Original Article

Fasting Serum Free Fatty Acid Composition, Waist/Hip Ratio and Insulin Activity in Essential Hypertensive Patients

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Abnormal fatty acid metabolism may play an important role in the pathogenesis of essential hypertension (EH). We compared fasting serum free fatty acid (FFA) composition between EH patients and nonhypertensive (NH) subjects, and examined the relationships between fasting serum FFA composition and waist/hip ratio, insulin activity, blood pressure, serum zinc, age, and sex in both groups. We conducted a cross-sectional study of 232 community-dwelling subjects aged between 35 and 60 years: 109 EH patients and 123 NH subjects. Serum FFA was determined by HPLC analysis. The data were analyzed by multivariate linear stepwise regression, stratified analysis, and correlation analysis. In men, EH patients had lower C22:6/C20:5 ratios ($n-3$ $\Delta 6$ -desaturase activity index, 7.96 ± 8.81 vs. 14.5 ± 13.1 , $p < 0.01$), polyunsaturated fatty acid (PUFA), $n-3$ PUFA and polyunsaturated/saturated (P/S) ratios compared with male subjects without hypertension. There were no differences in women. Fasting serum FFA composition was significantly related to many variables, including waist/hip ratio, insulin sensitivity index, and serum zinc (all $p < 0.05$ or < 0.01) for all the subjects, and included both age- and sex-related differences (both $p < 0.05$). Compared with NH, EH patients had lower levels of serum zinc ($14.9 \pm 5.00 \mu\text{mol L}^{-1}$ vs. $16.8 \pm 6.38 \mu\text{mol L}^{-1}$, $p < 0.05$). We concluded that EH patients had marked alteration in fasting serum FFA composition, which was affected by upper body obesity, insulin resistance, zinc deficiency, and high dietary fat, and that the differences were age- and sex-related. (*Hypertens Res* 2008; 31: 623–632)

Key Words: free fatty acid, essential hypertension, $\Delta 6$ -desaturase, α -linolenic acid

Introduction

Abnormal fatty acid metabolism may play an important role in the pathogenesis of essential hypertension (1–6), and fasting serum free fatty acid (FFA) composition is a good model for reflecting fatty acid metabolism (7). Serum FFA provide an important energy source as nutrients, and they also act as signaling molecules in various cellular processes relating to hypertension, such as endothelial cell endothelin-1 produc-

tion (8). Fasting serum FFA composition mainly reflects dietary fat intake, but also endogenous fatty acid synthesis catalyzed by Δ -desaturases. However, little is known about the alteration in the fasting FFA composition, the activity of Δ -desaturases in patients with essential hypertension (EH), or what factors have effects on desaturase activity and then on fasting serum FFA composition. Zinc has been identified as a constitutive cofactor of desaturase activity in human, and insulin not only is an exclusive hormone that enhances the activity of Δ -desaturase (9), but also decreases plasma levels

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and turnover rate of FFA. What are their effects on fasting FFA composition and desaturase activity in EH patients?

One cohort study shows that fatty acid composition of serum lipids predicts the development of the metabolic syndrome in men (10). In addition, EH development is associated with components of the metabolic syndrome, such as upper body obesity and insulin resistance. Therefore, the objects of this study are to compare serum FFA composition between EH patients and nonhypertensive (NH) control subjects, and to examine the relationship of fasting serum FFA composition with waist/hip ratio (WHR); body mass index (BMI); insulin activity; levels of serum zinc, triglycerides (TG), total cholesterol (TC), and high-density lipoprotein (HDL) cholesterol; age; and gender in EH patients and NH control subjects, and to explore impact factors of serum FFA composition and Δ -desaturase activity.

Methods

Two groups of 123 NH control subjects (mean age, 42.8 ± 4.45 years) and 109 EH matched for age and dietary habits were selected as described below.

For the NH subjects, we used data from a cross-sectional study in a population sample of 123 healthy subjects selected as previously described (11). Briefly, an initial selection of 540 healthy subjects was obtained by means of tables of random numbers; further selection was performed by three practitioners working in the area, who were familiar with the details of the clinical histories and lifestyles of the subjects. Exclusion criteria included a history of secondary hypertension, diabetes, coronary heart disease, chronic liver disease, and kidney disease, or the current use of steroids, nonsteroidal anti-inflammatory drugs, or antiplatelet and hypolipidemic or antihypertensive therapy.

EH patients were selected by cluster sampling. According to Hypertension Diagnoses Criterion of JNC 7 in 2003 ($SBP \geq 140$ mmHg and/or $DBP \geq 90$ mmHg) (12), and Obesity Diagnoses Criterion of the Asia Adult Obesity Task Force in 2000 ($BMI [kg/m^2] \geq 25$, obesity; ≥ 23 , overweight) (13), we selected 70 obese (including overweight) and 39 nonobese EH patients from all employees ($n=801$) in a large factory. Office blood pressure (BP) was evaluated by one practitioner.

None of the subjects were institutionalized or on a special diet, and none were taking trace elements or vitamin supplements. A 3-d food record was randomly obtained from a representative sample of subjects (50 EH patients and 30 NH subjects) to exclude any significant differences between EH and NH with respect to dietary intake of fatty acids. All procedures were reviewed and approved by the Medical Ethics Committee of Xi'an Jiaotong University, and all subjects gave informed consent.

Serum FFA Analysis

Blood samples were obtained after subjects had been seated for at least 5 min in the morning following a 10 h fast. It was confirmed that there was no sudden change in serum FFA level. Serum was separated from blood samples and then frozen at -80°C . HPLC analysis by the derivatization method was used to determine fasting serum FFA as previously described (14). Briefly, the use of ethanol (75%, v/v) as a precipitating agent for protein yielded FFA from protein-bound acids, then FFA was directly derivatized by treatment with acidic 2-nitrophenylhydrazine hydrochloride (2-NPH-HCl) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (1-EDC-HCl). The derivatized fatty acids were extracted into *n*-hexane and separated isocratically on a reversed-phase C_{18} column within 30 min. The detection wavelengths were 406 nm and 203 nm. Analytical recovery rates ranged from 87.8% to 120.5%. Assay coefficients of variation were less than 10%.

Determination of $\Delta 6$ -, $\Delta 5$ -Desaturase Activity

$\Delta 6$ - and $\Delta 5$ -desaturases, rate-limiting enzymes, catalyzed the desaturation processes of linoleic (18:2 n 6) and α -linolenic (18:3 n 3) acids. The $\Delta 6$ -desaturase converts linoleic acid (18:2 n 6) into γ -linolenic acid (DGLA, 18:3 n 6), α -linolenic acid (18:3 n 3) into stearidonic acid (18:4 n 3), and eicosapentaenoic acid (EPA) (20:5 n 3) into docosahexaenoic (DHA) (22:6 n 3). The $\Delta 5$ -desaturase is responsible for the biosynthesis of arachidonic acid (AA) (20:4 n 6) from DGLA and EPA (20:5 n 3) from stearidonic acid (18:4 n 3) (15). Desaturase activity is usually assayed *in vitro* or in animals by measuring the rate of conversion of radiolabeled precursor fatty acids to their respective products. Ethical and practical considerations prevent this method from being adopted in humans; thus, indirect information must be gathered from analysis of cell membrane lipid composition, which is known to reflect desaturation activities (16). Linoleic (18:2 n 6) and α -linolenic (18:3 n 3) acids are essential fatty acids because they cannot be synthesized *in vivo* and exclusively reflect dietary intake, where the fatty acids C20 and C22 derive from the diet as well as from essential fatty acid metabolism, *via* the above-mentioned pathway. Thus, the C20:4/C18:2, C20:5/C18:3 and C22:6/C20:5 ratios may be considered expressions of desaturase-elongase activity, when C20 and C22 fatty acid dietary intake is controlled.

Serum free polyunsaturated fatty acid (PUFA) composition is determined by three major factors: 1) the uptake of dietary essential fatty acids, 2) their elongation and desaturation, and 3) release from the cell membrane lipid pool induced by food intake. We therefore decided to regard the fatty acid C20:4/C18:2, C20:5/C18:3, and C22:6/C20:5 ratios of serum FFA under overnight postabsorptive conditions or fasted states as a desaturase activity index.

Table 1. Demographics of the Subjects Studied

Parameter	EH patients (n=109)	NH subjects (n=123)	p
Age, years	44.3±7.5	42.8±4.4	0.074
Sex, male/female	76/33	98/25	0.076
BMI, kg m ⁻²	26.8±3.67	24.8±2.83	<0.001
Waist circumference, cm	86.5±9.90	79.9±8.18	<0.001
WHR	0.87±0.06	0.84±0.07	<0.001
Systolic BP, mmHg	138.5±15.5	114.8±10.5	<0.001
Diastolic BP, mmHg	95.8±9.05	78.7±6.64	<0.001
Fasting insulin, mIU L ⁻¹	15.3±6.28	14.1±4.94	0.009
Fasting glucose, mmol L ⁻¹	4.54±0.559	4.49±0.588	0.55
ISI×10 ²	1.79±0.71	2.00±0.77	0.029
Triglycerides, mmol L ⁻¹	1.60±0.82	1.42±0.78	0.076
Total cholesterol, mmol L ⁻¹	4.16±0.69	3.96±0.80	0.042
LDL cholesterol, mmol L ⁻¹	2.47±0.67	2.25±0.69	0.019
HDL cholesterol, mmol L ⁻¹	1.55±0.46	1.65±0.42	0.098

Data are expressed as mean±SD. EH, essential hypertensive; NH, nonhypertensive; BMI, body mass index; WHR, waist/hip ratio; BP, blood pressure; ISI, insulin sensitivity index: ISI=1/[insulin (mIU L⁻¹)×FBG (mmol L⁻¹)]; FBG, fasting blood glucose; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Table 2. Fasting Serum Free Fatty Acid Composition and Zinc in Hypertensives, Compared with Nonhypertensives (Mean±SD)

Parameter (μmol L ⁻¹)	EH patients (n=109)	NH subjects (n=123)	p
C20:5n3	11.0±7.3	11.0±8.3	0.947
C22:6n3	64.7±39.2	94.2±31.3	0.000
C18:3n3	21.2±7.2	17.5±7.6	0.000
C20:4n6	34.3±27.8	31.6±21.7	0.465
C18:2n6	175.9±65.0	193.7±72.0	0.052
C20:5/C18:3 ratio	0.596±0.463	0.842±1.143	0.047
C22:6/C20:5 ratio	9.30±10.56	14.55±13.71	0.002
C20:4/C18:2 ratio	0.242±0.298	0.186±0.180	0.086
SFA	206.2±70.7	190.4±51.7	0.055
MUFA	181.8±76.7	166.1±63.6	0.097
PUFA	305.7±94.8	347.4±89.3	0.001
n-3	95.8±39.6	122.0±33.4	0.000
n-6	209.9±72.3	225.4±78.4	0.126
P/S ratio	1.57±0.55	1.88±0.45	0.000
Zinc	14.9±5.00	16.8±6.38	0.042

EH, essential hypertensive; NH, nonhypertensive; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; P/S ratio, PUFA/SFA ratio.

Determinations of Zinc in Serum and Other Biochemical Markers

Serum for zinc determination was diluted 1:10 with distilled-deionized water; serum zinc was measured by using flame atomic absorption spectrometry (NovAA model 300, Analytik Jena AG, Jena, Germany) (17).

Measurements were carried out in batches for plasma glucose, cholesterol, and triacylglycerol using enzymatic kits on a Cobas-Bio automated analyzer (Roche, Basel, Switzerland). HDL cholesterol was separated from plasma by selective precipitation of other lipoproteins. Plasma leptin and insulin were assayed with the Linco leptin and insulin assay kits (Linco Research Immunoassay, St. Charles, USA) by radioimmunoassay.

Blood Pressure

Seated BP was measured three times, at 30-s intervals, after the participants had been sitting for 5 min. Diastolic BP was defined as disappearance of the fifth Korotkoff sound and systolic BP as the appearance of the first Korotkoff sound. We took the average of the three readings.

Statistical Analysis

Data were expressed as means±SD for continuous variables and as a percentage for categorical variables. The significance of groups differences was assessed with Student's *t*-test or analysis of variance (ANOVA). Stratified analysis was used to assess the changes in fasting FFA composition in different groups, such as age, sex, and WHR. Simple nonparametric correlation (Spearman) was used to assess the statistical link between serum FFA composition and WHR, insulin sensitivity index (ISI), BMI, and serum zinc level. Multivariate linear stepwise regression models were used to examine the independence of associations, using the level of *n*-3 PUFA, C22:6/C20:5, C20:5/C18:3, or C20:4/C18:2 as the dependent variable, and using age, BMI, gender, living habits (including smoking, drinking, and diet), systolic BP, and diastolic BP as

Table 3. Pearson's Correlation Coefficients of Serum FFA with Serum Zinc, Triglycerides (TG), Total Cholesterol (TC), and High-Density Lipoprotein (HDL) Cholesterol, Insulin Sensitivity Index (ISI), Age, Waist/Hip Ratio, Body Mass Index (BMI), Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP)

Free fatty acids	Age	Zinc	ISI	WHR	BMI	SBP	DBP	TG	TC	HDL cholesterol
<i>n</i> -3 PUFA	0.18**	0.39**	0.23**	-0.38**	-0.19**	-0.07	-0.23*	0.01	0.24**	0.24**
P/S ratio	0.28**	0.46**	0.20*	-0.39**	-0.22**	-0.03	-0.18*	-0.09	0.19**	0.26**
C20:5/C18:3 ratio	-0.01	0.22**	0.19*	-0.09	-0.01	0.01	-0.07	0.06	0.13	0.10
C22:6/C20:5 ratio	0.02	-0.06	0.40**	-0.16*	-0.10	-0.14**	-0.15**	-0.18*	-0.15*	-0.01
C20:4/C18:2 ratio	0.02	-0.13	-0.24**	0.16*	0.10	0.16**	0.18**	0.26**	0.15*	-0.03

* $p < 0.05$, ** $p < 0.01$. WHR, waist/hip ratio; PUFA, polyunsaturated fatty acid; P/S ratio; PUFA/SFA ratio; ISI = $1/[\text{insulin (mIU L}^{-1}) \times \text{FBG (mmol L}^{-1})]$; FBG, fasting blood glucose.

independent variables. $p < 0.05$ was considered significant. All of the computations were performed with a commercial statistical package (SPSS version 13, Chicago, USA).

Results

Participants

The demographics for NH subjects and EH patients are summarized in Table 1. Age, sex, fasting glucose, triglycerides, and high-density lipoprotein were similar between groups (Table 1). As expected, EH patients had higher BMI, waist circumference, WHR, systolic and diastolic BP, total cholesterol, low-density lipoprotein, and insulin values ($p < 0.001$ or $p < 0.05$), but a lower ISI ($p < 0.05$). We found that EH patients have lower serum zinc values than NH subjects ($p < 0.05$, Table 1).

Serum FFA Composition in NH Subjects and EH Patients

Compared with NH subjects, EH patients had lower concentrations of PUFA ($p = 0.001$), but tended to have higher concentrations of saturated fatty acid (SFA, $p = 0.055$) and monounsaturated fatty acid (MUFA, $p = 0.097$), so the polyunsaturated/saturated (P/S) ratios were lower in EH patients ($p = 0.000$, shown in Table 2).

Table 2 also shows the means and standard deviations of individual fatty acids in fasting serum FFA. Compared with NH subjects, EH patients had lower concentrations of *n*-3 PUFA ($p = 0.000$). Among *n*-3 PUFA, concentrations of DHA (22:6 n 3), α -linolenic acid (18:3 n 3) metabolites, were lower ($p = 0.000$), while concentrations of α -linolenic acid (18:3 n 3) were higher ($p = 0.000$) in EH patients. Among *n*-6 PUFA, concentrations of linoleic acid (18:2 n 6) tended to be lower in EH patients ($p = 0.052$) than in NH subjects. In addition, compared with NH subjects, EH patients had significantly lower C20:5/C18:3 ratios (*n*-3 Δ 5-, Δ 6-desaturase activity index, $p = 0.047$) and C22:6/C20:5 ratios (*n*-3 Δ 6-desaturase activity index, $p = 0.002$), but tended to have higher C20:4/C18:2 ratios (*n*-6 Δ 5-, Δ 6-desaturase activity index, $p = 0.086$).

Pearson's Correlation Analysis of Serum FFA with Serum Zinc, TG, TC, and HDL Cholesterol, Age, ISI, WHR, BMI, and Diastolic BP

Table 3 shows Pearson's correlation coefficients of serum FFA with age, levels of serum zinc, TG, TC, and HDL cholesterol, ISI, WHR, BMI, and systolic and diastolic BP among participants. Diastolic BP was negatively associated with levels of *n*-3 PUFA ($r = -0.23$), P/S ratio ($r = -0.18$), and C22:6/C20:5 ratio ($r = -0.15$) and positively correlated with C20:4/C18:2 ratio ($r = 0.18$). The correlations of fatty acids with systolic BP were slightly weaker, which was negatively associated with C22:6/C20:5 ratio ($r = -0.14$) and positively correlated with C20:4/C18:2 ratio ($r = 0.16$). Similarly, BMI, WHR, and serum insulin level were negatively associated with levels of *n*-3 PUFA, P/S ratio, and C22:6/C20:5 ratio and positively correlated with C20:4/C18:2 ratio. For example, the correlation coefficient was -0.38 between WHR and *n*-3 PUFA, -0.39 between WHR and P/S ratio, -0.22 between BMI and P/S ratio, and 0.25 between serum insulin and C20:4/C18:2 ratio. In contrast, serum zinc concentration was positively associated with levels of *n*-3 PUFA ($r = 0.39$), P/S ratio ($r = 0.46$), and C22:5/C18:3 ratio ($r = 0.22$) and negatively correlated with C20:4/C18:2 ratio ($r = -0.13$). ISI was positively associated with levels of *n*-3 PUFA ($r = 0.23$), P/S ratio ($r = 0.20$), and C22:6/C20:5 ratio ($r = 0.40$) and negatively associated with C20:5/C18:2 ratio ($r = -0.24$). Age, TC, and HDL cholesterol were positively associated with levels of *n*-3 PUFA and P/S ratio. TG and TC were negatively associated with C22:6/C20:5 ratio while positively associated with C20:4/C18:2 ratio.

Impact Factors of Fasting FFA Composition by Stratified Analysis

Fasting Serum FFA Composition of NH Subjects and EH Patients Divided by Age Group

EH is related to changes in fasting serum FFA composition in participants ≤ 45 years old: EH patients ≤ 45 years old had significantly lower levels of PUFA, especially *n*-3 fatty acids, as well as P/S ratios, compared with corresponding NH sub-

Table 4. Essential Hypertensive Patients vs. Nonhypertensive Subjects: Effect of Aging (Mean±SD)

Fatty acids ($\mu\text{mol L}^{-1}$)	≤ 40 years		41 to 45 years		≥ 46 years	
	EH patients ($n=30$)	NH subjects ($n=35$)	EH patients ($n=39$)	NH subjects ($n=61$)	EH patients ($n=40$)	NH subjects ($n=27$)
C20:5n3	9.31±5.55	11.5±10.7	10.9±6.8	11.1±7.2	12.2±8.6	10.5±7.1
C22:6n3	56.9±38.9***	90.2±20.0	57.8±39.0 ^{†††}	95.2±32.6	77.2±37.9 [#]	97.0±40.6
C18:3n3	21.7±7.7*	18.0±8.1	22.2±7.1 ^{†††}	16.9±7.3	20.0±6.9	17.8±7.4
C20:4n6	38.3±27.3	30.8±21.7 [#]	32.8±30.8	26.1±21.3	32.5±25.8	45.0±40.9
C18:2n6	167.9±73.5	195.9±71.1	172.3±67.9	189.4±71.7	187.6±54.2	197.7±76.4
C20:5/C18:3	0.49±0.42	0.84±1.09	0.53±0.36	0.88±1.36	0.69±0.53	0.63±0.49
C22:6/C20:5	9.45±10.28	13.8±11.9	8.55±12.7 [†]	13.9±12.2	9.84±8.63 [#]	16.5±18.5
C20:4/C18:2	0.28±0.24	0.19±0.18	0.20±0.15	0.16±0.17	0.25±0.43	0.24±0.20
SFA	221.7±84.7 ^b	194.4±60.7	218.2±73.9 ^{†,b}	186.3±49.1	182.7±47.9	192.7±43.0
MUFA	191.6±96.0	166.0±62.5	186.4±80.2	163.9±63.8	171.3±53.6	169.1±66.9
PUFA	293.5±91.5*	345.4±85.7	293.0±100.0 [†]	337.9±82.6	329.3±100.0	368.0±106.6
n-3 PUFA	87.3±34.9*** ^b	118.8±25.3	88.7±39.8 ^{†††,b}	122.4±34.2	109.1±40.7	125.3±41.2
n-6 PUFA	206.2±75.7	226.7±71.9	204.2±80.4	215.5±72.6	220.1±61.5	242.7±97.0
P/S ratio	1.41±0.49*** [§]	1.87±0.51	1.39±0.43 ^{†††,§}	1.85±0.38	1.88±0.58	1.94±0.51

* $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared with NH subjects (≤ 40 years); [†] $p<0.05$, ^{††} $p<0.01$, ^{†††} $p<0.001$ compared with NH subjects (40–45 years); [#] $p<0.05$ compared with NH subjects (≥ 46 years); ^b $p<0.05$, [§] $p<0.001$ compared with EH patients (≥ 46 years). EH, essential hypertensive; NH, nonhypertensive; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; P/S ratio, PUFA/SFA ratio.

Table 5. Gender Comparison of Serum FFA Composition in Essential Hypertensive Patients and Nonhypertensive Subjects (Mean±SD)

Fatty acids ($\mu\text{mol L}^{-1}$)	Men		Women	
	EH patients ($n=75$)	NH subjects ($n=71$)	EH patients ($n=34$)	NH subjects ($n=52$)
C20:5n3	10.6±7.2	11.1±9.5	11.9±7.6	10.8±6.2
C22:6n3	56.6±36.7*** ^{††}	90.9±21.6	83.0±39.1	98.7±41.0
C18:3n3	20.5±7.5*	16.8±7.3	22.7±6.2	18.5±7.9
C20:4n6	34.0±28.6	31.5±25.8	34.9±26.4	31.7±30.4
C18:2n6	167.0±63.8	183.7±69.9	195.2±64.4	207.9±73.1
C20:5/C18:3	0.60±0.48	0.76±0.85	0.57±0.43	0.86±1.45
C22:6/C20:5	7.96±8.81**	14.5±13.1	11.7±13.2	14.6±14.7
C20:4/C18:2	0.26±0.33	0.20±0.19	0.20±0.20	0.17±0.16
SFA	209.5±74.6	190.7±54.5	199.1±61.9	189.9±48.2
MUFA	179.0±80.3	158.0±65.0	187.9±69.0	177.4±60.6
PUFA	293.6±105.7* [†]	333.5±83.9	344.2±89.7	366.6±93.7
n-3 PUFA	87.0±35.5*** ^{†††}	118.2±24.2	115.0±41.9	127.1±42.7
n-6 PUFA	201.1±73.0	215.2±72.3	229.1±68.0	239.6±84.7
P/S ratio	1.46±0.49*** ^{†††}	1.81±0.43	1.82±0.58	1.98±0.46

* $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared with NH subjects in men; [†] $p<0.05$, ^{††} $p<0.01$, ^{†††} $p<0.001$ compared with EH patients in women; [#] $p<0.05$ compared with NH subjects in women. EH, essential hypertensive; NH, nonhypertensive; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; P/S ratio, PUFA/SFA ratio.

jects. However, fasting serum FFA composition was similar between EH patients and NH subjects ≥ 46 years old, except for C22:6/C20:5 ratio ($n-3$ $\Delta 6$ -desaturase activity index). These results indicated that the degree of the changes of fasting serum FFA composition in EH patients decreased with age (shown in Table 4).

Fasting Serum FFA Composition of NH Subjects and EH Patients Divided by Sex

Table 5 shows that male EH patients had significantly lower levels of PUFA, especially $n-3$ fatty acids, P/S ratios, and C22:6/C20:5 ratios ($\Delta 6$ -desaturase activity index), compared with NH subjects in men. However, fasting FFA composition

Table 6. Essential Hypertensive Patients vs. Nonhypertensive Subjects: Effect of Upper Body Obesity (Mean±SD)

Fatty acids ($\mu\text{mol L}^{-1}$)	WHR $\geq$ 0.9		WHR<0.9	
	EH patients (n=38)	NH subjects (n=24)	EH patients (n=71)	NH subjects (n=99)
C20:5n3	10.9±7.22	11.1±7.99	10.5±8.22	10.0±8.41
C22:6n3	55.4±35.9***,†	94.0±34.5	72.1±40.2###	94.0±31.1
C18:3n3	20.9±7.23**	15.2±6.38	21.2±7.18#	18.0±7.79
C20:4n6	37.3±34.1	30.9±22.4	36.5±25.8	32.3±32.9
C18:2n6	175.8±77.3	185.0±78.4	173.4±56.6#	195.9±70.6
C20:5/C18:3	0.66±0.52	0.82±0.55	0.56±0.46	0.80±1.23
C22:6/C20:5	6.16±6.71††	9.15±5.20#	11.3±12.0#	15.6±14.6
C20:4/C18:2	0.28±0.40	0.19±0.16	0.24±0.20	0.18±0.20
SFA	232.3±86.0*,†††	200.0±59.0	190.2±53.5	187.5±49.2
MUFA	201.4±93.9††	165.0±70.2	170.5±62.3	165.1±61.2
PUFA	307.3±106.7	333.7±93.3	310.2±90.4#	347.7±87.8
n-3	85.9±34.7***,††	120.3±40.4	103.8±41.5##	121.9±32.2
n-6	221.5±88.4	213.4±77.4	206.4±62.2	225.8±78.2
P/S ratio	1.40±0.50*,††	1.72±0.43	1.70±0.56#	1.91±0.44

* $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared with NH subjects (WHR $\geq$ 0.9); † $p<0.05$, †† $p<0.01$, ††† $p<0.001$ compared with EH patients (WHR<0.9); # $p<0.05$, ## $p<0.01$, ### $p<0.001$ compared with NH subjects (WHR<0.9). EH, essential hypertensive; NH, nonhypertensive; WHR, waist/hip circumference ratio; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; P/S ratio, PUFA/SFA ratio.

Table 7. Linear Regression Models in All Subjects

Measurements	Normalized coefficients	<i>p</i>
Dependent variable: n-3 PUFA		
Waist/hip ratio	-0.328	0.000
Age	0.170	0.007
Diastolic blood pressure	-0.163	0.012
Dependent variable: P/S ratio		
Waist/hip ratio	-0.354	0.000
Age	0.254	0.006
Diastolic blood pressure	-0.134	0.038
Dependent variable: C22:6/C20:5		
Insulin sensitivity index	0.292	0.000
Dependent variable: C20:5/C18:3		
Insulin sensitivity index	0.139	0.047
Dependent variable: C20:4/C18:2		
Insulin sensitivity index	0.153	0.025
Diastolic blood pressure	0.149	0.026
Fish intake	0.141	0.039

PUFA, polyunsaturated fatty acid.

was similar between EH patients and NH subjects in women (Table 4). The results indicated that the changes in fasting FFA composition in EH patients differed according to sex, as they appeared in men only.

Fasting Serum FFA Composition of NH Subjects and EH Patients Divided by WHR Group

Table 6 shows that NH subjects (WHR $\geq$ 0.9) had lower

C22:6/C20:5 ratios (Δ 6-desaturase activity index) compared with NH subjects (WHR<0.9), and EH patients (WHR $\geq$ 0.9) had lower levels of n-3 fatty acids, P/S ratios and C22:6/C20:5 ratios (Δ 6-desaturase activity index) but higher levels of SFA and MUFA compared with EH patients (WHR<0.9). In addition, Table 5 shows that EH patients (WHR<0.9) had lower levels of n-3 fatty acids, P/S ratios and C22:6/C20:5 ratios (Δ 6-desaturase activity index) compared with NH subjects (WHR<0.9); EH patients (WHR $\geq$ 0.9) had lower levels of n-3 fatty acids, P/S ratios and C22:6/C20:5 ratios (Δ 6-desaturase activity index) but higher levels of SFA compared with NH subjects (WHR $\geq$ 0.9). These results indicated that, in addition to hypertension itself, upper body obesity is also associated with changes in fasting serum FFA composition, and that the two had synergistic effects on fasting serum FFA composition.

We also divided the population by BMI (BMI $\geq$ 23 and <23). In contrast, there were no statistically significant differences in the fasting FFA composition between EH patients with BMI $\geq$ 23 and those with BMI<23 or between NH subjects with BMI $\geq$ 23 and with BMI<23, although there were statistically significant differences between patients with essential hypertension and those without hypertension (data not shown).

Impact Factors of Fasting Serum FFA Composition in EH Patients

Table 7 shows the linear stepwise regression models in all the subjects. The table is divided into five parts for the five dependent variables: n-3 PUFA, P/S ratio, C22:6/C20:5,

Fig. 1. Insulin sensitivity index is positively correlated with serum C22:6/C20:5 (*n*-3 Δ 6-desaturase activity index) while negatively correlated with C20:4/C18:2 (*n*-6 Δ 5- and Δ 6-desaturase activity index) in 231 subjects. ISI, insulin sensitivity index: $1/[\text{insulin (mIU L}^{-1}) \times \text{FBG (mmol L}^{-1})]$.

C20:5/C18:3, and C20:4/C18:2. For the first two parts, the three lines show the results of the base model with the three independent predictors—WHR, age, and diastolic BP—which show significant association with the dependent variable. All other variables were forced into the model. The next three parts show that an independent predictor, ISI, is associated with the dependent variables. The last part also shows that independent predictors, diastolic BP and fish intake, are associated with the dependent variables. Therefore, the above results showed that WHR, insulin sensitivity, eating habit (such as fish intake) and age affect fasting serum FFA composition.

Impact Factors of Desaturase Activity in EH Patients

The ISI was independently positively associated with C22:6/C20:5 (*n*-3 Δ 6-desaturase activity index) and C20:5/C18:3 (*n*-3 Δ 6-desaturase activity index), while negatively associated with C20:4/C18:2 (*n*-6 Δ 6-desaturase activity index, Fig. 1). These results indicated that insulin has effects on fasting serum FFA composition by regulating desaturase activity. Hypertensive patients had lower ISI than NH subjects, so insulin resistance may be a factor in the decreased *n*-3 Δ 6-desaturase activity in EH patients.

Correlation analysis showed that WHR was negatively associated with C22:6/C20:5 (*n*-3 Δ 6-desaturase activity index) and positively associated with C20:4/C18:2 (*n*-6 Δ 6-desaturase activity index), which indicated that upper body obesity is associated with decreased *n*-3 Δ 6-desaturase activity in EH patients.

Linear stepwise regression models showed that serum zinc was independently positively associated with C20:5/C18:3 (*n*-3 Δ 6-desaturase activity index) (Table 3), and EH patients have lower serum zinc levels than NH subjects, so lower lev-

els of serum zinc may be a factor in the decreased *n*-3 Δ 6-desaturase activity in EH patients.

Linear stepwise regression models showed that C20:4/C18:2 (*n*-6 Δ 6-desaturase activity index) was independently associated with fish intake, which indicated that eating habits affect desaturase activity.

Therefore, the above results showed that upper body obesity, insulin resistance, serum zinc deficiency, and eating habits (such as fish intake) have effects on desaturase activity in EH patients.

Discussion

In this study, we measured fatty acid composition of serum in EH patients and NH subjects and correlated them with metabolic parameters. In men, EH patients had lower C22:6/C20:5, PUFA, *n*-3 PUFA, and P/S ratios compared with male NH subjects. There were no differences in women. Fasting serum FFA composition was significantly related to many variables including WHR, ISI, and serum zinc.

Changes in Fasting Serum FFA Composition in EH Patients

Fasting serum FFA composition is a biochemical marker reflecting fatty acid metabolism *in vivo* in the morning, and is in relatively steady state *in vivo*. Our results for the changes in fasting FFA composition in EH patients were consistent with those of several other reports. Clinical trials showed that *n*-3 PUFA supplementation can lower BP in EH patients (3, 4). In the Atherosclerosis Risk in the Communities (ARIC) study of middle-aged subjects, Zheng *et al.* found that EH patients had lower levels of PUFA and P/S ratios but higher concentrations of AA (20:4*n*6) in fasting plasma cholesterol esters, while they did not find significant changes in *n*-3 PUFA con-

centration (2). These differences may be associated with differences in the subjects' eating habits (such as intake of fish rich in *n*-3 PUFAs or fish oil); another possible explanation is that fasting serum FFA composition may be a better biochemical marker of fatty acid metabolism *in vivo* than fatty acid composition in fasting plasma cholesterol esters (7).

Impact Factors of Fasting Serum FFA Composition in EH Patients

FFA composition in serum is related to the composition of dietary fat and to the metabolic changes of fatty acids, *e.g.*, to *de novo* lipogenesis, β -oxidation and conversion accompanying the oxidative stress. Linoleic acid (18:2*n*6) and α -linolenic acid (18:3*n*3) are essential fatty acids because they cannot be synthesized *in vivo* and because they exclusively reflect dietary intake, whereas the fatty acids C20 and C22 derive from the diet as well as from essential fatty acid elongation and desaturation.

Insulin regulates fatty acid metabolism through three pathways. First, insulin plays an important role in the control of FFA release. Insulin is a major regulator of lipolysis. It inhibits lipolysis and FFA efflux into the circulation. The cellular mechanisms involved in the inhibition of lipolysis by insulin are to decrease intracellular cAMP levels through cyclic nucleotide phosphodiesterase-3B (PDE-3B) stimulation, which promotes cAMP hydrolysis, and then inhibition of lipolysis (18). Second, insulin stimulates fat storage (*i.e.*, increases the rate of resynthesis of triacylglycerol from non-esterified fatty acids; the reesterification effect). As a result, insulin significantly decreases the concentration of FFA in the circulation. Lastly, insulin regulates the activities of Δ 6- and Δ 5-desaturase activity in hepatocellular microsome, which affect the biosynthesis of fatty acids C20 and C22 from linoleic acid (18:2*n*6) and α -linolenic acid (18:3*n*3) and then affects FFA composition. The results of this study support the last pathway.

This study found that EH patients with upper body obesity (WHR $\geq$ 0.9) had higher levels of SFA and MUFA while lower levels of *n*-3 PUFA, P/S ratio, and C22:6/C20:5 (Δ 6-desaturase activity index), compared with lean hypertensive patients (WHR<0.9). In addition, we found that levels of *n*-3 PUFA and the P/S ratio are negatively associated with WHR. These results indicated that upper obesity (WHR $\geq$ 0.9) is associated with changes in fasting serum FFA composition. In fasting condition, circulating SFA and MUFA mainly derive from the lipolysis of abdominal adipose tissue, and PUFA mainly derives from the bioconversion of linoleic (C18:2*n*6) and α -linolenic (C18:3*n*3) acids through desaturation and elongation steps in hepatocytes when dietary consumption is controlled. Adipose tissue is heterogeneous with respect to lipolysis *in vivo*. Upper body nonvisceral fat or abdominal subcutaneous fat contributes the majority of circulating FFAs in lean and obese humans who are fasting, and increasing amounts of visceral fat probably result in greater

hepatic FFA delivery and insulin resistance (19); the latter decreased hepatic Δ 6- and Δ 5-desaturase activity and resulted in decreased production of PUFA, which altered fasting FFA composition.

Consistent with several other reports (20, 21), this study showed that dietary consumption, such as fish intake, had effects on fasting FFA composition. This offers evidence for improving abnormal FFA composition through dietary intervention in EH patients.

In addition, this study showed that fasting FFA composition in all subjects had age and sex differences. The changes of fasting FFA composition in EH patients decreased with age, compared with NH subjects of the same age. Significant changes in fasting FFA composition existed in men with hypertension but not in women with hypertension, compared with nonhypertensive men or women. A potential mechanism based on the age and sex differences of fasting FFA composition is not established yet.

Impact Factors of Desaturase Activity in EH Patients

This study showed that male EH patients had decreased bioconversion of α -linolenic acid (18:3*n*3) to EPA and DHA due to decreased *n*-3 desaturase activity, compared with male NH subjects. Besides insulin resistance, upper body obesity and unhealthy eating habits, namely zinc deficiency, may also be a factor in the decreased desaturase activity in EH patients.

Insulin is an exclusive hormone that stimulates Δ 6- and Δ 5-desaturase (8) and helps to restore levels of long-chain PUFAs to normalcy. Supporting this opinion, this study showed that the ISI was positively associated with C20:5/C18:3 (*n*-3 Δ 6- and Δ 5-desaturase activity index) and C22:6/C20:5 (*n*-3 Δ 6-desaturase activity index). However, this study also showed that ISI was negatively associated with C20:4/C18:2 (*n*-6 Δ 6- and Δ 5-desaturase activity index), which indicated that insulin enhances the affinities of Δ 6-desaturase only for *n*-3 fatty acids and then their affinities for *n*-6 fatty acids decreased. Another possible explanation was that two Δ 6-desaturase isozymes may exist in human hepatocytes (22) and that insulin plays opposite roles in them; that is to say, insulin enhances the activity of *n*-3 Δ 6-desaturase isozyme while inhibiting the activity of *n*-6 Δ 6-desaturase isozyme. Therefore, insulin resistance resulted in the increased production of AA (C20:4*n*6) and its metabolites and then contributed to hypertension development and progression, which may be a linking mechanism between insulin resistance and hypertension in upper body obese subjects.

Our study showed that serum zinc levels paralleled C20:5/C18:3 ratios (*n*-3 Δ 5-, Δ 6-desaturase activity index), as they were lower in EH patients than NH subjects. Zinc ions were found to be constitutive cofactors of desaturase activity in human (23). Together, these results indicated that zinc deficiency may be an impact factor in decreased desaturase activity in EH patients.

Most Chinese people prefer for vegetative food and plant-seed oils, so consumption of linoleic acid (18:2n6) and α -linolenic acid (18:3n3) derived from them is greater than in Western countries, whereas *n*-3 long-chain PUFA, EPA (C20:5n3), and DHA (C22:6n3), derived from oily fish, is lower than in Western countries. The total percent conversions of deuterated C18:3n3 to *n*-3 FAs and deuterated C18:2n6 to *n*-6 FAs were 11.0–18.5% and 1.0–2.2%, respectively, in the physiological state (24). Considering that fish stocks are declining and that the oils derived from fish are sometimes contaminated with a range of pollutants, such as heavy metals, could α -linolenic acid (18:3n3) be used as a substitute for EPA (C20:5n3) and DHA (C22:6n3) to lower BP and reduce the risk of cardiovascular disease in EH patients? Some clinical trials showed that plant-based α -linolenic acid lowered systolic BP and the prevalence of hypertension in normotensive subjects (5), but had no effects on BP in EH patients (6, 25). We presume that, to have such an effect would require the normal conversion of α -linolenic acid (18:3n3) to longer-chain *n*-3 PUFA catalyzed by *n*-3 desaturase in EH patients. Therefore, this study supports the need to find preliminary evidence for improving α -linolenic acid (18:3n3) metabolism by exploring impact factors of decreased *n*-3 desaturase activity in EH patients.

In conclusion, our study showed the marked alteration in fasting serum FFA composition in EH patients; this alteration was affected by upper body obesity, insulin resistance, zinc deficiency, and dietary fat, and differed according to age and sex.

Limitations

This study has a few limitations because of its cross-sectional design. The results showed not causal association but correlativity. Future studies should clarify impact factors of *n*-3 desaturase activity in EH patients, and investigate the changes in blood levels and in cardiovascular disease risk in EH patients, followed by a 5-year thorough intervention study for improving α -linolenic acid (18:3n3) metabolism.

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