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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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**Abstract**

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

**Design:** Cross-sectional study

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

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

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

**Conclusions:** Our results indicate that controlling SBP may lessen the CVD risk, and a diet rich in fruits can regulate SBP, which, in turn, reduces CVD risk.

**Keywords:** Cardiovascular disease, blood pressure, diet

## ARTICLE SUMMARY

### Strengths and limitations of this study

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

## INTRODUCTION

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

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

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

There is a need to study the degree to which these metabolic factors contribute to the relationship between risk factors and disease. Thus, using cross-sectional survey data from the 2013–2015 Korean National Health and Nutrition Examination Survey (KNHANES), we assessed the mediating effects of metabolic components applied to a confirmatory model. Furthermore, we assessed how the BMI contributes to the relationship between fruit or vegetable intake and CVD as a confounder or mediator.

## METHODS

### 1. Study subjects

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

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

### 2. Fruit and vegetable intake

The dish-based semi-quantitative FFQ was composed of 112 items and provided information on typical dietary consumption for 1 year 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). Based on a previous study [4], we excluded pickled and salted vegetables, including kimchi and fruit juice. Vegetable intake included bean sprouts (seasoned, soup); seasoned mung bean sprout; seasoned spinach; seasoned bellflower (boiled or not); pumpkin (seasoned, pan-fried); other seasoned vegetables; cucumber (seasoned, raw); radish (seasoned, pickled, dried); vegetable salad; seasoned green onion, and seasoned Chinese chives; raw vegetables (lettuce, sesame, Chinese cabbage, and pumpkin leaf); green pepper; boiled broccoli, boiled cabbage; garlic; tomato, and cherry tomato. Fruit intake was assessed based on 12 items: strawberry; melon; watermelon; peach; grape; apple; pear; persimmon, dried persimmon; tangerine; banana; orange; and kiwi. The frequency of fruit intake was used after adjusting for seasonal fruit. Estimated intakes of fruits and vegetables were calculated on the FFQ by multiplying the frequency of each food (as described above) by the selected amount consumed: small (0.5), medium (1), and large (1.5). Fruit and vegetable intake was expressed in four categories (< 1 time/d, 1 time/d, 2 times/d, and ≥ 3 times/d).

**3. Outcome and covariate data**

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

We used data on sex, age, quartiles of income, region (urban/rural), current smoker, and survey year as covariates through a literature review [8] and the results of a univariate analysis. We used quartile data for income instead of education level as a socioeconomic indicator because income may be directly linked to food purchases [9]. The question about physical activity was changed from the 2014 survey, so we did not consider physical activity.

**4. Statistical analysis**

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

We examined the association under the controlling covariates (sex, age, income, region [urban/rural], present smoking, and survey year) through four basic steps to assess mediation [10]. Step 1: association between dietary factors and CVD (X → Y; total effect and was marked path "c"); step 2: association between dietary factors and metabolic mediators (X → $M_i$; marked path "a"); step 3: association between metabolic mediators and CVD after controlling for metabolic mediators ($M_i$ → Y; marked path "b"); and step 4: association between dietary factors and CVD disease after controlling for metabolic mediators (direct effect; marked path "c"). We used the bootstrap method and the "process" macro suggested by Andrew to assess the mediating effects [11]. In this analysis, we applied 10,000 bootstraps. We separately or simultaneously assessed the indirect effect of the metabolic mediators on the association between dietary factors and CVD. The exponential regression coefficient is equal to the odds ratio (OR) when considering the CVD as an outcome variable. The percentage of risk mediated by the metabolic mediator was calculated as [12]: OR (confounder adjusted) − OR (confounder and mediator adjusted)/OR (confounder adjusted) − 1 × 100.

All statistical analyses were conducted under a random sampling condition excluding the basic characteristics given in Table 1 using SAS ver. 9.4 software (SAS Institute, Cary, NC, USA). A two-sided $p$-value $< 0.05$ was considered significant.

## RESULTS

The basic characteristics of the study subjects are presented in Table 1. Mean age was 43.7 years, and 1.81% of subjects had CVD. Subjects with a higher income ate more fruits or vegetables than those with a lower income. Those who ate more fruit were more likely to be non-smokers and female than their counterparts (Supplemental Tables 1, 2).

The total effect of fruit intake on CVD showed an inverse association without controlling for metabolic mediators (adjusted odds ratio [aOR], 0.86, 95% CI: 0.74–0.98), but the effect of vegetable intake was not significant (aOR, 0.93; 95% CI: 0.81–1.06) after controlling for sex, age, income, region (urban/rural), current smoker, and survey year.

The direct effect of fruit intake on CVD was borderline significant after further considering each metabolic mediator. The effect of SBP did not include zero in the 95% CI range as the other metabolic mediators. The effect of fruit intake on BMI showed borderline significance, and the effect of BMI on CVD was significant, but the indirect effect of BMI was not significant. Additionally, the effect of SBP was significant even after controlling for BMI as a covariate (Table 2). SBP, cholesterol, and BMI were associated with CVD, but vegetable intake did not contribute to either metabolic mediator or CVD (Table 3). The mediating effect of SBP on the association between fruit intake and outcome was dominant even when the outcome was restricted to those with a current illness or undergoing treatment.

The OR was attenuated to 0.89 (95% CI: 0.77–1.03) while simultaneously controlling for multiple metabolic mediators, indicating a 21.4% indirect effect for CVD. SBP showed an independent indirect effect. Higher fruit intake had a beneficial effect on fasting glucose, but its effect was not associated with CVD. The direct effect of fruit intake on CVD presented an inverse association, but it did not reach statistical significance (Figure 1). In addition, similar results were observed when adding BMI as covariate, with an OR of 0.90 (95% CI: 0.78–1.04; data not shown).

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

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

## DISCUSSION

In this study, we assessed how fruit or vegetable intake is related to CVD by assessing the indirect effect of metabolic mediators. Based on the established causal link, SBP, total cholesterol, and fasting glucose were considered metabolic mediators, and the effect of BMI was additionally assessed. Of them, the indirect effect of SBP on the relationship between fruit intake and CVD was significant even after considering BMI, but not vegetable intake. The indirect effect of the four metabolic factors accounted for 30.0% of the relationship between fruit intake and CVD.

The beneficial effects of high fruit or vegetable intake on CVD and the unfavorable effects of high blood pressure, glucose, and cholesterol on CVD are well known. Thus, previous studies considered metabolic factors together, and mediators were reported to attenuate the association of a direct effect [5]. One large prospective study conducted in 10 regions in China indicated that higher fresh fruit intake is linked to CVD death, and its effect was attenuated by hazard ratios from 0.63 (95% CI: 0.56–0.72) to 0.70 (95% CI: 0.61–0.79) after adjusting for BMI, blood pressure, glucose, and waist circumference [13]. Another study conducted in Shanghai, China showed an attenuated association between fruit intake and incident coronary heart disease after controlling for a history of diabetes, hypertension, or dyslipidemia, but not vegetable intake [5]. A women's health study reported by Liu et al. also showed that the effect of fruits and vegetables on CVD risk became stronger after excluding subjects with a history of diabetes, hypertension, and high cholesterol [14]. However, whether these metabolic factors were causal links between fruit and/or vegetable intake and CVD risk was not investigated.

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

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

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

The present study had some limitations. First, the results were derived from a cross-sectional study design, so causal relationships could not be effectively drawn. But, some parts of our results were consistent with previous studies [8, 19]. Furthermore, the results were consistent even after applying various definitions of outcome. Because the survey conducts general population except those in the hospital, subjects with diseases might be the relatively less moderate cases. In addition, unmeasured confounding factors may have influenced the association.

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

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

## Conflict of interest statement

None

## Contributor ship statement

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


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## Data availability

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

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**Figure Legends**

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

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

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

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

Table 1. Basic characteristics of the study subjects.

| | Weighted % (SE) |
|---|---|
| **Sex** | |
| Male | 49.92 (0.51) |
| Female | 50.08 (0.51) |
| **Age range** | 25–64 years |
| **Age (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) |

SE: Standard error.

†Weighted mean with standard error.

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

| | Fruit intake | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | X ⟶ M (a) | | | M ⟶ Y (b) | | | X ⟶ Y (c' = direct effect) | | | Indirect effect (a*b) | | |
| Metabolic Factors (M) | $\beta$ | SE | $p$ | $\beta$ | SE | $p$ | $\beta$ | SE | $p$ | $\beta$ | 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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469 SBP: systolic blood pressure, TC: total cholesterol, FPG: fasting plasma glucose, BMI: body mass
470 index, SE: standard error, 95% CI: 95% confidence interval.
471 [a]Adjusted for sex, age, income, region (urban/rural), current smoking, and survey year.
472 [b]Adjusted for sex, age, income, region (urban/rural), current smoking, survey year, and body mass
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Table 3. The effect of metabolic mediators (M) in the association between vegetable intake (X) and cardiovascular disease (Y).

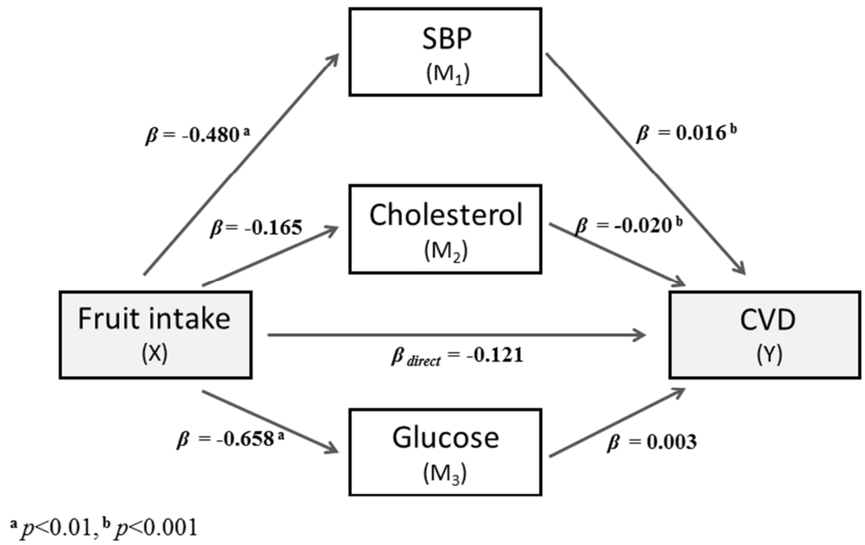
| | Vegetable intake | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | X ⟶ M (a) | | | M ⟶ Y (b) | | | X ⟶ Y (c' = direct effect) | | | Indirect effect (a*b) | | |
| Metabolic Factors (M) | *β* | SE | *p* | *β* | SE | *p* | *β* | SE | *p* | *β* | 95% CI | |
| SBP[a] | −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 |

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

[a]Adjusted for sex, age, income, region (urban/rural), current smoking, and survey year.

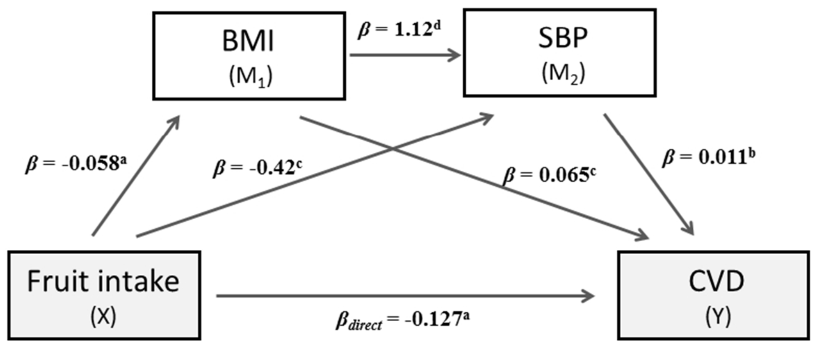
[b]Adjusted for sex, age, income, region (urban/rural), current smoking, survey year, and body mass index.

# Figure 1




254x190mm (96 x 96 DPI)

# Figure2




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

254x190mm (96 x 96 DPI)

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

| | Fruit intake | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | < 1 time/day | | 1 time/day | | 2 times/day | | 3+ times/day | | $p_{trend}$ |
| | 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 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | < 1 time/day | | 1 time/day | | 2 times/day | | 3+ times/day | | $p_{trend}$ |
| | 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).

| | Fruit intake | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | X ⟶ M (a) | | | M ⟶ Y (b) | | | X ⟶ Y (c' = direct effect) | | | Indirect effect (a*b) | | |
| Metabolic Factors (M) | β | SE | p | β | SE | p | β | SE | p | 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.

[a]Adjusted for sex, age, income, region (urban/rural), current smoking, and survey year.

[b]Adjusted 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).

| | Fruit intake | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | X ⟶ M (a) | | | M ⟶ Y (b) | | | X ⟶ Y (c' = direct effect) | | | Indirect effect (a*b) | | |
| Metabolic Factors (M) | *β* | SE | *p* | *β* | SE | *p* | *β* | SE | *p* | *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.

[a]Adjusted for sex, age, income, region (urban/rural), current smoking, and survey year.

[b]Adjusted for sex, age, income, region (urban/rural), current smoking, survey year, and body mass index.

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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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| | |

# 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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**Abstract**

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

**Design:** Cross-sectional study

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

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

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

**Conclusions:** Our results indicate that controlling SBP may lessen the CVD risk, and a diet rich in fruits can regulate SBP, which, in turn, reduces CVD risk.

**Keywords:** Cardiovascular disease, blood pressure, diet

## ARTICLE SUMMARY

### Strengths and limitations of this study

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

## INTRODUCTION

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

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

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

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

## METHODS

### 1. Study subjects

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

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

### 2. Fruit and vegetable intake

The dish-based semi-quantitative FFQ was composed of 112 items and provided information on typical dietary consumption for 1 year 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). Based on a previous study [4], we excluded pickled and salted vegetables, kimchi, and fruit juice. Vegetable intake and fruit intake were evaluated based on 15 items and 12 items, respectively (Supplemental Tables 1). The frequency of fruit intake was used after adjusting for seasonal fruit. Estimated intakes of fruits and vegetables were calculated on the FFQ by multiplying the frequency of each food (as described above) by the selected amount consumed: small (0.5), medium (1), and large (1.5). Fruit and vegetable intake was expressed in four categories (< 1 time/d, 1 time/d, 2 times/d, and ≥ 3 times/d).

**3. Outcome and covariate data**

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

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

We used data on sex, age, quartiles of income, region (urban/rural), current smoker, and survey year as covariates through a literature review [13] and the results of a univariate analysis. We used quartile data for income instead of education level as a socioeconomic indicator because income may be directly linked to food purchases [14]. The question about physical activity was changed from the 2014 survey, so we did not consider physical activity.

**4. Statistical analysis**

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

We examined the association under the controlling covariates (sex, age, income, region [urban/rural], present smoking, and survey year) through four basic steps to assess mediation [15]. Step 1: association between dietary factors and CVD (X → Y; total effect and was marked path "c"); step 2: association between dietary factors and metabolic mediators (X → $M_i$; marked path "a"); step 3: association between metabolic mediators and CVD after controlling for metabolic mediators ($M_i$ → Y; marked path "b"); and step 4: association between dietary factors and CVD disease after controlling for metabolic mediators (direct effect; marked path "c"). We used the bootstrap method and the "process" macro (ver. V2.16.3) suggested by Andrew to assess the mediating effects [16]. In this analysis, we applied 10,000 bootstraps. We separately or simultaneously assessed the indirect effect of the metabolic mediators on the association between dietary factors and CVD. The exponential regression coefficient is equal to the odds ratio (OR) when considering the CVD as an outcome variable. The percentage of risk mediated by the metabolic mediator was calculated as [17]: OR (confounder adjusted) − OR (confounder and mediator adjusted)/OR (confounder adjusted) − 1 × 100.

All statistical analyses were conducted under a random sampling condition excluding the basic characteristics given in Table 1 using SAS ver. 9.4 software (SAS Institute, Cary, NC, USA). A two-sided $p$-value < 0.05 was considered significant.

## RESULTS

The basic characteristics of the study subjects are presented in Table 1. Mean age was 43.7 years, and 1.81% of subjects (n=189) had CVD. In addition, 0.98% and 0.90% of subject had stroke (n=102) and ischemic heart disease (n=97), respectively. Subjects with a higher income ate more fruits or vegetables than those with a lower income. Those who ate more fruit were more likely to be non-smokers and female than their counterparts (Supplemental Tables 2, 3).

The total effect of fruit intake on CVD showed an inverse association without controlling for metabolic mediators (adjusted odds ratio [aOR], 0.86, 95% CI: 0.74–0.98), but the effect of vegetable intake was not significant (aOR, 0.93; 95% CI: 0.81–1.06) after controlling for sex, age, income, region (urban/rural), current smoker, and survey year.

The direct effect of fruit intake on CVD was borderline significant after further considering each metabolic mediator. The effect of SBP did not include zero in the 95% CI range as the other metabolic mediators. The effect of fruit intake on BMI showed borderline significance, and the effect of BMI on CVD was significant, but the indirect effect of BMI was not significant. Additionally, the effect of SBP was significant even after controlling for BMI as a covariate (Table 2). SBP, cholesterol, and BMI were associated with CVD, but vegetable intake did not contribute to either metabolic mediator or CVD (Table 3). The mediating effect of SBP on the association between fruit intake and outcome was dominant even when the outcome was restricted to those with a current illness or undergoing treatment.

The OR was attenuated to 0.89 (95% CI: 0.77–1.03) while simultaneously controlling for multiple 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 intake had a beneficial effect on fasting glucose, but its effect was not associated with CVD. The direct effect of fruit intake on CVD presented an inverse association, but it did not reach statistical significance (Figure 1). In addition, similar results were observed when adding BMI as covariate, with an OR of 0.90 (95% CI: 0.78–1.04; data not shown).

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

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

## DISCUSSION

In this study, we assessed how fruit or vegetable intake is related to CVD by assessing the indirect effect of metabolic mediators. Based on the suggested causal link, SBP, total cholesterol, and fasting glucose were considered metabolic mediators, and the effect of BMI was additionally assessed. Of them, the indirect effect of SBP on the relationship between fruit intake and CVD was significant even after considering BMI, but not vegetable intake. The indirect effect of the four metabolic factors accounted for 30.0% of the relationship between fruit intake and CVD (i.e. (0.8555-0.8989)/(0.8555-1)*100=30.0%).

The beneficial effects of high fruit or vegetable intake on CVD and the unfavorable effects of high blood pressure, glucose, and cholesterol on CVD are well known. Thus, previous studies considered metabolic factors together, and mediators were reported to attenuate the association of a direct effect [5]. One large prospective study conducted in 10 regions in China indicated that higher fresh fruit intake is linked to CVD death, and its effect was attenuated by hazard ratios from 0.63 (95% CI: 0.56–0.72) to 0.70 (95% CI: 0.61–0.79) after adjusting for BMI, blood pressure, glucose, and waist circumference [18]. Another study conducted in Shanghai, China showed an attenuated association between fruit intake and incident coronary heart disease after controlling for a history of diabetes, hypertension, or dyslipidemia, but no association or attenuation was observed for vegetable intake [5]. A women's health study reported by Liu et al. also showed that the effect of fruits and vegetables on CVD risk became stronger after excluding subjects with a history of diabetes, hypertension, and high cholesterol [19]. It seems that these mediators largely attribute to the relationship between fruit and/or vegetable intake and CVD risk. However, biological pathways by metabolic factors between fruit and/or vegetable intake and CVD risk have not been investigated.

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

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

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

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

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

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

## Conflict of interest statement

None

## Contributor ship statement

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

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## Data availability

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

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**Figure Legends**

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

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

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

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

486 Table 1. Basic characteristics of the study subjects.

| | Weighted % (SE) |
|---|---|
| **Sex** | |
| Male | 49.92 (0.51) |
| Female | 50.08 (0.51) |
| **Age range** | 25–64 years |
| **Age (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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Table 2. The effect of metabolic mediators (M) in the association between fruit intake (X) and cardiovascular disease (Y).

| | Fruit intake | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | X ⟶ M (a) | | | M ⟶ Y (b) | | | X ⟶ Y (c' = direct effect) | | | Indirect effect (a*b) | | |
| Metabolic Factors (M) | *β* | SE | *p* | *β* | SE | *p* | *β* | SE | *p* | *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 |

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

[a]Adjusted for sex, age, income, region (urban/rural), current smoking, and survey year.

[b]Adjusted for sex, age, income, region (urban/rural), current smoking, survey year, and body mass index.

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

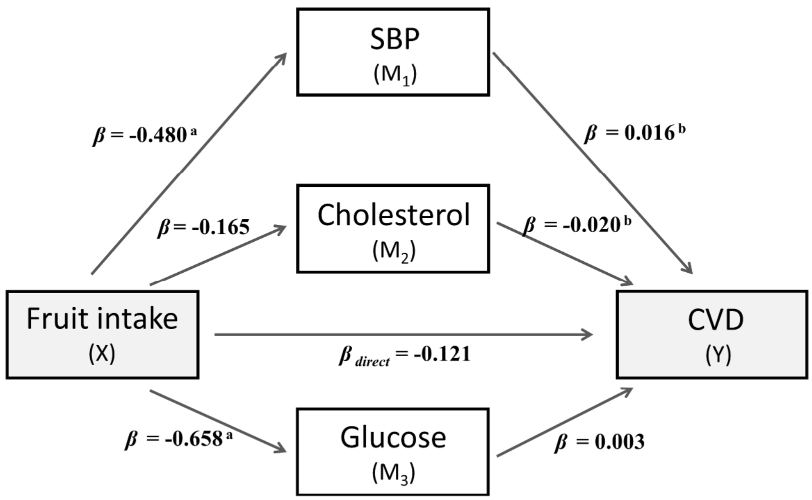
| | Vegetable intake | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | X ⟶ M (a) | | | M ⟶ Y (b) | | | X ⟶ Y (c' = direct effect) | | | Indirect effect (a*b) | | |
| Metabolic Factors (M) | *β* | SE | *p* | *β* | SE | *p* | *β* | SE | *p* | *β* | 95% CI | |
| SBP[a] | −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 |

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

[a]Adjusted for sex, age, income, region (urban/rural), current smoking, and survey year.

[b]Adjusted for sex, age, income, region (urban/rural), current smoking, survey year, and body mass index.

# Figure 1

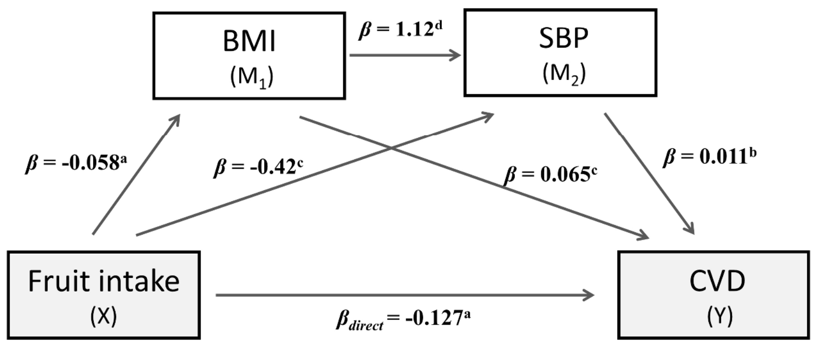



[a] $p$<0.01, [b] $p$<0.001

figure1

254x190mm (300 x 300 DPI)

# Figure2




[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 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | < 1 time/day | | 1 time/day | | 2 times/day | | 3+ times/day | | $p_{trend}$ |
| | 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 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | < 1 time/day | | 1 time/day | | 2 times/day | | 3+ times/day | | $p_{trend}$ |
| | 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).

| | Fruit intake | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | X ⟶ M (a) | | | M ⟶ Y (b) | | | X ⟶ Y (c' = direct effect) | | | Indirect effect (a*b) | | |
| Metabolic Factors (M) | *β* | SE | *p* | *β* | SE | *p* | *β* | SE | *p* | *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.

[a]Adjusted for sex, age, income, region (urban/rural), current smoking, and survey year.

[b]Adjusted 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).

| | Fruit intake | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | X ⟶ M (a) | | | M ⟶ Y (b) | | | X ⟶ Y (c' = direct effect) | | | Indirect effect (a*b) | | |
| Metabolic Factors (M) | *β* | SE | *p* | *β* | SE | *p* | *β* | SE | *p* | *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.

[a]Adjusted for sex, age, income, region (urban/rural), current smoking, and survey year.

[b]Adjusted 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 | 6 |
| | | (b) Give reasons for non-participation at each stage | |
| | | (c) Consider use of a flow diagram | |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 10 |
| | | (b) Indicate number of participants with missing data for each variable of interest | |
| 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 | 10-11 |
| | | (*b*) Report category boundaries when continuous variables were categorized | 7 |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | |
| 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.

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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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| | |

**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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**Abstract**

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

**Design:** Cross-sectional study

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

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

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

**Conclusions:** Our results indicate that controlling SBP may lessen the CVD risk, and a diet rich in fruits can regulate SBP, which, in turn, reduces CVD risk.

**Keywords:** Cardiovascular disease, blood pressure, diet

## ARTICLE SUMMARY

### Strengths and limitations of this study

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

## INTRODUCTION

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

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

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

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

## METHODS

### 1. Study subjects

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

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

### 2. Fruit and vegetable intake

The dish-based semi-quantitative FFQ was composed of 112 items and provided information on typical dietary consumption for 1 year 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). Based on a previous study [4], we excluded pickled and salted vegetables, kimchi, and fruit juice. Vegetable intake and fruit intake were evaluated based on 15 items and 12 items, respectively (Supplemental Tables 1). The frequency of fruit intake was used after adjusting for seasonal fruit. Estimated intakes of fruits and vegetables were calculated on the FFQ by multiplying the frequency of each food (as described above) by the selected amount consumed: small (0.5), medium (1), and large (1.5). Fruit and vegetable intake was expressed in four categories (< 1 time/d, 1 time/d, 2 times/d, and ≥ 3 times/d).

**3. Outcome and covariate data**

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

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

We used data on sex, age, quartiles of income, region (urban/rural), current smoker, and survey year as covariates through a literature review [13] and the results of a univariate analysis. We used quartile data for income instead of education level as a socioeconomic indicator because income may be directly linked to food purchases [14]. The question about physical activity was changed from the 2014 survey, so we did not consider physical activity.

**4. Statistical analysis**

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

We used the "process" macro based on the bootstrap method (ver. V2.16.3) suggested by Andrew to assess the mediating effects [15]. In this analysis, we applied 10,000 bootstraps. We separately or simultaneously assessed the indirect effect of the metabolic mediators on the association between dietary factors and CVD. Firstly, we examined the association under the controlling covariates (sex, age, income, region [urban/rural], present smoking, and survey year) through four basic steps to assess mediation [16]. Step 1: association between dietary factors and CVD ($X \rightarrow Y$; total effect and was marked path “c”); step 2: association between dietary factors and metabolic mediators ($X \rightarrow M_i$; marked path “a”); step 3: association between metabolic mediators and CVD after controlling for metabolic mediators ($M_i \rightarrow Y$;

marked path "b"); and step 4: association between dietary factors and CVD disease after controlling for metabolic mediators (direct effect; marked path "c"). Subsequently, we evaluated the multiple mediator model and the serial mediator model.

The exponential regression coefficient is equal to the odds ratio (OR) when considering the CVD as an outcome variable. The percentage of risk mediated by the metabolic mediator was calculated as [17]: OR (confounder adjusted) − OR (confounder and mediator adjusted)/OR (confounder adjusted) − 1 × 100.

All statistical analyses were conducted under a random sampling condition excluding the basic characteristics given in Table 1 using SAS ver. 9.4 software (SAS Institute, Cary, NC, USA). A two-sided $p$-value $< 0.05$ was considered significant.

## RESULTS

The basic characteristics of the study subjects are presented in Table 1. Mean age was 43.7 years, and 1.81% of subjects (n=189) had CVD. In addition, 0.98% and 0.90% of subject had stroke (n=102) and ischemic heart disease (n=97), respectively. Subjects with a higher income ate more fruits or vegetables than those with a lower income. Those who ate more fruit were more likely to be non-smokers and female than their counterparts (Supplemental Tables 2, 3).

The total effect of fruit intake on CVD showed an inverse association without controlling for metabolic mediators (adjusted odds ratio [aOR], 0.86, 95% CI: 0.74–0.98), but the effect of vegetable intake was not significant (aOR, 0.93; 95% CI: 0.81–1.06) after controlling for sex, age, income, region (urban/rural), current smoker, and survey year (data not shown).

The direct effect of fruit intake on CVD was borderline significant after further considering each metabolic mediator. The effect of fruit intake on SBP (X → M) and the effect of SBP on CVD (M → Y) were significant, and subsequently the indirect effect of SBP did not include zero in the 95% CI range, unlikely other metabolic mediators. The effect of fruit intake on BMI showed borderline significance, and the effect of BMI on CVD was significant, but the indirect effect of BMI was not significant. Additionally, the effect of SBP was significant even after controlling for BMI as a covariate (Table 2). SBP, cholesterol, and BMI were associated with CVD, but vegetable intake did not contribute to either metabolic mediator or CVD (Table 3). The mediating effect of SBP on the association between fruit intake and outcome was dominant even when the outcome was restricted to those with a current illness or undergoing treatment.

When the beta coefficient was expressed as OR, the OR of the effect of fruit intake on CVD was attenuated to 0.89 (95% CI: 0.77–1.03) while simultaneously controlling for three 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 intake had a beneficial effect on fasting glucose, but its effect was not associated with CVD. The direct effect of fruit intake on CVD presented an inverse association ($\beta$=-0.121, $p$=0.11), but it did not reach statistical significance (Figure 1). In addition, similar results were observed when adding BMI as covariate, with an OR (the effect of fruit intake on CVD) of 0.90 (95% CI: 0.78–1.04; data not shown). The indirect effect of the four metabolic factors accounted for 30.0% of the relationship between fruit intake and CVD (i.e. (0.8555-0.8989)/(0.8555-1)*100=30.0%).

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

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

**DISCUSSION**

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

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

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

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

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

The present study has some limitations. First, the results were derived from a cross-sectional study design, so causal relationships could not be effectively drawn. Our study design is also open to the problem of reverse causation. If the reverse causation affects the results, the association will appear to be null or reverse direction to what is expected. But, the indirect effect by SBP was significant and some parts of our results were consistent with previous studies [13, 24]. Furthermore, the results were also consistent when stroke and ischemic heart disease were analyzed separately. Because the survey was conducted through a household visit and excludes people in the hospital, subjects with diseases might be the relatively less serious cases. Measurement error in FFQ survey or self-reported disease status may influence the results. In addition, residual confounding factors such as physical activity may have influenced the association. Finally, because the number of participants with CVD was very low (1.8%), the study had inadequate statistical power which might explain some of the non-significant findings.

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

Taken together, our study suggests that diets rich in fruits may contribute to a lower CVD risk partly through lowered systolic blood pressure. Further prospective studies are needed for confirmation.

## Conflict of interest statement

None

## Contributor ship statement

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

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## Data availability

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

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## Figure Legends

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

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

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

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

487 Table 1. Basic characteristics of the study subjects.

| | Weighted % (SE) |
|---|---|
| **Sex** | |
| Male | 49.92 (0.51) |
| Female | 50.08 (0.51) |
| **Age range** | 25–64 years |
| **Age (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).

| | Fruit intake | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | X ⟶ M (a) | | | M ⟶ Y (b) | | | X ⟶ Y (c' = direct effect) | | | Indirect effect (a*b) | | |
| Metabolic Factors (M) | β | SE | p | β | SE | p | β | SE | p | 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 |

501
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 [a]Adjusted for sex, age, income, region (urban/rural), current smoking, and survey year.
505 [b]Adjusted 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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Table 3. The effect of metabolic mediators (M) in the association between vegetable intake (X) and cardiovascular disease (Y).

| | Vegetable intake | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | X ⟶ M (a) | | | M ⟶ Y (b) | | | X ⟶ Y (c' = direct effect) | | | Indirect effect (a*b) | | |
| Metabolic Factors (M) | *β* | SE | *p* | *β* | SE | *p* | *β* | SE | *p* | *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 |

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

[a]Adjusted for sex, age, income, region (urban/rural), current smoking, and survey year.

[b]Adjusted 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.

# Figure 1

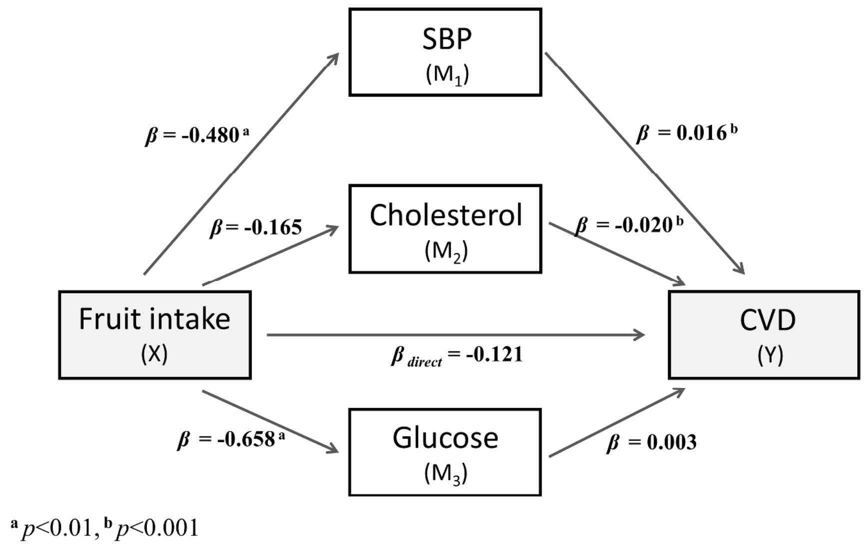
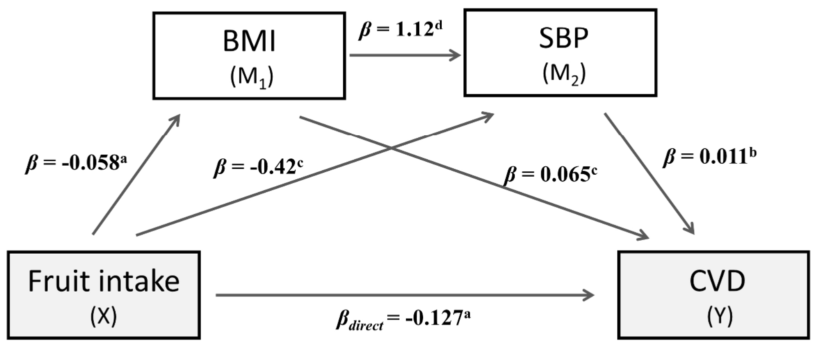



figure1

254x190mm (300 x 300 DPI)

# Figure2




[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 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | < 1 time/day | | 1 time/day | | 2 times/day | | 3+ times/day | | $p_{trend}$ |
| | 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 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | < 1 time/day | | 1 time/day | | 2 times/day | | 3+ times/day | | $p_{trend}$ |
| | 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).

| | Fruit intake | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | X ⟶ M (a) | | | M ⟶ Y (b) | | | X ⟶ Y (c' = direct effect) | | | Indirect effect (a*b) | | |
| Metabolic Factors (M) | *β* | SE | *p* | *β* | SE | *p* | *β* | SE | *p* | *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.

[a]Adjusted for sex, age, income, region (urban/rural), current smoking, and survey year.

[b]Adjusted 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).

| | Fruit intake | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | X ⟶ M (a) | | | M ⟶ Y (b) | | | X ⟶ Y (c' = direct effect) | | | Indirect effect (a*b) | | |
| Metabolic Factors (M) | *β* | SE | *p* | *β* | SE | *p* | *β* | SE | *p* | *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.

[a]Adjusted for sex, age, income, region (urban/rural), current smoking, and survey year.

[b]Adjusted 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 | 6 |
| | | (b) Give reasons for non-participation at each stage | |
| | | (c) Consider use of a flow diagram | |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 10 |
| | | (b) Indicate number of participants with missing data for each variable of interest | |
| 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 | 10-11 |
| | | (*b*) Report category boundaries when continuous variables were categorized | 7 |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | |
| 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.