Inverse Association of Serum Docosahexaenoic Acid With Newly Diagnosed Hypertension

A Community-based Case-control Study

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Abstract: Observational studies on circulating fatty acid (FA) and primary prevention of hypertension have yielded inconsistent results, and the association among the Chinese population is not fully clear. The aim of the study was to discern important FAs that can discriminate hypertensive patients from normotensive persons, and investigate associations between the important FAs and risk of hypertension.

We conducted a case-control study nested within a community-based cohort of 2447 individuals aged 35 to 79 years who completed a baseline assessment between October 2012 and April 2013. In all, 480 patients with newly diagnosed hypertension were identified at baseline and 480 normotensive individuals were randomly selected as matched normotensive controls. Controls were individually matched to cases by age (±2 y), sex, and recruitment center, with a 1:1 case-to-control ratio. Serum FA profile was compared between cases and controls by orthogonal partial least squares-discriminant analyses. Odds ratio (OR) with 95% confidence interval (CI) for newly diagnosed hypertension was estimated by a conditional logistical analysis.

After adjustment for body mass index, education, profession, family history of hypertension, salt intake, heart rate, blood lipids, and fasting glucose levels, serum FA profile in hypertensive patients was typically characterized by higher 16:0 and 16:1n-7, and lower 18:2n-6 and 22:6n-3, compared with normotensive controls. Docosahexaenoic acid (22:6n-3) and palmitoleic acid (16:1n-7) were identified as the important FA contributing most to the intergroup separations. When comparing the highest and lowest quartile of FA composition, newly diagnosed hypertension was negatively associated with 22:6n-3 (OR 0.65; 95% CI, 0.45–0.93; P for trend = 0.02), but positively associated with 16:1n-7 (OR 2.14; 95% CI, 1.46–3.12; P for trend < 0.001). The associations remained pronounced after multiple adjustments and in further stratified analyses.

In distinguishing hypertensive patients and normotensive persons, 22:6n-3 was considered as an important n-3 FA. Increased serum proportion of 22:6n-3 was associated with decreased odds of newly diagnosed hypertension, which suggests that high levels of 22:6n-3 in serum or perhaps in diet may be beneficial for prevention of hypertension in the Chinese population.

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INTRODUCTION

Hypertension is known as a strong modifiable risk factor for cardiovascular diseases (CVDs) and all-cause mortality worldwide. The American Heart Association (AHA) has recommended home blood pressure (BP) monitoring for patients diagnosed with hypertension, but the lack of effective molecular biomarker means its diagnosis is only based on the cut-off values of BP measured at the upper arm in a clinic environment. Evidence suggested that the early pathophysiological changes during the development of hypertension may occur before appreciable increases in BP. Thus, the identification of these metabolic perturbations may lead to a better understanding of a pathogenesis of hypertension and a new opportunity for the development of novel therapies and improved prognosis.

Experimental studies indicate that the alterations of fatty acid (FA) as constituents of membrane phospholipids have been implicated in the pathogenesis of hypertension by competing for eicosanoid metabolic pathway to change the balance of vasodilator/vasoconstrictor, the systemic arterial compliance mediated by the release of nitric oxide (NO), and the voltage-dependent L-type Ca2+ channel levels. Convincing evidence from meta-analyses and intervention trials suggests that increased consumption of n-3 polyunsaturated...
fatty acid (PUFA) has notably antihypertensive properties. FA intake can be estimated by a dietary questionnaire and weighed diet record, which may have led to measurement errors or bias. Compared with questionnaire estimates, FA biomarker can objectively reflect dietary consumption and biologically relevant processes (eg, absorption, incorporation, or metabolism), and also reliably distinguish among individual FA that may partially differ in physiological effects. Several epidemiological studies of FA biomarker have shown that a serum FA profile characterized by high 16:0, 16:1n-7, 20:3n-6, and low 18:2n-6 is associated with several cardiometabolic disorders. However, to date, little data have globally characterized serum/plasma FA profile in the study of hypertension among the Chinese population. To our knowledge, whether a potential FA biomarker can contribute to the discrimination between hypertensive patients and normotensive persons is still unclear in China.

Thus, we conducted a case-control study nested within a community-based cohort in Zhejiang Province, China. Serum FA profile was compared between hypertensive cases and matched normotensive controls to discern an important FA that contributed most to the intergroup separations. In addition, we attempted to investigate the association between the identified individual FA and newly diagnosed hypertension in this Chinese population.

METHODS
Study Design and Participants
The study protocols were approved by the Ethics Committee of Biosystem Engineering and Food Science College (Zhejiang University, Hangzhou, China), and were carried out in accordance with the approved guidelines. All participants provided written informed consent. In all, 3500 women and men aged 35 to 79 years free-living in 6 neighborhoods across 3 cities (Hangzhou, Jiaxin, and Shaoxin) located in Zhejiang Province, China, were invited to participate in a community-based cohort (Zhejiang Prospective Investigation into Hypertension and Lifestyle) between April and October 2012. Zhejiang province is a well development region with a good Medicare system even in remote areas. Participants are all local Chinese residents, and their complete healthy/medical records were documented in the local community hospitals. To define the study population, we excluded anyone who has no complete medical record, and meet any of the following characteristics: myocardial infarction, coronary heart disease, peripheral vascular disease, angina, cancer, dementia, schizophrenia, deaf, <35 years, and less than 6 months of living in the locality. Finally, a total of 3017 participants with complete health records agreed to participate. Baseline assessment included the collection of fasting blood samples, anthropometric measurements, and a personal interview using a validated questionnaire about medical history, and sociodemographic and lifestyle characteristics between October 2012 and April 2013. Of those, 2535 participants provided overnight fasting blood samples and completed a laboratory assessment, with the participation rate of 84.02% (2535/3017). Participants with missing values (n = 56) or implausible data (n = 19) on lifestyle factors from the self-administered questionnaire and with unreliable data on serum FA (n = 13) were excluded, and then 2447 participants (1153 men and 1294 women) finally remained for baseline analyses.

A total of 788 hypertensive patients were identified from 3017 participants who completed face-to-face interview and BP measurement, of which 480 cases were with newly diagnosed hypertension. Newly diagnosed hypertension was ascertained by meeting 1 of the 2 criteria: no hypertension previously, but reported newly diagnosed hypertension during the investigation based on a new physician’s diagnosis (an average systolic BP ≥140 mm Hg and/or an average diastolic BP ≥90 mm Hg); or newly initiated antihypertensive treatment (treatment duration ≤6 months). Normotensive controls were randomly chosen from individuals who were free of hypertension until the date of diagnosis of their matched case. Controls were individually matched to cases by age (±2 y), sex, and recruitment centers, with a 1:1 case-to-control ratio. Cases (n = 480) were comprised of 3 hypertension subtypes as follows: isolated systolic hypertension (ISH: SBP ≥140 mm Hg and DBP <90 mm Hg; n = 198), isolated diastolic hypertension (IDH: SBP <140 mm Hg and DBP ≥90 mm Hg; n = 89), and systolic-diastolic hypertension (SDH: SBP ≥140 mm Hg and DBP ≥90 mm Hg; n = 183), whereas normotensive controls were also divided into 3 subgroups as matched controls of ISH, IDH, and SDH, respectively.

Questionnaire Interview and Anthropometrical Measurements
A standard questionnaire was administered by trained investigators to collect information on social-demographic characteristics, lifestyle, family history of hypertension, and medical history. Anthropometrical measurements were performed by trained physicians using standard protocols, including height (m), weight (kg), heart rate (HR, beat/min), and BP (mm Hg). Body mass index (BMI, kg/m²) was calculated as the participant’s weight (kg) divided by the square of standing height (m²). Before BP measurements, participants were advised to avoid consuming alcohol or tobacco, ingesting tea or coffee, and engaging in exercise for at least 30 minutes. When measuring BP, participants were to refrain from talking and their arms were supported at the level of the heart. A standardized mercury sphygmomanometer was used to measure BP, and 1 of 3 cuff sizes (regular adult, large, or thigh) was chosen based on the circumference of the participant’s arm. BP for each participant was defined as the average of 3 measurements performed on the participant in a sitting position with 5-minute rest intervals at that visit.

Serum Sample Collection and FA Analyses
Fasting blood samples were collected in tubes with serum separator gel. They were left at room temperature for 30 minutes and then centrifuged at 2500 RCF (g) for 10 to 15 minutes to isolate serum. Measurement of serum triglycerides (TG), total cholesterol (TC), and fasting glucose (Fbg) levels were determined by standard procedures at the biochemistry laboratory in Zhejiang Hospital. The remaining serum samples were aliquoted into separate tubes (1 mL) and stored at −80°C until further FA analyses.

The FA composition in serum was analyzed between September 2013 and June 2014. The total lipid content of the serum was extracted with solvents. The methyl esters of the FAs in serum were prepared by saponification, and the serum composition of FA was determined by gas-liquid chromatography (GLC) as described previously. The main sources of serum FAs are derived from cholesterol esters, phospholipids, and triglycerides. The composition of individual FA was expressed as a percentage of total FAs in serum.
Statistical Analysis

Individual FAs were imported into SIMCA-P version 12.0 (Umetrics, Umea, Sweden) for multivariate pattern analyses. Principal component analysis (PCA) was firstly performed to examine the intrinsic variation of each subgroup, and then outliers were removed based on "Hotelling T2" from each subgroup. After unit variance (UV) scaling and mean-centering, orthogonal partial least squares-discriminate analysis (OPLS-DA) was employed to examine the distributions and discriminations between groups according to the differences in their global FA pattern in serum. The quality of OPLS-DA model was evaluated by $R^2$ and $Q^2$ values commonly. $R^2 (R^2X$ and $R^2Y$) represented the cumulative proportion of variance in the variable ($X$ and $Y$) explained by the model and indicated goodness of fit for the model, whereas $Q^2$ represented the cumulative predicted variation in the response $Y$ and indicated a predictive power of the constructed model. The values of $R^2$ and $Q^2$ more than 0.5 showed that an excellent discrimination (goodness of fit) and significant principal components (PCs) could be selected. Thus, the score plot of PC1 versus PC2 was utilized to detect separation between the 2 groups. In addition, S-plot and variable important for the plot of PC1 versus PC2 was utilized to detect separation between principal components (PCs) could be selected. Thus, the score plot of PC1 versus PC2 was utilized to detect separation between the 2 groups. In addition, S-plot and variable important for the projection (VIP) on the basis of the OPLS-DA model was constructed to identify the potential FA biomarker contributing most to the discrimination between the groups, respectively. S-plot integrated covariance and loading plot of OPLS-DA, in which the further a FA point departs from 0 of x-axis and y-axis, the more the FA contributed to intergroup separation. Individual FA with VIP >1.5 was regarded as a potential FA biomarker contributing most to intergroup discriminations.

Data analyses were performed by STATA version 11.0 (Stata CORP, College Station, TX). The distribution of continuous variables was examined for normality by Shapiro–Wilk test. Data with normal distribution were expressed as the mean (SD), whereas the skewed data were expressed as the median (inter-quartile rage [IQR]) and were log-transformed before statistic analyses. Baseline characteristics of study participants were compared by means of the McNemar test for categorical variables and the paired t test for continuous variables. Difference in serum FA between case and control was tested by a generalized linear model (GLM). The associations between newly diagnosed hypertension and the identified FA biomarkers were analyzed by fitting conditional logistic regression models. FA compositions were divided into quartiles (Q) based on their distribution in control individuals. Unadjusted odds ratio (OR) with 95% confidence interval (CI) was calculated for analyses of newly diagnosed hypertension across quartiles of FA composition, with the lowest quartile serving as the reference. Multivariate-adjusted OR with 95% CI was also estimated in a multivariable model adjusted for baseline variables that were unbalanced between the study groups ($P < 0.05$). The consistency of the overall findings was assessed in subgroups defined by the difference in baseline variables between case and control. Interaction tests were also conducted to measure whether multivariate-adjusted OR significantly differed between the strata analyzed by including simultaneously each strata factor, the quartiles of FA biomarker, and the respective interaction terms (the strata factor multiplied by quartiles of FA biomarker) in the models. Two-sided $P < 0.05$ was considered statistically significant.

RESULTS

Demographic and Clinical Characteristics

Demographic and clinical characteristics in case with newly diagnosed hypertension and matched controls are presented in Table 1. The proportion of primary education, nonmanual labor, and family history of hypertension was significantly higher in cases with newly diagnosed hypertension than in matched controls. Higher BMI, BP, HR, fasting serum TG, and TC were observed in newly diagnosed hypertension cases compared with controls.

FA Composition in Serum Between Case and Control

Difference in individual FA between case and matched control is presented in Table 2. Cases with newly diagnosed hypertension had significantly higher proportions of serum 16:0, 16:1n-7, 18:1n-9, 18:3n-3, 20:3n-3, and 20:3n-6, and also lower 18:2n-6, 22:6n-3, and 20:4n-6, compared with the matched controls (Table 2). Even after adjustment for BMI, education, profession, family history of hypertension, salt intake, TG, TC, and HR, the difference in 16:0, 16:1n-7, 22:6n-3, and 18:2n-6 became attenuated, but remained significant ($P < 0.05$, respectively). In addition, lower proportions of total n-3 PUFA and higher ratio of saturated FA (SFA)/PUFA were observed in hypertension cases than in matched controls.

Pattern Analyses of Individual FA

Firstly, OPLS-DA plot intuitively displayed an imperfect discriminant between cases with newly diagnosed hypertension ($n=480$) and controls ($n=480$) (Figure 1A). The cumulative $R^2Y$ and $Q^2$ were 0.538 and 0.294, which indicated that the OPLS-DA model has moderate fitness and prediction. Next, hypertension cases were divided into 3 subgroups: ISH ($n=168$), IDH ($n=119$), and SDH ($n=183$). OPLS-DA model was repeated to compare serum FA pattern between subgroup cases and their matched controls. SDH cases could be unambiguously separated from the matched controls ($R^2Y=0.727$, $Q^2=0.440$; Figure 2C), whereas ISH ($R^2Y=0.685$, $Q^2=0.249$; Figure 2A) and IDH cases ($R^2Y=0.715$, $Q^2=0.304$; Figure 2B) did not well differentiate from the matched controls, respectively.

The S-plot revealed that 22:6n-3 and 18:2n-6 were contributed most to the discrimination between hypertensive cases and the normotensive controls (Figure 1B). When comparing cases with hypertension phenotypes and their matched controls (Figure 2 D–F), 22:6n-3, 18:2n-6, 16:1n-7, and 20:3n-6 could be identified as the most important individual FAs in distinguishing hypertension phenotypes and their matched controls. Combined with the results from VIP (see Figures 1–4, http://links.lww.com/MD/A649, Supplemental Content, which illustrated variable important for the projection [VIP] following the OPLS-DA model in serum FA pattern), 22:6n-3 and 16:1n-7 were finally indentified as the important individual FAs for characteristics of newly diagnosed hypertension and its subtypes.

The Identified Individual FAs and Newly Diagnosed Hypertension

Table 3 presents logistic analysis results for the association of newly diagnosed hypertension with the identified individual FAs, by quartile of its distribution in controls. When comparing the upper quartile with the lowest quartile of individual FA composition in serum, newly diagnosed hypertension was positively associated with 16:0 (OR 1.59; 95% CI, 1.10–2.31; $P$ for trend = 0.011), 16:1n-7 (OR 2.14; 95% CI, 1.46–3.12; $P$ for trend < 0.001), and 20:3n-6 (OR 1.45; 95% CI, 1.01–2.07; $P$ for trend = 0.022), but negatively associated with
| Characteristics                        | Cases (n = 480) | Controls (n = 480) | P* |
|---------------------------------------|----------------|-------------------|----|
| Age, n (%)                            |                |                   | 0.767 |
| Middle-aged                           | 232 (48.30)    | 237 (49.37)       |    |
| Older                                 | 248 (51.70)    | 243 (50.63)       |    |
| Continuous, y                         | 55.49 (0.51)   | 55.35 (0.52)      | 0.852 |
| Sex, n (%)                            |                |                   | >0.99 |
| Male                                  | 240 (50%)      | 240 (50%)         |    |
| Female                                | 240 (50%)      | 240 (50%)         |    |
| BMI, n (%)                            |                |                   | <0.001 |
| Normal (≤24)                          | 209 (44.19)    | 284 (60.68)       |    |
| Overweight (24–28)                    | 193 (40.80)    | 146 (31.20)       |    |
| Obesity (>28)                         | 71 (15.01)     | 38 (8.12)         |    |
| Continuous, kg/m²                     | 24.79 (3.12)   | 23.42 (3.36)      | <0.001 |
| Lifestyle factors, n (%)              |                |                   |    |
| Smoking status                        |                |                   | 0.354 |
| No (<18 packs/y)                     | 363 (75.94)    | 349 (73.32)       |    |
| Yes (≥18 packs/y)                    | 117 (24.06)    | 131 (26.68)       |    |
| Drinking status                       |                |                   | 0.790 |
| No (<25g/d)                           | 343 (71.34)    | 338 (75.81)       |    |
| Yes (≥25g/d)                          | 137 (28.66)    | 142 (29.41)       |    |
| Education levels                      |                |                   | <0.001 |
| Primary (≤6 y)                        | 366 (76.37)    | 297 (62.80)       |    |
| Secondary (6–12 y)                    | 96 (20.89)     | 144 (30.97)       |    |
| High (>12 y)                          | 18 (2.74)      | 29 (6.23)         |    |
| Profession                            |                |                   | 0.023 |
| Nonmanual labor                       | 66 (13.77)     | 93 (19.27)        |    |
| Manual labor                          | 414 (86.23)    | 387 (80.73)       |    |
| Exercise habit                        |                |                   | 0.291 |
| No (<3 times/wk)                     | 376 (78.46)    | 358 (74.64)       |    |
| Yes (≥3 times/wk)                    | 104 (21.54)    | 122 (25.36)       |    |
| Salt intake                           |                |                   | 0.389 |
| High-salt (>10g/d)                    | 126 (26.25)    | 113 (23.89)       |    |
| Low-salt (≤10g/d)                     | 354 (73.75)    | 367 (76.11)       |    |
| Animal oil intake                     |                |                   | 0.435 |
| No (<2 times/wk)                     | 304 (63.26)    | 317 (65.68)       |    |
| Yes (≥2 times/wk)                    | 176 (36.74)    | 163 (34.42)       |    |
| Clinical parameter, n (%)             |                |                   | <0.001 |
| Family history                        |                |                   |    |
| No                                    | 334 (70.42)    | 387 (80.79)       |    |
| Yes                                   | 146 (29.58)    | 93 (19.21)        |    |
| Prevalent DM                          |                |                   | 0.121 |
| No                                    | 435 (90.62)    | 420 (87.50)       |    |
| Yes                                   | 45 (9.38)      | 60 (12.50)        |    |
| Systolic BP, mm Hg                    | 145.62 (14.11) | 119.29 (11.43)    | <0.001 |
| Diastolic BP, mm Hg                   | 90.24 (8.77)   | 77.81 (6.94)      | <0.001 |
| TG                                     |                |                   |    |
| Normal (≤1.7 mmol/L)                  | 313 (65.41)    | 357 (74.42)       | <0.001 |
| High (>1.7 mmol/L)                    | 167 (34.59)    | 123 (25.58)       |    |
| Continuous, mmol/L                    | 1.37 (0.96–2.02)| 1.19 (0.84–1.69)|    |
| Log-transformed                       | 0.35 (0.18)    | 0.22 (0.13)       | <0.001 |
| TC                                     |                |                   |    |
| Normal (≤5.18 mmol/L)                 | 330 (68.96)    | 364 (76.05)       | 0.010 |
| High (>5.18 mmol/L)                   | 150 (31.04)    | 116 (23.95)       |    |
| Continuous, mmol/L                    | 4.69 (4.14–5.36)| 4.48 (4.02–5.13)|    |
| Log-transformed                       | 1.55 (0.20)    | 1.50 (0.24)       | 0.020 |
| Fbg                                    |                |                   | 0.077 |
| Normal (≤6.1 mmol/L)                  | 426 (88.79)    | 441 (91.96)       |    |
| High (>6.1 mmol/L)                    | 54 (11.21)     | 39 (8.04)         |    |
| Continuous, mmol/L                    | 4.70 (4.31–5.12)| 4.62 (4.30–4.96)|    |
| Log-transformed                       | 1.56 (0.18)    | 1.55 (0.16)       | 0.092 |
18:2n-6 (OR 0.66; 95% CI, 0.49–0.94; P for trend = 0.041) and 22:6n-3 (OR 0.65; 95% CI, 0.45–0.93; P for trend = 0.018).

After adjustment for BMI, family history of hypertension, education, profession, salt intake, HR, blood lipid, and fasting glucose levels, these associations were attenuated and remained significant only for 16:1n-7 (OR 1.74; 95% CI, 1.05–2.89; P for trend = 0.040) and 22:6n-3 (OR 0.68; 95% CI, 0.47–0.98; P for trend = 0.048). The multivariate-adjusted associations for 16:1n-7 and 22:6n-3 remained pronounced in most subgroups examined (Figure 3). No significant interactions were observed between the odds of newly diagnosed hypertension and the strata analyzed.

**TABLE 2. Serum Fatty Acid Composition at Baseline Between Cases and Matched Controls**

| Fatty Acid (%) | Cases (n = 480) | Controls (n = 480) | P* | P† |
|---------------|----------------|--------------------|----|----|
| 12:0          | 2.58           | 1.83–3.61          | 2.60| 1.73–3.74 | 0.933 | 0.650 |
| 14:0          | 1.74           | 1.47–1.98          | 1.68| 1.41–1.96 | 0.652 | 0.282 |
| 16:0          | 20.95          | 19.22–22.86        | 20.44| 18.85–20.21 | 0.001 | 0.040 |
| 17:0          | 0.22           | 0.19–0.28          | 0.23| 0.18–0.28 | 0.355 | 0.886 |
| 18:0          | 6.36           | 5.89–6.84          | 6.33| 5.90–6.90 | 0.439 | 0.821 |
| 20:0          | 0.46           | 0.30–0.70          | 0.46| 0.29–0.75 | 0.448 | 0.300 |
| 22:0          | 0.45           | 0.32–0.70          | 0.48| 0.35–0.70 | 0.218 | 0.727 |
| 24:0          | 0.35           | 0.26–0.46          | 0.38| 0.27–0.51 | 0.067 | 0.267 |
| 16:1n-7       | 1.56           | 1.01–2.20          | 1.32| 0.93–1.94 | <0.001 | 0.021 |
| 18:1n-9       | 19.61          | 17.13–21.93        | 18.96| 16.24–21.51 | 0.031 | 0.155 |
| 20:1n-9       | 0.19           | 0.13–0.30          | 0.18| 0.12–0.28 | 0.598 | 0.453 |
| 22:1n-9       | 0.20           | 0.12–0.32          | 0.20| 0.11–0.32 | 0.384 | 0.639 |
| 24:1n-9       | 1.05           | 0.75–0.42          | 1.03| 0.76–1.42 | 0.820 | 0.611 |
| 18:3n-3       | 0.88           | 0.67–1.15          | 0.84| 0.63–1.08 | 0.021 | 0.312 |
| 20:3n-3       | 0.10           | 0.04–0.52          | 0.08| 0.04–0.38 | 0.047 | 0.151 |
| 20:5n-3       | 3.07           | 2.05–4.15          | 3.09| 2.06–4.48 | 0.203 | 0.115 |
| 22:5n-3       | 0.47           | 0.38–0.56          | 0.47| 0.38–0.57 | 0.721 | 0.627 |
| 22:6n-3       | 1.59           | 1.21–1.96          | 1.69| 1.32–2.11 | <0.001 | 0.051 |
| 18:2n-6       | 27.33          | 24.12–31.09        | 28.72| 24.47–32.65 | 0.019 | 0.045 |
| 18:3n-6       | 0.36           | 0.25–0.50          | 0.34| 0.22–0.47 | 0.436 | 0.505 |
| 20:3n-6       | 1.15           | 0.96–1.37          | 1.05| 0.92–1.33 | 0.039 | 0.108 |
| 20:4n-6       | 5.85           | 4.72–6.85          | 6.07| 5.02–7.15 | 0.012 | 0.103 |
| 22:2n-6       | 0.13           | 0.08–0.19          | 0.14| 0.08–0.20 | 0.184 | 0.426 |
| 22:4n-6       | 0.16           | 0.12–0.19          | 0.16| 0.13–0.20 | 0.666 | 0.211 |
| SFA           | 30.50          | 29.10–32.45        | 30.26| 28.66–32.05 | 0.035 | 0.163 |
| MUFA          | 22.53          | 19.77–25.24        | 21.49| 18.65–24.75 | 0.011 | 0.139 |
| PUFA          | 41.51          | 37.90–48.20        | 42.82| 38.84–46.66 | <0.001 | 0.024 |
| n-3 PUFA      | 6.28           | 5.07–7.30          | 6.33| 5.08–7.66 | <0.001 | 0.019 |
| n-6 PUFA      | 34.97          | 31.53–38.75        | 36.07| 31.98–40.02 | 0.010 | 0.211 |
| n-3/n-6       | 0.18           | 0.14–0.22          | 0.18| 0.14–0.23 | 0.547 | 0.247 |
| SFA/PUFA      | 0.72           | 0.65–0.84          | 0.70| 0.62–0.81 | <0.001 | 0.018 |

MUF A = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid, QR = quartile range, SFA = saturated fatty acid.

*P for difference between groups was tested by a nonadjusted generalized linear model.

†P for difference between groups was tested by a generalized linear model adjusted for age (y), sex, body mass index (kg/m²), duration of education (y), profession (manual/nonmanual), family history of hypertension (yes/no), salt intake (>10/≤10 g/d), triglyceride (mmol/L), total cholesterol (mmol/L), fasting blood glucose (mmol/L), and heart rate (beats/min).
DISCUSSION

The present study indicated that serum FA profile in cases with newly diagnosed hypertension was typically characterized by higher 16:0 and 16:1n-7, and also lower 18:2n-6 and 22:6n-3, compared with matched controls, which was similar to the results from 2 nested case-control studies conducted in the United States. Pattern analyses suggested that 22:6n-3 was identified as an important n-3 FA in distinguishing hypertensive cases (n = 480), whereas the gray dots represented the matched controls (n = 480). In S-plot (B), the black triangle denoted each individual FA. The further a black triangle departed from zero of x axis and y axis, the more individual FA represented by the black triangle contributed to the intergroup separation.

Extensive evidence from meta-analyses, clinical trials, and experimental studies suggest that high consumption of fatty fish or fish oil rich in 20:5n-3 (eicosapentaenoic acid [EPA]) and 22:6n-3 (docosahexaenoic acid [DHA]) may be protective against CVD. Dietary n-3 PUFA can be incorporated into serum/plasma, platelets, and tissue lipids to change biomembrane fluidity, increase the production of vasodilators, reduce cardiac adrenergic activity, and lower BP. The antihypertensive effects may be attributable to 22:6n-3, but not 20:5n-3, which has been supported by most previous studies. A meta-analysis of clinical studies showed 22:6n-3 had a slightly greater dose–response effect on BP levels than 20:5n-3 (−1.5/[−0.77 mm Hg vs −0.93/[−0.53 mm Hg per gram]). Mori et al found that 22:6n-3, but not 20:5n-3 supplementation, reduced the 24-hour and daytime ambulatory BP in mildly hyperlipidemic men. A 8-week fatty seafood trial conducted in Europe showed that 22:6n-3 content in erythrocyte at baseline was associated with a greater DBP reduction in overweight young adults. In agreement with evidence from the prior studies, our data also supported that participants in the highest quartile of serum 22:6n-3 composition had a notably lower risk of hypertension compared with those in the lowest quartile. Several possible mechanisms can explain the antihypertensive property of 22:6n-3. Firstly, the 22:6n-3 can be more preferentially incorporated into the biomembrane than 20:5n-3. A decrease in beta-adrenergic signaling through increased GRK2 phosphorylation would reduce the vasodilatative response, thereby increasing BP. The incorporation of DHA into cardiomyocyte membranes can inhibit the beta-adrenergic
system, which may help to explain its antiarrhythmic and BP-lowering effects. Additionally, calcium/calmodulin-dependent kinase 4 (CaMK4) gene deletion can impair CaMK-mediated activation of eNOS, which induces hypertension in the mice null for CaMK4. The 22:6n-3 can be incorporated into endothelial membranes to stimulate ATP release from the endothelium, which leads to vasodilatation mediated by NO release. The induction of NO release, together with the decrease in noradrenaline levels, is likely to be responsible for BP-lowering effect of 22:6n-3. Finally, adverse BP levels has been a potential risk factor for coronary heart disease (CHD). Platelet antigen 2 (PlA2) polymorphism of the GPIIIa gene may be associated with a more aggressive atherothrombotic disease, especially with high risk of myocardial infarction, CHD, and ischemic stroke in hypertensive patients. The 22:6n-3 has been found to especially have anti-atherothrombotic and antiinflammatory properties, which were speculated to alleviate the progression of cardio-cerebral vascular adverse events through increasing antiplatelet activity and decreasing the infiltration of inflammatory cells around the plaque.

Serum FA can be affected not only by dietary fat intake but also by relevant biological processes. Taking into account the amount of bioavailable 20:5n-3 and 22:6n-3 within human body mainly derived from the diet, n-3 FA levels in circulating blood can be regarded as a biomarker to closely reflect diet consumption. Nevertheless, because of lower contents of 16:1n-7 in diet, serum 16:1n-7 may be derived mainly from the endogenous metabolism of 16:0 catalyzed by stearoyl-CoA desaturase (SCD), which perhaps complicates the use of serum non-essential FA as a biomarker. Feeding animals with SFA can impair endothelial function, induce an up-regulation of SCD expression within liver, enhance sympathetic nervous system activity, and increase BP. In the present study, serum ratio of SFA/PUFA was significantly higher in hypertension cases than in controls, and the difference remained significant even after multiple adjustments. Thus, serum 16:1n-7 cannot perfectly reflect diet intake, but may be a consequence of high consumptions in SFA relative to a low intake of PUFA. Considering that data on FA consumption were scarce in our study, it is difficult to find firm evidence that the high proportion of 16:1n-7 in

**FIGURE 2.** The OPLS-DA plot and corresponding S-plot derived from the serum FA profile in hypertension phenotype case compared with their matched controls. The black circles denoted isolated systolic hypertension (ISH, n = 198) in (A), isolated diastolic hypertension (IDH, n = 99) in (B), and systolic-diastolic hypertension (SDH, n = 183) in (C), whereas the gray dots represented their respective matched controls. SDH cases were clearly differentiated from the matched controls in (C) ($R^2_Y = 0.727$, $Q^2 = 0.440$). The S-plot of ISH, IDH, and SDH was displayed in (D), (E), and (F), respectively. FA = fatty acid, OPLS-DA = orthogonal partial least squares-discriminate analysis.
# TABLE 3. Odds Ratios With 95% Confidence Interval for Newly Diagnosed Hypertension by Quartile of Serum Fatty Acid

| FA        | Quartiles of FA |     |     |     |        |        |
|-----------|-----------------|-----|-----|-----|-------|-------|
|           | Q1              | Q2  | Q3  | Q4  | P for trend | Per Quartile Increment |
| 16:0      | Range (% of total FA) | <19.06 | 19.06–20.66 | 20.66–22.56 | ≥22.56 | 0.010 | 1.18 (1.05–2.32) |
|           | Case/control    | 106/133 | 110/131 | 129/113 | 135/103 |       |       |
|           | Crude OR (95% CI) | 1 (Ref.) | 1.00 (0.69–1.46) | 1.34 (0.93–1.92) | 1.59 (1.10–2.31) |       |       |
|           | Adjusted OR (95% CI) | 1 (Ref.) | 0.99 (0.62–1.61) | 1.20 (0.74–1.97) | 1.26 (0.77–2.07) | 0.331 | 1.09 (0.92–2.27) |
| 16:1n-7   | Range (% of total FA) | <1.00 | 1.00–1.42 | 1.42–2.07 | ≥2.07 | <0.001 | 1.28 (1.13–1.44) |
|           | Case/control    | 91/147 | 119/119 | 132/107 | 138/103 |       |       |
|           | Crude OR (95% CI) | 1 (Ref.) | 1.63 (1.12–2.37) | 2.03 (1.39–2.97) | 2.14 (1.46–3.12) |       |       |
|           | Adjusted OR (95% CI) | 1 (Ref.) | 1.55 (0.94–2.55) | 1.68 (1.01–2.80) | 1.76 (1.05–2.95) | 0.040 | 1.18 (1.01–1.39) |
| 18:2n-6   | Range (% of total FA) | <24.28 | 24.28–27.58 | 27.58–31.74 | ≥31.74 |       |       |
|           | Case/control    | 128/112 | 127/117 | 122/114 | 103/137 |       |       |
|           | Crude OR (95% CI) | 1 (Ref.) | 0.92 (0.64–1.31) | 0.99 (0.68–1.43) | 0.66 (0.49–0.94) | 0.041 | 0.88 (0.79–0.99) |
|           | Adjusted OR (95% CI) | 1 (Ref.) | 1.00 (0.62–1.62) | 0.93 (0.57–1.50) | 0.87 (0.53–1.41) | 0.527 | 0.95 (0.81–1.11) |
| 20:3n-6   | Range (% of total FA) | <0.94 | 0.94–1.12 | 1.12–1.35 | ≥1.35 |       |       |
|           | Case/control    | 107/132 | 114/124 | 128/114 | 131/110 |       |       |
|           | Crude OR (95% CI) | 1 (Ref.) | 1.13 (0.79–1.62) | 1.42 (0.99–2.04) | 1.45 (1.01–2.07) | 0.02 | 1.14 (1.02–1.28) |
|           | Adjusted OR (95% CI) | 1 (Ref.) | 0.97 (0.60–1.58) | 1.24 (0.76–2.03) | 1.05 (0.64–1.69) | 0.64 | 1.03 (0.88–1.21) |
| 22:6n-3   | Range (% of total FA) | <1.26 | 1.26–1.64 | 1.64–2.04 | ≥2.04 |       |       |
|           | Case/control    | 130/104 | 122/121 | 118/125 | 110/130 |       |       |
|           | Crude OR (95% CI) | 1 (Ref.) | 0.74 (0.51–1.06) | 0.73 (0.51–1.04) | 0.65 (0.45–0.93) | 0.018 | 0.67 (0.51–0.89) |
|           | Adjusted OR (95% CI) | 1 (Ref.) | 0.78 (0.54–1.33) | 0.76 (0.53–1.10) | 0.65 (0.44–0.97) | 0.048 | 0.90 (0.81–1.00) |

CI = confidence interval, FA = fatty acid, OR = odds ratio, Ref = reference.

*Crude OR was estimated in an initial conditional logistic regression model.

*Adjusted OR was estimated in a multivariate-adjusted conditional logistic regression model adjusted for body mass index (<24/≥24), duration of education (<6/≥6 y), profession (manual/nonmanual), family history of hypertension (yes/no), salt intake (<10/≥10 g/d), serum triglyceride levels (<1.7/≥1.7 mmol/L), serum total cholesterol levels (<5.18/≥5.18 mmol/L), fasting glucose level (<6.1/≥6.1 mmol/L), and heart rate (<72/≥72 beats/min).
hypertension cases compared with normotensive controls only resulted from endogenous synthesis driven by SCD. Serum 16:1n-7 as potential biomarker in distinguishing hypertensive patients and normotensive controls should be interpreted with caution.

Several strengths should be highlighted in our study. To begin with, adopting a population-based case-control design is considered more reliable because their participants are more representative than those of hospital-based case-control studies. In addition, pattern analyses of individual FA were employed to discern important individual FAs contributing most to the discrimination between cases and controls. Thirdly, serum n-3 FA can be regarded as a helpfully complementary tool for the Food Frequency Questionnaire (FFQ) to closely reflect the true dietary intake of an individual; thus the possibility of recall bias from participants can be eliminated. Finally, the inverse association between 22:6n-3 and new diagnosed hypertension was consistent for most subgroups examined. Nevertheless, the potential limitations should also be considered in our study. Firstly, the possibility of selection bias cannot be minimized, which perhaps distorts the true association. Secondly, inference on temporality of associations cannot be allowed, though the use of biomarkers of dietary FA intake counterbalanced this point. Thus, the observed findings need to be verified in prospective cohort studies. Thirdly, despite comprehensive adjustment for demographic characteristics, multiple lifestyle and clinical factors to reduce the potential for confounders, we cannot exclude the possibility of residual confounding caused by imprecisely measured or unmeasured risk factors. In particular, we were unable to adjust our results for total energy intake or the consumption of specific foods, as dietary intakes were not assessed at baseline. Fourthly, hypertension was defined primarily by

![FIGURE 3](image-url)

**FIGURE 3.** Associations between identified fatty acid biomarker and newly diagnosed hypertension stratified by unbalanced variables between cases and controls. Associations of 16:1n-7 (A) and 22:6n-3 (B) with risk of hypertension when comparing the highest quartiles of FA composition with the lowest were stratified by body mass index (BMI), family history of hypertension, duration of education, profession, serum triglyceride (TG), total cholesterol (TC), and heart rate (HR), respectively. \( P \) for interaction was calculated to determine whether multivariate-adjusted odds ratio (OR) for newly diagnosed hypertension significantly differed between the subgroups examined. The black squares represented multivariate-adjusted OR in each strata, and the solid horizontal lines denoted the corresponding 95% confidence interval (CI).
3 readings on 1 occasion or by information on oral antihypertensive drugs. Thus, some misclassifications likely occurred. Fifthly, serum fatty acid represents a combination of triacylglycerol, cholesterol esters, and phospholipids found in lipoproteins, which can only reflect concentration over medium term (several weeks to days) and easily affected by the postprandial status of the individual. Thus, it may be an untypical representative of sensitive biomarkers indicating postabsorptive amounts and change at the circulating blood. Sixthly, the levels of serum FA may be subject to laboratory and biological variation; however, we measured FA in BP cases and their matched controls in the same chromatography, thus the possibility of measurement variation between cases and controls may be minimized. Finally, all participants from the present study were of Chinese Han ethnicity, which minimized the effects of confounding by ethnic background. Our study population did not perfectly represent a random sample of Chinese adults with diverse ethnicity, and thus caution is needed for generalizing the present findings to the whole Chinese population.

In summary, the 22:6n-3 as an important n-3 PUFA may play a pivotal role in discriminating hypertensive patients from normotensive persons. Increased serum proportion of 22:6n-3 was associated with decreased odds of newly diagnosed hypertension. The present findings suggest that increased levels of 22:6n-3 in serum or perhaps in diet may be beneficial for prevention of hypertension in Chinese population.

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