

N-3 POLYUNSATURATED ACIDS IN ERYTHROCYTE PHOSPHOLIPIDS ARE ASSOCIATED WITH INSULIN SENSITIVITY IN OBESE PATIENTS ON A TYPICAL SERBIAN DIET

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Abstract — This study examines the relationship between erythrocyte phospholipid FA composition and insulin sensitivity in obese patients on a typical Serbian diet. In 30 patients, two insulin sensitivity groups were formed and their FAs analyzed. The shares of 22:5n-3, 22:6n-3, and total n-3 PUFAs, as well as a measure of $\Delta 5$ desaturase activity (the 20:4n-6/20:3n-6 ratio) were lower in the insulin resistance group compared to the normal glucose tolerance group. The fasting insulin level and HOMA value were inversely related to the percentage of 22:5n-3, 22:6n-3, and total n-3 PUFAs. Our data indicate that the percentage of n-3 PUFAs in erythrocyte phospholipids is linked with insulin sensitivity parameters in obese patients.

Key words: n-3 PUFA, insulin resistance, obesity, hyperlipidemia, dietary fat

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INTRODUCTION

Obesity is one of the most important risk factors for the development of type 2 diabetes (Colditz et al., 1990). Insulin sensitivity can be modulated by different lifestyle factors, and the quality of dietary fat seems to be an important one (Vessby et al., 2001; Allen et al., 2003).

Epidemiological studies in humans based on dietary questionnaires have generally found a positive association between intake of saturated fatty acids (SFA) and risk of diabetes (Feskens and Kromhout, 1990; Salmeron et al., 2001). However, data on relationships between polyunsaturated fatty acid (PUFA) intake and insulin sensitivity are less clear (Feskens et al., 1995; Salmeron et al. 2001). Dietary fat intake is difficult to estimate, and the amount of different fatty acids in the phospholipids of serum or cell membrane appears to be a more objective marker of dietary intake than dietary questionnaire measurements (Rivellese et al., 2002). The concentrations of essential n-6 and n-3 PUFAs in cell membranes or body tissues are highly correlated

with dietary intake (Ma et al., 1995) and blood cell membrane composition is an objective biomarker of longer-term dietary fatty acid intake (~120 days) (Arab, 2003).

Results of some cross-sectional studies that investigated the correlation between erythrocyte membrane fatty acid composition and insulin sensitivity (Agostoni et al., 1994; Clifton and Nestel, 1998; Enriquez et al., 2004; Rodriguez and Christophe, 2005) indicate that this relation may differ, depending on the type of population studied. Clifton and Nestel (1998) found an inverse relationship between fasting plasma insulin and the erythrocyte percentage of arachidonic acid (AA, 20:4n-6) and total n-6 PUFAs, and a positive relationship between it and the percentage of SFAs in healthy men (Clifton and Nestel, 1998). In obese type 2 diabetics receiving metformin therapy, different relationships were observed: the fasting insulin level correlated positively with alpha-linolenic (18:3n-3) and dihomo-gamma-linolenic (20:3n-6) acids and negatively with the fraction of palmitic acid (16:0) in erythro-

cyte phospholipids (Enriquez et al., 2004).

The effects of n-3 long-chain PUFAs on insulin sensitivity in humans also need to be clarified, as some n-3 LC-PUFA supplementation studies reported a beneficial effect (Pop-Snijders et al., 1987) and others a lack of effects on insulin sensitivity (Brady et al., 2004), and this variability may also be related to metabolic characteristics of the study population (Browning, 2003). It could be important to investigate a population on a typical Serbian diet, with high intake of SFAs and low intake of PUFAs, especially ones of the n-3 PUFA family (Simopoulos, 2005).

To the best of our knowledge, there are no published reports on the relationship between erythrocyte fatty acid composition and insulin sensitivity in nondiabetic, normoglycemic, and overweight/obese subjects with hyperlipidemia. There is very limited information about the FA intake of patients on a typical Serbian diet and related health consequences. Using membrane fatty acid composition as an objective index of dietary fatty acid intake, we obtained results in a cross-sectional study that may aid the planning of nutritional strategies designed to prevent the development of type 2 diabetes in obesity.

MATERIALS AND METHODS

Subjects

A total of 30 free-living overweight/obese patients were selected on the basis of the following criteria: body mass index (BMI) greater than 25 kg/m² and weight stable (± 2 kg) for 6 months prior to the beginning of the study; normal fasting glucose levels according to World Health Organization criteria (fasting glucose < 6.1 mmol/l); hyperlipidemia, as defined by total serum cholesterol ≥ 5.2 mmol/l, serum LDL-cholesterol ≥ 3.4 mmol/l, and normal or increased triglyceride levels (the lipid status was based on data from two fasting serum lipid profiles). Exclusion criteria were: hepatic dysfunction; renal dysfunction; uncontrolled hypertension; hypo- or hyperthyroidism; unstable angina pectoris; myocardial infarction within the previous 6 months; and alcohol consumption. None of the eligible subjects used any lipid-lowering drugs, immunosuppressive

agents, anticoagulants, hormone substitutes, beta-blockers, or anti-platelet aggregation medications for 12 weeks prior to the start of the study. All of the subjects were free-living on their habitual diet, typical of the Serbian population, with low fish and soy consumption (one meal in two weeks) and high animal fat intake (they consumed meat, eggs, and whole milk products every day). They engaged in low regular physical activity (light physical exertion such as walking or biking for less than 2 hours/week). **None of the patients had a family history of diabetes mellitus.**

All patients signed an informed consent document; the study was approved by the Medical Ethics Committee (Institute for Medical Research, Belgrade) and conducted according to principles of good scientific practice.

Methods

Body weight was measured on a balance calibrated to 0.1 kg. Standing height was measured barefoot to the nearest 0.5 cm using a wall-mounted stadiometer. All measurements were obtained after an overnight fast of at least 12 h.

The fasting serum insulin level and homeostasis model assessment (HOMA) values were selected as insulin resistance markers. The HOMA value was calculated as the fasting glucose level (mmol/l) \times the fasting insulin level (mU/l)/22.5. A HOMA value > 2.5 was considered indicative of insulin resistance, as suggested in the Bruneck study (Bonora et al., 1998). Using the HOMA value, we formed two insulin sensitivity groups: a normal glucose tolerance (HOMA value < 2.5) or NGT group; and an insulin resistance (HOMA value > 2.5) or IR group of overweight/obese patients with hyperlipoproteinemia.

Glucose, total cholesterol, and triglyceride levels were measured in serum after a 12-h fast using enzymatic methods with glucose oxidase, cholesterol oxidase, and glycerol oxidase, respectively (EliTech Diagnostic, Sées, France). HDL-Cholesterol was determined in the supernatant liquid after precipitation with phosphotungstic acid and magnesium chloride. LDL-Cholesterol was estimated using the Friedewald formula. The serum insulin level was

determined with a radioimmunoassay kit (INEP, Zemun, Serbia).

Red blood cell lipids were extracted by the method of Harth (Harth et al., 1978). The erythrocyte phospholipid fraction was isolated by one-dimensional thin-layer chromatography (TLC) in a neutral hexane-diethyl ether-acetic acid (87:12:1; v/v/v) solvent system using silica gel GF plates (C. Merck, Darmstadt, Germany). Following methylation, fatty acid ester derivatives were analyzed by gas chromatography using a Varian gas chromatograph (model 3400). Individual fatty acid methyl esters were identified using a fatty acid standard mixture (PUFA-2, Sigma, Germany). The share of 18:3 n-3 was too low to be detected and was not included in the sum of n-3 PUFA.

Statistical analyses

All results are expressed as a means \pm SD. Differences between the formed groups were analyzed using the unpaired Student's t-test, accepting an alpha significance level of $p \leq 0.05$. Pearson correlation coefficients were computed for examining the relations between BMI, insulin, HOMA value, and erythrocyte fatty acid concentrations.

Abbreviations — FA – fatty acid; LDL – low-density lipoproteins; HOMA – homeostasis model assessment; NGT – normal glucose tolerance; IR – insulin

resistance; TLC – thin-layer chromatography; PUFA – polyunsaturated fatty acids; BMI – body mass index; SFA – saturated fatty acids; AA – arachidonic acid; HDL – high-density lipoproteins; MUFA – monounsaturated fatty acids; LC-PUFA – long-chain polyunsaturated fatty acids.

RESULTS

The mean clinical and metabolic parameters are shown in Table 1. The BMI ranged from 26 to 45 kg/m², serum fasting insulin from 9.1 to 31.2 mU/ml, and fasting glucose from 3.92 to 5.24 mmol/l. No differences of lipid parameters were found between groups. In the IR group, the fasting insulin level (20.1 \pm 6.0 mU/ml) was higher than in the NGT group (10.8 \pm 1.3 mU/ml) ($p < 0.001$). The BMI value was also significantly higher in the IR group ($p < 0.05$).

Results on erythrocyte phospholipid fatty acid composition are shown in Table 2. Levels of 22:5n-3 ($p < 0.01$), 22:6n-3 ($p < 0.01$), and total n-3 PUFAs ($p < 0.001$) were lower in the IR group than in the NGT group. Higher n-6 PUFA/n-3 PUFA, SFA/n-3 PUFA, and MUFA/n-3 PUFA ratios were also observed in IR patients compared to the NGT group of patients ($p < 0.01$). There was no difference in the percentage of individual or total n-6 PUFAs between groups, although the ratio between arachidonic acid (20:4n-

Table 1. Clinical and biochemical data for all subjects grouped in glucose tolerance categories. All data represented as means \pm SE. * $p < 0.05$; *** $p < 0.001$ compared to NGT group.

	all patients n=30	NGT group n=12	IR group n=18
age (years)	56 \pm 12	58 \pm 9	54 \pm 14
male/female	7/23	2/10	5/13
BMI (kg/m ²)	32.9 \pm 5.0	30.3 \pm 2.4	34.7 \pm 5.6*
fasting glucose (mmol/l)	4.76 \pm 0.50	4.63 \pm 0.54	4.84 \pm 0.46
triglycerides (mmol/l)	2.15 \pm 0.99	2.21 \pm 1.03	2.12 \pm 1.00
total cholesterol (mmol/l)	7.62 \pm 1.02	7.51 \pm 1.06	7.68 \pm 1.02
LDL-cholesterol (mmol/l)	5.28 \pm 1.06	5.16 \pm 1.11	5.35 \pm 1.05
HDL-cholesterol (mmol/l)	1.32 \pm 0.13	1.34 \pm 0.14	1.30 \pm 0.13
fasting insulin (mU/ml)	16.4 \pm 6.6	10.8 \pm 1.3	20.1 \pm 6.0***
HOMA value	3.50 \pm 1.63	2.21 \pm 0.6	4.37 \pm 1.58***

Table 2. Erythrocyte fatty acid composition for all subjects grouped in glucose tolerance categories. All data represented as means±SE. Fatty acid concentration is expressed in % of totally detected fatty acids. SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids. *p < 0.05; **p < 0.01; ***p < 0.001 compared to NGT group.

	all patients n=30	NGT group n=12	IR group n=18
16:0	22.63±1.39	22.49±1.67	22.73±1.21
18:0	18.77±2.08	18.25±1.07	19.11±2.51
SFA	41.40±2.54	40.74±1.73	41.83±2.93
16:1n-7	0.31±0.11	0.29±0.13	0.33±0.10
18:1n-9	16.78±1.29	16.30±0.71	17.10±1.50
MUFA	17.10±1.05	16.59±0.81	17.44±1.55
18:2n-6	12.66±1.69	12.20±1.60	12.97±1.73
20:3n-6	1.85±0.42	1.68±0.32	1.96±0.44
20:4n-6	16.67±1.92	17.29±1.73	16.26±1.98
22:4n-6	3.83±0.72	3.85±0.75	3.82±0.72
n-6	35.1±2.98	35.02±2.36	35.01±3.40
20:5n-3	0.43±0.23	0.51±0.23	0.38±0.22
22:5n-3	1.71±0.39	1.97±0.43	1.55±0.26**
22:6n-3	4.33±1.25	5.17±1.29	3.77±0.86**
n-3	6.48±1.70	7.65±1.70	5.69±1.19***
PUFA	41.49±3.18	42.67±1.91	40.70±3.63
n-6/n-3	5.77±1.56	4.86±1.41	6.38±1.37**
SFA/PUFA	1.01±0.14	0.96±0.08	1.04±0.17
SFA/n-6	1.20±0.17	1.17±0.13	1.21±0.20
SFA/n-3	6.83±1.85	5.61±1.51	7.65±1.61**
MUFA/PUFA	0.42±0.06	0.39±0.03	0.43±0.07
MUFA/n-6	0.49±0.07	0.48±0.04	0.50±0.09
MUFA/n-3	2.84±0.83	2.31±0.72	3.19±0.72**
SFA/MUFA	2.43±0.20	2.46±0.16	2.41±0.23
20:4/20:3	9.44±2.32	10.67±2.28	8.63±2.03*
C20-22	28.83±2.80	30.26±1.96	27.74±2.80**

Table 2. Pearson correlation coefficients between insulin resistance markers and erythrocyte fatty acid percentages.

	insulin (mU/ml)		HOMA value		BMI (kg/m ²)	
	R	P	r	P	r	P
20:5n-3	- 0.34	0.062	- 0.31	0.093	- 0.40	0.057
22:5n-3	- 0.42	< 0.05	- 0.41	< 0.05	- 0.40	< 0.05
22:6n-3	- 0.51	< 0.01	- 0.46	< 0.01	- 0.30	0.089
n-3	- 0.52	< 0.01	- 0.47	< 0.01	- 0.40	< 0.05

6) and dihomo-gamma linolenic acid (20:3n-6), as a measure of $\Delta 5$ desaturase activity, was significantly lower in the IR group ($p < 0.05$).

A positive correlation was discovered between the fasting insulin level and the resistance parameter (HOMA value) and BMI ($r = 0.60$ and $r = 0.58$, respectively; $p < 0.001$). Correlations were determined between insulin resistance parameters (the fasting insulin level and HOMA value), BMI, and the percentage of erythrocyte n-3 PUFAs (Table 3). The fasting insulin level and HOMA value were inversely related to the percentage of 22:5n-3 ($p < 0.05$) and 22:6n-3 ($p < 0.01$) individual fatty acids, as well as total n-3 ($p < 0.01$). In erythrocyte membrane phospholipids, a negative correlation was also found between BMI and the percentage of 22:5n-3 ($p < 0.05$) and total n-3 PUFAs ($p < 0.01$).

DISCUSSION

We have demonstrated that membrane fatty acid composition is related to insulin sensitivity in overweight/obese patients with hyperlipidemia. Our data indicate a previously unreported strong correlation between erythrocyte phospholipid n-3 PUFA levels and insulin sensitivity in obesity. Since erythrocyte fatty acid composition reflects dietary essential FA intake, this suggests that increased PUFA intake, especially uptake of long-chain n-3 PUFAs, could provide a simple mechanism for increased insulin responsiveness in obese patients on a typical Serbian diet low in n-3 PUFAs.

Since a measure of $\Delta 5$ desaturase activity (the ratio of 20:4n-6 to 20:3n-6) was lower in the IR compared to the NGT group, it follows that alteration of fatty acid metabolism is a factor related to insulin responsiveness in our study. Similar results indicating association of insulin action with $\Delta 5$ desaturase activity have previously been reported in healthy individuals, type 2 diabetic patients, and type 1 diabetic patients (Tilvis and Miettinen, 1985; Clifton and Nestel, 1998; Rodriguez and Christophe, 2005).

The effects of long-chain (LC) n-3 PUFAs on insulin sensitivity were mostly evaluated in human studies. Beneficial effects of n-3 LC-PUFA supple-

mentation on insulin sensitivity were reported in some patient groups (diabetic patients, persons with impaired glucose tolerance), but positive effects were not observed in others (healthy subjects) (Pop-Snijders et al., 1987; Brady et al., 2004). In studies that investigated the relation between erythrocyte fatty acid composition and insulin sensitivity, no correlation was found between the erythrocyte percentage of n-3 PUFAs and fasting insulin levels in healthy individuals and non-obese type 2 diabetic patients (Enriquez et al., 2004; Rodriguez and Christophe, 2005), although the erythrocyte 20:5n-3 level was negatively correlated with insulin levels in obese children (Agostoni et al., 1994). Haugaard et al. (2006) examined the impact of a hypocaloric low-fat dietary intervention on membrane fatty acid composition and insulin sensitivity. They found that skeletal muscle long-chain n-3 PUFAs were an independent predictor of HOMA values in obese subjects (Haugaard et al., 2006).

Differences in the share of n-3 PUFAs in erythrocyte phospholipids between the NGT and IR groups were demonstrated in our study for obese patients with increased LDL-cholesterol levels. We also found that the percentage of some long-chain n-3 PUFAs was in negative correlation with the fasting insulin level and HOMA value, as well as with BMI.

Our results show that the erythrocyte membrane n-3 PUFA level and insulin sensitivity are linked in obese patients with high LDL-cholesterol levels on a typical Serbian diet. Since erythrocyte phospholipid n-3 PUFA levels are also correlated with BMI, it follows that characteristics of the population studied may be highly significant for the effect of n-3 PUFAs on insulin sensitivity. Our results suggest that the erythrocyte membrane n-3 PUFA status could be an important factor determining the influence of n-3 PUFA supplementation on insulin resistance.

A major finding of our cross-sectional study was the inverse relationship observed in the studied population group between erythrocyte n-3 PUFA levels and insulin sensitivity, which could be a characteristic of obesity and low dietary n-3 PUFA intake. Since erythrocyte phospholipid fatty acid composition can be modified with dietary long-chain n-3

PUFAs, their increased intake could be a beneficial nutritional measure in preventing the development of type 2 diabetes in overweight/obese patients on a typical Serbian diet suffering from hyperlipidemia accompanied by high LDL-cholesterol levels (even if their triglyceride levels are not increased).

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ЗАСТУПЉЕНОСТ N-3 ПОЛИНЕЗАСИЋЕНИХ МАСНИХ КИСЕЛИНА У ФОСФОЛИПИДИМА ЕРИТРОЦИТА И ИНСУЛИНСКА СЕНЗИТИВНОСТ КОД ГОЈАЗНИХ ОСОБА ПРИ ТИПИЧНОЈ ИСХРАНИ ПОДНЕБЉА СРБИЈЕ

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Маснокиселински састав мембрана, који се бар делимично може модификовати дијетарним уносом масних киселина, игра важну улогу у сензитивности на инсулин. Циљ ове студије био је испитивање везе између сензитивности на инсулин и маснокиселинског састава фосфолипида еритроцита код гојазних особа. Тридесет умерено гојазних/гојазних недијабетичара са хиперлипипропротеинемијама (укупни холестерол > 5.2 mmol/l, LDL-холестерол > 3.4 mmol/l) подељени су у две групе, користећи НОМА вредност као параметар сензитивности на инсулин: групу са нормалном толеранцом на глюкозу (NTG) и групу са резистенцијом на инсулин (IR). Укупни липиди еритроцита су естраховани и фосфолипиди изоловани танкослојном хрома-

тографијом. После метиловања масне киселине анализирани су гасно-течном хроматографијом. Заступљеност 22:5n-3 ($p < 0.01$), 22:6n-3 ($p < 0.01$), укупних n-3 полинезасићених масних киселина ($p < 0.001$) и однос заступљености 20:4n-6/20:3n-6 (показатеља активности делта-5 десатуразе) ($p < 0.05$) су снижени у IR групи у поређењу са NGT групом. Ниво инсулина наташте и НОМА вредност инвертно су повезани са заступљеношћу 22:5n-3 ($p < 0.05$), 22:6n-3 ($p < 0.01$) и укупним n-3 полинезасићеним киселинама ($p < 0.01$). Наши резултати показују да је ниво n-3 масних киселина у фосфолипидима еритроцита повезан са сензитивношћу на инсулин код гојазних особа на исхрани типичној за поднебље Србије.