



# Relationship between n-3 and n-6 plasma fatty acid levels and insulin resistance in coronary patients with and without metabolic syndrome<sup>☆</sup>

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## KEYWORDS

Metabolic syndrome;  
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**Abstract** *Background and aims:* Animal studies show that eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are effective for the prevention and treatment of insulin resistance (IR). Data from human studies are contradictory. We sought to determine whether the relationships between plasma n-3 and n-6 polyunsaturated fatty acid (FA) levels and IR differ according to the presence or absence of metabolic syndrome (MS) in a coronary heart disease sample.

*Method and results:* Clinical, metabolic parameters, plasma phospholipid FA profiles and indirect measurement of IR (homeostatic model assessment-HOMA) were measured in 734 subjects, 8 weeks following acute coronary syndrome. FA levels and their correlations with IR were compared in subjects with and without MS. MS patients had higher saturated (16:0, 18:0) and n-6 (18:3n-6, 20:3n-6, 22:4n-6, 22:5n-6) FA levels, and lower EPA and DHA levels. HOMA-IR correlated positively with total saturated ( $r = 0.13$ ,  $P = 0.017$ ) and n-6 ( $r = 0.17$ ,  $P = 0.001$ ) FA levels and negatively with total n-3 FA levels ( $r = -0.13$ ,  $P = 0.012$ ), in MS subjects only. Total n-3 and n-6 FAs and n-6/n-3 ratio were associated with HOMA-IR levels

*Abbreviations:* FA, fatty acid; MS, metabolic syndrome; HOMA-IR, homeostasis model of insulin resistance; PUFA, polyunsaturated fatty acid; CHD, coronary heart disease; AMI, acute myocardial infarction; ATP III, adult treatment panel III.

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in MS subjects independent of total saturated FA levels, age, sex, sedentary behaviour, smoking, waist circumference, triglycerides, HDL-cholesterol, and systolic blood pressure.

**Conclusions:** Relationships between polyunsaturated FA type and IR vary according to the presence or absence of MS. N-3 FAs including EPA and DHA are associated with lower HOMA-IR, while the opposite is true for n-6 FAs. Prospective studies are required to address the potential effects of intermediate dose EPA and DHA on glucose handling in MS patients.

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## Introduction

A dietary pattern rich in saturated fat and processed foods and low in fruits and vegetables along with a low physical activity level are thought to play important roles in the pathogenesis of the metabolic syndrome (MS). Mirroring dietary habits, subjects with insulin resistance syndromes including MS, have been shown to possess tissue and plasma fatty acid profiles characterized by a relative predominance of the saturated fatty acids palmitic (16:0) and myristic acid (14:0) and the n-6 polyunsaturated fatty acids (PUFAs)  $\gamma$ -linoleic (18:3n-6), dihomo- $\gamma$ -linolenic (20:3n-6) and arachidonic acid (AA 20:4n-6) [1–3]. This fatty acid pattern appears to confer a higher risk of both diabetes [4] and coronary heart disease (CHD) events [5].

Dietary supplementation with the long-chain n-3 PUFAs eicosapentaenoic acid (EPA 20:5n-3) and docosahexaenoic acid (DHA 22:6n-3) has been shown to reduce recurrent cardiac events in victims of acute myocardial infarction (AMI) [6]. Mechanisms that may contribute to the cardioprotective effects of EPA and DHA include anti-arrhythmic effects, anti-inflammatory effects, and beneficial effects on endothelial function [7]. N-3 PUFAs may also have beneficial effects on glucose homeostasis. Data from animal studies suggest that EPA and DHA may reduce or prevent insulin resistance [8]. However, data from human studies are conflicting, with some studies suggesting benefit [9,10], while most showed no effect [11,12] or even deleterious effects on glucose metabolism [13,14]. These conflicting results are likely due to differences in study design, sample size and the sample studied (presence or absence of insulin resistance and background diet) [12]. In addition, it has generally been assumed that both n-3 and n-6 PUFAs have similar effects on insulin resistance. In an attempt to better understand the relationship between plasma n-3 and n-6 PUFA levels and insulin resistance, we used a relatively large North American secondary CHD prevention sample to (1) compare plasma fatty acid profiles in subjects with and without MS, (2) compare the relationship between n-3 and n-6 PUFA levels and insulin resistance (homeostatic assessment model HOMA-IR) in those with and without MS, and (3) determine whether in subjects with MS, n-3 and n-6 PUFA levels are related to insulin resistance independently of other correlates of MS.

## Methods

### Study population

The ESCAPE project (Epidemiologic Study of Acute Coronary syndromes and the Pathophysiology of Emotions) is

a prospective cohort study assessing the pathophysiologic mechanisms and prognostic implications of depression in subjects with recent AMI, including the relationship between plasma fatty acid levels and post-AMI depression [15]. Between August 31, 1999 and August 2, 2001, patients at the Montreal Heart Institute and the Hôpital du Sacre-Coeur de Montreal with documented AMI based upon clinical and enzyme markers (elevated troponin-T levels) who underwent coronary angiography were potentially eligible for study participation. The exclusion criteria were a secondary cause of AMI, estimated survival less than 2 years from a non-cardiac condition, living too far from the research centre to be able to come back for assessment, inability to speak and read French or English, and a lack of informed consent. Patients were sent letters describing the study. Those not refusing additional contact were telephoned approximately 6 weeks after hospital discharge to arrange for a baseline evaluation. A total of 812 subjects were enrolled in the study representing 51.5% of eligible patients, of which 734 had complete data and are included in the present study. The protocol was approved by the Research Ethics Committees of both participating hospitals.

Clinical variables recorded at baseline include history of hypertension and diabetes, smoking status, physical activity level, previous cardiac history, discharge diagnosis, revascularization procedures during index admission, number of major cardiac vessels with  $\geq 50\%$  blockage according to cardiac catheterization, left ventricular ejection fraction, and current use of medications. No patients were taking n-3 PUFA supplements at study entry. Physical examination parameters included heart rate, blood pressure, height, weight, body-mass index and waist circumference.

The metabolic syndrome was defined using the National Cholesterol Education Program Adult Treatment Panel III (ATP III) criteria [16]. The ATP III definition of MS requires the presence of  $\geq 3$  of 5 criteria, namely abdominal obesity (waist circumference  $>102$  cm in men and  $>88$  cm in women), triglycerides  $\geq 1.70$  mmol/l, decreased HDL-cholesterol ( $<1.0$  mmol/l in men and  $<1.3$  mmol/l in women), blood pressure  $\geq 130/85$  mmHg, and FPG  $\geq 6.1$  mmol/l. In the current study, for blood pressure, we used a modified criterion of treated hypertension or blood pressure  $\geq 130/85$  mmHg. For dysglycemia, we used a modified criterion of FPG  $\geq 6.1$  mmol/l or anti-diabetic therapy.

Biological markers measured included fasting glucose, insulin, lipid profile (LDL-cholesterol, HDL-cholesterol, triglycerides), and FA profile. Insulin resistance was estimated using the homeostatic assessment model (HOMA-IR) and calculated according to the formula  $\text{HOMA-IR} = \text{fasting plasma insulin } (\mu\text{U/ml}) \times \text{fasting plasma glucose}$

(mmol/l)/22.5 [17]. Insulin resistance was defined as HOMA-IR  $\geq$ 4.0 [18].

Plasma fatty acid analyses were performed as previously described [15], using a previously validated technique [19,20]. Briefly, after an overnight fast, venous blood was drawn into ethylenediamine tetraacetate (EDTA) tubes, and plasma was immediately separated by centrifugation stored at  $-80^{\circ}\text{C}$  for subsequent analyses. Plasma lipids were extracted with chloroform:methanol (2:1, by volume). Total phospholipids were then isolated with isopropyl ether:acetic acid (96:4) by thin layer chromatography. Isolated phospholipids were methylated. The fatty acid profiles were obtained by capillary gas chromatography (HP 5890 gas chromatograph equipped with an automated injector 7673A and a flame ionization detector, Hewlett Packard, Toronto, Canada) using a capillary column DB-23 (Agilent Technologies, Oakville, Ontario, Canada) with nitrogen as the carrier. The fatty acid composition of total plasma phospholipids was expressed as the percent of the total area of all fatty acids (C14:0–C24:1).

## Statistical analyses

Baseline characteristics and fatty acid profiles of those with and without MS were compared using independent *t*-tests for continuous variables and Pearson Chi-square tests for categorical variables. When the expected frequencies were less than 5 in any cell, we calculated Fisher's Exact tests. Since distributions for fatty acid levels were often skewed, these analyses were based on arcsine transformations. Because distributions of insulin levels and HOMA-IR values were also skewed, these analyses were based on natural log transformations. Crude correlations were evaluated between fatty acid levels and HOMA-IR levels in those with and without MS. Differences in correlation coefficients between the two groups were compared using Fisher *r*-to-*z* transformations. Separate multivariate models were created to evaluate the relationships between n-3 and n-6 PUFA levels and the n-6/n-3 ratio and HOMA-IR values after adjustment for potential confounders (other observed correlates of MS) in patients with MS. *P*-values  $\leq$ 0.05 were considered statistically significant. Statistical analyses were performed with SPSS version 14.0 (Statistical Product and Service Solutions, Chicago, Illinois).

## Results

Patients with MS were significantly more likely to be female, sedentary, and have a prior history of CHD relative to those without MS (Table 1). They also had significantly higher BMIs, waist circumferences, blood pressure readings, fasting lipid, glucose and insulin levels, and higher HOMA-IR values versus non-MS patients.

Table 2 includes the serum fatty acid profile according to the presence or absence of MS. Metabolic syndrome patients had significantly higher levels of palmitic (16:0) and stearic acid (18:0) as well as  $\gamma$ -linoleic (18:3n-6), dihomo- $\gamma$ -linolenic (20:3n-6), adrenic (22:4n-6) and docosapentaenoic acid (22:5n-6) versus non-MS patients. Total n-3 PUFA levels were significantly lower in MS patients, reflecting lower levels of EPA and DHA.

**Table 1** Baseline characteristics according to the presence or absence of metabolic syndrome

	No metabolic syndrome (n = 381)	Metabolic syndrome (n = 353)	<i>P</i>
	Mean $\pm$ SD	Mean $\pm$ SD	
<i>Demographic variables</i>			
Age (years)	59.4 $\pm$ 10.5	60.3 $\pm$ 10.7	0.28
Female (%)	14.4	23.2	0.002
<i>Risk factors and cardiac history</i>			
Sedentary (%)	45.7	54.5	0.016
Current daily smoker (%)	13.9	18.4	0.097
Diabetes mellitus (%)	7.3	32.6	<0.001
Previous MI, coronary bypass or angioplasty (%)	29.4	38.0	0.014
Coronary bypass surgery at index admission (%)	21.5	16.7	0.098
Coronary angioplasty at index admission (%)	59.8	64.0	0.24
<i>Clinical and metabolic variables</i>			
Body-mass index (kg/m <sup>2</sup> )	26.6 $\pm$ 3.3	30.2 $\pm$ 4.6	<0.001
Waist circumference (cm)	96.1 $\pm$ 10.1	105.5 $\pm$ 11.3	<0.001
Systolic blood pressure (mmHg)	130.5 $\pm$ 21.9	140.5 $\pm$ 23.7	<0.001
Diastolic blood pressure (mmHg)	72.8 $\pm$ 10.0	76.8 $\pm$ 10.8	<0.001
Triglycerides (mmol/l)	1.47 $\pm$ 0.72	2.29 $\pm$ 1.14	<0.001
HDL-cholesterol (mmol/l)	1.17 $\pm$ 0.25	1.01 $\pm$ 0.20	<0.001
LDL-cholesterol (mmol/l)	2.57 $\pm$ 0.82	2.70 $\pm$ 0.84	0.039
Fasting plasma glucose (mmol/l)	5.69 $\pm$ 1.22	6.87 $\pm$ 2.04	<0.001
Fasting plasma insulin (pmol/l) <sup>a</sup>	68.6 $\pm$ 31.2	106.6 $\pm$ 59.6	<0.001
HOMA-IR (units)	2.44 $\pm$ 1.25	4.61 $\pm$ 3.30	<0.001
<i>Medications</i>			
Aspirin (%)	89.5	89.0	0.81
Beta-blockers (%)	78.0	77.3	0.84
Angiotensin converting enzyme inhibitors (%)	44.4	58.9	<0.001
Angiotensin-II receptor antagonists (%)	1.0	1.1	0.91
Calcium channel blockers (%)	14.2	28.3	<0.001
Statins (%)	75.9	70.8	0.12
Fibrates (%)	1.3	3.7	0.054
Oral hypoglycemics (%)	6.3	27.2	<0.001
Insulin (%)	1.0	5.4	0.001
Metformin (%)	4.2	22.4	<0.001
Glitazones (%)	0.5	0.6	1.00

HOMA-IR indicates homeostasis model assessment of insulin resistance.

<sup>a</sup> Based on natural log-transformed data.

**Table 2** Plasma fatty acid levels according to the presence or absence of metabolic syndrome

Fatty acid <sup>a</sup>	No metabolic syndrome	Metabolic syndrome	<i>P</i> <sup>b</sup>
	( <i>n</i> = 381)	( <i>n</i> = 353)	
	Mean ± SD	Mean ± SD	
<i>Saturated fatty acids</i>			
Myristic 14:0	0.34 ± 0.11	0.35 ± 0.11	0.10
Palmitic 16:0	25.74 ± 1.55	25.98 ± 1.52	0.036
Stearic 18:0	13.66 ± 1.05	13.88 ± 1.11	<0.001
Total saturated fatty acids	44.2 ± 1.46	44.6 ± 1.23	<0.001
<i>Monounsaturated fatty acids</i>			
Palmitoleic 16:1n-7	0.47 ± 0.11	0.52 ± 0.12	0.003
Oleic 18:1n-9	10.02 ± 1.72	9.99 ± 1.72	0.65
<i>n-3 Fatty acids</i>			
Alpha-linolenic 18:3n-3	0.23 ± 0.08	0.23 ± 0.08	0.53
Stearidonic 18:4n-3	0.05 ± 0.03	0.05 ± 0.04	0.50
Eicosatetraenoic 20:4n-3	0.16 ± 0.08	0.18 ± 0.15	0.008
Eicosapentaenoic (EPA) 20:5n-3	1.27 ± 0.71	1.09 ± 0.44	<0.001
Docosapentaenoic (n-3) 22:5n-3	1.13 ± 0.21	1.12 ± 0.21	0.45
Docosahexaenoic (DHA) 22:6n-3	3.83 ± 1.20	3.58 ± 1.09	0.004
Total EPA and DHA n-3 Total	5.10 ± 1.65	4.67 ± 1.31	<0.001
	6.69 ± 1.70	6.27 ± 1.37	<0.001
<i>n-6 Fatty acids</i>			
Linoleic 18:2n-6	17.50 ± 2.40	17.70 ± 2.40	0.28
Gamma-linoleic 18:3n-6	0.10 ± 0.07	0.11 ± 0.06	0.002
Dihomo-gamma-linolenic 20:3n-6	3.23 ± 0.62	3.52 ± 0.68	<0.001
Arachidonic (AA) 20:4n-6	11.59 ± 2.04	11.52 ± 1.99	0.68
Adrenic 22:4n-6	0.40 ± 0.10	0.42 ± 0.10	0.016
Docosapentaenoic (n-6) 22:5n-6	0.30 ± 0.09	0.32 ± 0.09	0.001
n-6 Total	33.68 ± 2.23	34.16 ± 2.00	0.002
<i>Ratios</i>			
n-6/n-3 Ratio	5.39 ± 1.52	5.71 ± 1.31	0.002
AA/EPA ratio	11.22 ± 5.58	12.15 ± 5.10	0.019

SD indicates standard deviation.

<sup>a</sup> Fatty acid levels expressed as the percent of the total area of all fatty acids (C14:0 to C24:1).

<sup>b</sup> *P*-values based on analyses with arcsine transformations.

Insulin resistance as defined by HOMA-IR  $\geq 4.0$  represented the 74th percentile for HOMA-IR in our cohort. Subjects with insulin resistance had a similar fatty acid profile to those with MS, with significantly lower total n-3 PUFA levels and higher total saturated fatty acid and n-6 PUFA levels relative to those without insulin resistance (data not shown).

Associations between fatty acid levels and HOMA-IR (continuous values) are presented in Table 3 according to the presence or absence of MS. Overall, the relationships

**Table 3** Univariate correlations (*R*) between fatty acids levels and HOMA-IR in subjects with and without metabolic syndrome

Variable	HOMA-IR		<i>P</i> for difference between <i>R</i> values		
	No metabolic syndrome	Metabolic syndrome			
	( <i>n</i> = 381)	( <i>n</i> = 353)	<i>R</i>	<i>P</i>	<i>P</i>
<i>Saturated fatty acids</i>					
16:0	-0.083	0.11	0.029	0.59	0.13
18:0	0.152	0.003	0.089	0.095	0.39
Total Saturated	0.064	0.21	0.127	0.017	0.39
<i>n-3 Fatty acids</i>					
18:3n-3	-0.023	0.66	-0.091	0.088	0.36
20:5n-3	0.093	0.069	-0.122	0.022	0.69
22:5n-3	-0.108	0.035	-0.146	0.006	0.60
22:6n-3	0.056	0.27	-0.094	0.077	0.042
Total n-3	0.060	0.25	-0.134	0.012	0.0085
<i>n-6 Fatty acids</i>					
18:2n-6	-0.008	0.88	0.145	0.006	0.038
20:3n-6	0.201	<0.001	0.149	0.005	0.47
20:4n-6	0.003	0.95	-0.037	0.49	0.59
Total n-6	0.032	0.53	0.171	0.001	0.057
<i>Ratios</i>					
AA/EPA	-0.060	0.25	0.090	0.090	0.042
AA/DHA	-0.064	0.21	0.067	0.21	0.077
n-6/n-3	-0.017	0.75	0.173	0.001	0.0096

between total saturates, total n-3 PUFA, and total n-6 PUFA levels and HOMA-IR were significant only in individuals with MS. Among the n-6 fatty acids, linoleic (18:2n-6) and dihomo- $\gamma$ -linolenic acid (20:3n-6) correlated positively with HOMA-IR among patients with MS. In contrast, EPA (20:5n-3) and docosapentaenoic acid (22:5n-3) showed inverse correlations with HOMA-IR in this same population. Statistical trends were noted for the inverse relationships between HOMA-IR and both alpha-linolenic acid (ALA 18:3n-3) and DHA. The n-6/n-3 ratio also correlated positively with HOMA-IR in MS patients only.

Within the MS group, we created multivariate models to assess the relationship between HOMA-IR levels and total n-3 PUFA levels, total n-6 PUFA levels and the n-6/n-3 ratio respectively, after adjusting for background variables (Table 4). Each of these parameters contributed significantly to the explained variance of HOMA-IR independent of age, sex, sedentary behaviour, smoking, waist circumference, triglyceride and HDL levels, systolic blood pressure and total saturated fatty acids. Although the greatest portion of the variance in HOMA-IR was explained by waist circumference followed by female sex and HDL-cholesterol, the contribution of n-3 PUFA levels to variance of HOMA-IR surpassed that of triglycerides and other covariates.

## Discussion

The main findings of this study are that patients with MS possessed higher plasma levels of palmitic (16:0) and

**Table 4** Multivariate predictors of HOMA-IR levels in 353 patients with metabolic syndrome

Variables	Total n-3 fatty acids <sup>a</sup>		Total n-6 fatty acids <sup>b</sup>		n-6/n-3 Ratio <sup>c</sup>	
	R <sup>2</sup>	P	R <sup>2</sup>	P	R <sup>2</sup>	P
Age	0.004	0.18	0.004	0.18	0.004	0.15
Female sex	0.021	0.002	0.019	0.003	0.020	0.002
Sedentary	0.005	0.13	0.004	0.15	0.005	0.11
Daily smoker	0.001	0.54	<0.001	0.67	0.001	0.48
Waist circumference	0.205	<0.001	0.187	<0.001	0.196	<0.001
Triglycerides	0.010	0.030	0.009	0.034	0.011	0.026
HDL-cholesterol	0.020	0.002	0.019	0.003	0.019	0.003
Systolic blood pressure	<0.001	0.84	<0.001	0.76	<0.001	0.74
Total saturated fatty acids	0.001	0.46	0.008	0.051	0.002	0.31
PUFA measure <sup>a,b</sup> or <sup>c</sup>	0.013	0.016	0.025	0.001	0.021	0.002
Total adjusted R <sup>2</sup>	0.256	<0.001	0.269	<0.001	0.265	<0.001

HOMA-IR levels were analyzed as natural logarithmic transformation.

<sup>a</sup> R<sup>2</sup> value for total n-3 fatty acids.

<sup>b</sup> R<sup>2</sup> value for total n-6 fatty acids.

<sup>c</sup> R<sup>2</sup> value for n-6/n-3 ratio.

stearic acid (18:0) and the n-6 fatty acids  $\gamma$ -linoleic (18:3n-6), dihomo- $\gamma$ -linolenic (20:3n-6), adrenic (22:4n-6) and docosapentaenoic acid (22:5n-6), whereas levels of the long-chain n-3 fatty acids EPA and DHA were lower relative to those without MS. The relationship between fatty acid levels and HOMA-IR varied according to the absence or presence of MS, whereby subjects without MS showed no significant relationship between n-6 or n-3 PUFA levels and HOMA-IR. In contrast, HOMA-IR correlated negatively with EPA and docosapentaenoic acid (22:5n-3), and positively with linoleic (18:2n-6) and dihomo- $\gamma$ -linolenic acid (20:3n-6) in MS subjects. Similarly, the n-6/n-3 ratio also correlated positively with HOMA-IR values, but only in MS subjects. Finally, total n-3 and n-6 FA levels as well n-6/n-3 ratio all contributed significantly to the variance of HOMA-IR in subjects with MS after adjusting for potential confounding variables including total saturated fatty acid levels.

Our study contributes to the published literature in several important ways. This is the first study to evaluate fatty acid profile according to the presence or absence of MS in a patient sample with underlying CHD. The results we obtained were consistent with previous data in a non-coronary sample showing that individuals with MS possess higher levels of saturated fatty acids (14:0, 16:0) and n-6 plasma fatty acids (18:3n-6, 20:3n-6, 20:4n-6) [3], suggesting that plasma fatty acid levels continued to reflect dietary fatty acid intake over the preceding weeks in our sample and were not significantly affected by a recent AMI. Secondly, our data confirm the positive relationship existing between saturated plasma fatty acid levels and measures of insulin resistance; this relationship was evident, however, only in subjects with MS, a result consistent with a diet higher in saturated fat in individuals with this condition. Thirdly, this study demonstrates that the relationships between n-6 and n-3 PUFA levels and HOMA-IR may differ between subjects with and without MS, this was the case for linoleic (18:2n-6) and dihomo- $\gamma$ -linolenic (20:3n-6), EPA (20:5n-3) and docosapentaenoic acid (22:5n-3) in our study. Fourthly, our data suggest that n-3 and n-6

PUFAs may have opposing effects on insulin resistance. Finally, EPA and DHA appeared to be more related to glucose metabolism than ALA (18:3n-3).

Dietary fat quality is known to influence glucose handling and the development of insulin resistance. Consumption of saturated fat has been shown to reduce insulin sensitivity according to large epidemiological studies [12,21,22]. Data from mechanistic studies in humans also support an inverse relationship between tissue content of saturated fatty acids and insulin sensitivity [3,12]. Similarly, insulin resistance syndromes including MS are associated with a plasma fatty acid profile characterized by a relative predominance of saturated (14:0, 16:0) and n-6 PUFAs (18:3n-6, 20:3n-6, 20:4n-6) [1–3]. The relationship between individual classes of PUFAs and insulin resistance, however, is unclear. Animal studies have consistently shown that dietary supplementation with EPA and DHA not only prevents the development of insulin resistance in response to a high-sucrose or high-saturated fat diet, but that these long-chain n-3 PUFAs may also reverse insulin resistance in liver, muscle and adipose tissue after exposure to a long-term high-sucrose diet [11]. Data from human studies, however, have failed to show similarly consistent results [8,12]. Interventions undertaken in type 2 diabetic subjects have generally failed to show beneficial effects of EPA and DHA supplementation on glucose disposal [23,24]. In contrast, one study in 6 type 2 diabetic patients demonstrated that treatment with 3 g EPA + DHA per day for 8 weeks improved insulin sensitivity and lowered triglyceride levels [9]. Methodological limitations in these studies, including small sample size, short study duration, use of a cross-over design with potential carry-over effects, and potential differences in background diet, limit the interpretation of the results [12]. Paradoxically, high-dose EPA and DHA were shown to modestly increase plasma glucose levels and reduce insulin sensitivity in type 2 diabetic subjects without hypertriglyceridemia [14]. This data is consistent with other studies in type 2 diabetic patients treated with high-dose EPA + DHA (5–8 g/day) [8]. Conversely, a study in 162 healthy individuals randomized

to high-saturated or high-monounsaturated fat diet and subsequently to 3.6 g/day EPA + DHA or placebo for a total of 3 months did not show any effect of n-3 PUFAs on insulin secretion or sensitivity [25]. Finally, in 258 men and postmenopausal women aged 45–70 years free of diabetes mellitus or coronary disease, treatment with diets rich in n-3 PUFAs with n-6/n-3 ratios varying from 5:1 to 3:1 for 6 months had no effect on insulin sensitivity despite significant triglyceride-lowering effects [26].

Our data are consistent with a recently published study in Alaskan Inuit showing an inverse relationship between MS components, measures of insulin resistance and plasma n-3 PUFA (C20–C22) levels [10]. Our data are also consistent with findings from the KANWU study showing the lack of effect of 3.6 g EPA + DHA on glucose metabolism in healthy non-obese individuals independent of n-3 or n-6 fatty acid intake [25]. We believe that we were able to highlight the different relationships between PUFA classes and HOMA-IR in subjects with and without MS due to the relatively large size of our study sample. In addition, a diet low in n-3 PUFA content but high in saturated and n-6 fatty acid content in MS subjects, presumably also allowed us to detect the beneficial effect of n-3 PUFAs on HOMA-IR. Subjects with MS might therefore be sensitive to longer-term supplementation with moderate doses of long-chain n-3 PUFAs for reducing insulin resistance. Such a study has yet to be performed.

Total n-3 PUFA levels did not show any significant relationships with triglycerides or HDL-cholesterol. These data are consistent with previous studies showing a lack of significant triglyceride-lowering or HDL-raising effect with low-intermediate doses of long-chain n-3 PUFAs [27]. The beneficial effects of n-3 PUFAs on insulin sensitivity in MS patients could be independent of effects on triglyceride levels. Potential mechanisms put forth include effects on cell membrane fatty acid composition of insulin target tissues leading to enhanced insulin secretion and biological action in target tissues through improved insulin signalling [8].

Limitations to the current study include its cross-sectional design. Therefore, a relationship of causality cannot be established between MS, insulin resistance and the observed fatty acid levels. Secondly, information on dietary intake or alcohol intake was not available. However, plasma PUFA levels are known to reflect dietary PUFA consumption over the preceding weeks [28]. Thirdly, while we adjusted for potential confounders in our multivariate models, other unmeasured confounders may have influenced the relationships between HOMA-IR and fatty acid levels. Fourthly, the relationship between individual MS criteria and fatty acid levels may have been altered as a result of pharmacological therapy with anti-diabetic agents, fibrates and anti-hypertensive medication. While fibrates were recently shown to increase plasma AA (20:4n-6) levels and decrease linoleic acid (18:2n-6) and DHA levels [29], only a minority of patients in either of our groups were receiving fibrate therapy, and levels of the two n-6 FAs in question were similar between those with and without MS. Statin therapy which most patients were receiving, may have also modestly affected HDL-cholesterol and triglyceride levels, but was balanced in the two groups. Additionally, the effect of statins on plasma fatty acid levels

remain unclear; available data suggest they may result in a relative decrease in linoleic acid (18:2n-6) and a relative increase in AA (20:4n-6) levels without significant effects on n-3 PUFA levels [29,30]. Despite the use of these medications, however, patients in our MS sample did have insulin resistance based upon a mean HOMA-IR of  $4.61 \pm 3.30$ . We do not believe that the above-mentioned medications, however, had a significant impact on the associations between fatty acid levels and HOMA-IR which we were most interested in. Finally, we used the 6.1 mmol/l cutpoint in our MS definition rather than the new cutpoint of 5.6 mmol/l, which allowed us to study an MS population with a higher degree of insulin resistance and which permitted us to better highlight the differences in the associations between HOMA-IR and fatty acid levels in our two groups.

In conclusion, the relationships between n-3 and n-6 PUFA levels and HOMA-IR varied according to the presence or absence of MS, and were independent of other factors known to be associated with MS and insulin resistance. In this large sample, EPA and docosapentaenoic acid (22:5n-3) were associated with better glucose homeostasis while n-6 PUFAs (18:3n-6, 20:3n-6, 22:4n-6) were associated with opposite effects. This finding puts into question previous small studies suggesting a lack of beneficial effects of long-chain n-3 PUFAs for the treatment of insulin resistant states. Larger randomized trials with appropriate methodology including studying patients with insulin resistance, employing an intermediate dose of EPA and DHA over a longer treatment period are now required to confirm or discount the results of our study.

## References

- [1] Salomaa V, Ahola I, Tuomilehto J, Aro Am Pietinen P, Korhonen HJ, Penttila I. Fatty acid composition of serum cholesterol esters in different degrees of glucose intolerance. *Metabolism* 1990;39:1285–91.
- [2] Borkman M, Storlien LH, Pan DA, Jenkins AB, Chisholm DJ, Campbell LV. The relationship between insulin sensitivity and the fatty acid composition of skeletal muscle phospholipids. *N Engl J Med* 1993;328:238–44.
- [3] Warensjo E, Riserus U, Vessby B. Fatty acid composition predicts the development of the metabolic syndrome in men. *Diabetologia* 2005;48:1999–2005.
- [4] Vessby B, Aro A, Skarfors E, Berglund L, Salminen I, Lithell H. The risk to develop NIDDM is related to the fatty acid composition of the serum cholesterol esters. *Diabetes* 1994; 43:1353–7.
- [5] Ohrvall M, Berglund L, Salminen I, Lithell H, Aro A, Vessby B. The serum cholesterol ester fatty acid composition, but not the serum concentration of alpha tocopherol predicts the development of myocardial infarction in 50-year old men, 19 years follow up. *Atherosclerosis* 1996;127:65–71.
- [6] Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI)-Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. *Lancet* 1999;354:447–55.
- [7] Harris WS. Omega-3 fatty acids and cardiovascular disease: a case for omega-3 index as a new risk factor. *Pharmacol Res* 2007;55:217–23.
- [8] Lombardo YB, Chicco AG. Effects of dietary polyunsaturated n-3 fatty acids on dyslipidemia and insulin resistance in rodents and humans. A review. *J Nutr Biochem* 2006;17:1–13.

- [9] Popp-Snijders C, Schouten JA, Heine RJ, van derMeer J, van der Veen EA. Dietary supplementation of omega-3 polyunsaturated fatty acids improves insulin sensitivity in non-insulin-dependent diabetes. *Diabetes Res* 1987;4:141–7.
- [10] Ebbesson SO, Risica PM, Ebbesson LO, Kennish JM, Tejero ME. Omega-3 fatty acids improve glucose tolerance and components of the metabolic syndrome in Alaskan Eskimos: the Alaska Siberia project. *Int J Circumpolar Health* 2005;64:396–408.
- [11] Friedberg CE, Janssen MJ, Heine RJ, Grobbee DE. Fish oil and glycemic control in diabetes. A meta-analysis. *Diabetes Care* 1998;21:494–500.
- [12] Riccardi G, Giacco R, Rivellese AA. Dietary fat, insulin sensitivity and the metabolic syndrome. *Clin Nutr* 2004;23:447–56.
- [13] Friday KE, Childs MT, Tsunehara CH, Fujimoto WY, Bierman EL, Ensink JW. Elevated plasma glucose and lowered triglyceride levels from omega 3 fatty acid supplementation in type II diabetes. *Diabetes Care* 1989;12:276–81.
- [14] Mostad IL, Bjerve KS, Bjorgaas MR, Lydersen S, Grill V. Effects of n-3 fatty acids in subjects with type 2 diabetes: reduction of insulin sensitivity and time-dependent alteration from carbohydrate to fat oxidation. *Am J Clin Nutr* 2006;84:540–50.
- [15] Frasure-Smith N, Lespérance F, Julien P. Major depression is associated with lower omega-3 fatty acid levels in patients with recent acute coronary syndromes. *Biol Psychiatry* 2004;55:891–6.
- [16] Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA* 2001;285:2486–97.
- [17] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Insulin resistance and B-cell function from fasting plasma glucose and insulin concentration in man. *Diabetologia* 1985;28:412–9.
- [18] Muntner P, He J, Chen J, Fonseca V, Whelton PK. Prevalence of non-traditional cardiovascular disease risk factors among persons with impaired fasting glucose, impaired glucose tolerance, diabetes, and the metabolic syndrome: analysis of the Third National Health and Nutrition Examination Survey (NHANES III). *Ann Epidemiol* 2004;14:686–95.
- [19] Vidgren HM, Agren JJ, Schwab U, Rissanen T, Hänninen O, Uusitupa M. Incorporation of n-3 fatty acids into plasma lipid fractions, and erythrocyte membranes and platelets during dietary supplementation with fish, fish oil, and docosahexaenoic acid-rich oil among healthy young men. *Lipids* 1997;32:697–705.
- [20] Harris WS. Fish oils and plasma lipid and lipoprotein metabolism in humans: a critical review. *J Lipid Res* 1989;30:785–807.
- [21] Maron DJ, Fair JM, Haskell WL. Saturated fat intake and insulin resistance in men with coronary heart disease. *Circulation* 1991;84:2020–7.
- [22] Parker DR, Weiss ST, Troisi R, Cassano PA, Vokonas PS, Landsberg L. Relationship of dietary saturated fatty acids and body habitus to serum insulin concentrations: the normative aging study. *Am J Clin Nutr* 1993;58:129–36.
- [23] Borkman M, Chisholm DJ, Furler SM, Strolin LH, Kraegen EW, Simons LA, et al. Effects of fish oil supplementation on glucose and lipid metabolism in NIDDM. *Diabetes* 1989;38:1314–9.
- [24] Rivellese AA, Maffettone A, Lovine C, Di Marino L, Annuzzi G, Mancini M. Long term effects of fish oil on insulin resistance and plasma lipoproteins in NIDDM patients with hypertriglyceridemia. *Diabetes Care* 1996;19:1207–13.
- [25] Giacco R, Cuomo V, Vessby B, Uusitupa M, Hermansen K, Meyer BJ, et al. KANWU Study Group. Fish oil, insulin sensitivity, insulin secretion and glucose tolerance in healthy people: is there any effect of fish oil supplementation in relation to the type of background diet and habitual dietary intake of n-6 and n-3 fatty acids? *Nutr Metab Cardiovasc Dis* 2007;17:572–80.
- [26] Griffin MD, Sanders TA, Davies IG, Morgan LM, Millward DJ, Lewis F, et al. Effects of altering the ratio of dietary n-6 to n-3 fatty acids on insulin sensitivity, lipoprotein size, and postprandial lipemia in men and postmenopausal women aged 45–70 y: the OPTILIP Study. *Am J Clin Nutr* 2006;84:1290–8.
- [27] Harris WS. n-3 Fatty acids and serum lipoproteins: human studies. *Am J Clin Nutr* 1997;65(5 Suppl.):1645S–54S.
- [28] Katan MB, Deslypere JP, van Birgelen AP, Penders M, Zegwaard M. Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study. *J Lipid Res* 1997;38:2012–22.
- [29] de Lorgeril M, Salen P, Guiraud A, Zeghichi S, Boucher F, de Leiris J. Lipid-lowering drugs and essential omega-6 and omega-3 fatty acids in patients with coronary heart disease. *Nutr Metab Cardiovasc Dis* 2005;15:36–41.
- [30] Harris JI, Hibbeln JR, Mackey RH, Muldoon MF. Statin treatment alters serum n-3 and n-6 fatty acids in hypercholesterolemic patients. *Prostaglandins Leukot Essent Fatty Acids* 2004;71:263–9.