

# Does Protein Intake Affect Metabolic Risk Factors among Older Adults in Korea?

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**Background:** Carbohydrate intake can impact metabolic risk factors, but related research on protein intake in the elderly is rare. Our purpose was to estimate protein intake and explore how different levels of protein intake influence metabolic risk factors in Korean older adults.

**Methods:** Data were obtained from men aged 51–70 years (n=1,735), men aged ≥71 years (n=700), women aged 51–70 years (n=2,305), and women aged ≥71 years (n=957). Health and dietary data were obtained from the Korean National Health and Nutrition Examination Survey of 2010–2011.

**Results:** Relative to the recommended protein intake based on the Dietary Reference Intakes for Koreans, inadequate intake was higher in women and super-aging groups than in others. Women had an increased risk of having metabolic risk factors. After adjustment for age and sex, compared with those in the highest quartile of protein intake levels (>1.2 g protein/kg body weight/day), participants in the lowest quartile (<0.8 g protein/kg body weight/day) had increased odds ratios (ORs) for abdominal obesity (men: OR, 2.67; 95% confidence interval [CI], 2.00–3.56; women: OR, 3.42; 95% CI, 2.64–4.43), hypertriglyceridemia (men: OR, 1.44; 95% CI, 1.08–1.93; women: OR, 1.82; 95% CI, 1.45–2.29), and high fasting blood glucose (men: OR, 1.41; 95% CI, 1.07–1.87; women: OR, 2.03; 95% CI, 1.48–2.79).

**Conclusion:** Protein intake (<0.8 g protein/kg body weight/day) lower than the recommend level was associated with a higher risk of metabolic abnormalities in Korean older adults. In particular, lower intake of protein contributed to a higher prevalence of metabolic risk factors in women than in men.

**Key words:** Protein intake, Metabolic abnormality, Body composition, Adults

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## INTRODUCTION

Metabolic syndrome (MS) is closely linked with an increased risk of chronic diseases such as cardiovascular disease and all-cause mortality both in Korea and worldwide.<sup>1-3</sup> Among Korean adults, the prevalence of MS was 28.1% in 2001, 29.2% in 2005, and 31.3% in 2007.<sup>4</sup> The prevalence is even higher in the elderly. According to a report<sup>5</sup>, Korea has become a super-aging society like Japan, and the proportion of the elderly has been increasing rapidly, with the elderly projected to account for 14.3% of the population by 2018. Therefore, MS is one of the major public health and aging

concerns among Korean older adults.

A number of studies have found that impaired glucose metabolism, such as insulin resistance (IR), is the core cause of metabolic risk factors<sup>6-8</sup>, and associations between body composition changes and IR are fundamental to metabolic risk factors.<sup>9</sup> With aging, body composition changes, and loss of skeletal muscle and/or increased fat mass may increase the risk of functional impairment and chronic metabolic disease. Skeletal muscle produces cytokines and myokines, and is a primary organ for insulin-stimulated glucose utilization.<sup>9</sup> According to Eckel et al.<sup>10</sup>, an overabundance of fatty acids from high intake of carbohydrates and fat is a major contributor to

the development of IR. The most efficient strategy to prevent MS in the elderly is to decrease weight by decreasing total caloric, fat, and carbohydrate intake and increasing physical activity.<sup>11,12</sup> However, the relationship between protein requirements and metabolic risk factors in older adults has not been well explored.

Especially in older adults, for whom progressive loss of muscle mass and increased fat mass are natural phenomena, it is inappropriate to focus on decreased fat and carbohydrate intake to prevent metabolic risk without considering protein-related muscle synthesis.<sup>13</sup> In addition, many studies have questioned whether protein recommendations for older adults are adequate or not.<sup>14,15</sup> Adequate protein intake could be a specific intervention to reduce the risk of metabolic risk factors by increasing muscle mass, which could improve insulin sensitivity, particularly in the elderly. From this perspective, it is important to investigate the possibility of insufficient intake of protein in some Korean older adults. Using national data, we estimated protein intake levels/status and explored how suboptimal intake of protein can influence metabolic risk factors in Korean older adults.

## METHODS

### Study population

We examined cross-sectional associations between protein intake and metabolic risk factors in Korean older adults by analysis of data obtained from the Korean National Health and Nutrition Examination Survey (KNHANES) 2010–2011. The protocol of this study was approved by the Research Ethics Review Committee of the Korean Centers for Disease Control and Prevention Institutional Review Board (IRB No. 2010-02CON-21-C and 2011-02CON-06-C). All the participants signed the informed consent form. A rolling sampling design involving a complex, stratified, multistage probability-cluster survey of a representative sample of the noninstitutionalized civilian population was used to assess the health and nutritional status of the Korean population.<sup>16</sup> Subjects in the current cross-sectional study ( $n = 5,697$ ) were men aged 51–70 years ( $n = 1,735$ ), men aged  $\geq 71$  years ( $n = 700$ ), women aged 51–70 years ( $n = 2,305$ ), and women aged  $\geq 71$  years ( $n = 957$ ) who completed both the health and dietary surveys of the KNHANES.

### Procedure

#### Assessment of risk factors

We examined the following metabolic risk factors: central obesity (waist circumference  $\geq 90$  cm in men and  $\geq 85$  cm in women), body mass index (BMI,  $\geq 25$  kg/m<sup>2</sup>), hypertriglyceridemia (blood triglyceride  $\geq 150$  mg/dL), hyperglycemia (fasting plasma glucose  $\geq 100$  mg/dL), HbA1c ( $\geq 7\%$ ), and total cholesterol ( $\geq 200$  mg/dL). BMI of 25 kg/m<sup>2</sup> or higher indicates a status of overweight or obese according to the standards of the International Obesity Task Force, the World Health Organization and the Korean Society for the Study of Obesity.<sup>17</sup> The equation to calculate BMI is as follows: BMI (kg/m<sup>2</sup>) = weight (kg) ÷ height (m)<sup>2</sup>. To determine how different quantities of protein intake influence metabolic risk factors in Korean older adults, daily protein intake was classified into four levels as follows: less than 0.8 g protein/kg body weight per day, 0.8–1.0 g protein/kg body weight per day, 1.0–1.2 g protein/kg body weight per day, and 1.2 g protein/kg body weight per day or more. We hypothesized that protein intake of 1.2 g protein/kg body weight per day or more would be associated with fewer metabolic risk factors than lower levels of protein intake.

#### Statistical analyses

All statistical analyses were conducted using IBM SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics and the percentage of the population with abnormalities were used to test for protein intake-related factors and biochemical parameters of metabolic risk factors by both sex and age. Values are reported as mean  $\pm$  standard error. Logistic regression analyses were used to calculate odds ratio (OR) with 95% confidence interval (CI) for the different levels of protein intake and metabolic abnormality factors after adjusting for age and sex. Statistical significance was accepted at  $P < 0.05$ .

## RESULTS

Table 1 summarizes the protein intake and percentage of the population below the Dietary Reference Intakes for Koreans. Mean grams per day (g/day) of protein intake was lower in women in the oldest age group (aged  $\geq 71$  years) and the 51–70 age group ( $41.53 \pm 0.88$  and  $56.28 \pm 0.79$ , respectively) than men in these two

**Table 1.** Usual protein intake among Korean elderly and the percentage of the population below the estimated average requirement\*

Protein measure		Percentile						% < EAR
		Mean±SE	5th	25th	50th	75th	95th	
Gram per day								-
Men	51–70 yr	82.66±1.25	34.43	53.98	74.63	100.16	153.54	
	≥71 yr	57.76±1.39	24.40	39.63	52.78	70.95	114.18	
Women	51–70 yr	56.28±0.79	23.25	38.11	51.70	69.00	107.80	
	≥71 yr	41.53±0.88	16.87	27.82	38.83	53.75	82.51	
% Energy								-
Men	51–70 yr	13.91±0.12	8.73	11.42	13.57	16.01	20.92	
	≥71 yr	12.66±0.17	8.16	10.37	12.30	14.88	19.43	
Women	51–70 yr	13.42±0.11	8.60	10.89	13.05	15.29	20.08	
	≥71 yr	12.04±0.13	7.93	9.72	11.56	13.81	18.58	
Gram per kg body weight								
Men	51–70 yr	1.24±0.02	0.52	0.83	1.12	1.52	2.32	22.7
	≥71 yr	0.96±0.03	0.41	0.64	0.87	1.18	1.89	44.2
Women	51–70 yr	0.98±0.01	0.41	0.64	0.90	1.21	1.94	42.4
	≥71 yr	0.79±0.02	0.31	0.54	0.74	1.01	1.59	60.5

\*Values were obtained from men aged 51–70 yr, n=1,735; men aged ≥71 yr, n=700; women aged 51–70 yr, n=2,305; women aged ≥71 yr, n=957. SE, standard error; EAR, estimated average requirement.

age groups ( $57.76 \pm 1.39$  and  $82.66 \pm 1.25$ , respectively). Typical mean g/kg body weight protein intake and percentage energy intake showed the same patterns among age and sex subgroups, as did the g/day amount of protein intake. The percentage (%) of energy from protein did not vary much by sex or age (men:  $13.91 \pm 0.12$ ,  $12.66 \pm 0.17$  vs. women:  $13.42 \pm 0.11$ ,  $12.04 \pm 0.13$  for those aged 51–70 and aged ≥71, respectively). Table 2 shows the metabolic risk factors and abnormality estimates by percentage of the population according to sex and age. Mean waist circumference (cm) measurements, serum triglycerides (mg/dL), and glucose (mg/dL) levels were lower in women than men. However, mean BMI (kg/m<sup>2</sup>) and HbA1c (%) levels were higher in women than men.

Logistic regression results and OR (95% CI) for metabolic risk factors according to protein intake level are presented in Table 3. Risk of developing a waist abnormality was significantly higher in the lowest protein intake group (<0.8 g protein/kg body weight/day) in both men (OR, 2.67; 95% CI, 2.00–3.56;  $P < 0.001$ ) and women (OR, 3.42; 95% CI, 2.64–4.43;  $P < 0.001$ ). The group with the lowest protein intake (<0.8 g protein/kg body weight/day) had a significantly higher likelihood of risk factors for BMI abnormalities in both men (OR, 1.90; 95% CI, 1.42–2.54;  $P < 0.001$ ) and women (OR, 2.72; 95% CI, 2.07–3.58;  $P < 0.001$ ). There was a significant association between the lowest protein intake group

(<0.8 g protein/kg body weight/day) and risk of triglycerides abnormality in both men (OR, 1.44; 95% CI, 1.08–1.93;  $P < 0.05$ ) and women (OR, 1.82; 95% CI, 1.45–2.29;  $P < 0.001$ ). The group with the lowest protein intake (<0.8 g protein/kg body weight/day) had a significantly higher likelihood of elevated fasting blood glucose in both men (OR, 1.41; 95% CI, 1.07–1.87;  $P < 0.05$ ) and women (OR, 2.03; 95% CI, 1.48–2.79;  $P < 0.001$ ). The group with the lowest protein intake (<0.8 g protein/kg body weight/day) were also significantly more likely to have an elevated HbA1c level in both men (OR, 1.86; 95% CI, 1.27–2.72;  $P < 0.01$ ) and women (OR, 1.78; 95% CI, 1.19–2.68;  $P < 0.01$ ). Total cholesterol was not associated with protein intake.

## DISCUSSION

We investigated the association between dietary intake of protein and metabolic risk factors in Korean older adults based on analysis of KNHANES data. The study was conducted under the assumption that insufficient protein intake could significantly impact metabolic risk factors and that the currently recommend protein intake levels are not sufficient for Korean older adults. To improve basic knowledge about healthy aging, it is important to explore how different amounts of protein intake influence metabolic risk factors in

**Table 2.** Metabolic risk factors among Korean elderly and percentage of the population\*

Metabolic risk factor		Mean ± SE	Percentile					% < Criteria <sup>†</sup>
			5th	25th	50th	75th	95th	
Waist circumference (cm) <sup>‡</sup>								
Men	51–70 yr	85.56 ± 0.25	71.40	80.58	85.50	90.93	98.20	28.30
	≥ 71 yr	83.99 ± 0.49	67.45	77.50	84.70	91.25	99.15	29.40
Women	51–70 yr	82.32 ± 0.27	68.00	76.00	82.05	88.20	97.07	37.40
	≥ 71 yr	82.82 ± 0.42	65.64	75.80	82.40	89.30	99.12	41.80
Body mass index (kg/m <sup>2</sup> ) <sup>§</sup>								
Men	51–70 yr	23.89 ± 0.08	19.10	22.00	23.89	25.67	28.57	35.60
	≥ 71 yr	22.71 ± 0.14	18.00	20.71	22.65	24.89	27.95	23.50
Women	51–70 yr	24.36 ± 0.08	19.56	22.21	24.10	26.23	29.82	39.20
	≥ 71 yr	23.88 ± 0.16	18.26	21.55	23.71	26.04	30.17	34.50
Serum triglycerides (mg/dL) <sup>  </sup>								
Men	51–70 yr	169.90 ± 4.16	52.00	89.00	131.00	195.00	361.00	43.00
	≥ 71 yr	130.28 ± 4.01	49.00	74.75	109.00	155.00	287.00	28.10
Women	51–70 yr	134.16 ± 2.23	50.00	80.00	113.00	166.00	286.00	31.00
	≥ 71 yr	146.27 ± 3.76	54.60	87.50	123.00	172.50	289.70	35.80
Glucose (mg/dL) <sup>¶</sup>								
Men	51–70 yr	106.13 ± 0.83	82.00	91.00	98.00	110.00	161.00	25.10
	≥ 71 yr	102.11 ± 1.14	82.00	90.00	97.00	109.00	145.00	20.70
Women	51–70 yr	99.54 ± 0.62	81.00	88.00	94.00	103.00	137.00	15.50
	≥ 71 yr	104.10 ± 1.09	81.00	89.00	97.00	108.00	149.00	23.20
HbA1c (%) <sup>**</sup>								
Men	51–70 yr	6.18 ± 0.05	5.10	5.50	5.80	6.50	8.60	23.00
	≥ 71 yr	6.16 ± 0.07	5.20	5.50	5.90	6.50	8.38	21.60
Women	51–70 yr	6.08 ± 0.04	5.20	5.60	5.80	6.20	8.20	17.30
	≥ 71 yr	6.22 ± 0.05	5.20	5.60	5.90	6.48	8.70	22.30
Total cholesterol (mg/dL) <sup>††</sup>								
Men	51–70 yr	188.82 ± 1.10	131.00	164.00	187.00	211.00	250.00	36.10
	≥ 71 yr	179.76 ± 1.65	124.00	157.00	177.00	200.00	240.00	25.50
Women	51–70 yr	203.35 ± 1.07	144.00	178.00	202.00	226.00	264.00	51.80
	≥ 71 yr	198.63 ± 1.62	140.00	172.00	196.00	221.00	264.70	45.90

\*Values were obtained from men aged 51–70 yr, n = 1,735; men aged ≥ 71 yr, n = 700; women aged 51–70 yr, n = 2,305; women aged ≥ 71 yr, n = 957; <sup>†</sup>Percent of the population with metabolic abnormalities; <sup>‡</sup>Criteria: waist circumference, ≥ 90 cm in men and ≥ 85 cm in women; <sup>§</sup>Criteria: ≥ 25 kg/m<sup>2</sup>; <sup>||</sup>Criteria of hypertriglyceridemia: ≥ 150 mg/dL; <sup>¶</sup>Criteria of hyperglycemia (fasting plasma glucose): ≥ 110 mg/dL; <sup>\*\*</sup>Criteria: ≥ 7%; <sup>††</sup>Criteria: ≥ 200 mg/dL. SE, standard error.

the elderly. Lim et al.<sup>4</sup> reported that the prevalence of MS in Korea has increased more rapidly than in the USA, and the Ministry of Health and Welfare<sup>18</sup> reported that it increased to 46.3% among elderly aged 65 and over of both genders in Korea. Adequate intake of protein, which could improve IR by maintaining muscle mass, could be a strategy for preventing diseases in seniors.<sup>19</sup> According to Lu et al.'s research<sup>20</sup>, low muscle mass and a form of obesity called sarcopenia<sup>21</sup> are associated with MS in the American elderly. In the present study, we found evidence of associations between protein intake and metabolic risk factors in both genders, and

women were more at risk than men. The same findings emerged in Lim et al.'s study<sup>4</sup>, in which the prevalence of MS in young people aged 20–49 years was higher in men than women, but for the elderly (aged ≥ 50 years), it increased more notably in women than men. This suggests that gender-specific treatments should be provided in older adults to prevent MS, such as different amounts of protein intake.

Our analysis showed that the ranges of suboptimal protein intake differed according to gender and metabolic risk factors. As protein intake decreased to less than 1.2 g/kg, abnormalities in waist cir-

**Table 3.** ORs for metabolic risk factors according to protein intake (men = 2,435, women = 3,262)

Variable	Protein intake (g/body weight [kg]/day)			
	>1.2	1.0–1.2	0.8–1.0	<0.8
Abdominal obesity*				
Men ≥ 51 yr	1.00	1.91 (1.37–2.67) <sup>§</sup>	1.49 (1.04–2.13) <sup>†</sup>	2.67 (2.00–3.56) <sup>§</sup>
Women ≥ 51 yr	1.00	1.61 (1.19–2.18) <sup>†</sup>	2.13 (1.60–2.83) <sup>§</sup>	3.42 (2.64–4.43) <sup>§</sup>
Body mass index ≥ 25 kg/m <sup>2</sup>				
Men ≥ 51 yr	1.00	1.56 (1.13–2.14) <sup>†</sup>	1.51 (1.07–2.11) <sup>†</sup>	1.90 (1.42–2.54) <sup>§</sup>
Women ≥ 51 yr	1.00	1.49 (1.07–2.08) <sup>†</sup>	1.88 (1.37–2.58) <sup>§</sup>	2.72 (2.07–3.58) <sup>§</sup>
Triglycerides ≥ 150 mg/dL				
Men ≥ 51 yr	1.00	1.29 (0.91–1.82)	1.17 (0.87–1.56)	1.44 (1.08–1.93) <sup>†</sup>
Women ≥ 51 yr	1.00	1.34 (0.95–1.88)	1.76 (1.30–2.38) <sup>§</sup>	1.82 (1.45–2.29) <sup>§</sup>
Total cholesterol ≥ 200 mg/dL				
Men ≥ 51 yr	1.00	1.16 (0.84–1.60)	1.13 (0.82–1.57)	1.02 (0.78–1.34)
Women ≥ 51 yr	1.00	0.98 (0.72–1.34)	1.03 (0.75–1.42)	0.95 (0.75–1.20)
High fasting blood glucose ≥ 110 mg/dL				
Men ≥ 51 yr	1.00	1.12 (0.78–1.61)	1.28 (0.90–1.80)	1.41 (1.07–1.87) <sup>†</sup>
Women ≥ 51 yr	1.00	1.40 (0.92–2.14)	1.52 (1.01–2.28) <sup>†</sup>	2.03 (1.48–2.79) <sup>§</sup>
HbA1c ≥ 7%				
Men ≥ 51 yr	1.00	1.19 (0.70–2.01)	1.59 (1.00–2.53)	1.86 (1.27–2.72) <sup>†</sup>
Women ≥ 51 yr	1.00	1.34 (0.79–2.26)	1.58 (0.96–2.60)	1.78 (1.19–2.68) <sup>†</sup>

Values are presented as OR (95% confidence interval). After adjustment for age, sex, statistical significance was accepted at < 0.05.

\*Waist circumference, ≥ 90 cm in men and ≥ 85 cm in women; <sup>†</sup>*P* < 0.05; <sup>‡</sup>*P* < 0.01; <sup>§</sup>*P* < 0.001.

OR, odds ratio.

cumference and BMI increased significantly more rapidly in women than men. Aging-related hormonal changes influence biological changes and functions. In postmenopausal women, as age increases, body composition changes; there is 1%–2% muscle mass loss and fat mass increase due to reduced estrogen secretion.<sup>22</sup> However, the increase in fat mass may stop or decline as women age further.<sup>22</sup> According to Lu et al.<sup>20</sup>, the group with sarcopenic obesity showed a higher risk for MS than other groups and this was independently associated with individual components of MS including waist circumference, high-density lipoprotein cholesterol, triglyceride, and fasting glucose levels. In other research on protein and skeletal muscle, insufficient protein intake was associated with various abnormal body composition phenotypes.<sup>23</sup> High protein intake is associated with reduced body weight, fat mass, and triglyceride level and increased muscle mass and resting energy expenditure.<sup>24</sup> Clearly, too much protein intake in elderly individuals with poor kidney function is also not desirable. The risks of having triglycerides and fasting glucose level abnormalities were significantly higher when protein intake was less than 0.8 g/kg body weight per day compared to higher daily protein intake levels.

Our findings suggest that 1.2 g protein/kg body weight per day or more of protein consumption could reduce metabolic risk. How much protein is appropriate for older adults? Currently, the protein intake levels considered to be adequate for seniors vary among studies. A substantial amount of evidence suggests that the recommended dietary allowances (RDA) of 0.8 g/kg of protein per day is not optimal for muscle and bone health in elderly people in the United States. In the United States, 40% of the elderly aged ≥ 70 years have been shown to have sufficient protein intake according to the recommended criteria.<sup>19</sup> Among the Korean elderly, typical protein intake is lower than among the American elderly.<sup>25</sup> In the present study, the prevalence of intake of less than 0.8 g protein/kg body weight per day was higher in women than in men, which may have contributed to the higher prevalence of MS in women than men. Additionally, more people in the older age group were found to have poor protein intake than those in the younger age group. When we investigated mean protein intake by sex and age, all groups except the oldest women had a protein intake of at least 0.8 g protein/kg body weight per day. However, almost half of the population are likely to have insufficient protein intake because of de-

creased relaxation of the fundus, increased release of cholecystokinin and leptin, reduced appetite due to changes in taste and smell, social changes, and economic limits associated with aging.<sup>26,27</sup> Therefore, for the elderly, more individual and specific criteria and training are needed.

Research on nitrogen balance in the elderly conducted by Kim et al.<sup>28</sup> showed that intake of 0.8 g protein/kg body weight per day was not sufficient for the elderly, and those authors suggested that protein intake be increased to 1.14 g/kg body weight per day. Research by Dutheil et al.<sup>29</sup> conducted on 28 average people with MS aged 61.8 years revealed that the lower threshold of protein intake to maintain blood protein homeostasis was 1.2 g protein/kg body weight per day. Another study reported a required intake level of approximately 0.9 g protein/kg body weight per day.<sup>30</sup> The recommended intake of dietary protein based on achieving nitrogen equilibrium varies according to age because energy requirements decline with age. It was originally 1.0 g protein/kg body weight per day until the 1980 RDA, but it was thereafter decreased to 0.8 g protein/kg body weight per day, which is the current RDA for all adults (aged  $\geq 19$  years). A moderate increase in daily protein intake to 1.0 to 1.3 g protein/kg might be needed to lower energy intake, improve IR function, and reduce the progressive loss of muscle mass in elderly individuals.<sup>15,31</sup>

Our study has both strengths and limitations, which could be addressed by additional investigations. One limitation was the use of 24-hour recall data to estimate nutrition intake. Memory errors in older adults may have resulted in over- or under-reporting of protein intake, which may have impacted the accuracy of our data. In addition, it was difficult to accurately assess individuals' daily protein intake. Another limitation was that the cross-sectional design of this study precluded our ability to make causal inferences between level of protein intake and metabolic risk factors. Determination, treatment, and the presence of MS in the KNHANES should be explored in the future studies. We could not control for all epigenetic influences; thus, these results need to be confirmed by additional studies with longitudinal designs if the results of this study are to be generalized. Advantages of our study include the fact that we explored the relationships between defined optimal levels of dietary protein and metabolic risk factors in a large number of Korean older individuals whereas most previous studies on strategies to

prevent MS have been conducted in Western countries, even though the prevalence of MS has been increasing rapidly and steadily in Korea. Additionally, only a few studies have focused on protein intake level and MS among older adults in Korea.

In conclusion, our findings suggest that elderly women who eat less than 0.8 g protein/kg body weight per day have higher metabolic risk factors than elderly men who eat less than 0.8 g protein/kg body weight per day. Greater emphasis should be placed on a protein intake over 0.8 g protein/kg body weight to decrease metabolic risk, and efforts should be made to educate older adults about the importance of protein intake and to provide them with protein-rich foods. Most Korean older adults appeared to consume more than enough protein in their daily diets when compared with the mean value, but among them, specific people were at risk for being protein-deficient, including older women and very old individuals. Therefore, the current recommendations for protein intake for optimal health in Korean older adults should be reconsidered to potentially decrease metabolic risk in Korean older adults.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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CO designed and carried out the study, analyzed the data, and drafted the manuscript. JN contributed to study design and article revision. All authors read and approved the final manuscript.

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