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The mediating effects of metabolic factors on the association between fruit or vegetable intake and cardiovascular disease: the Korean National Health and Nutrition Examination Survey

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Manuscripts

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4 1 **The mediating effects of metabolic factors on the association between fruit or vegetable**
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6 2 **intake and cardiovascular disease: the Korean National Health and Nutrition**
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8 3 **Examination Survey**
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4 21 **Abstract**

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7 22 **Objective:** We assessed the mediating effects of metabolic components on the relationship
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9 23 between fruit or vegetable intake and cardiovascular disease (CVD).
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12 24 **Design:** Cross-sectional study
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15 25 **Setting:** This study was conducted using data from the 2013–2015 Korean National Health
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17 26 and Nutrition Examination Survey, which is a national representative cross-sectional survey
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19 27 to assess health and nutritional status in the Korean population.
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21
22 28 **Method and analysis:** A total of 9,040 subjects (3,555 males and 5,485 females) aged ≥ 25
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24 29 years were included in the study. Physician-diagnosed CVD was used as the outcome. Fruit
25
26 30 or vegetable intake was measured via a dish-based semi-quantitative food frequency
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28 31 questionnaire and grouped into categories (< 1 time/d, 1 time/d, 2 times/d, and ≥ 3 times/d).
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30 32 Systolic blood pressure (SBP), cholesterol, and fasting glucose were considered metabolic
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32 33 mediators, and the bootstrap method was used to assess mediating effect.
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36 34 **Results:** About 1.8% of adults aged 25–64years had CVD. The risk for CVD decreased by
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38 35 14% as fruit, but not vegetable, intake was increased by one unit per day. After additional
39
40 36 adjustment for metabolic factors, the odds ratio was attenuated to 0.89 (95% confidence
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42 37 interval; 0.77–1.03). This result indicates that the indirect effect of three metabolic factors
43
44 38 accounted for 21.4% of the relationship between fruit intake and CVD. SBP was a more
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46 39 important metabolic mediator than the other factors. The indirect effect accounted for 30.0%
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48 40 when body mass index was additionally controlled as a mediator, and SBP still had an
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50 41 independent effect compared to the other mediators.
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4 42 **Conclusions:** Our results indicate that controlling SBP may lessen the CVD risk, and a diet
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6 43 rich in fruits can regulate SBP, which, in turn, reduces CVD risk.
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9 44 **Keywords:** Cardiovascular disease, blood pressure, diet
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15 46 **ARTICLE SUMMARY**

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19 48 **Strengths and limitations of this study**

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21 49 - In this study, we assessed how fruit or vegetable intake is related to CVD by assessing
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23 50 the indirect effect of systolic blood pressure (SBP), total cholesterol, and fasting
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25 51 glucose. Of them, the mediating effect of SBP on the association between fruit intake
26
27 52 and CVD was dominant.
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30 53 - Our study suggests that controlling SBP might lessen CVD risk, and a diet rich in fruits
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32 54 can be used to regulate SBP, which, in turn, reduces CVD risk.
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34 55 - The results were derived from a cross-sectional study design, so causal relationships
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36 56 could not be effectively drawn.
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64 INTRODUCTION

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66 Cardiovascular diseases (CVDs) are responsible for mortality worldwide; a report from
67 the World Health Organization stated that CVDs accounted for 31% of all deaths worldwide
68 in 2015 [1]. Although mortality from ischemic heart disease has shown a flat trend and that
69 from cerebrovascular disease has shown a declining trend in the Republic of Korea since
70 2005, these causes of death remain highly ranked [2].

71 Several risk factors for CVDs, including metabolic factors, such as high glucose, high
72 blood pressure, and high cholesterol, have been suggested [3]. Several studies have suggested
73 that these metabolic factors are also linked to risk factors (e.g., body mass index [BMI] and
74 dietary factors) and CVD risk as mediators [4, 5]. The causal link between these mediators
75 and disease risk must be identified for an effective public health intervention. However,
76 previous studies focused on a single relationship between a risk factor and a disease rather
77 than the mediating effects.

78 Excessive risk for CVD caused by poor diet and chronic diseases was reported from a
79 study of global burden of disease (GBD). In addition, the GBD study established causal
80 mediating relationships between a diet poor in fruits or vegetables, metabolic mediators
81 (blood pressure, cholesterol, and glucose), and disease [4]. These metabolic mediators have
82 also been linked to BMI and CVD [4]. The effect of a diet rich in fruits and vegetables on
83 BMI has been reported through epidemiological studies [6], but few studies have assessed
84 BMI as a mediator.

85 There is a need to study the degree to which these metabolic factors contribute to the
86 relationship between risk factors and disease. Thus, using cross-sectional survey data from

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87 the 2013–2015 Korean National Health and Nutrition Examination Survey (KNHANES), we
88 assessed the mediating effects of metabolic components applied to a confirmatory model.
89 Furthermore, we assessed how the BMI contributes to the relationship between fruit or
90 vegetable intake and CVD as a confounder or mediator.

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For peer review only

105 **METHODS**

107 **1. Study subjects**

108 This study was conducted using data from the 2013–2015 KNHANES, which is a
109 national representative cross-sectional survey to assess health and nutritional status in the
110 Korean population. It consists of a health interview, health examination, and a nutrition
111 survey. A number of variables were collected by trained staff, including physicians, medical
112 technicians, and dietitians. The detailed KNHANES survey method has already been
113 described [7].

114 The food frequency questionnaire (FFQ) was changed to a dish-based semi-quantitative
115 FFQ based on a 2012 survey. The survey assessed subjects 19–64 years of age. We used the
116 sixth survey from 2013 to 2015 by sampling according to the survey cycle. This study
117 included subjects ≥ 25 years. Additionally, the eligible study population included the
118 respondents with data from all three parts of the survey. A total of 9,040 subjects (3,555
119 males and 5,485 females) were included in the study.

121 **2. Fruit and vegetable intake**

122 The dish-based semi-quantitative FFQ was composed of 112 items and provided
123 information on typical dietary consumption for 1 year using a 9-point scale (less than once
124 per month or never, once per month, 2–3 times per month, once per week, 2–4 times per
125 week, 5–6 times per week, once per day, twice per day, and three times per day) and three
126 levels to represent the amount consumed by referring to a standard amount (less, standard,
127 and more). Based on a previous study [4], we excluded pickled and salted vegetables,

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4 128 including kimchi and fruit juice. Vegetable intake included bean sprouts (seasoned, soup);
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6 129 seasoned mung bean sprout; seasoned spinach; seasoned bellflower (boiled or not); pumpkin
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8 130 (seasoned, pan-fried); other seasoned vegetables; cucumber (seasoned, raw); radish (seasoned,
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10 131 pickled, dried); vegetable salad; seasoned green onion, and seasoned Chinese chives; raw
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12 132 vegetables (lettuce, sesame, Chinese cabbage, and pumpkin leaf); green pepper; boiled
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14 133 broccoli, boiled cabbage; garlic; tomato, and cherry tomato. Fruit intake was assessed based
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16 134 on 12 items: strawberry; melon; watermelon; peach; grape; apple; pear; persimmon, dried
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18 135 persimmon; tangerine; banana; orange; and kiwi. The frequency of fruit intake was used after
19
20 136 adjusting for seasonal fruit. Estimated intakes of fruits and vegetables were calculated on the
21
22 137 FFQ by multiplying the frequency of each food (as described above) by the selected amount
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24 138 consumed: small (0.5), medium (1), and large (1.5). Fruit and vegetable intake was expressed
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26 139 in four categories (< 1 time/d, 1 time/d, 2 times/d, and ≥ 3 times/d).
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32 141 **3. Outcome and covariate data**

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34 142 We used data from the health-related questionnaire for the diseases diagnosed by
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36 143 physicians. We selected the questions about stroke, myocardial infarction, and angina pectoris
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38 144 for the CVD-related diseases. If a subject answered “yes” to any of the three diseases, we
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40 145 considered that the subject had CVD. Additionally, we separately considered subjects who
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42 146 answered “yes” on the question about current illness with a physician’s diagnosis and those
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44 147 who responded “yes” to a question about receiving treatment for a disease.
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47 148 We used data on sex, age, quartiles of income, region (urban/rural), current smoker, and
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49 149 survey year as covariates through a literature review [8] and the results of a univariate
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51 150 analysis. We used quartile data for income instead of education level as a socioeconomic
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151 indicator because income may be directly linked to food purchases [9]. The question about
152 physical activity was changed from the 2014 survey, so we did not consider physical activity.

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154 **4. Statistical analysis**

155 The basic characteristics of the study subjects are presented as weighted percentages or
156 weighted means with standard errors by considering the multi-stage sampling survey method.
157 The distributions of the basic characteristics according to fruit or vegetable intake level were
158 assessed using the trend test under the random sampling condition. In the main analysis, CVD
159 was considered the outcome (Y) and fruit or vegetable intake was considered an independent
160 variable (X). Systolic blood pressure (SBP) (M_1), total cholesterol (M_2), and fasting glucose
161 (M_3) were applied as metabolic mediators (M). Additionally, BMI was considered as either a
162 covariate or mediator.

163 We examined the association under the controlling covariates (sex, age, income, region
164 [urban/rural], present smoking, and survey year) through four basic steps to assess mediation
165 [10]. Step 1: association between dietary factors and CVD ($X \rightarrow Y$; total effect and was
166 marked path “c”); step 2: association between dietary factors and metabolic mediators ($X \rightarrow$
167 M_i ; marked path “a”); step 3: association between metabolic mediators and CVD after
168 controlling for metabolic mediators ($M_i \rightarrow Y$; marked path “b”); and step 4: association
169 between dietary factors and CVD disease after controlling for metabolic mediators (direct
170 effect; marked path “c”). We used the bootstrap method and the “process” macro suggested
171 by Andrew to assess the mediating effects [11]. In this analysis, we applied 10,000 bootstraps.
172 We separately or simultaneously assessed the indirect effect of the metabolic mediators on the
173 association between dietary factors and CVD. The exponential regression coefficient is equal

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4 174 to the odds ratio (OR) when considering the CVD as an outcome variable. The percentage of
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6 175 risk mediated by the metabolic mediator was calculated as [12]: $OR(\text{confounder adjusted}) -$
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8 176 $OR(\text{confounder and mediator adjusted})/OR(\text{confounder adjusted}) - 1 \times 100.$
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10 177 All statistical analyses were conducted under a random sampling condition excluding the
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12 178 basic characteristics given in Table 1 using SAS ver. 9.4 software (SAS Institute, Cary, NC,
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14 179 USA). A two-sided p -value < 0.05 was considered significant.
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4 197 **RESULTS**
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10 199 The basic characteristics of the study subjects are presented in Table 1. Mean age was
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12 200 43.7 years, and 1.81% of subjects had CVD. Subjects with a higher income ate more fruits or
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14 201 vegetables than those with a lower income. Those who ate more fruit were more likely to be
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16 202 non-smokers and female than their counterparts (Supplemental Tables 1, 2).
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19 203 The total effect of fruit intake on CVD showed an inverse association without controlling
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21 204 for metabolic mediators (adjusted odds ratio [aOR], 0.86, 95% CI: 0.74–0.98), but the effect
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23 205 of vegetable intake was not significant (aOR, 0.93; 95% CI: 0.81–1.06) after controlling for
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25 206 sex, age, income, region (urban/rural), current smoker, and survey year.
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28 207 The direct effect of fruit intake on CVD was borderline significant after further
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30 208 considering each metabolic mediator. The effect of SBP did not include zero in the 95% CI
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32 209 range as the other metabolic mediators. The effect of fruit intake on BMI showed borderline
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34 210 significance, and the effect of BMI on CVD was significant, but the indirect effect of BMI
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36 211 was not significant. Additionally, the effect of SBP was significant even after controlling for
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38 212 BMI as a covariate (Table 2). SBP, cholesterol, and BMI were associated with CVD, but
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40 213 vegetable intake did not contribute to either metabolic mediator or CVD (Table 3). The
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42 214 mediating effect of SBP on the association between fruit intake and outcome was dominant
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44 215 even when the outcome was restricted to those with a current illness or undergoing treatment.
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48 216 The OR was attenuated to 0.89 (95% CI: 0.77–1.03) while simultaneously controlling for
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50 217 multiple metabolic mediators, indicating a 21.4% indirect effect for CVD. SBP showed an
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52 218 independent indirect effect. Higher fruit intake had a beneficial effect on fasting glucose, but
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4 219 its effect was not associated with CVD. The direct effect of fruit intake on CVD presented an
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6 220 inverse association, but it did not reach statistical significance (Figure 1). In addition, similar
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8 221 results were observed when adding BMI as covariate, with an OR of 0.90 (95% CI: 0.78–
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10 222 1.04; data not shown).

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13 223 We analyzed the serial mediator model to assess whether BMI influenced SBP (Figure 2).
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15 224 Although the effect of fruit intake on BMI showed borderline significance, the influence of
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17 225 BMI on SBP, and the effect of SBP on CVD reached statistical significance. Of the three
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19 226 possible indirect paths, the fruit intake path → SBP → CVD was the only one to show an
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21 227 independent association.

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25 228 Fruit intake was directly linked to subjects who suffered a stroke, but not ischemic heart
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27 229 disease, regardless of which metabolic factors were controlled. In addition, the mediating
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29 230 effect of SBP was dominant in patients who suffered a stroke or ischemic heart disease even
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31 231 after controlling for BMI (Supplemental Tables 3, 4).

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4 239 **DISCUSSION**
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9 241 In this study, we assessed how fruit or vegetable intake is related to CVD by assessing
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11 242 the indirect effect of metabolic mediators. Based on the established causal link, SBP, total
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13 243 cholesterol, and fasting glucose were considered metabolic mediators, and the effect of BMI
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15 244 was additionally assessed. Of them, the indirect effect of SBP on the relationship between
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17 245 fruit intake and CVD was significant even after considering BMI, but not vegetable intake.
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19 246 The indirect effect of the four metabolic factors accounted for 30.0% of the relationship
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21 247 between fruit intake and CVD.
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24 248 The beneficial effects of high fruit or vegetable intake on CVD and the unfavorable
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26 249 effects of high blood pressure, glucose, and cholesterol on CVD are well known. Thus,
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28 250 previous studies considered metabolic factors together, and mediators were reported to
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30 251 attenuate the association of a direct effect [5]. One large prospective study conducted in 10
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32 252 regions in China indicated that higher fresh fruit intake is linked to CVD death, and its effect
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34 253 was attenuated by hazard ratios from 0.63 (95% CI: 0.56–0.72) to 0.70 (95% CI: 0.61–0.79)
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36 254 after adjusting for BMI, blood pressure, glucose, and waist circumference [13]. Another study
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38 255 conducted in Shanghai, China showed an attenuated association between fruit intake and
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40 256 incident coronary heart disease after controlling for a history of diabetes, hypertension, or
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42 257 dyslipidemia, but not vegetable intake [5]. A women's health study reported by Liu et al. also
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44 258 showed that the effect of fruits and vegetables on CVD risk became stronger after excluding
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46 259 subjects with a history of diabetes, hypertension, and high cholesterol [14]. However,
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48 260 whether these metabolic factors were causal links between fruit and/or vegetable intake and
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50 261 CVD risk was not investigated.
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4 262 The assessment of a mediating effect could help understand how fruit and/or vegetable
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6 263 intake affects CVDs. In addition, an effect of poor dietary risk by metabolic mediators on
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8 264 CVD was suggested by the GBD study, so that was considered to estimate the disease burden.
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10 265 The meditating effect of blood pressure on the association between fruit and/or vegetable
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12 266 intake and CVD was suggested by a prospective cohort study of patients in the first National
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14 267 Health and Nutrition Examination Survey [8]. Blood pressure contributed 22.2% to the
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16 268 relationship between fruit and vegetable intake and CVD death. This was similar to the
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18 269 results adjusted for BMI, cholesterol, and blood pressure. That study also showed that the
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20 270 direct effect of fruit and vegetable intake was notable in patients who suffered a stroke but
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22 271 not those with ischemic heart disease. These results are in line with those of the present study.

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25 272 We assessed a potential role for BMI on the association between fruit intake and CVD
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27 273 using various models. Several reports, including the above-mentioned study, considered BMI
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29 274 as a potential mediator [8, 13]. Additionally, a causal link between BMI and CVD risk is
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31 275 mediated through metabolic factors. Two pooled studies of prospective cohorts assessed the
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33 276 effect of BMI on coronary heart disease and stroke as mediated by metabolic components.
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35 277 They reported that blood pressure was a more important mediator compared to cholesterol
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37 278 and glucose [12, 15]. Other pooled data from an Asian cohort also indicate that estimated
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39 279 mediating proportions through hypertension were 62.3, 35.7, and 92.4% for the association
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41 280 between BMI and death due to CVD, coronary heart disease, and stroke, respectively, but not
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43 281 by diabetes [16]. The GBD study restricted total calories to 2,000 kcal instead of considering
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45 282 BMI [17]. In the present study, higher fruit intake was inversely associated with BMI, but it
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47 283 was borderline significant ($\beta = -0.06$, $p = 0.08$), which affected the results of the fruit intake
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49 284 path \rightarrow BMI \rightarrow SBP \rightarrow CVD in the serial multiple mediator model. Our study found that the
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4 285 mediating effect due to BMI was about 7.9%, but previous studies showed a < 3.0%
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6 286 mediating effect by BMI on the association between fruit only or fruit and vegetable intake
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8 287 and CVD deaths by presenting little change in the adjusted risk value [8, 13]. However, it is
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10 288 difficult to make a direct comparison due to discrepancies in study design, study populations,
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12 289 the definition of disease, and fruit and/or vegetable intake.

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15 290 Eating more vegetables was not significantly associated with either a direct or indirect
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17 291 effect. In Korea, vegetables in the general population are easily accessible by a side dish.
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19 292 Indeed, statistics from the Organisation for Economic Co-operation and Development
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21 293 (OECD) have reported that daily vegetable consumption among adults was the highest in
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23 294 Korea [18]. However, the manner of preparation and/or cooking can influence nutrient
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25 295 content [6]. The favorable effects of fruit and vegetable intake can be explained by nutrients,
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27 296 such as dietary fiber, folate, potassium, and antioxidant vitamins (i.e., vitamin E, vitamin C,
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29 297 polyphenols, flavonoids, and carotenoids) and other components. These nutrients might be
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31 298 involved with controlling glucose, lipid level, and blood pressure, and reduce the risk of CVD
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33 299 along with weight control [6]. However, because foods contain various nutrients, food
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35 300 recommendations help subjects follow a prevention strategy. In addition, healthy eating is
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37 301 also associated with other health behaviors, such as not smoking and regular physical activity
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39 302 [8, 19].

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41
42 303 The present study had some limitations. First, the results were derived from a cross-
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44 304 sectional study design, so causal relationships could not be effectively drawn. But, some parts
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46 305 of our results were consistent with previous studies [8, 19]. Furthermore, the results were
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48 306 consistent even after applying various definitions of outcome. Because the survey conducts
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50 307 general population except those in the hospital, subjects with diseases might be the relatively

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4 308 less moderate cases. In addition, unmeasured confounding factors may have influenced the
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6 309 association.

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8 310 Nevertheless, our study focused on the mediating effects of metabolic factors on CVD
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10 311 and assessed which metabolic factors affect CVD. Our results were produced using the
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12 312 bootstrapping method and did not impose the assumption of normality of the sampling
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14 313 distribution; thus, it was an appropriate design for multiple mediations [10]. The given
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16 314 evidence was conceptually approached and was not statistically tested for an indirect effect.

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19 315 Taken together, our study suggests that controlling SBP might lessen CVD risk, and a
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21 316 diet rich in fruits can be used to regulate SBP, which, in turn, reduces CVD risk.

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4 329 **Conflict of interest statement**

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12 332 **Contributor ship statement**

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15 333 HA Lee wrote the manuscript and performed the statistical analyses; D Lim, K Oh, and EJ Kim,
16
17 334 provided advice about writing the manuscript, and H Park helped interpret the data.

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28 338 Health & Welfare, Republic of Korea (HI13C0729).

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33 340 **Data availability**

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36 341 The National Health and Nutrition Examination Survey files are available from the Korea Centers for Disease
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38 342 Control and Prevention database (URL https://knhanes.cdc.go.kr/knhanes/sub03/sub03_02_02.do). If you
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40 343 register your e-mail on this site, you can freely download the raw data.

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4 435 **Figure Legends**
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10 437 Figure 1. The effect of multiple metabolic factor (M_i) mediators in the association between
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12 438 fruit intake (X) and cardiovascular diseases (Y).
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15 439 ^a $p < 0.01$, ^b $p < 0.001$, SBP: systolic blood pressure. Coefficients were adjusted for sex, age,
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17 440 income, region (urban/rural), current smoker, and survey year using the bootstrapping method.
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22 442 Figure 2. The effect of multiple serial mediators of metabolic factors (M_i) in the association
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24 443 between fruit intake (X) and cardiovascular diseases (Y).
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27 444 ^a $p < 0.1$, ^b $p < 0.05$, ^c $p < 0.01$, ^d $p < 0.001$, BMI: body mass index, SBP: systolic blood pressure.
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29 445 Coefficients were adjusted for sex, age, income, region (urban/rural), current smoker, and
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31 446 survey year using the bootstrapping method.
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454 Table 1. Basic characteristics of the study subjects.

	Weighted % (SE)
Sex	
Male	49.92 (0.51)
Female	50.08 (0.51)
Age range	
Age (years)†	25–64 years
	43.68 (0.18)
Region	
Urban	83.87 (1.52)
Rural	16.13 (1.52)
Income level (quartiles)	
Q1	23.46 (0.73)
Q2	25.61 (0.72)
Q3	25.03 (0.71)
Q4	25.90 (0.97)
Current smoking	
No	75.83 (0.60)
Yes	24.17 (0.60)
Disease	
Cardiovascular disease	1.81 (0.16)
Stroke	0.98 (0.13)
Ischemic heart disease	0.90 (0.10)
Metabolic Factors†	
Systolic blood pressure (mmHg)	115.01 (0.21)
Total cholesterol (mg/dL)	190.98 (0.47)
Fasting plasma glucose (mg/dL)	98.58 (0.30)
Body mass index (kg/m ²)	23.92 (0.05)

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456 SE: Standard error.

457 †Weighted mean with standard error.

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466 Table 2. The effect of metabolic mediators (M) in the association between fruit intake (X) and
467 cardiovascular disease (Y).

Metabolic Factors (M)	Fruit intake										
	X → M (a)			M → Y (b)			X → Y (c' = direct effect)			Indirect effect (a*b)	
	β	SE	<i>p</i>	β	SE	<i>p</i>	β	SE	<i>p</i>	β	95% CI
SBP ^a	-0.484	0.144	0.001	0.013	0.004	0.002	-0.137	0.072	0.06	-0.007	-0.014 -0.002
TC ^a	-0.156	0.357	0.66	-0.019	0.003	<.0001	-0.144	0.075	0.05	0.003	-0.011 0.017
FPG ^a	-0.665	0.217	<.01	0.004	0.003	0.20	-0.144	0.074	0.05	-0.002	-0.006 0.001
BMI ^a	-0.059	0.034	0.08	0.078	0.022	0.001	-0.143	0.072	<.05	-0.005	-0.012 0.001
SBP ^b	-0.420	0.139	<.01	0.011	0.005	0.01	-0.127	0.072	0.08	-0.005	-0.011 -0.0004
TC ^b	-0.064	0.352	0.86	-0.019	0.003	<.0001	-0.126	0.075	0.09	0.001	-0.012 0.015
FPG ^b	-0.614	0.214	<.01	0.002	0.003	0.42	-0.130	0.074	0.08	-0.002	-0.005 0.002

468 SBP: systolic blood pressure, TC: total cholesterol, FPG: fasting plasma glucose, BMI: body mass
469 index, SE: standard error, 95% CI: 95% confidence interval.

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471 ^aAdjusted for sex, age, income, region (urban/rural), current smoking, and survey year.

472 ^bAdjusted for sex, age, income, region (urban/rural), current smoking, survey year, and body mass
473 index.

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488 Table 3. The effect of metabolic mediators (M) in the association between vegetable intake
 489 (X) and cardiovascular disease (Y).

Metabolic Factors (M)	Vegetable intake										
	X → M (a)			M → Y (b)			X → Y (c' = direct effect)			Indirect effect (a*b)	
	β	SE	<i>p</i>	β	SE	<i>p</i>	β	SE	<i>p</i>	β	95% CI
SBP ^a	-0.042	0.169	0.80	0.014	0.004	0.002	-0.132	0.086	0.13	-0.001	-0.006 0.004
TC ^a	0.236	0.420	0.57	-0.019	0.003	<.0001	-0.121	0.089	0.18	-0.005	-0.021 0.012
FPG ^a	-0.054	0.256	0.83	0.004	0.003	0.18	-0.132	0.088	0.14	-0.0002	-0.003 0.002
BMI ^a	0.057	0.040	0.16	0.080	0.022	<.001	-0.145	0.086	0.09	0.005	-0.002 0.013
SBP ^b	-0.114	0.163	0.48	0.012	0.005	0.01	-0.131	0.086	0.13	-0.001	-0.006 0.002
TC ^b	0.142	0.415	0.73	-0.019	0.003	<.0001	-0.122	0.089	0.17	-0.003	-0.019 0.014
FPG ^b	-0.121	0.252	0.63	0.003	0.003	0.4	-0.132	0.088	0.14	-0.0003	-0.003 0.002

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491 SBP: systolic blood pressure, TC: total cholesterol, FPG: fasting plasma glucose, BMI: body mass
 492 index, SE: standard error, 95% CI: 95% confidence interval.

493 ^aAdjusted for sex, age, income, region (urban/rural), current smoking, and survey year.

494 ^bAdjusted for sex, age, income, region (urban/rural), current smoking, survey year, and body mass
 495 index.

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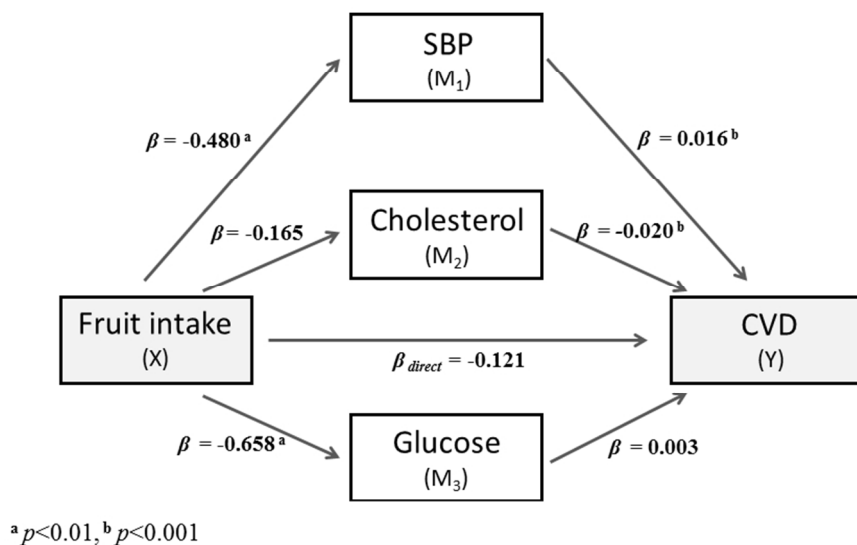
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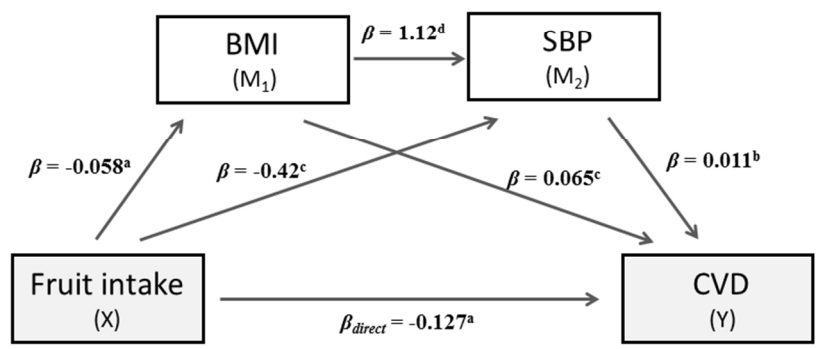
Figure 1



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Figure 2



^a $p < 0.1$, ^b $p < 0.05$, ^c $p < 0.01$, ^d $p < 0.001$

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Supplemental Table 1. Distribution of basic characteristics by fruit intake.

	Fruit intake								<i>p</i> _{trend}
	< 1 time/day		1 time/day		2 times/day		3+ times/day		
	n	%	n	%	n	%	n	%	
Survey year									
2013	961	29.61	935	28.8	629	19.38	721	22.21	0.03
2014	945	31.81	825	27.77	538	18.11	663	22.32	
2015	916	32.45	810	28.69	488	17.29	609	21.57	
Sex									
Male	1519	42.73	983	27.65	551	15.5	502	14.12	<.0001
Female	1303	23.76	1587	28.93	1104	20.13	1491	27.18	
Age (years)	45.83	10.97	45.38	10.94	45.76	10.69	46.53	10.81	0.02
Region									
Urban	2280	30.62	2118	28.44	1389	18.65	1660	22.29	<.01
Rural	542	34.02	452	28.37	266	16.7	333	20.9	
Income level (quartiles)									
Q1	863	40.29	574	26.8	354	16.53	351	16.39	<.0001
Q2	827	36.38	634	27.89	359	15.79	453	19.93	
Q3	612	26.9	702	30.86	435	19.12	526	23.12	
Q4	510	22.02	649	28.02	500	21.59	657	28.37	
Current smoking									
No smoking	1826	26.21	2030	29.14	1377	19.77	1733	24.88	<.0001
Smoking	833	50.24	431	26	210	12.67	184	11.1	

Supplemental Table 2. Distribution of basic characteristics by vegetable intake.

	Vegetable intake								<i>p</i> _{trend}
	< 1 time/day		1 time/day		2 times/day		3+ times/day		
	n	%	n	%	n	%	n	%	
Survey year									
2013	357	11	711	21.9	674	20.76	1504	46.33	<.0001
2014	374	12.59	725	24.4	585	19.69	1287	43.32	
2015	382	13.53	724	25.65	574	20.33	1143	40.49	
Sex									
Male	428	12.04	892	25.09	709	19.94	1526	42.93	0.38
Female	685	12.49	1268	23.12	1124	20.49	2408	43.9	
Age (years)	45.54	11.70	45.11	10.93	45.49	10.93	46.49	10.55	<.0001
Region									
Urban	910	12.22	1775	23.84	1526	20.49	3236	43.45	0.73
Rural	203	12.74	385	24.17	307	19.27	698	43.82	
Income level (quartiles)									
Q1	182	21.19	221	25.73	133	15.48	323	37.6	<.0001
Q2	337	15.56	546	25.21	438	20.22	845	39.01	
Q3	325	11.33	708	24.68	612	21.33	1224	42.66	
Q4	264	8.48	677	21.75	642	20.63	1529	49.13	
Current smoker									
No	810	11.63	1668	23.94	1421	20.4	3067	44.03	<.01
Yes	248	14.96	392	23.64	325	19.6	693	41.8	

Supplemental Table 3. The effect of metabolic mediators (M) in the association between fruit intake (X) and stroke (Y).

Metabolic Factors (M)	Fruit intake										
	X → M (a)			M → Y (b)			X → Y (c' = direct effect)			Indirect effect (a*b)	
	β	SE	<i>p</i>	β	SE	<i>p</i>	β	SE	<i>p</i>	β	95% CI
SBP ^a	-0.484	0.144	<.001	0.015	0.006	<.01	-0.242	0.100	0.02	-0.007	-0.017 -0.001
TC ^a	-0.156	0.357	0.66	-0.018	0.003	<.0001	-0.268	0.105	0.01	0.003	-0.009 0.016
FPG ^a	-0.665	0.217	<.01	0.005	0.004	0.19	-0.269	0.105	0.01	-0.003	-0.008 0.002
BMI ^a	-0.059	0.034	0.08	0.074	0.029	0.01	-0.249	0.100	0.01	-0.004	-0.013 0.001
SBP ^b	-0.420	0.139	<.01	0.013	0.006	0.03	-0.238	0.100	0.02	-0.005	-0.014 0.001
TC ^b	-0.064	0.352	0.86	-0.018	0.003	<.0001	-0.255	0.105	0.02	0.001	-0.011 0.015
FPG ^b	-0.614	0.214	<.01	0.003	0.004	0.37	-0.260	0.105	0.01	-0.002	-0.007 0.004

SBP: systolic blood pressure, TC: total cholesterol, FPG: fasting plasma glucose, BMI: body mass index, SE: standard error, 95% CI: 95% confidence interval.

^aAdjusted for sex, age, income, region (urban/rural), current smoking, and survey year.

^bAdjusted for sex, age, income, region (urban/rural), current smoking, survey year, and body mass index.

Supplemental Table 4. The effect of metabolic mediators (M) in the association between fruit intake (X) and ischemic heart disease (Y).

Metabolic Factors (M)	Fruit intake										
	X → M (a)			M → Y (b)			X → Y (c' = direct effect)			Indirect effect (a*b)	
	β	SE	<i>p</i>	β	SE	<i>p</i>	β	SE	<i>p</i>	β	95% CI
SBP ^a	-0.484	0.144	<.001	0.011	0.006	0.06	-0.065	0.097	0.51	-0.006	-0.013 -0.0001
TC ^a	-0.156	0.357	0.66	-0.021	0.003	<.0001	-0.042	0.100	0.67	0.003	-0.012 0.019
FPG ^a	-0.665	0.217	<.01	0.002	0.004	0.65	-0.048	0.099	0.63	-0.001	-0.006 0.004
BMI ^a	-0.059	0.034	0.08	0.079	0.031	0.01	-0.069	0.097	0.48	-0.005	-0.012 0.001
SBP ^b	-0.420	0.139	<.01	0.010	0.006	0.12	-0.047	0.097	0.63	-0.004	-0.011 0.001
TC ^b	-0.064	0.352	0.86	-0.020	0.003	<.0001	-0.018	0.100	0.86	0.001	-0.013 0.016
FPG ^b	-0.614	0.214	<.01	0.001	0.004	0.88	-0.028	0.099	0.78	0.000	-0.004 0.005

SBP: systolic blood pressure, TC: total cholesterol, FPG: fasting plasma glucose, BMI: body mass index, SE: standard error, 95% CI: 95% confidence interval.

^aAdjusted for sex, age, income, region (urban/rural), current smoking, and survey year.

^bAdjusted for sex, age, income, region (urban/rural), current smoking, survey year, and body mass index.

BMJ Open

The mediating effects of metabolic factors on the association between fruit or vegetable intake and cardiovascular disease: the Korean National Health and Nutrition Examination Survey

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Primary Subject Heading:	Public health
Secondary Subject Heading:	Epidemiology
Keywords:	Cardiovascular disease, blood pressure, NUTRITION & DIETETICS

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Manuscripts

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4 1 **The mediating effects of metabolic factors on the association between fruit or vegetable**
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6 2 **intake and cardiovascular disease: the Korean National Health and Nutrition**
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8 3 **Examination Survey**
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52 20 **Word count:** 2,911
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4 21 **Abstract**

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7 22 **Objective:** We assessed the mediating effects of metabolic components on the relationship
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9 23 between fruit or vegetable intake and cardiovascular disease (CVD).

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12 24 **Design:** Cross-sectional study

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15 25 **Setting:** This study was conducted using data from the 2013–2015 Korean National Health
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17 26 and Nutrition Examination Survey, which is a national representative cross-sectional survey
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19 27 to assess health and nutritional status in the Korean population.

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22 28 **Method and analysis:** A total of 9,040 subjects (3,555 males and 5,485 females) aged ≥ 25
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24 29 years were included in the study. Physician-diagnosed CVD via self-report was used as the
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26 30 outcome. Fruit or vegetable intake was measured via a dish-based semi-quantitative food
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28 31 frequency questionnaire and grouped into categories (< 1 time/d, 1 time/d, 2 times/d, and ≥ 3
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30 32 times/d). Systolic blood pressure (SBP), cholesterol, and fasting glucose were considered
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32 33 metabolic mediators, and the bootstrap method was used to assess mediating effect.

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35 34 **Results:** About 1.8% of adults aged 25–64years had CVD. The risk for CVD decreased by
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37 35 14% as fruit, but not vegetable, intake was increased by one unit per day. After additional
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39 36 adjustment for metabolic factors, the odds ratio was attenuated to 0.89 (95% confidence
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41 37 interval; 0.77–1.03). This result indicates that the indirect effect of three metabolic factors
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43 38 accounted for 21.4% of the relationship between fruit intake and CVD. SBP was a more
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45 39 important metabolic mediator than the other factors. The indirect effect accounted for 30.0%
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47 40 when body mass index was additionally controlled as a mediator, and SBP still had an
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49 41 independent effect compared to the other mediators.

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4 42 **Conclusions:** Our results indicate that controlling SBP may lessen the CVD risk, and a diet
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6 43 rich in fruits can regulate SBP, which, in turn, reduces CVD risk.
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9 44 **Keywords:** Cardiovascular disease, blood pressure, diet
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15 46 **ARTICLE SUMMARY**

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19 48 **Strengths and limitations of this study**

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21 49 - In this study, we assessed how fruit or vegetable intake is related to cardiovascular
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23 50 disease by assessing the indirect effect of systolic blood pressure, total cholesterol, and
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25 51 fasting glucose, including body mass index. This topic was a less interesting part so far,
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27 52 so the study has scientific value.
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30 53 - Using national representative data source, we sought to generalize the research findings.
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32 54 - But, this results were derived from a cross-sectional study design, so causal
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34 55 relationships could not be effectively drawn. Therefore, it is necessary to pay attention
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36 56 to interpretation of research results.
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64 INTRODUCTION

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66 Cardiovascular diseases (CVDs) are responsible for mortality worldwide; a report from
67 the World Health Organization stated that CVDs accounted for 31% of all deaths worldwide
68 in 2015 [1]. Although mortality from ischemic heart disease has shown a flat trend and that
69 from cerebrovascular disease has shown a declining trend in the Republic of Korea since
70 2005, these causes of death remain highly ranked [2].

71 Several risk factors for CVDs, including metabolic factors, such as high glucose, high
72 blood pressure, and high cholesterol, have been suggested [3]. Several studies have suggested
73 that these metabolic factors are also linked to risk factors (e.g., body mass index [BMI] and
74 dietary factors) and CVD risk as mediators [4, 5]. The causal link between these mediators
75 and disease risk must be identified for an effective public health intervention. The mediators
76 can help explain how intervention of risk factors works. However, previous studies focused
77 on a single relationship between a risk factor and a disease rather than the mediating effects.

78 Excessive risk for CVD caused by poor diet and chronic diseases was reported from a
79 study of global burden of disease (GBD). In addition, the GBD study established possible
80 causal mediating relationships between a diet poor in fruits or vegetables, metabolic
81 mediators (blood pressure, cholesterol, and glucose), and disease [4]. Moreover, a recent
82 meta-analysis reported that the beneficial effects of fruits and vegetables intake were also
83 shown in CVD, as well as in cancer and all-cause mortality [6]. The metabolic mediators
84 mentioned above have also been linked to BMI and CVD [4]. The effect of a diet rich in
85 fruits and vegetables on BMI has been reported through epidemiological studies [7], but few
86 studies have assessed BMI as a mediator.

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4 87 There is a need to study the degree to which these metabolic factors contribute to the
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6 88 relationship between risk factors and disease. Although the evidence for the association
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8 89 between fruit/vegetable intake and CVD is relatively strong [8, 9], clarifying the potential
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10 90 biological pathway mechanisms could substantially add to our knowledge. Thus, using cross-
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12 91 sectional survey data from the 2013–2015 Korean National Health and Nutrition Examination
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14 92 Survey (KNHANES), we assessed the mediating effects of metabolic components applied to
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16 93 a confirmatory model. Furthermore, we assessed how the BMI contributes to the relationship
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18 94 between fruit or vegetable intake and CVD as a confounder or mediator.
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107 **METHODS**

109 **1. Study subjects**

110 This study was conducted using data from the 2013–2015 KNHANES, which is a
111 national representative cross-sectional survey to assess health and nutritional status in the
112 Korean population (response rate=78.3%). It consists of a health interview, health
113 examination, and a nutrition survey. A number of variables were collected by trained staff,
114 including physicians, medical technicians, and dieticians. The detailed KNHANES survey
115 method has already been described [10].

116 The food frequency questionnaire (FFQ) was changed to a dish-based semi-quantitative
117 FFQ based on a 2012 survey. The survey assessed subjects 19–64 years of age. Details
118 regarding the development process and validation results of the FFQ tool have been
119 previously published elsewhere [11, 12]. We used the sixth survey from 2013 to 2015 by
120 sampling according to the survey cycle. This study included subjects ≥ 25 years.
121 Additionally, the eligible study population included the respondents with data from all three
122 parts of the survey. Of the subjects aged 25-64 who participated in the survey (n=12,258), 73.7%
123 participated in all three parts of the survey. A total of 9,040 subjects (3,555 males and 5,485
124 females) were included in the study. The study protocol was approved by the Institutional
125 Review Board of the Ewha Womans University Hospital.

127 **2. Fruit and vegetable intake**

128 The dish-based semi-quantitative FFQ was composed of 112 items and provided
129 information on typical dietary consumption for 1 year using a 9-point scale (less than once

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4 130 per month or never, once per month, 2–3 times per month, once per week, 2–4 times per
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6 131 week, 5–6 times per week, once per day, twice per day, and three times per day) and three
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8 132 levels to represent the amount consumed by referring to a standard amount (less, standard,
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10 133 and more). Based on a previous study [4], we excluded pickled and salted vegetables, kimchi,
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12 134 and fruit juice. Vegetable intake and fruit intake were evaluated based on 15 items and 12
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14 135 items, respectively (Supplemental Tables 1). The frequency of fruit intake was used after
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16 136 adjusting for seasonal fruit. Estimated intakes of fruits and vegetables were calculated on the
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18 137 FFQ by multiplying the frequency of each food (as described above) by the selected amount
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20 138 consumed: small (0.5), medium (1), and large (1.5). Fruit and vegetable intake was expressed
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22 139 in four categories (< 1 time/d, 1 time/d, 2 times/d, and ≥ 3 times/d).
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141 **3. Outcome and covariate data**

142 We used data from the health-related questionnaire for the diseases diagnosed by
143 physicians. We selected the questions about stroke, myocardial infarction, and angina pectoris
144 for the CVD-related diseases. If a subject answered “yes” to any of the three diseases, we
145 considered that the subject had CVD. Additionally, we separately considered subjects who
146 answered “yes” on the question about current illness with a physician’s diagnosis and those
147 who responded “yes” to a question about receiving treatment for a disease.

148 Using the measured height and weight information, BMI was calculated in units of kg/m^2 .
149 Blood pressure was measured three times in total and the average value of the second and
150 third measurements was used. Total cholesterol and glucose were measured by taking blood
151 from fasting state.

152 We used data on sex, age, quartiles of income, region (urban/rural), current smoker, and

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4 153 survey year as covariates through a literature review [13] and the results of a univariate
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6 154 analysis. We used quartile data for income instead of education level as a socioeconomic
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8 155 indicator because income may be directly linked to food purchases [14]. The question about
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10 156 physical activity was changed from the 2014 survey, so we did not consider physical activity.
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15 158 **4. Statistical analysis**

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17 159 The basic characteristics of the study subjects are presented as weighted percentages or
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19 160 weighted means with standard errors by considering the multi-stage sampling survey method.
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21 161 The distributions of the basic characteristics according to fruit or vegetable intake level were
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23 162 assessed using the trend test under the random sampling condition. In the main analysis, CVD
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25 163 was considered the outcome (Y) and fruit or vegetable intake was considered an independent
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27 164 variable (X). Systolic blood pressure (SBP) (M_1), total cholesterol (M_2), and fasting glucose
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29 165 (M_3) were applied as metabolic mediators (M). Additionally, BMI was considered as either a
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31 166 covariate or mediator.
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34 167 We examined the association under the controlling covariates (sex, age, income, region
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36 168 [urban/rural], present smoking, and survey year) through four basic steps to assess mediation
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38 169 [15]. Step 1: association between dietary factors and CVD ($X \rightarrow Y$; total effect and was
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40 170 marked path “c”); step 2: association between dietary factors and metabolic mediators ($X \rightarrow$
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42 171 M_i ; marked path “a”); step 3: association between metabolic mediators and CVD after
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44 172 controlling for metabolic mediators ($M_i \rightarrow Y$; marked path “b”); and step 4: association
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46 173 between dietary factors and CVD disease after controlling for metabolic mediators (direct
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48 174 effect; marked path “c”). We used the bootstrap method and the “process” macro (ver.
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50 175 V2.16.3) suggested by Andrew to assess the mediating effects [16]. In this analysis, we
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4 176 applied 10,000 bootstraps. We separately or simultaneously assessed the indirect effect of the
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6 177 metabolic mediators on the association between dietary factors and CVD. The exponential
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8 178 regression coefficient is equal to the odds ratio (OR) when considering the CVD as an
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10 179 outcome variable. The percentage of risk mediated by the metabolic mediator was calculated
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12 180 as [17]: $OR(\text{confounder adjusted}) - OR(\text{confounder and mediator adjusted}) / OR(\text{confounder}$
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14 181 $\text{adjusted}) - 1 \times 100$.

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17 182 All statistical analyses were conducted under a random sampling condition excluding the
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19 183 basic characteristics given in Table 1 using SAS ver. 9.4 software (SAS Institute, Cary, NC,
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21 184 USA). A two-sided p -value < 0.05 was considered significant.
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4 199 **RESULTS**
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10 201 The basic characteristics of the study subjects are presented in Table 1. Mean age was
11
12 202 43.7 years, and 1.81% of subjects (n=189) had CVD. In addition, 0.98% and 0.90% of
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14 203 subject had stroke (n=102) and ischemic heart disease (n=97), respectively. Subjects with a
15
16 204 higher income ate more fruits or vegetables than those with a lower income. Those who ate
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18 205 more fruit were more likely to be non-smokers and female than their counterparts
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20 206 (Supplemental Tables 2, 3).
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23 207 The total effect of fruit intake on CVD showed an inverse association without controlling
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25 208 for metabolic mediators (adjusted odds ratio [aOR], 0.86, 95% CI: 0.74–0.98), but the effect
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27 209 of vegetable intake was not significant (aOR, 0.93; 95% CI: 0.81–1.06) after controlling for
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29 210 sex, age, income, region (urban/rural), current smoker, and survey year.
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33 211 The direct effect of fruit intake on CVD was borderline significant after further
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35 212 considering each metabolic mediator. The effect of SBP did not include zero in the 95% CI
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37 213 range as the other metabolic mediators. The effect of fruit intake on BMI showed borderline
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39 214 significance, and the effect of BMI on CVD was significant, but the indirect effect of BMI
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41 215 was not significant. Additionally, the effect of SBP was significant even after controlling for
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43 216 BMI as a covariate (Table 2). SBP, cholesterol, and BMI were associated with CVD, but
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45 217 vegetable intake did not contribute to either metabolic mediator or CVD (Table 3). The
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47 218 mediating effect of SBP on the association between fruit intake and outcome was dominant
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49 219 even when the outcome was restricted to those with a current illness or undergoing treatment.
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53 220 The OR was attenuated to 0.89 (95% CI: 0.77–1.03) while simultaneously controlling for
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4 221 multiple metabolic mediators, indicating a 21.4% indirect effect for CVD (i.e. $(0.8555-$
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6 222 $0.8864)/(0.8555-1)*100=21.4\%$). SBP showed an independent indirect effect. Higher fruit
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8 223 intake had a beneficial effect on fasting glucose, but its effect was not associated with CVD.
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10 224 The direct effect of fruit intake on CVD presented an inverse association, but it did not reach
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12 225 statistical significance (Figure 1). In addition, similar results were observed when adding
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14 226 BMI as covariate, with an OR of 0.90 (95% CI: 0.78–1.04; data not shown).

17 227 We analyzed the serial mediator model to assess whether BMI influenced SBP (Figure 2).
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19 228 Although the effect of fruit intake on BMI showed borderline significance, the influence of
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21 229 BMI on SBP, and the effect of SBP on CVD reached statistical significance. Of the three
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23 230 possible indirect paths, the fruit intake path \rightarrow SBP \rightarrow CVD was the only one to show an
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25 231 independent association.

29 232 Fruit intake was directly linked to subjects who suffered a stroke, but not ischemic heart
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31 233 disease, regardless of which metabolic factors were controlled. In addition, the mediating
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33 234 effect of SBP was dominant in patients who suffered a stroke or ischemic heart disease even
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35 235 after controlling for BMI (Supplemental Tables 4, 5).

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4 242 **DISCUSSION**
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9 244 In this study, we assessed how fruit or vegetable intake is related to CVD by assessing
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11 245 the indirect effect of metabolic mediators. Based on the suggested causal link, SBP, total
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13 246 cholesterol, and fasting glucose were considered metabolic mediators, and the effect of BMI
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15 247 was additionally assessed. Of them, the indirect effect of SBP on the relationship between
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17 248 fruit intake and CVD was significant even after considering BMI, but not vegetable intake.
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19 249 The indirect effect of the four metabolic factors accounted for 30.0% of the relationship
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22 250 between fruit intake and CVD (i.e. $(0.8555-0.8989)/(0.8555-1)*100=30.0\%$).
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24 251 The beneficial effects of high fruit or vegetable intake on CVD and the unfavorable
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26 252 effects of high blood pressure, glucose, and cholesterol on CVD are well known. Thus,
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28 253 previous studies considered metabolic factors together, and mediators were reported to
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30 254 attenuate the association of a direct effect [5]. One large prospective study conducted in 10
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32 255 regions in China indicated that higher fresh fruit intake is linked to CVD death, and its effect
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34 256 was attenuated by hazard ratios from 0.63 (95% CI: 0.56–0.72) to 0.70 (95% CI: 0.61–0.79)
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36 257 after adjusting for BMI, blood pressure, glucose, and waist circumference [18]. Another study
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38 258 conducted in Shanghai, China showed an attenuated association between fruit intake and
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40 259 incident coronary heart disease after controlling for a history of diabetes, hypertension, or
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42 260 dyslipidemia, but no association or attenuation was observed for vegetable intake [5]. A
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44 261 women's health study reported by Liu et al. also showed that the effect of fruits and
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46 262 vegetables on CVD risk became stronger after excluding subjects with a history of diabetes,
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48 263 hypertension, and high cholesterol [19]. It seems that these mediators largely attribute to the
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50 264 relationship between fruit and/or vegetable intake and CVD risk. However, biological
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4 265 pathways by metabolic factors between fruit and/or vegetable intake and CVD risk have not
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6 266 been investigated.

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8 267 The assessment of a mediating effect could help understand how fruit and/or vegetable
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10 268 intake affects CVDs. In addition, an effect of poor dietary risk by metabolic mediators on
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12 269 CVD was suggested by the GBD study, so that was considered to estimate the disease burden.
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14 270 The mediating effect of blood pressure on the association between fruit and/or vegetable
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16 271 intake and CVD was suggested by a prospective cohort study of patients in the first National
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18 272 Health and Nutrition Examination Survey [13]. Blood pressure contributed 22.2% to the
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20 273 relationship between fruit and vegetable intake and CVD death. This was similar to the
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22 274 results adjusted for BMI, cholesterol, and blood pressure. That study also showed that the
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24 275 direct effect of fruit and vegetable intake was notable in patients who suffered a stroke but
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26 276 not those with ischemic heart disease. These results are in line with those of the present study.

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29 277 We assessed a potential role for BMI on the association between fruit intake and CVD
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31 278 using various models. Several reports, including the above-mentioned study, considered BMI
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33 279 as a potential mediator [13, 18]. Additionally, a causal link between BMI and CVD risk is
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35 280 mediated through metabolic factors. Two pooled studies of prospective cohorts assessed the
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37 281 effect of BMI on coronary heart disease and stroke as mediated by metabolic components.
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39 282 They reported that blood pressure was a more important mediator compared to cholesterol
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41 283 and glucose [17, 20]. Other pooled data from an Asian cohort also indicate that estimated
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43 284 mediating proportions through hypertension were 62.3, 35.7, and 92.4% for the association
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45 285 between BMI and death due to CVD, coronary heart disease, and stroke, respectively, but not
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47 286 by diabetes [21]. The GBD study restricted total calories to 2,000 kcal instead of considering
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49 287 BMI [22]. In the present study, higher fruit intake was inversely associated with BMI, but it
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4 288 was borderline significant ($\beta = -0.06$, $p = 0.08$), which affected the results of the fruit intake
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6 289 path \rightarrow BMI \rightarrow SBP \rightarrow CVD in the serial multiple mediator model. Our study found that the
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8 290 mediating effect due to BMI was about 7.9%, but previous studies showed a $< 3.0\%$
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10 291 mediating effect by BMI on the association between fruit only or fruit and vegetable intake
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12 292 and CVD deaths by presenting little change in the adjusted risk value [13, 18]. However, it is
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14 293 difficult to make a direct comparison due to discrepancies in study design, study populations,
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16 294 the definition of disease, and fruit and/or vegetable intake.

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19 295 Eating more vegetables was not significantly associated with either a direct or indirect
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21 296 effect. In Korea, vegetables in the general population are easily accessible by a side dish.
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23 297 Indeed, statistics from the Organisation for Economic Co-operation and Development
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25 298 (OECD) have reported that daily vegetable consumption among adults was the highest in
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27 299 Korea [23]. However, the manner of preparation and/or cooking can influence nutrient
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29 300 content [7]. The favorable effects of fruit and vegetable intake can be explained by nutrients,
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31 301 such as dietary fiber, folate, potassium, and antioxidant vitamins (i.e., vitamin E, vitamin C,
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33 302 polyphenols, flavonoids, and carotenoids) and other components. These nutrients might be
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35 303 involved with controlling glucose, lipid level, and blood pressure, and reduce the risk of CVD
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37 304 along with weight control [7]. However, because foods contain various nutrients, food
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39 305 recommendations help subjects follow a prevention strategy. In addition, healthy eating is
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41 306 also associated with other health behaviors, such as not smoking and regular physical activity
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43 307 [13, 24].

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47 308 The present study has some limitations. First, the results were derived from a cross-
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49 309 sectional study design, so causal relationships could not be effectively drawn. Our study
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51 310 design is also open to the problem of reverse causation. If the reverse causation affects the
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4 311 results, the association will appear to be null or reverse direction to what is expected. But, the
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6 312 indirect effect by SBP was significant and some parts of our results were consistent with
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8 313 previous studies [13, 24]. Furthermore, the results were consistent even after applying stroke
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10 314 or ischemic heart disease. Because the survey is conducted through a household visit and
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12 315 excludes people in the hospital, subjects with diseases might be the relatively less serious
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14 316 cases. Measurement error in FFQ survey or self-reported disease status may influence the
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16 317 results. In addition, residual confounding factors such as physical activity may have
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18 318 influenced the association. Finally, owing to the number of participants with CVD is very low
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20 319 (1.8%), the study has inadequate statistical power which might explain some of the non-
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22 320 significant findings.

25 321 Nevertheless, our study focused on the mediating effects of metabolic factors on CVD
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27 322 and assessed which metabolic factors affect CVD. Our results were produced using the
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29 323 bootstrapping method and did not impose the assumption of normality of the sampling
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31 324 distribution; thus, it was an appropriate design for multiple mediations [15]. The given
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33 325 evidence was conceptually approached and was not statistically tested for an indirect effect.

36 326 Taken together, our study suggests that controlling SBP might lessen CVD risk, and a
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38 327 diet rich in fruits can be used to regulate SBP, which, in turn, reduces CVD risk.

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4 334 **Conflict of interest statement**
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13 337 **Contributor ship statement**
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15 338 HA Lee wrote the manuscript and performed the statistical analyses; D Lim, K Oh, and EJ Kim,
16
17 339 provided advice about writing the manuscript, and H Park helped interpret the data.
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27
28 343 Health & Welfare, Republic of Korea (HI13C0729).
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34 345 **Data availability**
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37 346 The National Health and Nutrition Examination Survey files are available from the Korea Centers for Disease
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39 347 Control and Prevention database (URL https://knhanes.cdc.go.kr/knhanes/sub03/sub03_02_02.do). If you
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41 348 register your e-mail on this site, you can freely download the raw data.
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4 467 **Figure Legends**

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9
10 469 Figure 1. The effect of multiple metabolic factor (M_i) mediators in the association between
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12 470 fruit intake (X) and cardiovascular diseases (Y).

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15 471 ^a $p < 0.01$, ^b $p < 0.001$, SBP: systolic blood pressure. Coefficients were adjusted for sex, age,
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17 472 income, region (urban/rural), current smoker, and survey year using the bootstrapping method.

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21
22 474 Figure 2. The effect of multiple serial mediators of metabolic factors (M_i) in the association
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24 475 between fruit intake (X) and cardiovascular diseases (Y).

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27 476 ^a $p < 0.1$, ^b $p < 0.05$, ^c $p < 0.01$, ^d $p < 0.001$, BMI: body mass index, SBP: systolic blood pressure.
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29 477 Coefficients were adjusted for sex, age, income, region (urban/rural), current smoker, and
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31 478 survey year using the bootstrapping method.

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486 Table 1. Basic characteristics of the study subjects.

	Weighted % (SE)
Sex	
Male	49.92 (0.51)
Female	50.08 (0.51)
Age range	
Age (years)†	25–64 years
	43.68 (0.18)
Region	
Urban	83.87 (1.52)
Rural	16.13 (1.52)
Income level (quartiles)	
Q1	23.46 (0.73)
Q2	25.61 (0.72)
Q3	25.03 (0.71)
Q4	25.90 (0.97)
Current smoking	
No	75.83 (0.60)
Yes	24.17 (0.60)
Disease	
Cardiovascular disease	1.81 (0.16)
Stroke	0.98 (0.13)
Ischemic heart disease	0.90 (0.10)
Metabolic Factors†	
Systolic blood pressure (mmHg)	115.01 (0.21)
Total cholesterol (mg/dL)	190.98 (0.47)
Fasting plasma glucose (mg/dL)	98.58 (0.30)
Body mass index (kg/m ²)	23.92 (0.05)

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488 SE: Standard error.

489 †Weighted mean with standard error.

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498 Table 2. The effect of metabolic mediators (M) in the association between fruit intake (X) and
 499 cardiovascular disease (Y).

Metabolic Factors (M)	Fruit intake										
	X → M (a)			M → Y (b)			X → Y (c' = direct effect)			Indirect effect (a*b)	
	β	SE	<i>p</i>	β	SE	<i>p</i>	β	SE	<i>p</i>	β	95% CI
SBP ^a	-0.484	0.144	0.001	0.013	0.004	0.002	-0.137	0.072	0.06	-0.007	-0.014 -0.002
TC ^a	-0.156	0.357	0.66	-0.019	0.003	<.0001	-0.144	0.075	0.05	0.003	-0.011 0.017
FPG ^a	-0.665	0.217	<.01	0.004	0.003	0.20	-0.144	0.074	0.05	-0.002	-0.006 0.001
BMI ^a	-0.059	0.034	0.08	0.078	0.022	0.001	-0.143	0.072	<.05	-0.005	-0.012 0.001
SBP ^b	-0.420	0.139	<.01	0.011	0.005	0.01	-0.127	0.072	0.08	-0.005	-0.011 -0.0004
TC ^b	-0.064	0.352	0.86	-0.019	0.003	<.0001	-0.126	0.075	0.09	0.001	-0.012 0.015
FPG ^b	-0.614	0.214	<.01	0.002	0.003	0.42	-0.130	0.074	0.08	-0.002	-0.005 0.002

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501 SBP: systolic blood pressure, TC: total cholesterol, FPG: fasting plasma glucose, BMI: body mass
 502 index, SE: standard error, 95% CI: 95% confidence interval.

503 ^aAdjusted for sex, age, income, region (urban/rural), current smoking, and survey year.

504 ^bAdjusted for sex, age, income, region (urban/rural), current smoking, survey year, and body mass
 505 index.

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520 Table 3. The effect of metabolic mediators (M) in the association between vegetable intake
521 (X) and cardiovascular disease (Y).

Metabolic Factors (M)	Vegetable intake										
	X → M (a)			M → Y (b)			X → Y (c' = direct effect)			Indirect effect (a*b)	
	β	SE	<i>p</i>	β	SE	<i>p</i>	β	SE	<i>p</i>	β	95% CI
SBP ^a	-0.042	0.169	0.80	0.014	0.004	0.002	-0.132	0.086	0.13	-0.001	-0.006 0.004
TC ^a	0.236	0.420	0.57	-0.019	0.003	<.0001	-0.121	0.089	0.18	-0.005	-0.021 0.012
FPG ^a	-0.054	0.256	0.83	0.004	0.003	0.18	-0.132	0.088	0.14	-0.0002	-0.003 0.002
BMI ^a	0.057	0.040	0.16	0.080	0.022	<.001	-0.145	0.086	0.09	0.005	-0.002 0.013
SBP ^b	-0.114	0.163	0.48	0.012	0.005	0.01	-0.131	0.086	0.13	-0.001	-0.006 0.002
TC ^b	0.142	0.415	0.73	-0.019	0.003	<.0001	-0.122	0.089	0.17	-0.003	-0.019 0.014
FPG ^b	-0.121	0.252	0.63	0.003	0.003	0.4	-0.132	0.088	0.14	-0.0003	-0.003 0.002

522 SBP: systolic blood pressure, TC: total cholesterol, FPG: fasting plasma glucose, BMI: body mass
523 index, SE: standard error, 95% CI: 95% confidence interval.

524 ^aAdjusted for sex, age, income, region (urban/rural), current smoking, and survey year.

525 ^bAdjusted for sex, age, income, region (urban/rural), current smoking, survey year, and body mass
526 index.

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Figure 1

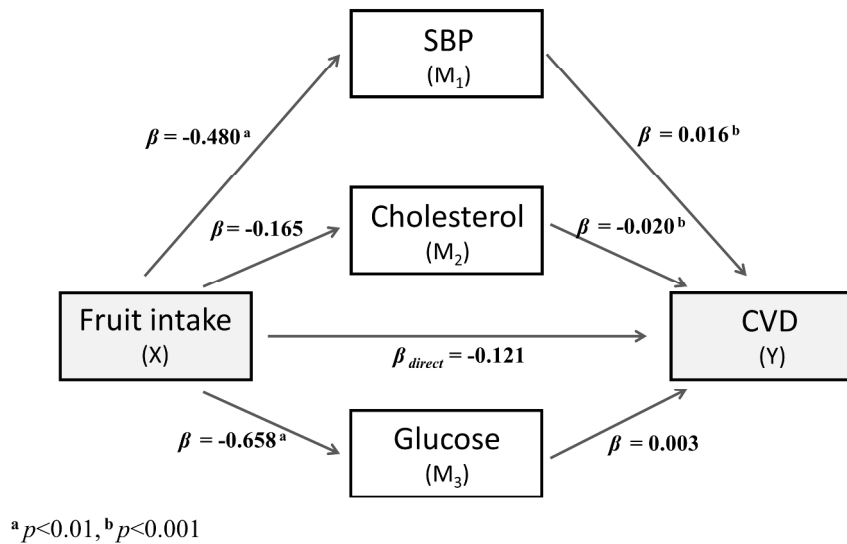
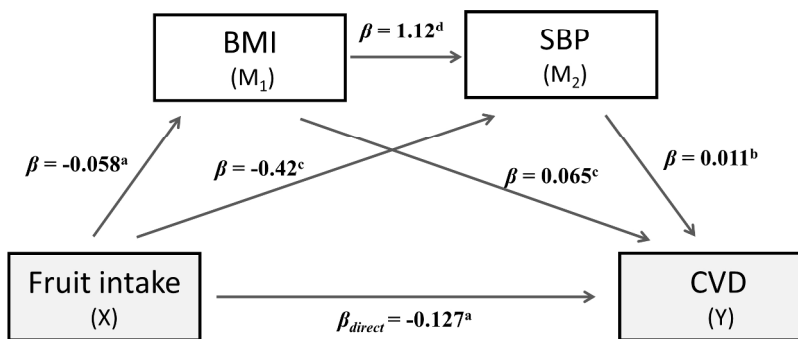


figure1

254x190mm (300 x 300 DPI)

Figure 2



^a $p < 0.1$, ^b $p < 0.05$, ^c $p < 0.01$, ^d $p < 0.001$

figure2

254x190mm (300 x 300 DPI)

Supplemental Table 1. List of fruit or vegetable related food items

Fruit	Vegetable
Strawberry	Bean sprouts (seasoned, soup)
Melon	Seasoned mung bean sprout
Watermelon	Seasoned spinach
Peach	Seasoned bellflower (boiled or not)
Grape	Pumpkin (seasoned, pan-fried)
Apple	Other seasoned vegetables
Pear	Cucumber (seasoned, raw)
Persimmon, dried persimmon	Radish (seasoned, pickled, dried)
Tangerine	Vegetable salad
Banana	Seasoned green onion, and seasoned Chinese chives
Orange	Raw vegetables (lettuce, sesame, Chinese cabbage, and pumpkin leaf)
Kiwi	Green pepper
	Boiled broccoli, boiled cabbage
	Garlic
	Tomato, and cherry tomato

The food frequency questionnaire consists of dietary consumption using a 9-point scale (less than once per month or never, once per month, 2–3 times per month, once per week, 2–4 times per week, 5–6 times per week, once per day, twice per day, and three times per day) and three levels to represent the amount consumed by referring to a standard amount (less, standard, and more).

Supplemental Table 2. Distribution of basic characteristics by fruit intake.

	Fruit intake								<i>p</i> _{trend}
	< 1 time/day		1 time/day		2 times/day		3+ times/day		
	n	%	n	%	n	%	n	%	
Survey year									
2013	961	29.61	935	28.8	629	19.38	721	22.21	0.03
2014	945	31.81	825	27.77	538	18.11	663	22.32	
2015	916	32.45	810	28.69	488	17.29	609	21.57	
Sex									
Male	1519	42.73	983	27.65	551	15.5	502	14.12	<.0001
Female	1303	23.76	1587	28.93	1104	20.13	1491	27.18	
Age (years)	45.83	10.97	45.38	10.94	45.76	10.69	46.53	10.81	0.02
Region									
Urban	2280	30.62	2118	28.44	1389	18.65	1660	22.29	<.01
Rural	542	34.02	452	28.37	266	16.7	333	20.9	
Income level (quartiles)									
Q1	863	40.29	574	26.8	354	16.53	351	16.39	<.0001
Q2	827	36.38	634	27.89	359	15.79	453	19.93	
Q3	612	26.9	702	30.86	435	19.12	526	23.12	
Q4	510	22.02	649	28.02	500	21.59	657	28.37	
Current smoking									
No	1826	26.21	2030	29.14	1377	19.77	1733	24.88	<.0001
Yes	833	50.24	431	26	210	12.67	184	11.1	

Supplemental Table 3. Distribution of basic characteristics by vegetable intake.

	Vegetable intake								<i>p</i> _{trend}
	< 1 time/day		1 time/day		2 times/day		3+ times/day		
	n	%	n	%	n	%	n	%	
Survey year									
2013	357	11	711	21.9	674	20.76	1504	46.33	<.0001
2014	374	12.59	725	24.4	585	19.69	1287	43.32	
2015	382	13.53	724	25.65	574	20.33	1143	40.49	
Sex									
Male	428	12.04	892	25.09	709	19.94	1526	42.93	0.38
Female	685	12.49	1268	23.12	1124	20.49	2408	43.9	
Age (years)	45.54	11.70	45.11	10.93	45.49	10.93	46.49	10.55	<.0001
Region									
Urban	910	12.22	1775	23.84	1526	20.49	3236	43.45	0.73
Rural	203	12.74	385	24.17	307	19.27	698	43.82	
Income level (quartiles)									
Q1	394	18.39	550	25.68	391	18.25	807	37.68	<.0001
Q2	288	12.67	587	25.82	481	21.16	917	40.34	
Q3	239	10.51	529	23.25	485	21.32	1022	44.92	
Q4	187	8.07	486	20.98	468	20.21	1175	50.73	
Current smoker									
No	810	11.63	1668	23.94	1421	20.4	3067	44.03	<.01
Yes	248	14.96	392	23.64	325	19.6	693	41.8	

Supplemental Table 4. The effect of metabolic mediators (M) in the association between fruit intake (X) and stroke (Y).

Metabolic Factors (M)	Fruit intake											
	X → M (a)			M → Y (b)			X → Y (c' = direct effect)			Indirect effect (a*b)		
	β	SE	<i>p</i>	β	SE	<i>p</i>	β	SE	<i>p</i>	β	95% CI	
SBP ^a	-0.484	0.144	<.001	0.015	0.006	<.01	-0.242	0.100	0.02	-0.007	-0.017	-0.001
TC ^a	-0.156	0.357	0.66	-0.018	0.003	<.0001	-0.268	0.105	0.01	0.003	-0.009	0.016
FPG ^a	-0.665	0.217	<.01	0.005	0.004	0.19	-0.269	0.105	0.01	-0.003	-0.008	0.002
BMI ^a	-0.059	0.034	0.08	0.074	0.029	0.01	-0.249	0.100	0.01	-0.004	-0.013	0.001
SBP ^b	-0.420	0.139	<.01	0.013	0.006	0.03	-0.238	0.100	0.02	-0.005	-0.014	0.001
TC ^b	-0.064	0.352	0.86	-0.018	0.003	<.0001	-0.255	0.105	0.02	0.001	-0.011	0.015
FPG ^b	-0.614	0.214	<.01	0.003	0.004	0.37	-0.260	0.105	0.01	-0.002	-0.007	0.004

SBP: systolic blood pressure, TC: total cholesterol, FPG: fasting plasma glucose, BMI: body mass index, SE: standard error, 95% CI: 95% confidence interval.

^aAdjusted for sex, age, income, region (urban/rural), current smoking, and survey year.

^bAdjusted for sex, age, income, region (urban/rural), current smoking, survey year, and body mass index.

Supplemental Table 5. The effect of metabolic mediators (M) in the association between fruit intake (X) and ischemic heart disease (Y).

Metabolic Factors (M)	Fruit intake											
	X → M (a)			M → Y (b)			X → Y (c' = direct effect)			Indirect effect (a*b)		
	β	SE	<i>p</i>	β	SE	<i>p</i>	β	SE	<i>p</i>	β	95% CI	
SBP ^a	-0.484	0.144	<.001	0.011	0.006	0.06	-0.065	0.097	0.51	-0.006	-0.013	-0.0001
TC ^a	-0.156	0.357	0.66	-0.021	0.003	<.0001	-0.042	0.100	0.67	0.003	-0.012	0.019
FPG ^a	-0.665	0.217	<.01	0.002	0.004	0.65	-0.048	0.099	0.63	-0.001	-0.006	0.004
BMI ^a	-0.059	0.034	0.08	0.079	0.031	0.01	-0.069	0.097	0.48	-0.005	-0.012	0.001
SBP ^b	-0.420	0.139	<.01	0.010	0.006	0.12	-0.047	0.097	0.63	-0.004	-0.011	0.001
TC ^b	-0.064	0.352	0.86	-0.020	0.003	<.0001	-0.018	0.100	0.86	0.001	-0.013	0.016
FPG ^b	-0.614	0.214	<.01	0.001	0.004	0.88	-0.028	0.099	0.78	0.000	-0.004	0.005

SBP: systolic blood pressure, TC: total cholesterol, FPG: fasting plasma glucose, BMI: body mass index, SE: standard error, 95% CI: 95% confidence interval.

^aAdjusted for sex, age, income, region (urban/rural), current smoking, and survey year.

^bAdjusted for sex, age, income, region (urban/rural), current smoking, survey year, and body mass index.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-8
Data sources/ measurement	8*	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	6-8
Bias	9	Describe any efforts to address potential sources of bias	15
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	8-9
		(b) Describe any methods used to examine subgroups and interactions	
		(c) Explain how missing data were addressed	
		(d) If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	8
Results			

Participants	13*	(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 (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	6
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest	10
Outcome data	15*	Report numbers of outcome events or summary measures	10
Main results	16	(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 (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	10-11 7
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
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	16

*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.

BMJ Open

The mediating effects of metabolic factors on the association between fruit or vegetable intake and cardiovascular disease: the Korean National Health and Nutrition Examination Survey

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Manuscript ID	bmjopen-2017-019620.R2
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Primary Subject Heading:	Public health
Secondary Subject Heading:	Epidemiology
Keywords:	Cardiovascular disease, blood pressure, NUTRITION & DIETETICS

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Manuscripts

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4 1 **The mediating effects of metabolic factors on the association between fruit or vegetable**
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6 2 **intake and cardiovascular disease: the Korean National Health and Nutrition**
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8 3 **Examination Survey**
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11 4

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52 20 **Word count:** 2,986
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4 21 **Abstract**

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7 22 **Objective:** We assessed the mediating effects of metabolic components on the relationship
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9 23 between fruit or vegetable intake and cardiovascular disease (CVD).

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12 24 **Design:** Cross-sectional study

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15 25 **Setting:** This study was conducted using data from the 2013–2015 Korean National Health
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17 26 and Nutrition Examination Survey, which is a national representative cross-sectional survey
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19 27 to assess health and nutritional status in the Korean population.

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22 28 **Method and analysis:** A total of 9,040 subjects (3,555 males and 5,485 females) aged ≥ 25
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24 29 years were included in the study. Physician-diagnosed CVD via self-report was used as the
25
26 30 outcome. Fruit or vegetable intake was measured via a dish-based semi-quantitative food
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28 31 frequency questionnaire and grouped into categories (< 1 time/d, 1 time/d, 2 times/d, and ≥ 3
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30 32 times/d). Systolic blood pressure (SBP), cholesterol, and fasting glucose were considered
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32 33 metabolic mediators, and the bootstrap method was used to assess mediating effect.

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36 34 **Results:** About 1.8% of adults aged 25–64years had CVD. According to the result of
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38 35 "process" macro, the confounder adjusted risk for CVD decreased by 14% (odds ratio (OR) =
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40 36 0.86, 95 % confidence interval (CI): 0.74–0.98) as fruit, but not vegetable, intake was
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42 37 increased by one unit per day. After additional adjustment for three metabolic factors
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44 38 simultaneously, the OR was attenuated to 0.89 (95% CI; 0.77–1.03). This result indicates that
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46 39 the indirect effect of three metabolic factors accounted for 21.4% of the relationship between
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48 40 fruit intake and CVD. SBP was a more important metabolic mediator than the other factors.
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50 41 The indirect effect by metabolic factors accounted for 30.0% when body mass index was

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4 42 additionally controlled as a mediator, and SBP still had an independent effect compared to the
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6 43 other mediators.
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9 44 **Conclusions:** Our results indicate that controlling SBP may lessen the CVD risk, and a diet
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11 45 rich in fruits can regulate SBP, which, in turn, reduces CVD risk.
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14 46 **Keywords:** Cardiovascular disease, blood pressure, diet
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20 48 **ARTICLE SUMMARY**
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24 50 **Strengths and limitations of this study**
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- 26 51 - In this study, we assessed how fruit or vegetable intake is related to cardiovascular
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28 52 disease by assessing the indirect effect of systolic blood pressure, total cholesterol, and
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30 53 fasting glucose, including body mass index. This topic was a less interesting part so far,
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32 54 so the study has scientific value.
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34 55 - Using national representative data source, we sought to generalize the research findings.
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36 56 - But, this results were derived from a cross-sectional study design, so causal
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38 57 relationships could not be effectively drawn. Therefore, it is necessary to pay attention
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40 58 to interpretation of research results.
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64 INTRODUCTION

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66 Cardiovascular diseases (CVDs) are responsible for mortality worldwide; a report from
67 the World Health Organization stated that CVDs accounted for 31% of all deaths worldwide
68 in 2015 [1]. Although mortality from ischemic heart disease has shown a flat trend and that
69 from cerebrovascular disease has shown a declining trend in the Republic of Korea since
70 2005, these causes of death remain highly ranked [2].

71 Several risk factors for CVDs, including metabolic factors, such as high glucose, high
72 blood pressure, and high cholesterol, have been suggested [3]. Several studies have suggested
73 that these metabolic factors are also linked to risk factors (e.g., body mass index [BMI] and
74 dietary factors) and CVD risk as mediators [4, 5]. The causal link between these mediators
75 and disease risk can help explain how intervention of risk factors works. However, previous
76 studies focused on a single relationship between a risk factor and a disease rather than the
77 mediating effects.

78 Excessive risk for CVD caused by poor diet and chronic diseases was reported from a
79 study of global burden of disease (GBD). In addition, the GBD study established possible
80 causal mediating relationships between a diet poor in fruits or vegetables, metabolic
81 mediators (blood pressure, cholesterol, and glucose), and disease [4]. Moreover, a recent
82 meta-analysis reported that the beneficial effects of fruits and vegetables intake were also
83 shown in CVD, as well as in cancer and all-cause mortality [6]. The metabolic mediators
84 mentioned above have also been linked to BMI and CVD [4]. The effect of a diet rich in
85 fruits and vegetables on BMI has been reported through epidemiological studies [7], but few
86 studies have assessed BMI as a mediator.

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4 87 There is a need to study the degree to which these metabolic factors contribute to the
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6 88 relationship between risk factors and disease. Although the evidence for the association
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8 89 between fruit/vegetable intake and CVD is relatively strong [8, 9], clarifying the potential
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10 90 biological pathway mechanisms could substantially add to our knowledge. Thus, using cross-
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12 91 sectional survey data from the 2013–2015 Korean National Health and Nutrition Examination
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14 92 Survey (KNHANES), we assessed the mediating effects of metabolic components applied to
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16 93 a confirmatory model. Furthermore, we assessed how the BMI contributes to the relationship
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18 94 between fruit or vegetable intake and CVD as a confounder or mediator.
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107 **METHODS**

109 **1. Study subjects**

110 This study was conducted using data from the 2013–2015 KNHANES, which is a
111 national representative cross-sectional survey to assess health and nutritional status in the
112 Korean population (response rate=78.3%). It consists of a health interview, health
113 examination, and a nutrition survey. A number of variables were collected by trained staff,
114 including physicians, medical technicians, and dieticians. The detailed KNHANES survey
115 method has already been described [10].

116 The food frequency questionnaire (FFQ) was changed to a dish-based semi-quantitative
117 FFQ based on a 2012 survey. The survey assessed subjects 19–64 years of age. Details
118 regarding the development process and validation results of the FFQ tool have been
119 previously published elsewhere [11, 12]. We used the sixth survey from 2013 to 2015 by
120 sampling according to the survey cycle. This study included subjects ≥ 25 years.
121 Additionally, the eligible study population included the respondents with data from all three
122 parts of the survey. Of the subjects aged 25-64 who participated in the survey (n=12,258), 73.7%
123 participated in all three parts of the survey. A total of 9,040 subjects (3,555 males and 5,485
124 females) were included in the study. The study protocol was approved by the Institutional
125 Review Board of the Ewha Womans University Hospital.

127 **2. Fruit and vegetable intake**

128 The dish-based semi-quantitative FFQ was composed of 112 items and provided
129 information on typical dietary consumption for 1 year using a 9-point scale (less than once

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4 130 per month or never, once per month, 2–3 times per month, once per week, 2–4 times per
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6 131 week, 5–6 times per week, once per day, twice per day, and three times per day) and three
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8 132 levels to represent the amount consumed by referring to a standard amount (less, standard,
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10 133 and more). Based on a previous study [4], we excluded pickled and salted vegetables, kimchi,
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12 134 and fruit juice. Vegetable intake and fruit intake were evaluated based on 15 items and 12
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14 135 items, respectively (Supplemental Tables 1). The frequency of fruit intake was used after
15
16 136 adjusting for seasonal fruit. Estimated intakes of fruits and vegetables were calculated on the
17
18 137 FFQ by multiplying the frequency of each food (as described above) by the selected amount
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20 138 consumed: small (0.5), medium (1), and large (1.5). Fruit and vegetable intake was expressed
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22 139 in four categories (< 1 time/d, 1 time/d, 2 times/d, and ≥ 3 times/d).
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141 **3. Outcome and covariate data**

142 We used data from the health-related questionnaire for the diseases diagnosed by
143 physicians. We selected the questions about stroke, myocardial infarction, and angina pectoris
144 for the CVD-related diseases. If a subject answered “yes” to any of the three diseases, we
145 considered that the subject had CVD. Additionally, we separately considered subjects who
146 answered “yes” on the question about current illness with a physician’s diagnosis and those
147 who responded “yes” to a question about receiving treatment for a disease.

148 Using the measured height and weight information, BMI was calculated in units of kg/m^2 .
149 Blood pressure was measured three times in total and the average value of the second and
150 third measurements was used. Total cholesterol and glucose were measured by taking blood
151 from fasting state.

152 We used data on sex, age, quartiles of income, region (urban/rural), current smoker, and

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4 153 survey year as covariates through a literature review [13] and the results of a univariate
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6 154 analysis. We used quartile data for income instead of education level as a socioeconomic
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8 155 indicator because income may be directly linked to food purchases [14]. The question about
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10 156 physical activity was changed from the 2014 survey, so we did not consider physical activity.
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15 158 **4. Statistical analysis**

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17 159 The basic characteristics of the study subjects are presented as weighted percentages or
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19 160 weighted means with standard errors by considering the multi-stage sampling survey method.
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21 161 The distributions of the basic characteristics according to fruit or vegetable intake level were
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23 162 assessed using the trend test under the random sampling condition. In the main analysis, CVD
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25 163 was considered the outcome (Y) and fruit or vegetable intake was considered an independent
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27 164 variable (X). Systolic blood pressure (SBP) (M_1), total cholesterol (M_2), and fasting glucose
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29 165 (M_3) were applied as metabolic mediators (M). Additionally, BMI was considered as either a
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31 166 covariate or mediator.
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34 167 We used the "process" macro based on the bootstrap method (ver. V2.16.3) suggested by
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36 168 Andrew to assess the mediating effects [15]. In this analysis, we applied 10,000 bootstraps.
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38 169 We separately or simultaneously assessed the indirect effect of the metabolic mediators on the
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40 170 association between dietary factors and CVD. Firstly, we examined the association under the
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42 171 controlling covariates (sex, age, income, region [urban/rural], present smoking, and survey
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44 172 year) through four basic steps to assess mediation [16]. Step 1: association between dietary
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46 173 factors and CVD ($X \rightarrow Y$; total effect and was marked path "c"); step 2: association between
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48 174 dietary factors and metabolic mediators ($X \rightarrow M_i$; marked path "a"); step 3: association
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50 175 between metabolic mediators and CVD after controlling for metabolic mediators ($M_i \rightarrow Y$;
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4 176 marked path “b”); and step 4: association between dietary factors and CVD disease after
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6 177 controlling for metabolic mediators (direct effect; marked path “c”). Subsequently, we
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8 178 evaluated the multiple mediator model and the serial mediator model.
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10 179 The exponential regression coefficient is equal to the odds ratio (OR) when considering
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12 180 the CVD as an outcome variable. The percentage of risk mediated by the metabolic mediator
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14 181 was calculated as [17]: $OR(\text{confounder adjusted}) - OR(\text{confounder and mediator}$
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16 182 $\text{adjusted}) / OR(\text{confounder adjusted}) - 1 \times 100$.
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19 183 All statistical analyses were conducted under a random sampling condition excluding the
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21 184 basic characteristics given in Table 1 using SAS ver. 9.4 software (SAS Institute, Cary, NC,
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23 185 USA). A two-sided p -value < 0.05 was considered significant.
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4 199 **RESULTS**
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10 201 The basic characteristics of the study subjects are presented in Table 1. Mean age was
11
12 202 43.7 years, and 1.81% of subjects (n=189) had CVD. In addition, 0.98% and 0.90% of
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14 203 subject had stroke (n=102) and ischemic heart disease (n=97), respectively. Subjects with a
15
16 204 higher income ate more fruits or vegetables than those with a lower income. Those who ate
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18 205 more fruit were more likely to be non-smokers and female than their counterparts
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20 206 (Supplemental Tables 2, 3).
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23 207 The total effect of fruit intake on CVD showed an inverse association without controlling
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25 208 for metabolic mediators (adjusted odds ratio [aOR], 0.86, 95% CI: 0.74–0.98), but the effect
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27 209 of vegetable intake was not significant (aOR, 0.93; 95% CI: 0.81–1.06) after controlling for
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29 210 sex, age, income, region (urban/rural), current smoker, and survey year (data not shown).
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33 211 The direct effect of fruit intake on CVD was borderline significant after further
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35 212 considering each metabolic mediator. The effect of fruit intake on SBP ($X \rightarrow M$) and the
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37 213 effect of SBP on CVD ($M \rightarrow Y$) were significant, and subsequently the indirect effect of SBP
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39 214 did not include zero in the 95% CI range, unlikely other metabolic mediators. The effect of
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41 215 fruit intake on BMI showed borderline significance, and the effect of BMI on CVD was
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43 216 significant, but the indirect effect of BMI was not significant. Additionally, the effect of SBP
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45 217 was significant even after controlling for BMI as a covariate (Table 2). SBP, cholesterol, and
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47 218 BMI were associated with CVD, but vegetable intake did not contribute to either metabolic
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49 219 mediator or CVD (Table 3). The mediating effect of SBP on the association between fruit
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51 220 intake and outcome was dominant even when the outcome was restricted to those with a
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221 current illness or undergoing treatment.

222 When the beta coefficient was expressed as OR, the OR of the effect of fruit intake on
223 CVD was attenuated to 0.89 (95% CI: 0.77–1.03) while simultaneously controlling for three
224 metabolic mediators, indicating a 21.4% indirect effect for CVD (i.e. $(0.8555-0.8864)/(0.8555-1)*100=21.4\%$). SBP showed an independent indirect effect. Higher fruit
225 intake had a beneficial effect on fasting glucose, but its effect was not associated with CVD.
226 The direct effect of fruit intake on CVD presented an inverse association ($\beta=-0.121, p=0.11$),
227 but it did not reach statistical significance (Figure 1). In addition, similar results were
228 observed when adding BMI as covariate, with an OR (the effect of fruit intake on CVD) of
229 0.90 (95% CI: 0.78–1.04; data not shown). The indirect effect of the four metabolic factors
230 accounted for 30.0% of the relationship between fruit intake and CVD (i.e. $(0.8555-0.8989)/(0.8555-1)*100=30.0\%$).
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233 We analyzed the serial mediator model to assess whether BMI influenced SBP (Figure 2).
234 Although the effect of fruit intake on BMI showed borderline significance, the influence of
235 BMI on SBP, and the effect of SBP on CVD reached statistical significance. Of the three
236 possible indirect paths, the fruit intake path \rightarrow SBP \rightarrow CVD was the only one to show an
237 independent association.

238 Fruit intake was directly linked to subjects who suffered a stroke, but not ischemic heart
239 disease, regardless of which metabolic factors were controlled. In addition, the mediating
240 effect of SBP was dominant in patients who suffered a stroke or ischemic heart disease even
241 after controlling for BMI (Supplemental Tables 4, 5).

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4 243 **DISCUSSION**

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9 245 In this study, we assessed how fruit or vegetable intake is related to CVD by assessing
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11 246 the indirect effect of metabolic mediators. Based on the suggested causal link, SBP, total
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13 247 cholesterol, and fasting glucose were considered metabolic mediators, and the effect of BMI
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15 248 was additionally assessed. Of them, the indirect effect of SBP on the relationship between
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17 249 fruit intake and CVD was significant even after considering BMI, but not vegetable intake.
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19 250 The indirect effect of the four metabolic factors accounted for 30.0% of the relationship
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21 251 between fruit intake and CVD.

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24 252 The beneficial effects of high fruit or vegetable intake on CVD and the unfavorable
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26 253 effects of high blood pressure, glucose, and cholesterol on CVD are well known. Thus,
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28 254 previous studies considered metabolic factors together, and mediators were reported to
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30 255 attenuate the association of a direct effect [5]. One large prospective study conducted in 10
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32 256 regions in China indicated that higher fresh fruit intake is linked to CVD death, and its effect
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34 257 was attenuated by hazard ratios from 0.63 (95% CI: 0.56–0.72) to 0.70 (95% CI: 0.61–0.79)
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36 258 after adjusting for BMI, blood pressure, glucose, and waist circumference [18]. Another study
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38 259 conducted in Shanghai, China showed an attenuated association between fruit intake and
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40 260 incident coronary heart disease after controlling for a history of diabetes, hypertension, or
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42 261 dyslipidemia, but no association or attenuation was observed for vegetable intake [5]. The
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44 262 Women's Health Study reported by Liu et al. also showed that the effect of fruits and
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46 263 vegetables on CVD risk became stronger after excluding subjects with a history of diabetes,
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48 264 hypertension, and high cholesterol [19]. It seems that these mediators largely attribute to the
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50 265 relationship between fruit and/or vegetable intake and CVD risk. However, biological
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4 266 pathways by metabolic factors between fruit and/or vegetable intake and CVD risk have not
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6 267 been investigated.

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8 268 The assessment of a mediating effect could help understand how fruit and/or vegetable
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10 269 intake affects CVDs. In addition, an effect of poor dietary risk by metabolic mediators on
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12 270 CVD was suggested by the GBD study, so that was considered to estimate the disease burden.
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14 271 The mediating effect of blood pressure on the association between fruit and/or vegetable
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16 272 intake and CVD was suggested by a prospective cohort study of patients in the first National
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18 273 Health and Nutrition Examination Survey [13]. Blood pressure contributed 22.2% to the
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20 274 relationship between fruit and vegetable intake and CVD death. This was similar to the
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22 275 results adjusted for BMI, cholesterol, and blood pressure. That study also showed that the
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24 276 direct effect of fruit and vegetable intake was notable in patients who suffered a stroke but
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26 277 not those with ischemic heart disease. These results are in line with those of the present study.

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29 278 We assessed a potential role for BMI on the association between fruit intake and CVD
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31 279 using various models. Several reports, including the above-mentioned study, considered BMI
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33 280 as a potential mediator [13, 18]. Additionally, a causal link between BMI and CVD risk is
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35 281 mediated through metabolic factors. Two pooled studies of prospective cohorts assessed the
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37 282 effect of BMI on coronary heart disease and stroke as mediated by metabolic components.
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39 283 They reported that blood pressure was a more important mediator compared to cholesterol
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41 284 and glucose [17, 20]. Other pooled data from an Asian cohort also indicate that estimated
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43 285 mediating proportions through hypertension were 62.3, 35.7, and 92.4% for the association
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45 286 between BMI and death due to CVD, coronary heart disease, and stroke, respectively, but not
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47 287 by diabetes [21]. The GBD study restricted total calories to 2,000 kcal instead of considering
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49 288 BMI [22]. In the present study, higher fruit intake was inversely associated with BMI, but it
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4 289 was borderline significant ($\beta = -0.06$, $p = 0.08$), which affected the results of the fruit intake
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6 290 path \rightarrow BMI \rightarrow SBP \rightarrow CVD in the serial multiple mediator model. Our study found that the
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8 291 mediating effect due to BMI was about 7.9%, but previous studies showed a $< 3.0\%$
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10 292 mediating effect by BMI on the association between fruit only or fruit and vegetable intake
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12 293 and CVD deaths by presenting little change in the adjusted risk value [13, 18]. However, it is
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14 294 difficult to make a direct comparison due to discrepancies in study design, study populations,
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16 295 the definition of disease, and fruit and/or vegetable intake.

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19 296 Eating more vegetables was not significantly associated with either a direct or indirect
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21 297 effect. In Korea, vegetables in the general population are easily accessible by a side dish.
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23 298 Indeed, statistics from the Organisation for Economic Co-operation and Development
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25 299 (OECD) have reported that daily vegetable consumption among adults was the highest in
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27 300 Korea [23]. However, the manner of preparation and/or cooking can influence nutrient
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29 301 content [7]. The favorable effects of fruit and vegetable intake can be explained by nutrients,
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31 302 such as dietary fiber, folate, potassium, and antioxidant vitamins (i.e., vitamin E, vitamin C,
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33 303 polyphenols, flavonoids, and carotenoids) and other components. These nutrients might be
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35 304 involved with controlling glucose, lipid level, and blood pressure, and reduce the risk of CVD
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37 305 along with weight control [7]. However, because foods contain various nutrients, food
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39 306 recommendations help subjects follow a prevention strategy. In addition, healthy eating is
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41 307 also associated with other health behaviors, such as not smoking and regular physical activity
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43 308 [13, 24].

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47 309 The present study has some limitations. First, the results were derived from a cross-
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49 310 sectional study design, so causal relationships could not be effectively drawn. Our study
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51 311 design is also open to the problem of reverse causation. If the reverse causation affects the
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4 312 results, the association will appear to be null or reverse direction to what is expected. But, the
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6 313 indirect effect by SBP was significant and some parts of our results were consistent with
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8 314 previous studies [13, 24]. Furthermore, the results were also consistent when stroke and
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10 315 ischemic heart disease were analyzed separately. Because the survey was conducted through
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12 316 a household visit and excludes people in the hospital, subjects with diseases might be the
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14 317 relatively less serious cases. Measurement error in FFQ survey or self-reported disease status
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16 318 may influence the results. In addition, residual confounding factors such as physical activity
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18 319 may have influenced the association. Finally, because the number of participants with CVD
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20 320 was very low (1.8%), the study had inadequate statistical power which might explain some of the
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22 321 non-significant findings.
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25 322 Nevertheless, our study focused on the mediating effects of metabolic factors on CVD
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27 323 and assessed which metabolic factors affect CVD. Our results were produced using the
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29 324 bootstrapping method and did not impose the assumption of normality of the sampling
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31 325 distribution; thus, it was an appropriate design for multiple mediations [16]. The given
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33 326 evidence was conceptually approached and was not statistically tested for an indirect effect.
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36 327 Taken together, our study suggests that diets rich in fruits may contribute to a lower
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38 328 CVD risk partly through lowered systolic blood pressure. Further prospective studies are
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40 329 needed for confirmation.
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4 335 **Conflict of interest statement**
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7 336 None
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13 338 **Contributor ship statement**
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15 339 HA Lee wrote the manuscript and performed the statistical analyses; D Lim, K Oh, and EJ Kim,
16
17 340 provided advice about writing the manuscript, and H Park helped interpret the data.
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34 346 **Data availability**
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37 347 The National Health and Nutrition Examination Survey files are available from the Korea Centers for Disease
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39 348 Control and Prevention database (URL https://knhanes.cdc.go.kr/knhanes/sub03/sub03_02_02.do). If you
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41 349 register your e-mail on this site, you can freely download the raw data.
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4 468 **Figure Legends**

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10 470 Figure 1. The effect of multiple metabolic factor (M_i) mediators in the association between
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12 471 fruit intake (X) and cardiovascular diseases (Y).

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15 472 ^a $p < 0.01$, ^b $p < 0.001$, SBP: systolic blood pressure. Coefficients were adjusted for sex, age,
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17 473 income, region (urban/rural), current smoker, and survey year using the bootstrapping method.

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22 475 Figure 2. The effect of multiple serial mediators of metabolic factors (M_i) in the association
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24 476 between fruit intake (X) and cardiovascular diseases (Y).

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27 477 ^a $p < 0.1$, ^b $p < 0.05$, ^c $p < 0.01$, ^d $p < 0.001$, BMI: body mass index, SBP: systolic blood pressure.
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29 478 Coefficients were adjusted for sex, age, income, region (urban/rural), current smoker, and
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31 479 survey year using the bootstrapping method.

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487 Table 1. Basic characteristics of the study subjects.

	Weighted % (SE)
Sex	
Male	49.92 (0.51)
Female	50.08 (0.51)
Age range	
Age (years)†	25–64 years
	43.68 (0.18)
Region	
Urban	83.87 (1.52)
Rural	16.13 (1.52)
Income level (quartiles)	
Q1	23.46 (0.73)
Q2	25.61 (0.72)
Q3	25.03 (0.71)
Q4	25.90 (0.97)
Current smoking	
No	75.83 (0.60)
Yes	24.17 (0.60)
Disease	
Cardiovascular disease	1.81 (0.16)
Stroke	0.98 (0.13)
Ischemic heart disease	0.90 (0.10)
Metabolic Factors†	
Systolic blood pressure (mmHg)	115.01 (0.21)
Total cholesterol (mg/dL)	190.98 (0.47)
Fasting plasma glucose (mg/dL)	98.58 (0.30)
Body mass index (kg/m ²)	23.92 (0.05)

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489 SE: Standard error.

490 †Weighted mean with standard error.

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499 Table 2. The effect of metabolic mediators (M) in the association between fruit intake (X) and
500 cardiovascular disease (Y).

Metabolic Factors (M)	Fruit intake											
	X → M (a)			M → Y (b)			X → Y (c' = direct effect)			Indirect effect (a*b)		
	β	SE	<i>p</i>	β	SE	<i>p</i>	β	SE	<i>p</i>	β	95% CI	
SBP ^a	-0.484	0.144	0.001	0.013	0.004	0.002	-0.137	0.072	0.06	-0.007	-0.014	-0.002
TC ^a	-0.156	0.357	0.66	-0.019	0.003	<.0001	-0.144	0.075	0.05	0.003	-0.011	0.017
FPG ^a	-0.665	0.217	<.01	0.004	0.003	0.20	-0.144	0.074	0.05	-0.002	-0.006	0.001
BMI ^a	-0.059	0.034	0.08	0.078	0.022	0.001	-0.143	0.072	<.05	-0.005	-0.012	0.001
SBP ^b	-0.420	0.139	<.01	0.011	0.005	0.01	-0.127	0.072	0.08	-0.005	-0.011	-0.0004
TC ^b	-0.064	0.352	0.86	-0.019	0.003	<.0001	-0.126	0.075	0.09	0.001	-0.012	0.015
FPG ^b	-0.614	0.214	<.01	0.002	0.003	0.42	-0.130	0.074	0.08	-0.002	-0.005	0.002

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502 SBP: systolic blood pressure, TC: total cholesterol, FPG: fasting plasma glucose, BMI: body mass
503 index, SE: standard error, 95% CI: 95% confidence interval.

504 ^aAdjusted for sex, age, income, region (urban/rural), current smoking, and survey year.

505 ^bAdjusted for sex, age, income, region (urban/rural), current smoking, survey year, and body mass
506 index.

507 All analyzes were performed separately according to each metabolic mediator.

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521 Table 3. The effect of metabolic mediators (M) in the association between vegetable intake
522 (X) and cardiovascular disease (Y).

Metabolic Factors (M)	Vegetable intake										
	X → M (a)			M → Y (b)			X → Y (c' = direct effect)			Indirect effect (a*b)	
	β	SE	<i>p</i>	β	SE	<i>p</i>	β	SE	<i>p</i>	β	95% CI
SBP ^a	-0.042	0.169	0.80	0.014	0.004	0.002	-0.132	0.086	0.13	-0.001	-0.006 0.004
TC ^a	0.236	0.420	0.57	-0.019	0.003	<.0001	-0.121	0.089	0.18	-0.005	-0.021 0.012
FPG ^a	-0.054	0.256	0.83	0.004	0.003	0.18	-0.132	0.088	0.14	-0.0002	-0.003 0.002
BMI ^a	0.057	0.040	0.16	0.080	0.022	<.001	-0.145	0.086	0.09	0.005	-0.002 0.013
SBP ^b	-0.114	0.163	0.48	0.012	0.005	0.01	-0.131	0.086	0.13	-0.001	-0.006 0.002
TC ^b	0.142	0.415	0.73	-0.019	0.003	<.0001	-0.122	0.089	0.17	-0.003	-0.019 0.014
FPG ^b	-0.121	0.252	0.63	0.003	0.003	0.4	-0.132	0.088	0.14	-0.0003	-0.003 0.002

523 SBP: systolic blood pressure, TC: total cholesterol, FPG: fasting plasma glucose, BMI: body mass
524 index, SE: standard error, 95% CI: 95% confidence interval.

525 ^aAdjusted for sex, age, income, region (urban/rural), current smoking, and survey year.

526 ^bAdjusted for sex, age, income, region (urban/rural), current smoking, survey year, and body mass
527 index.

528 All analyzes were performed separately according to each metabolic mediator.

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Figure 1

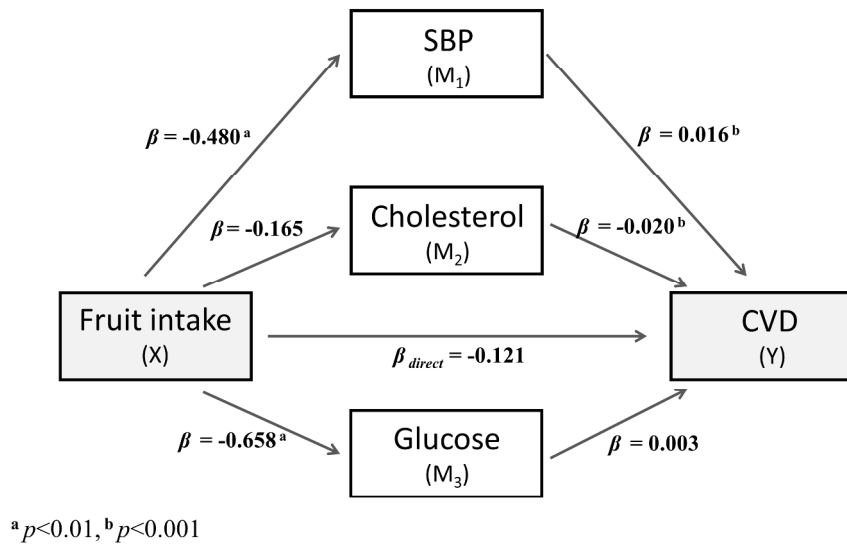
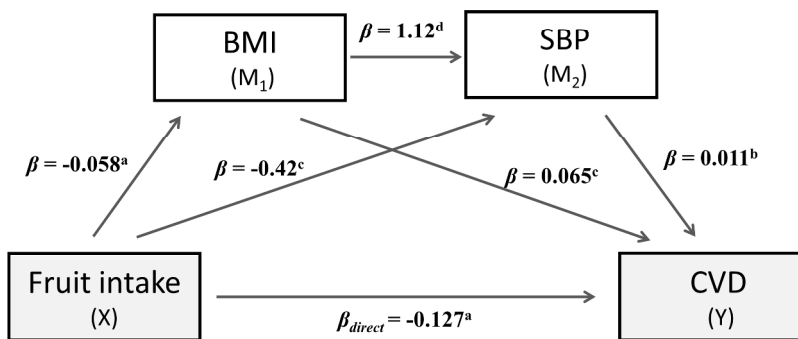


figure1

254x190mm (300 x 300 DPI)

Figure 2



^a $p < 0.1$, ^b $p < 0.05$, ^c $p < 0.01$, ^d $p < 0.001$

figure2

254x190mm (300 x 300 DPI)

Supplemental Table 1. List of fruit or vegetable related food items

Fruit	Vegetable
Strawberry	Bean sprouts (seasoned, soup)
Melon	Seasoned mung bean sprout
Watermelon	Seasoned spinach
Peach	Seasoned bellflower (boiled or not)
Grape	Pumpkin (seasoned, pan-fried)
Apple	Other seasoned vegetables
Pear	Cucumber (seasoned, raw)
Persimmon, dried persimmon	Radish (seasoned, pickled, dried)
Tangerine	Vegetable salad
Banana	Seasoned green onion, and seasoned Chinese chives
Orange	Raw vegetables (lettuce, sesame, Chinese cabbage, and pumpkin leaf)
Kiwi	Green pepper
	Boiled broccoli, boiled cabbage
	Garlic
	Tomato, and cherry tomato

The food frequency questionnaire consists of dietary consumption using a 9-point scale (less than once per month or never, once per month, 2–3 times per month, once per week, 2–4 times per week, 5–6 times per week, once per day, twice per day, and three times per day) and three levels to represent the amount consumed by referring to a standard amount (less, standard, and more).

Supplemental Table 2. Distribution of basic characteristics by fruit intake.

	Fruit intake								<i>p</i> _{trend}
	< 1 time/day		1 time/day		2 times/day		3+ times/day		
	n	%	n	%	n	%	n	%	
Survey year									
2013	961	29.61	935	28.8	629	19.38	721	22.21	0.03
2014	945	31.81	825	27.77	538	18.11	663	22.32	
2015	916	32.45	810	28.69	488	17.29	609	21.57	
Sex									
Male	1519	42.73	983	27.65	551	15.5	502	14.12	<.0001
Female	1303	23.76	1587	28.93	1104	20.13	1491	27.18	
Age (years)	45.83	10.97	45.38	10.94	45.76	10.69	46.53	10.81	0.02
Region									
Urban	2280	30.62	2118	28.44	1389	18.65	1660	22.29	<.01
Rural	542	34.02	452	28.37	266	16.7	333	20.9	
Income level (quartiles)									
Q1	863	40.29	574	26.8	354	16.53	351	16.39	<.0001
Q2	827	36.38	634	27.89	359	15.79	453	19.93	
Q3	612	26.9	702	30.86	435	19.12	526	23.12	
Q4	510	22.02	649	28.02	500	21.59	657	28.37	
Current smoking									
No	1826	26.21	2030	29.14	1377	19.77	1733	24.88	<.0001
Yes	833	50.24	431	26	210	12.67	184	11.1	

Supplemental Table 3. Distribution of basic characteristics by vegetable intake.

	Vegetable intake								<i>p</i> _{trend}
	< 1 time/day		1 time/day		2 times/day		3+ times/day		
	n	%	n	%	n	%	n	%	
Survey year									
2013	357	11	711	21.9	674	20.76	1504	46.33	<.0001
2014	374	12.59	725	24.4	585	19.69	1287	43.32	
2015	382	13.53	724	25.65	574	20.33	1143	40.49	
Sex									
Male	428	12.04	892	25.09	709	19.94	1526	42.93	0.38
Female	685	12.49	1268	23.12	1124	20.49	2408	43.9	
Age (years)	45.54	11.70	45.11	10.93	45.49	10.93	46.49	10.55	<.0001
Region									
Urban	910	12.22	1775	23.84	1526	20.49	3236	43.45	0.73
Rural	203	12.74	385	24.17	307	19.27	698	43.82	
Income level (quartiles)									
Q1	394	18.39	550	25.68	391	18.25	807	37.68	<.0001
Q2	288	12.67	587	25.82	481	21.16	917	40.34	
Q3	239	10.51	529	23.25	485	21.32	1022	44.92	
Q4	187	8.07	486	20.98	468	20.21	1175	50.73	
Current smoker									
No	810	11.63	1668	23.94	1421	20.4	3067	44.03	<.01
Yes	248	14.96	392	23.64	325	19.6	693	41.8	

Supplemental Table 4. The effect of metabolic mediators (M) in the association between fruit intake (X) and stroke (Y).

Metabolic Factors (M)	Fruit intake											
	X → M (a)			M → Y (b)			X → Y (c' = direct effect)			Indirect effect (a*b)		
	β	SE	<i>p</i>	β	SE	<i>p</i>	β	SE	<i>p</i>	β	95% CI	
SBP ^a	-0.484	0.144	<.001	0.015	0.006	<.01	-0.242	0.100	0.02	-0.007	-0.017	-0.001
TC ^a	-0.156	0.357	0.66	-0.018	0.003	<.0001	-0.268	0.105	0.01	0.003	-0.009	0.016
FPG ^a	-0.665	0.217	<.01	0.005	0.004	0.19	-0.269	0.105	0.01	-0.003	-0.008	0.002
BMI ^a	-0.059	0.034	0.08	0.074	0.029	0.01	-0.249	0.100	0.01	-0.004	-0.013	0.001
SBP ^b	-0.420	0.139	<.01	0.013	0.006	0.03	-0.238	0.100	0.02	-0.005	-0.014	0.001
TC ^b	-0.064	0.352	0.86	-0.018	0.003	<.0001	-0.255	0.105	0.02	0.001	-0.011	0.015
FPG ^b	-0.614	0.214	<.01	0.003	0.004	0.37	-0.260	0.105	0.01	-0.002	-0.007	0.004

SBP: systolic blood pressure, TC: total cholesterol, FPG: fasting plasma glucose, BMI: body mass index, SE: standard error, 95% CI: 95% confidence interval.

^aAdjusted for sex, age, income, region (urban/rural), current smoking, and survey year.

^bAdjusted for sex, age, income, region (urban/rural), current smoking, survey year, and body mass index.

All analyzes were performed separately according to each metabolic mediator.

Supplemental Table 5. The effect of metabolic mediators (M) in the association between fruit intake (X) and ischemic heart disease (Y).

Metabolic Factors (M)	Fruit intake											
	X → M (a)			M → Y (b)			X → Y (c' = direct effect)			Indirect effect (a*b)		
	β	SE	<i>p</i>	β	SE	<i>p</i>	β	SE	<i>p</i>	β	95% CI	
SBP ^a	-0.484	0.144	<.001	0.011	0.006	0.06	-0.065	0.097	0.51	-0.006	-0.013	-0.0001
TC ^a	-0.156	0.357	0.66	-0.021	0.003	<.0001	-0.042	0.100	0.67	0.003	-0.012	0.019
FPG ^a	-0.665	0.217	<.01	0.002	0.004	0.65	-0.048	0.099	0.63	-0.001	-0.006	0.004
BMI ^a	-0.059	0.034	0.08	0.079	0.031	0.01	-0.069	0.097	0.48	-0.005	-0.012	0.001
SBP ^b	-0.420	0.139	<.01	0.010	0.006	0.12	-0.047	0.097	0.63	-0.004	-0.011	0.001
TC ^b	-0.064	0.352	0.86	-0.020	0.003	<.0001	-0.018	0.100	0.86	0.001	-0.013	0.016
FPG ^b	-0.614	0.214	<.01	0.001	0.004	0.88	-0.028	0.099	0.78	0.000	-0.004	0.005

SBP: systolic blood pressure, TC: total cholesterol, FPG: fasting plasma glucose, BMI: body mass index, SE: standard error, 95% CI: 95% confidence interval.

^aAdjusted for sex, age, income, region (urban/rural), current smoking, and survey year.

^bAdjusted for sex, age, income, region (urban/rural), current smoking, survey year, and body mass index.

All analyzes were performed separately according to each metabolic mediator.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-8
Data sources/ measurement	8*	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	6-8
Bias	9	Describe any efforts to address potential sources of bias	15
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	8-9
		(b) Describe any methods used to examine subgroups and interactions	
		(c) Explain how missing data were addressed	
		(d) If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	8
Results			

Participants	13*	(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 (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	6
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest	10
Outcome data	15*	Report numbers of outcome events or summary measures	10
Main results	16	(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 (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	10-11 7
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
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	16

*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.