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RESEARCH ARTICLE

Increased plasma n-3 polyunsaturated fatty acid is associated with improved insulin sensitivity in type 2 diabetes in China

Tao Huang[1,2], Mark L. Wahlqvist[1,2,3], Tongcheng Xu[1,2], Amei Xu[1,2], Aizhen Zhang[4] and Duo Li[1,2,5]

[1] Department of Food Science and Nutrition, Zhejiang University, Hangzhou, P. R. China
[2] APCNS Centre of Nutrition and Food Safety, Hangzhou, P. R. China
[3] National Health Research Institutes, Zhunan, Taiwan
[4] School of Medicine, Zhejiang University, Hangzhou, P. R. China
[5] IAES, Zhejiang University, Hangzhou, P. R. China

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Increased tissue n-3 polyunsaturated fatty acid (PUFA) is associated with improved insulin sensitivity in type 2 diabetes. However, this relationship among Chinese is not clear. To investigate the relationship between plasma phospholipids (PL) fatty acid composition and insulin resistance (IR) in type 2 diabetes mellitus, 186 type 2 diabetes and 180 healthy subjects were studied in this case-control study. In the sex, age and BMI controlled partial correlation, homeostasis model assessment (HOMA)-IR and blood glucose was significantly negatively correlated with plasma PL n-3 PUFA, 20:5n-3 and ratio of n-3:n-6 ($p<0.01$), and positively correlated with n-6 PUFA ($p<0.001$) and saturated fatty acid ($p<0.05$) in the diabetes patients. PL 22:6n-3 was also significantly negatively correlated with HOMA-IR ($p<0.01$), but not with blood glucose. Fasting insulin was significantly negatively correlated with plasma PL n-3 PUFA, 20:5n-3, 22:6n-3 and ratio of n-3:n-6 ($p<0.01$). The 18:3n-3 was not associated with HOMA-IR and fasting insulin. The results suggested that increased plasma PL n-3 PUFA, 20:5n-3, 22:6n-3 and ratio of n-3:n-6 PUFA was associated with decreased HOMA-IR in type 2 diabetes. Increased plasma PL n-3 PUFA improves insulin sensitivity in type 2 diabetes.

Keywords:
Blood glucose / Insulin resistance / Insulin sensitivity / n-3 polyunsaturated fatty acid / Type 2 diabetes

1 Introduction

n-3 polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3), play a key role in the prevention and progression of human diseases *via* different mechanisms, such as modulating membrane lipid composition and affecting metabolic and signal-transduction pathways, reduction in serum/plasma triacylglycerol levels, anti-thrombotic effects, anti-inflammatory effects and secondary prevention of cardiovascular disease [1]. However, few studies have examined the role of n-3 PUFA in the development of type 2 diabetes mellitus [2, 3].

Obesity and lack of physical activity are known to be strongly associated with type 2 diabetes [4], but evidence also suggests that dietary factors play a role in the development of this disease. In 12 years of follow-up in the cohort of male health professionals, significant associations were observed

Correspondence: Professor Duo Li, Department of Food Science and Nutrition, Zhejiang University, 268 Kaixuan Road, Hangzhou 310029, P. R. China
E-mail: duoli@zju.edu.cn
Fax: +86-571-86971024

Abbreviations: GLU, fasting glucose; **HOMA,** homeostasis model assessment; **IR,** insulin resistance; **MUFA,** monounsaturated fatty acid; **PL,** phospholipids; **PUFA,** polyunsaturated fatty acid; **SFA,** saturated fatty acid

between the types of fat and risk of type 2 diabetes [5]. A higher proportion of unsaturated fat may improve insulin signaling by increasing membrane fluidity [6]. Consistent with this mechanism, the proportion of unsaturated fat in skeletal muscle membrane phospholipids (PL) was positively associated with insulin sensitivity in humans [7]. Studies have also shown that changes in fatty acid composition play a role in modulating insulin action in peripheral tissues [8], but this has never been examined in detail.

An imbalance in dietary n-6 and n-3 PUFA is a contributory factor to insulin resistance, and related blood lipid abnormalities of the metabolic syndrome [9]. Storlien *et al*. [10] showed high skeletal muscle membrane n-6 PUFA to n-3 PUFA ratio in the skeletal muscle membrane to be adversely related to insulin sensitivity, which supports the hypothesis that a high ratio of n-6:n-3 dietary PUFA contributes to the higher prevalence of insulin resistance. To further support this, animal studies reported that feeding n-3 PUFA resulted in improvements in insulin sensitivity [11], whereas a human study showed that feeding n-6 PUFA led to deterioration in insulin sensitivity [12].

While these effects of fatty acids have not been seen universally, the prospective studies of type 2 diabetes reported no association between total dietary fat [13, 14] or specific types of fatty acids and risk of diabetes [14]. Previous studies have also found no relationship between 20:5n-3 in serum [15, 16] and insulin resistance or diabetes risk. Interestingly, Lovejoy *et al*. reported a positive association between serum 20:5n-3 and insulin levels in men and women with a range of glucose tolerance [17]. Storlien *et al*. reported that high-fat diets enriched in 18:3n-3 completely prevented insulin resistance induced by a saturated-fat diet [18]. However, the moderate amounts of dietary 18:3n-3 administered did not significantly affect blood concentrations of glucose, insulin in healthy people [19]. Therefore, the effect of 18:3n-3 on insulin resistance is still controversial [18–20].

Considering the relationship between dietary fatty acid composition and biomarkers for insulin resistance among Chinese is not clear. We conducted this study to investigate the correlation of plasma PL fatty acid composition (as a biomarker of dietary intake of fatty acids) with 186 diabetes patients and 180 healthy subjects in Zhejiang province, China.

2 Materials and methods

2.1 Subjects

The study protocol was approved by the Ethics Committee, College of Biosystem Engineering and Food Science, Zhejiang University, China, and all subjects were volunteers who gave their written consent prior to participation in the study.

After careful screening, 186 type 2 diabetes mellitus patients (103 males, 83 females), aged 40 to 70 (59.75 ± 8.38 years), whose blood sugar levels were stabilized between 7.0 and 10.0 mmol/L, were recruited from outpatient in the 2nd affiliated Hospital, Zhejiang University, Hangzhou, China. Subjects with type 1 diabetes mellitus, patients with a history of cardiovascular disease and/or arteriosclerotic disease, cerebrovascular disease, serious hepatic disease and/or renal disease and hematological disorders were excluded from the study. All the subjects were taking antidiabetic medications with 31% of the subjects taking glipizide, 24% of the subjects taking acarbose, 22% of the subjects using insulin or protamine zinc insulin and the remaining 23% taking other antidiabetic medications such as metformin, gliquidon and repaglinide.

Age- and sex-matched healthy control subjects were recruited through a health check program during the period of March 2006 through October 2006 in the 2nd affiliated Hospital, Zhejiang University, Hangzhou, China. After careful screening for hypertension, renal disease, hyperlipemia, hematological disorders, diabetes, family history of cardiovascular disease, excessive alcohol intake and drug use, 180 subjects (98 males, 82 females, aged 60.08 ± 9.79 year) were accepted.

2.2 Blood collection

Subjects attended the Zhejiang Hospital in the morning following an overnight fast. Subjects were allowed to sit relaxed for 10 min; the subject's weight, height, waist to hip ratio and blood pressure were measured. Then venous blood was taken in plain and EDTA vacuum tubes with 21-gauge needles. After blood collection, plasma samples were prepared quickly after blood was drawn, aliquoted into separate tubes and stored at -20°C until analysis.

2.3 Laboratory measurements

Plasma lipids were determined on an autoanalyzer (Olympus AU2700, Tokyo, Japan), *via* commercially available kits (Olympus). Fasting serum insulin and glucose were measured by standard methods as previously described [21]. Homeostasis model assessment (HOMA) insulin resistance (IR) (HOMA-IR) was calculated using the mathematical approximations described by Matthews *et al*. [22]. Specically, HOMA-IR = (fasting insulin (FINS) × fasting glucose)/22.5. Total lipid content of plasma was extracted with solvents, the PL fraction was separated by TLC and the fatty acid methyl esters were prepared and separated by gas-liquid chromatography as described previously [23].

2.4 Statistical analysis

The data analyses were performed using an SPSS version 12 (SPSS, Chicago, IL, USA) software program. All the data

were checked for normal distribution. Difference between the two groups in each parameter was analyzed using ANOVA. The relationship between plasma PL fatty acid composition and HOMA-IR FINS and blood glucose was determined by partial correlation analysis, controlled for sex, age and BMI. Data was reported as mean ± SD. The level of significance was $p < 0.05$.

3 Results

Compared with the healthy control group, the diabetes patient group showed significantly higher plasma concentrations of total triacylglycerol ($p < 0.01$) and total cholesterol ($p < 0.05$); however, there were no significant differences between the two groups in plasma concentrations of high-density lipoprotein cholesterol or low-density lipoprotein cholesterol (Table 1). Compared with the healthy control group, the plasma PL 20:5n-3, 22:6n-3, n-3:n-6, n-3 PUFA in the diabetes patient group were significantly lower, while the plasma PL monounsaturated fatty acid (MUFA) and n-6 PUFA in the diabetes patient group were significantly higher (Table 2). There was no significant difference in plasma PL fatty acid compositions and other parameters insofar as medication usage in these diabetes patients was concerned.

In sex, age and BMI controlled partial correlation, HOMA-IR and blood glucose concentration were significantly negatively correlated with plasma PL n-3 PUFA, 20:5n-3, and ratio of n-3:n-6 ($p < 0.01$), and positively correlated with n-6 PUFA ($p < 0.001$) and saturated fatty acid (SFA) ($p < 0.05$) (Figs. 1–3) in the diabetes patient group. PL 22:6n-3 was also significantly negatively correlated with HOMA-IR ($r = -0.415$, $p = 0.001$) (Table 3), but not with blood glucose in diabetes patient group. FINS was significantly negatively correlated with plasma PL n-3 PUFA ($r = -0.508$, $p = 0.006$), 20:5n-3 ($r = -0.378$, $p = 0.001$), 22:6n-3 ($r = -0.502$, $p = 0.008$) and ratio of n-3:n-6 ($r = -0.492$, $p < 0.001$) in diabetes patient group (Table 3). There was no significant association between 18:3n-3, MUFA or HOMA-IR and fasting serum glucose in the diabetes patient group.

In the control subjects, total n-3 PUFA was significantly negatively correlated with HOMA-IR ($r = -0.198$, $p = 0.040$) and FINS concentration ($r = -0.197$, $p = 0.041$) and 22:6n-3 was significantly negatively correlated with HOMA-IR ($r = -0.233$, $p = 0.015$) and FINS concentration ($r = -0.221$, $p = 0.021$) after controlled sex, age (Table 4). We did not find other fatty acids to be correlated with FINS, HOMA-IR or fast glucose in control group.

4 Discussions

Type 2 diabetes results from the association of a defect in insulin secretion from β-cells and IR in muscle, adipose tissue and the liver [8]. IR, which is a reduced efficacy of insulin *in vivo* to inhibit hepatic glucose production and to stimulate glucose utilization in skeletal muscle and adipose tissue, is a common characteristic of type 2 diabetes, obesity and the metabolic syndrome [24]. However, the specific mechanisms of IR are not fully known.

Evidence has shown that the fatty acid composition of plasma and membrane lipids plays a major role in insulin action and blood glucose regulation [25]. Because it is difficult to estimate dietary fatty acid pattern intake with traditional dietary assessment methods, biomarkers can improve the accuracy measure of the assessment of long term intake by dietary questionnaires [26]. In recent years, tissue fatty acids (serum/plasma, adipose, platelet and erythrocytes) have been demonstrated to provide suitable biomarkers for long-term fatty acid pattern intake [26–28]. In the present study we have used plasma PL fatty acid patterns as a biomarker to examine the association between plasma PL fatty acids and type 2 diabetes.

The present study demonstrates that total n-3 PUFA, 20:5n-3 and 22:6n-3 is significantly and negatively correlated with HOMA-IR and serum glucose, while n-6 PUFA is

Table 1. Plasma parameters of the type 2 diabetes and the healthy control groups

Plasma parameters	Healthy control group ($n = 180$)	Diabetes patient group ($n = 186$)	*p*-Value
Age (year)	60.1 ± 9.8	59.8 ± 8.4	0.102
BMI (kg/m^2)	23.2 ± 2.9	22.8 ± 3.2	0.454
SBP (mm HG)	131.0 ± 23.2	136.7 ± 20.8	0.120
DBP (mm HG)	80.1 ± 11.5	79.9 ± 11.7	0.915
TC (mmol/L)	4.6 ± 0.5	4.9 ± 0.9	0.020
TG (mmol/L)	1.2 ± 0.4	1.5 ± 0.8	0.002
HDL-C (mmol/L)	1.4 ± 0.2	1.4 ± 0.4	0.781
LDL-C (mmol/L)	2.1 ± 0.3	2.1 ± 0.7	0.516
GLU (mmol/L)	4.8 ± 0.5	8.2 ± 2.7	< 0.001
FINS (μU/mL)	5.8 ± 0.8	9.9 ± 1.9	< 0.001
HOMA-IR	1.2 ± 0.2	3.1 ± 1.1	< 0.001

All the data were presented as Mean ± SD. SB, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, total triaclyglycerol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Table 2. Plasma PL fatty acid compositions of the type 2 diabetes group and healthy control group

PL fatty acid (%)	Healthy control group ($n = 180$)	Diabetes patient group ($n = 186$)	p-Value
14:0	0.27±0.1	0.25±0.1	0.163
16:0	33.5±7.4	26.5±3.1	0.000
16:n-7	0.26±0.2	0.36±0.2	0.000
18:0	20.3±5.3	13.9±1.5	0.000
18:1	8.12±2.6	10.8±2.1	0.000
18:2n-6	17.1±5.6	22.3±3.1	0.000
18:3n-3	0.70±0.2	0.36±0.1	0.000
20:0	0.31±0.1	0.30±0.1	0.942
20:n-11	0.42±0.1	0.21±0.1	0.000
20:2n-6	0.25±0.2	0.46±0.1	0.000
20:3n-6	1.79±0.8	2.12±0.7	0.005
20:4n-6	7.12±3.3	9.12±2.0	0.000
20:5n-3	2.12±0.7	0.99±0.5	0.000
22:2n-6	0.17±0.1	0.19±0.1	0.457
23:0	0.64±0.2	0.28±0.1	0.000
22:4n-6	0.21±0.1	0.23±0.1	0.130
22:5n-6	0.18±0.0	0.21±0.1	0.031
22:5n-3	0.58±0.3	0.72±0.3	0.004
22:6n-3	5.80±2.0	2.46±2.2	0.000
Total SFA	55.1±12.6	46.9±2.8	0.000
Total MUFA	8.81±2.8	13.9±2.1	0.000
Total PUFA	39.9±5.4	38.9±3.7	0.136
Total n-6 PUFA	26.9±9.1	34.6±2.8	0.000
Total n-3 PUFA	9.22±1.8	4.52±2.8	0.000
n-3:n-6	0.39±0.1	0.13±0.1	0.000

All the data were presented as Mean±SD.

positively correlated with HOMA-IR. Similar relationships between the proportion of n-3 PUFA and insulin sensitivity have also been reported by others [7, 8]. Insulin levels in mothers were inversely associated with the child's muscle membrane total n-3 PUFA ($r = 0.23$, $p = 0.0016$) and 22:6 n-3 ($r = 0.29$, $p = 0.0006$), but positively associated with the total n-6 PUFA ($r = 0.05$, $p = 0.03$) [29]. An epidemiologic study showed that lower prevalence of type 2 diabetes in Alaskan natives was attributed mainly to diets rich in n-3 PUFA [30]. An 8-wk daily supplementation of 3 g of the n-3 fatty acids rich in 22:6n-3 and 20:5n-3 has improved insulin sensitivity in diabetic patients [31]. We have found that the

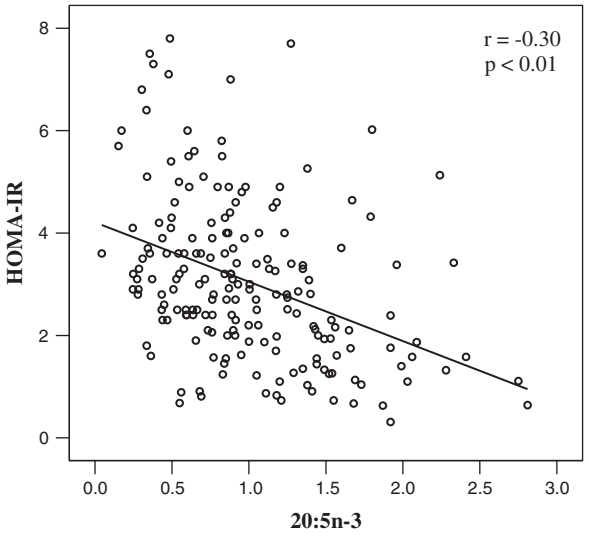

Figure 1. Correlation of HOMA-IR concentration with plasma PL 20:5n-3 composition.

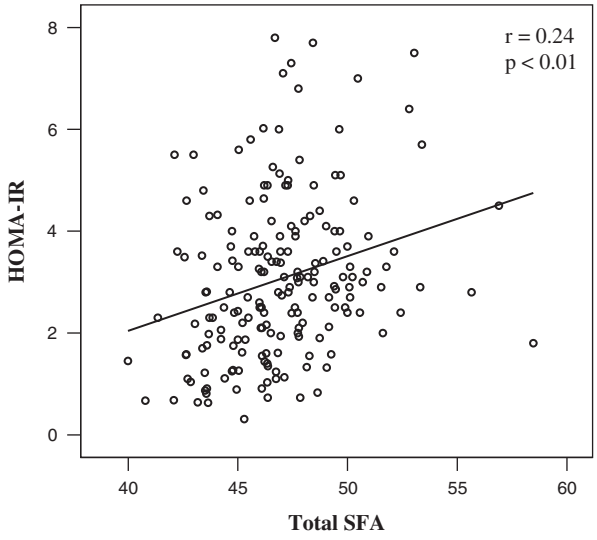

Figure 2. Correlation of HOMA-IR concentration with plasma PL SFA composition.

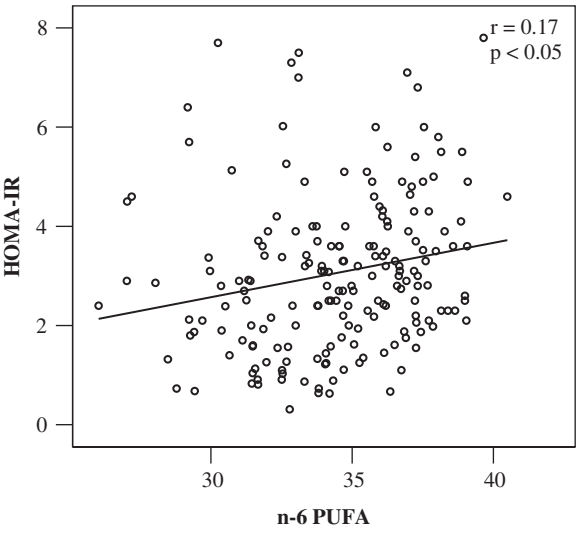

Figure 3. Correlation of HOMA-IR concentration with plasma PL n-6 PUFA composition.

plasma PL 20:5n-3, 22:6n-3, n-3:n-6 and n-3 PUFA in diabetes patients are significantly lower than in healthy controls. In fact, a lower plasma/tissue 22:6n-3 content could be a consequence of IR, because IR changes lipid composition, for the formation of which, the main enzymes (delta5 and 6-desaturases) responsible are activated by insulin [32]. Therefore, insulin-resistant subjects could well have impaired conversion of 18:3n-3 to 22:6n-3. Genetic variants in fatty acid metabolism, dietary intake, the insulin or oral agents used in therapy may also have contributed to the lower concentration of n-3 PUFA in the diabetes patient group.

There are several apparently conflicting studies. For example, Lovejoy reported a positive relationship between log fasting serum insulin and serum 20:5n-3; IR was assessed by HOMA and showed a similar pattern to FINS, since it was signicantly and positively correlated with 20:5n-3 [17]. Furthermore, the US Health Professionals Follow-Up Study showed that n-3 PUFA intake was not associated with the risk of diabetes in more than 42 000 men [5]. In a study of nearly 36, 000 older Iowa women who did not have type 2 diabetes at enrollment, diabetes incidence after 11 years was positively associated with n-3 PUFA consumption [33]. In a randomized double-blind placebo-controlled design with a parallel group sequence, 16 type 2 diabetes patients with hypertriglyceridemia (triacylglycerol, 2.25–5.65 mmol/L) were randomly assigned to either fish oil (2.7 g/day 20: 5n-3 plus 22:6n-3 for 2 months, then 1.7 g/day for 4 more months) ($n = 8$) or placebo (hypoglycemic drugs) ($n = 8$). The amount of n-3 PUFA used in the study was unable to improve insulin sensitivity in the patients [34]. While the reason for these discrepancies are not clear, explanations might be found in differences in subject health status in their habitual dietary intakes especially total fat, PUFA and other fatty acid composition; *inter alia*, there has been considerable interest in *trans* fatty acids and the pathogenesis of diabetes [35], which maybe also contribute to these discrepancies.

The present study showed that total SFA is significantly and positively associated with HOMA-IR. It is consistent with the results reported by several previous studies. Several studies of hyperinsulinemia and hyperglycemia suggested a detrimental effect of SFA [36] and of *trans* fatty acids [16, 37]. Higher dietary SFA intake was associated with hyperinsulinemia; SFA was a significant independent predictor of both fasting and postprandial insulin in middle-aged men [38]. A cross-sectional study has also reported that HOMA-IR is positively associated with SFA and MUFA [39]. A negative association between insulin sensitivity and SFA has also been reported [8]. However, another study reported that total fat, SFA and MUFA intakes are not associated with a higher risk of type 2 diabetes in women, but *trans* fatty acids increase the risk substantially [33]. In the present study, we did not find significant relationship between MUFA and

Table 3. Partial correlations between plasma PL fatty acid compositions and FINS, HOMA-IR, blood glucose in diabetes patient group, controlled for confounding factors

PL fatty acids	Controlled for age, gender and BMI					
	HOMA-IR		FINS		Glucose	
	Std. coeff.	*p*-Value	Std. coeff.	*p*-Value	Std. coeff.	*p*-Value
18:3n-3	−0.023	0.848	−0.047	0.702	−0.094	0.207
20:4n-6	0.005	0.942	−0.002	0.982	0.289	0.000
20:5n-3	−0.301	0.007	−0.378	0.001	−0.301	0.000
22:6n-3	−0.415	0.001	−0.502	0.008	−0.118	0.162
Total SFA	0.237	0.002	0.267	0.000	−0.300	0.000
Total PUFA	−0.216	0.006	−0.291	0.000	0.132	0.115
Total n-3 PUFA	−0.410	0.000	−0.508	0.006	−0.214	0.010
Total n-6 PUFA	0.171	0.020	0.129	0.081	0.315	0.000
n-3:n-6	−0.394	0.000	−0.492	0.000	−0.338	0.000

Std. coeff. = standardized coefficients.

Table 4. Partial correlations between plasma PL fatty acid compositions and FINS, HOMA-IR, blood glucose in control group, controlled for confounding factors

PL fatty acids	Controlled for age, gender and BMI					
	HOMA-IR		FINS		Glucose	
	Std. coeff.	*p*-Value	Std. coeff.	*p*-Value	Std. coeff.	*p*-Value
18:3n-3	0.075	0.443	0.044	0.648	0.111	0.251
20:4n-6	−0.071	0.463	−0.061	0.540	−0.018	0.854
20:5n-3	−0.022	0.822	−0.047	0.629	0.175	0.070
22:6n-3	−0.233	0.015	−0.221	0.021	−0.030	0.758
Total SFA	0.024	0.806	0.039	0.688	−0.031	0.752
Total PUFA	0.046	0.638	0.030	0.755	0.122	0.210
Total n-3 PUFA	−0.198	0.040	−0.197	0.041	0.035	0.719
Total n-6 PUFA	0.102	0.293	0.087	0.372	0.109	0.260
n-3:n-6	−0.086	0.377	−0.082	0.402	−0.046	0.633

Std. coeff. = standardized coefficients.

HOMA-IR. The apparent inconsistencies observed in different populations may be due to lack of adjustment for confounding by other dietary or non-dietary risk factors and population characteristics, such as age, BMI and physical activity [40]. In addition, the genetic variation of some critical genes involved among population could be another reason accounting for the inconsistencies observed in different studies.

The present study showed that the plasma PL ratio of n-3:n-6 PUFA was significantly and negatively correlated with HOMA-IR and blood glucose concentration ($p<0.01$). This result is consistent with a previous study, which showed the ratio of n-6:n-3 PUFA in skeletal muscle membrane to be inversely related to insulin sensitivity [10]. Insulin levels have been positively associated with the ratio of n-6:n-3 PUFA in children's muscle membrane ($r=0.20$, $p=0.007$) [29]. However, one study showed that decreasing the ratio of n-6:n-3 in diets, chiefly by altering the mass of n-3 PUFA, did not influence insulin sensitivity [41]. In an intervention study, 29 males were assigned to either a moderate or high ratio of n-6:n-3 PUFA (9 or 16) diet for 6 wk; the results showed that FINS and HOMA-IR were increased in the high n-6:n-3 ratio PUFA diet [42].

A previous study demonstrated that the small amount of 18:3n-3 (0.3g/100 g diet) from flaxseed oil completely prevented conjugated linoleic acid-induced IR and significantly decreased the fasting plasma glucose concentration in mice [43]. Studies also suggest that 18:3n-3 may be direct and more effective than 22:6n-6 or sh oil in preventing IR [20, 44, 45]. However, we did not find an association between 18:3n-3 and HOMA-IR. A randomized strictly controlled dietary study has suggested that moderate amounts of dietary 18:3n-3, 20:5n-3 or 22:6n-3 administered do not significantly affect blood concentrations of glucose, insulin or HbA1c in 48 healthy normal-weight men and women over a time course of 3 wk [19]. This disparity may reflect the different research models, different populations studied, sample size or genetic polymorphisms like those involved in insulin signal pathway.

The molecular mechanisms, which could account for the relationship between fatty acids and type 2 diabetes, have not been clarified. Previous studies have showed that the fatty acid profile of type 2 diabetes patients is characterized by increased SFA and n-6 PUFA, and decreased n-3 PUFA, which is an indicator of high activities of $\triangle 9$ and $\triangle 6$ desaturases and low activity of $\triangle 5$ desaturase [15, 46]. Therefore, we hypothesized that a defect of desaturase activity alters cell membrane lipid composition, which could in turn alter cell membrane fluidity, the insulin signaling pathway, expression of genes or generate lipid signals and finally interfere with glucose metabolism. Based on the animal study by Taouis [47], the protective effect of n-3 PUFA against HOMA-IR could be explained by the prevention of alterations in insulin signaling: (i) the prevention of the decrease of phosphatidylinositol 3-kinase activity and the depletion of glucose transporter protein GLUT4 in the muscle and the decreased mRNA expression of GLUT4 in adipose tissue [47], (ii) n-3 PUFA inhibits both the activity and mRNA expression of liver glucose-6-phosphatase, which could account for the beneficial effect with respect to the excessive hepatic glucose output induced by a high-fat diet [24, 47].

In conclusion, type 2 diabetes patients in Zhejiang province, China, exhibit a decreased plasma PL n-3 PUFA, and increased SFA and n-6 PUFA compared with healthy control subjects, and increased tissue n-3 PUFA is associated with improved insulin sensitivity in these Chinese patients with type 2 diabetes. Although a number of studies have reported this favorable relationship between n-3 PUFA and type 2 diabetes, it is still controversial as to whether increased intakes of n-3 PUFA are beneficial. The apparent discrepancies between different studies may be partly a result of whether the methodology has been dietary or biomarker or both, what the background dietary

exposure has been (even since conception given the role of n-3PUFA in gene expression) or whether there are relevant genetic polymorphisms involved in the insulin signal pathways with specific responses to the nutritional factors in question or to other lifestyle factors. Further research should involve a nutrigenomics approach to investigate the interactions between n-3 PUFA and evolving insulin insensitivity in the pathogenesis of type 2 diabetes in longitudinal studies.

The authors have declared no conflict of interest.

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