Material**).

136 STATA version 16.0 (Stata Corp., College Station, TX, USA) was used for data analysis.
137 All *P*-values were two-tailed, and *P*-values <0.05 were considered statistically significant.

138

139 RESULTS

140 The median age of the participants was 36.9 years (interquartile range, 32.4-41.8 years),
141 and 44.5% of patients were females. At baseline, the proportions of participants with 25(OH)D
142 levels <25, 25-<50, 50-<75, and ≥ 75 nmol/L were 16.2%, 56.6%, 21.9%, and 5.3%,
143 respectively (**Table 1**). Serum 25(OH)D levels were positively associated with age, alcohol
144 intake, physical activity, education level, medication use for hyperlipidaemia, and use of
145 multivitamin, vitamin D, and/or calcium supplements (**Table 1**). Baseline characteristics of the
146 study participants are also presented according to 25(OH)D levels at baseline and subsequent
147 visits (**eTable 1**).

148 Within 720,713.2 person-years of follow-up (median, 3.9 years; interquartile range, 2.1-
149 5.0 years; maximum, 7.3 years), 19,526 participants developed LMM (incidence rate, 27.1 per
150 1,000 person-years) (**Table 2**). Overall, baseline 25(OH)D levels were inversely associated
151 with the risk of incident LMM. After adjusting for age, sex, physical activity, and other
152 potential confounders (Model 1), HRs (95% CI) for incident LMM at baseline 25(OH)D levels
153 of 25-<50, 50-<75, and ≥ 75 nmol/L (compared to the reference, <25 nmol/L) were 0.93 (0.90-
154 0.97), 0.83 (0.79-0.88), and 0.67 (0.62-0.73), respectively. Further adjustment for either BMI

155 or waist circumference attenuated the association, which remained significant (**Table 2**, Model
156 2, and **eTable 2**). The inverse association was consistently observed in men and women but
157 with a slightly stronger effect in men than in women (P for interaction=0.025). The association
158 between 25(OH) level and incident LMM became stronger in time-dependent analyses than in
159 the original analyses. Corresponding HRs (95% CI) comparing 25(OH)D levels of 25-<50, 50-
160 <75, and ≥ 75 nmol/L to <25 nmol/L were 0.79 (0.76-0.83), 0.65 (0.62-0.68), and 0.52 (0.48-
161 0.56), respectively. In spline regression models, the LMM risk decreased across the range of
162 the 25(OH) level in both men and women (**Figure 1**). Similar results were observed in a
163 sensitivity analysis using LMM defined as SMI less than minus two standard deviations below
164 the mean values of young adults (**eTable 3**).

165 Changes in 25(OH)D levels from baseline to follow-up were significantly associated with
166 the risk of incident LMM without any significant interaction by sex (p for interaction=0.326)
167 (**Table 3**). The multivariable-adjusted HRs (95% CI) for the “decreased,” “increased,” and
168 “persistently adequate” groups versus the “persistently low” group for LMM development were
169 0.84 (0.77-0.92), 0.85 (0.79-0.91), and 0.81 (0.75-0.87), respectively (Model 2). The
170 significant associations persisted after serum 25(OH)D levels and other confounders were
171 considered time-varying variables.

172 In subgroup analyses (**eTable 4**), the association between 25(OH)D level and incident
173 LMM differed with respect to hypertension, insulin resistance, and inflammation status; the
174 association was evident in participants without either homeostasis model assessment of insulin
175 resistance (HOMA-IR) of ≥ 2.5 or hypertension but was attenuated in those with either insulin
176 resistance or hypertension (p for interaction <0.001 and 0.002, respectively). The graded dose-
177 response association between 25(OH)D levels and incident LMM was slightly stronger in those
178 with hsCRP <1.0 mg/L than in those with hsCRP ≥ 1.0 mg/L (p for interaction=0.018).

179 Otherwise, there were no other significant interactions by subgroup, including the age group
180 (<40 vs. ≥40 years). Participants taking vitamin D supplements tended to engage in a healthier
181 lifestyle including physical activity and less smoking (**eTable 5**); however, after adjustments
182 for physical activity and smoking status, there was an independent and inverse association
183 between serum 25(OH)D levels and incident LMM. Additionally, in subgroup analyses, these
184 associations were similarly observed, and there was no significant interaction, by smoking
185 status, alcohol intake, and physical activity.

186 DISCUSSION

187 In this large cohort study of young Korean adults without LMM at baseline, serum
188 25(OH)D levels were inversely associated with LMM development in a dose-response manner.
189 The protective association between higher serum 25(OH)D levels and decreased LMM
190 incidence was consistently observed irrespective of sex and age. Furthermore, increases in
191 25(OH)D levels from insufficient levels at baseline to 50 nmol/L at follow-up and adequate
192 25(OH)D levels over time were associated with lower risk of incident LMM; these associations
193 were independent of factors such as vitamin D supplementations, exercise, BMI, or season of
194 the blood draw.

195 Most studies exploring the link between vitamin D and sarcopenia/LMM by far were
196 almost exclusively focused on older adults, the effect of vitamin D in the risk of LMM among
197 younger adults is unknown. There are few cross-sectional studies that have explored the effects
198 of serum vitamin D levels in muscle mass in younger individuals. A study of 667 community-
199 dwelling adults aged 21-97 years showed significant associations between 25(OH)D levels and
200 muscle mass only in participants younger than 65 years⁸. Some other studies have also reported
201 the potential benefit of serum vitamin D on muscle mass; however, these studies were
202 undertaken in specific population subgroups (e.g., obese men)^{16, 17}. To our knowledge, our

203 study is the first cohort study showing that adequate serum 25(OH)D levels confer decreased
204 risk of incident LMM in young and middle-aged individuals without comorbidities. Also, while
205 there is scarce data on the prevalence of sarcopenia or LMM in young populations, a previous
206 report has estimated that, among adults aged 21-59 years, up to 32% have LMM and 7% have
207 sarcopenia, suggesting that it is already prevalent among younger adults¹⁸. Likewise, our
208 findings on the incident rate of LMM (27.1 per 1,000 person-years) further supports the notion
209 that LMM in young adults is no longer an uncommon condition.

210 In our study, persistently adequate serum 25(OH)D levels over time and increases in
211 serum 25(OH)D levels from being insufficient to sufficient were significantly associated with
212 decreased LMM risk. The effect of time-dependent changes of serum 25(OH)D levels on
213 preserving muscle mass has been uncertain, with a lack of comparable data. Two previous
214 RCTs evaluated the benefit of vitamin D supplementation and changes in vitamin D levels on
215 muscle mass and strength in young and middle-aged individuals, but neither found any
216 significant benefits of vitamin D in improving muscle mass^{9, 10}. However, it is difficult to
217 directly compare these study results with ours because these trials were underpowered with a
218 sample size <40 and had a very short-term follow-up (12 weeks) in a setting of resistance
219 training, wherein vitamin D was supplemented only as an adjunct intervention. In our large
220 sample of 192,908 healthy participants free of LMM at baseline, we could account for various
221 known confounders, as well as time-dependent variables. Also, the extended follow-up
222 duration of approximately 4 years allowed the extended time frame for us to better observe the
223 development of LMM over time. Although the possibility of residual confounding remains due
224 to unmeasured factors including sun exposure or outdoor physical activity, our findings suggest
225 that improved or persistently adequate serum 25(OH)D status over time may have benefit in
226 reducing the risk of incident LMM.

227 The present study has several important clinical implications. Earlier onset of sarcopenia
228 has constantly been increasing, especially in developed countries, possibly owing to changes
229 in lifestyle and diets¹⁸, and there is an emerging need for taking a life-course approach to
230 sarcopenia prevention during early years^{18, 19}. Given the progressive nature of, and the
231 seriousness of disability and complications associated with sarcopenia¹, preventing mild LMM
232 may in turn delay further loss of muscle mass, consequently lowering the risk of sarcopenia as
233 well as sarcopenia-related health consequences in later life. In light of this, our findings suggest
234 that the prevention of early unfavourable changes in muscle mass and mild LMM may be
235 achievable in young individuals by maintaining sufficient serum 25(OH)D levels. In addition,
236 the proportion of our study participants with sub-optimal 25(OH)D levels (<50 nmol/L) at
237 baseline (approximately 74%) is considerably higher than that in the United States and Europe
238 (24-40%)²⁰, although it is comparable to the previously reported national prevalence in the
239 Korean population²¹. We assume that a high proportion white-collar workers in our population
240 who are likely to have less sun exposure may have contributed to the relatively high prevalence
241 of sub-optimal serum 25(OH)D levels. Our findings thus highlight the importance of
242 maintaining adequate serum 25(OH)D levels to reduce the risk of LMM in populations with a
243 high prevalence of low vitamin D status. Large and well-designed intervention trials are
244 necessary to confirm our findings.

245 The mechanism by which serum 25(OH)D reduces LMM risk is not completely
246 understood, but recent studies confirm that VDR is expressed in skeletal muscle and that a
247 substantial level of signalling via VDR is required for normal muscle growth and muscle mass
248 maintenance²². In animal studies, VDR knockout mice had small and variable muscle fibers²³;
249 vitamin D deficiency in rats inhibited mammalian target of rapamycin complex 1 (mTORC1)
250 signalling and contributed to decreased protein synthesis in skeletal muscles²⁴, while VDR

251 overexpression induced muscle hypertrophy⁵. In human muscle tissue, VDR expression levels,
252 which decline with age, can be altered using vitamin D supplementation²⁵, indicating that
253 maintaining adequate 25(OH)D levels could reduce LMM risk. 25(OH)D may also stimulate
254 protein synthesis through mTORC1 signalling; this mechanism may play an important role in
255 muscle hypertrophy and muscle loss prevention²².

256 According to our subgroup analyses, the association between serum 25(OH)D and LMM
257 was attenuated in participants with insulin resistance defined as HOMA-IR ≥ 2.5 and/or
258 hypertension. Skeletal muscle is the key tissue responsible for insulin-stimulated glucose
259 disposal and is the major site of peripheral insulin resistance²⁶. Muscle mass is determined by
260 the balance between protein synthesis and breakdown in the tissue, and particularly in younger
261 people, insulin has a predominant role in inhibiting protein catabolism, thereby preventing
262 muscle atrophy²⁷. Insulin resistance thus may represent a state of “anabolic resistance” in
263 skeletal muscle, wherein the insulin-mediated suppression of muscle breakdown is inhibited,
264 potentially leading to increased proteolysis that may eventually result in sarcopenia²⁸. VDRs
265 are also involved in the pathogenesis of insulin resistance²⁹; a recent report showed that
266 decreased glucose uptake reduced VDR expression in a diabetic mouse model³⁰. Therefore, a
267 series of these interactive processes may act synergistically to attenuate the effect of vitamin
268 D. The reason for the null association observed in the presence of hypertension is unclear.
269 Hypertension is pathologically related to hyperactivity of the renin-angiotensin system (RAS),
270 and animal studies show the involvement of VDR activation in downregulating RAS³¹. High
271 circulating levels of angiotensin II decrease muscle protein homeostasis and accelerate
272 proteolysis, thereby promoting skeletal muscle fibre atrophy³². Thus, in hypertension, vitamin
273 D metabolism may not compensate for the effects of RAS overactivation. Future studies are
274 warranted to better elucidate the role of insulin resistance and hypertension in the association

275 between 25(OH)D and LMM.

276 This study had some limitations. First, we used bioimpedance analysis instead of dual-
277 energy X-ray absorptiometry (DEXA), which is the gold standard body composition
278 measurement for assessing muscle mass. DEXA, however, may expose participants to low
279 level ionizing radiation and is expensive to perform in large cohort studies. Second, we did not
280 collect information on variables that could influence the serum 25(OH)D levels such as vitamin
281 D intake via food consumption, details of amount of and frequency of vitamin D
282 supplementation (e.g., dose, frequency, and duration), outdoor activities, or sunlight exposure,
283 or presence of genetic polymorphism. Therefore, the potential for residual confounding
284 remains. Third, the reference values used in our study in defining LMM were derived from the
285 young adults in this study population since there is no available value derived from
286 representative sample of Korean population based on bio-impedance analysis. According to
287 the 4th and 5th KNHANES, the cutoff values of 1SD below the mean for DEXA-based SMI of
288 young adults were 32.2 % and 29.9 % respectively for men and 25.6 and 23.5% respectively
289 for women^{33,34}, which were similar to the cutoff values used in our study (30.2 % for men and
290 26.1% for women). Finally, our study participants represented a relatively young and healthy
291 Korean working population. Although this could be perceived as a limitation, it also
292 represented a strength of our study as relatively few study participants had existing
293 comorbidities that are associated with low serum 25(OH)D levels. Nevertheless, the
294 generalizability of our findings to other populations with comorbidities or different
295 sociodemographic characteristics may be limited.

296 In conclusion, we demonstrated that serum 25(OH)D levels are inversely associated with
297 LMM risk in young adults. Favorable changes in serum 25(OH)D levels from insufficient to
298 sufficient were associated with reduced LMM risk. Considering the importance of attaining

299 high peak muscle mass during adulthood for sarcopenia prevention, maintaining sufficient
300 serum 25(OH)D levels, which may be easily achieved by sun exposure or vitamin D
301 supplementation, could be an effective primary prevention strategy to slow muscle loss and its
302 associated consequences in later years.

303

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309

310 **Author Contributions:**

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312 **Yoonsoo Chang:** Study concept and design, acquisition of data, interpretation of data, and
313 drafting and critical revision of the manuscript

314 **Seungho Ryu:** Study concept and design, acquisition of data, analysis and interpretation of
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FIGURE LEGENDS

Figure 1 Multivariable-adjusted hazard ratios for the development of low muscle mass.

Curves represent adjusted hazard ratios for low muscle mass based on restricted cubic splines with knots at the 5th, 35th, 65th, and 95th percentiles of serum 25(OH)D distribution. Models were adjusted for age, sex (only for total), centre, year of screening examination, alcohol consumption, smoking, physical activity, total energy intake, education level, ongoing medication for hypertension and/or diabetes, multivitamin and/or calcium supplementation, season, and body mass index.