

Fatty Acid Composition Indicates Two Types of Metabolic Syndrome Independent of Clinical and Laboratory Parameters

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Summary

Dietary composition and metabolism of fatty acids (FA) influence insulin resistance, atherogenic dyslipidemia and other components of the metabolic syndrome (MS). It is known that patients with MS exhibit a heterogeneous phenotype; however, the relationships of individual FA to MS components have not yet been consistently studied. We examined the plasma phosphatidylcholine FA composition of 166 individuals (68F/98M) with MS and of 188 (87F/101M) controls. Cluster analysis of FA divided the groups into two clusters. In cluster 1, there were 65.7 % of MS patients and 37.8 % of controls, cluster 2 contained 34.3 % of patients and 62.2 % of controls ($P<0.001$). Those with MS within cluster 1 (MS1) differed from individuals with MS in cluster 2 (MS2) by concentrations of glucose ($P<0.05$), NEFA ($P<0.001$), HOMA-IR ($P<0.05$), and levels of conjugated dienes in LDL ($P<0.05$). The FA composition in MS1 group differed from MS2 by higher contents of palmitoleic (+30 %), γ -linolenic (+22 %), dihomo- γ -linolenic (+9 %) acids and by a lower content of linoleic acid (-25 %) (all $P<0.01$). These FA patterns are supposed to be connected with the progression and/or impaired biochemical measures of MS (lipolysis, oxidative stress, dysglycemia, and insulin resistance).

Key words

Metabolic syndrome • Fatty acids • Delta desaturase activities • Cluster analysis

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Introduction

Metabolic syndrome (MS) is one of the most important health issues in developed countries over recent decades. Its increasing prevalence ranges between 20-30 % in the middle aged (Bruce and Byrne 2009, Ervin 2009, Sethom *et al.* 2011). MS significantly increases the risk for cardiovascular diseases, type 2 diabetes mellitus and other diseases (neuropsychiatric disorders and some cancers) (Bruce and Byrne 2009).

MS represents a cluster of cardiovascular risk factors connected with insulin resistance, visceral obesity, disturbed glucose metabolism, atherogenic dyslipidemia and arterial hypertension. These components of MS are complemented with chronic low-grade inflammation, coagulopathy, endothelial dysfunction and oxidative stress. It is supposed that the link between visceral obesity and metabolic disturbances, such as insulin resistance, impaired secretion of insulin or dyslipidemia, is caused by dysregulation of FA metabolism and/or chronic low-grade inflammation of the adipose tissue (Kalupahana *et al.* 2012).

MS develops in consequence of an increased energy intake and physical inactivity resulting in overweight and obesity; other important factors include composition of the diet, aging of the population and genetic background (Bruce and Byrne 2009, Lottenberg *et al.* 2012, Murphy *et al.* 2013, Walsh *et al.* 2014).

Epidemiological data indicate that increased fat intake is connected with a higher prevalence of overweight and obesity. The individual components of MS are variably influenced by saturated fatty acids

(SFA), monounsaturated fatty acids (MFA) and polyunsaturated fatty acids (PUFA) of n-3 as well as n-6 series, probably *via* their effects on plasma lipids (lipoproteins), blood pressure, insulin secretion and its action on target tissues and low-grade inflammation (Králová Lesná *et al.* 2013). Also, recent findings attribute an important influence on MS components to *trans* isomers of FA (Lottenberg *et al.* 2012).

Fatty acid profiles of individual lipid classes, especially those of cholesteryl esters and phosphatidylcholine, reflect dietary FA intake over several-weeks (i), FA metabolism (SFA synthesis, desaturation and elongation processes) (ii), as well as both enzymatic (β -oxidation) and nonenzymatic (lipoperoxidation) degradation (iii). The resulting profiles of FA are also influenced by racial, ethnic, geographic, genetic factors and concomitant diseases (Hodson *et al.* 2008).

The FA profiles in MS are characterized by an increased content of SFA [especially palmitic acid (16:0)], palmitoleic (16:1n-7), γ -linolenic (18:3n-6) and dihomo- γ -linolenic (20:3n-6) acids, accompanied by a lower concentration of linoleic acid (18:2n-6). Moreover, there is enhanced activity of delta 9 desaturase (D9D, synonym for stearoyl-CoA desaturase 1, SCD-1) and delta 6 desaturase (D6D) together with lower activity of delta 5 desaturase (D5D) (Warensjö *et al.* 2005, Žák *et al.* 2007, Paillard *et al.* 2008, Kawashima *et al.* 2009, Mayneris-Perxachs *et al.* 2014).

Lower content of linoleic acid, higher ratios of both γ -linolenic and dihomo- γ -linolenic acids reflecting increased activities of D6D, as well as a decreased activity of D5D were described in obese children with other components of MS, but not in simple obesity. Analogous changes in FA profiles resembling insulin resistance were described in patients with recent myocardial infarction (Marangoni *et al.* 2014) and depressive disorder (Vařeka *et al.* 2012). FA profiles in adolescents with MS correlate not only with insulin resistance, but also with systemic markers of inflammation (Decsi *et al.* 2000, Klein-Platat *et al.* 2005). It must be stated that none of the cited papers have presented all these changes simultaneously.

Since MS is a heterogeneous group of diseases, with a different genetic background, as well as absence of a uniting definition based on various pathophysiological mechanisms (e.g. from interaction of genetic and environmental factors), the serum FA profile is not consistent. The aim of this study was to analyze the FA

composition in the MS and control groups with the help of cluster analysis. We tried to characterize the patients with MS by cluster analysis of FA profile independently of clinical and laboratory parameters.

Methods

Study design

This study was carried out at the 4th Department of Internal Medicine of General University Hospital from January 2009 to September 2013. The study protocol was approved by the Joint Ethical Committee of the General University Hospital and the 1st Medical Faculty, Charles University in Prague. Written informed consent was obtained from all participants. In this period of time, a total of 354 persons were examined at the Lipid Clinic of the 4th Department of Medicine, the 1st Faculty of Medicine, Charles University and the General University Hospital in Prague.

Participants

The study group consisted of 188 (101 men and 87 women) controls (CON) and 166 patients (98 men and 68 women) with MS. MS were diagnosed according to the International Diabetes Federation criteria (Alberti *et al.* 2006). Combinations of individual MS components and their prevalence are shown in Table 1. All samples were marked with unique anonymized identification numbers, and the data was merged only after the assays had been completed.

The control group consisted of 42 healthy subjects (22 men and 20 women) that were recruited from employees of the General University Hospital, and 146 probands (79 men and 67 women) with at least one component of MS who failed to fulfil the diagnostic criteria for MS (see Table 1). None of control subjects was treated for dyslipidemia, hypertension or diabetes mellitus (prediabetic state, respectively).

Exclusion criteria for both groups were the following: current antioxidant, lipid-lowering and antidiabetic medication, excessive alcohol consumption (>30 g/day), hormonal replacement therapy, supplementation with polyunsaturated fatty acids (both of n-3 and n-6 families), manifestation of cardiovascular and/or cerebrovascular diseases, type 1 diabetes mellitus, liver (with exception of nonalcoholic fatty liver disease) and kidney diseases (creatinine >130 μ mol/l), microalbuminuria (urinary albumin 30–300 mg/day),

hypothyroidism as well as recent infections and malignancies.

Table 1. Components of the metabolic syndrome in studied groups.

Control group (n=188)	
<i>without any component^a</i>	42 (22.3) ^b
<i>W</i>	44 (23.4)
<i>TG</i>	11 (5.8)
<i>HDL-C</i>	5 (2.7)
<i>fs-G</i>	4 (2.1)
<i>W + TG</i>	42 (22.3)
<i>W + BP</i>	22 (11.7)
<i>W + fs-G</i>	7 (3.7)
<i>W + HDL-C</i>	6 (3.2)
<i>BP + fs-G</i>	5 (2.6)
Metabolic syndrome (n=166)	
<i>W + TG + HDL-C</i>	44 (26.5)
<i>W + TG + BP</i>	29 (17.5)
<i>W + TG + BP + fs-G</i>	19 (11.4)
<i>W + TG + HDL-C + fs-G</i>	18 (10.8)
<i>W + TG + fs-G</i>	17 (10.2)
<i>W + BP + fs-G</i>	13 (7.8)
<i>W + TG + HDL-C + BP</i>	12 (7.2)
<i>W + TG + HDL-C + BP + fs-G</i>	11 (6.6)
<i>W + HDL-C + BP</i>	2 (1.2)
<i>W + HDL-C + fs-G</i>	1 (0.6)

^a – presence (absence, respectively) of MS component(-s);
^b – number of cases (%); fs-G – fasting serum glucose; BP – blood pressure; W – waist circumference; TG – triglycerides; HDL-C – cholesterol in HDL. Metabolic syndrome: waist circumference >94 (88, respectively) cm for men (women, respectively), and further at least 2 factors of following: triglycerides >1.70 mmol/l; HDL-C<1.04 (1.29, respectively) mmol/l for men (women, respectively); fasting serum glucose ≥5.60 mmol/l (or presence of diabetes mellitus type 2); elevated BP >130/85 mm Hg (or antihypertensive therapy).

Blood sampling

Blood samples were taken after 12 h of fasting. Routine biochemical and hematological analyses were performed immediately; samples for special analyses were stored at -70 °C until use.

Dietary habits

The nutritional intake of the main dietary components, based on a regular 7-day dietary

questionnaire, was assessed in all study subjects. The nutritional data were analyzed by Nutrimaster SE software, version 1.0.

Anthropometry

Basal clinical and anthropometrical data, including assessment of body fat, were examined using standard methods, as described previously (Žák *et al.* 2007).

Laboratory measurements

Plasma concentrations of total cholesterol and triglycerides were measured by enzymatic-colorimetric methods (Boehringer, Mannheim, Germany). HDL-C was determined in the supernatant after precipitation of lipoproteins B by PTA/Mg²⁺, using the kit from the same manufacturer. Concentration of apolipoprotein (apo) B was measured by a Laurell rocket electroimmunoassay using standard and specific antibodies (Behringwerke, Marburg, Germany). Immunoreactive insulin was determined by a RIA method using double monoclonal antibodies (Insulin IRMA, Imunotech Prague, Czech Republic). Concentrations of conjugated dienes in precipitated LDL (CD-LDL) were determined spectrophotometrically (Ahotupa *et al.* 1996). The concentrations of non-esterified fatty acids (NEFA) were determined by enzymatic-colorimetric method (NEFA, Randox Laboratories, U.K.).

Fatty acid patterns in main plasma lipid classes were examined by analytical procedures described previously (Tvrzická *et al.* 2002). The method variability presented as relative standard deviation (RSD) ranged from 1.07 % for palmitic acid (16:0) to 8.60 % for 16:1n-9. The relevant variability data (RSD) were for dihomoo-γ-linolenic 1.25 %; stearic 0.76 %, myristic 8.06 %; docosahexaenoic 2.38 %; docosapentaenoic 1.91 %, and linoleic acids 0.725 %.

Calculated parameters

The Homeostasis model assessment method, HOMA-IR, was used as an index of insulin resistance (Matthews *et al.* 1985). Desaturase activities were estimated using FA product/precursor ratios (Žák *et al.* 2007).

Statistical analyses

Prior analyses, all data were cleaned and pre-processed: extreme values were examined and double-checked. After that, a power-transformation was

performed to achieve symmetry and constant variance. Data are expressed as mean and standard deviation or median and inter-quartile range (IQR, 25th-75th percentile) in cases of non-Gaussian distribution of data. Normality of the distribution was tested by the Shapiro-Wilks W test. Comparisons between the groups were carried out by the independent t-test, and the Wilcoxon test, respectively. P-value was adjusted for multiple comparisons using Benjamini-Hochberg correction.

Cluster analysis

Cluster analysis was performed in two steps. In the first step, the number of individual fatty acids in plasma phosphatidylcholine was reduced, and in the second step the grouping of subjects into the clusters was carried out.

To reduce number of individual fatty acids, a linear discriminant analysis was performed with stepwise forward variable selection, using the Wilk's lambda criterion: an initial model was created from the variable that mostly separated the groups. This model was then iteratively extended by including further variables depending on the Wilk's lambda criterion. Incorporation of additional variables stopped when the newly added variable did not show a statistically significant improvement ($P>0.05$). We used 22 initially analyzed FAs of all the probands to separate CON and MS groups using linear discriminant analysis. This resulted in overall 69.8 % correct classification. Final correct classification was 74.5 % for CON, 64.5 % for MS, respectively. Variables subjected into the linear discriminant analysis were dihomo- γ -linolenic (20:3n-6; $F=30.41$), stearic (18:0; $F=24.2$), myristic (14:0; $F=21.55$), docosahexaenoic (22:6n-3; $F=17.66$), docosapentaenoic (22:5n-3; $F=14.92$), and linoleic (18:2n-6, $F=13.19$) acids. These six individual fatty acids were included into the cluster analysis. On the selected six fatty acids, a hierarchical clustering was applied using the Ward's method with Euclidean measure (Ward 1963).

All the statistical analyses were performed using the R software version 3.1.0 (The R Development Core Team 2014).

Results

Basic clinical and biochemical parameters of the subjects with MS and in the CON group are shown in Table 2. As expected, subjects with MS differed from controls in nearly all parameters examined. There was no

difference in sex ratio between MS and CON. Table 3 shows the composition of FA in plasma phosphatidylcholine and the corresponding derived parameters. In MS patients, significantly increased concentrations of palmitoleic (16:1n-7), stearic (18:0), dihomo- γ -linolenic (20:3n-6) acids, and the sum of SFA (Σ SFA) were found. In contrast, patients with the MS had a decreased content of linoleic acid (18:2n-6), and the sum of PUFA n-6 (Σ PUFAn-6).

Table 2. Clinical and biochemical characteristics of the studied groups.

	Metabolic syndrome	Controls
Number of persons	166	188
Gender (M/F)	98/68	101/87
Age (years)	55.2 ± 10.6 ^a	54.5 ± 11.9
Weight (kg)	88.4 ± 15.6 ^a ***	77.4 ± 14.4
BMI (kg.m ⁻²)	29.8 ± 4.0 ^a ***	26.5 ± 4.1
Waist circumference (cm)	103 ± 10 ^a ***	91 ± 12
Systolic BP (mm Hg)	141 ± 17 ^a ***	129 ± 14
Diastolic BP (mm Hg)	88 ± 10 ^a ***	80 ± 9
Relative fat mass (%)	33.9 ± 6.9 ^a ***	30.0 ± 7.6
Fat mass (kg)	29.8 ± 7.6 ^a ***	23.3 ± 7.9
Glucose (mmol/l)	5.96 ± 1.92 ^a ***	4.99 ± 1.27
Insulin (mU/l)	10.70/7.24 ^b ***	7.70/5.56
HOMA-IR (ratio)	2.59/2.14 ^b ***	1.62/1.20
TC (mmol/l)	6.42 ± 1.43 ^{a*}	6.05 ± 1.26
TG (mmol/l)	2.69/2.09 ^b ***	1.40/0.83
HDL-C (mmol/l)	1.27 ± 0.35 ^a ***	1.53 ± 0.37
NEFA (mmol/l)	0.60/0.36 ^b ***	0.53/0.36
Apo B (g/l)	1.36 ± 0.34 ^a ***	1.21 ± 0.37
CD-LDL (μmol/l)	66.7/23.7 ^b ***	56.4/22.9

^a – mean ± SD; ^b – median/IQR. Statistical analysis: Student's t-test, or Wilcoxon test (in cases of non-Gaussian distribution of data); p-values were adjusted for multiple comparisons using Benjamini-Hochberg corrections: * $P<0.05$, ** $P<0.01$, *** $P<0.001$. M – males; F – females; BMI – body mass index; BP – blood pressure; NEFA – nonesterified fatty acids; CD-LDL – conjugated dienes in LDL; TC – total cholesterol; TG – triglycerides; LDL – low density lipoproteins; HDL – high density lipoproteins; Apo – apolipoprotein; HOMA-IR – homeostasis model assessment for insulin resistance (f-insulin (μU/ml) × f-glucose (mmol/l)/22.5); IQR – interquartile range.

A significant increase in activities of delta 9 desaturase for palmitoleic (D9D16) and delta 6 desaturase (D6D), as well as a decreased activity of delta 5 desaturase (D5D), were found in MS.

Table 3. Plasma phosphatidylcholine fatty acid composition of the studied groups.

Fatty acid ^c	Metabolic syndrome	Controls
<i>Number of persons</i>	166	188
14:0	0.28 ± 0.08 ^a	0.28 ± 0.09
16:0	29.68 ± 1.73 ^a	29.4 ± 1.60
16:In-9	0.11 ± 0.03 ^a	0.11 ± 0.03
16:In-7	0.59/24 ^{b** g}	0.52/0.20
18:0	14.44 ± 1.28 ^{a ***}	13.84 ± 1.14
18:In-9	10.06 ± 1.62 ^a	10.03 ± 1.73
18:In-7	1.56 ± 0.36 ^a	1.61 ± 0.37
18:2n-6	21.94 ± 3.16 ^{a ***}	23.54 ± 3.00
18:3n-6	0.08/0.05 ^b	0.08/0.05
18:3n-3	0.19/0.08 ^b	0.21/0.10
20:In-9	0.14 ± 0.04 ^a	0.14 ± 0.04
20:2n-6	0.41 ± 0.12	0.40 ± 0.11
20:3n-6	3.35 ± 0.70 ^{a ***}	2.98 ± 0.56
20:4n-6	10.99 ± 2.05 ^a	10.91 ± 1.83
20:5n-3	0.94/0.50 ^b	0.92/0.48
22:4n-6	0.31 ± 0.08 ^a	0.32 ± 0.07
22:5n-6	0.20 ± 0.06 ^a	0.20 ± 0.05
22:5n-3	0.90 ± 0.18 ^a	0.91 ± 0.17
22:6n-3	3.56 ± 1.11 ^a	3.36 ± 0.95
ΣSFA	44.47 ± 1.68 ^{a ***}	43.59 ± 1.43
ΣMFA	12.48 ± 2.04 ^a	12.45 ± 2.12
ΣPUFA n-6	37.22 ± 3.20 ^{a **}	38.38 ± 2.77
ΣPUFA n-3	5.79 ± 1.77 ^a	5.55 ± 1.53
D9D16 ^d	0.020/0.008 ^{b**}	0.018/0.007
D9D18 ^e	0.700 ± 0.120 ^a	0.730 ± 0.150
D6Dn6 ^e	0.004/0.003 ^{b**}	0.003/0.002
D5Dn6 ^f	3.45 ± 1.08 ^{a **}	3.79 ± 0.98

^a – mean ± SD (mol%); ^b – median/IQR; ^c – shorthand notation of fatty acids: number of carbon atoms: number of double bonds n – number of carbon atom from the methyl end to the nearest double bond. Only relevant fatty acids are presented; ^d – D9D16, delta 9 desaturase for palmitoleic acid (16:1n-7/16:0); ^e – D9D18, delta 9 desaturase for oleic (18:1n-9/18:0); ^f – D6D, delta 6 desaturase (18:3n-6/18:2n-6); ^g – D5D, delta 5 desaturase (20:4n-6/20:3n-6); statistical analysis: Wilcoxon test; p – values were adjusted for multiple comparisons using Benjamini-Hochberg corrections: * P<0.05, ** P<0.01, *** P<0.001. IQR – interquartile range; ΣSFA – total content (the sum) of saturated fatty acids; ΣMFA – the sum of monounsaturated fatty acids; Σn-6 PUFA – the sum of polyunsaturated fatty acids n-6 family; Σn-3 PUFA – the sum of polyunsaturated fatty acids n-3 family.

Based on the cluster analysis that includes six selected FA obtained from linear discriminant analysis, two clusters were found both in the CON and MS groups. In the MS group, 109 patients (65.7 %) were classified as

Table 4. Clinical and biochemical characteristics of metabolic syndrome according to cluster analysis.

	Cluster 1	Cluster 2
<i>Number of persons</i>	109	57
<i>Gender (M/F)</i>	67/42	31/26
<i>Age (years)</i>	54.6 ± 11.1 ^a	56.3 ± 9.5
<i>Weight (kg)</i>	89.9 ± 15.7 ^a	85.5 ± 15.0
<i>BMI (kg.m⁻²)</i>	30.2 ± 4.1 ^a	29.1 ± 3.8
<i>Waist circumference (cm)</i>	104 ± 11 ^{a*}	101 ± 9
<i>Systolic BP (mm Hg)</i>	141 ± 15 ^a	142 ± 15
<i>Diastolic BP (mm Hg)</i>	88 ± 9 ^a	89 ± 10
<i>Relative fat mass (%)</i>	33.3 ± 6.8 ^a	35.3 ± 6.9
<i>Fat mass (kg)</i>	29.7 ± 7.7 ^a	29.9 ± 7.4
<i>Phenotypes of metabolic syndrome</i>		
<i>MS_{gly}</i>	6 (5.5) ^{c, d *}	7 (12.3)
<i>MS_{glylip}</i>	50 (45.9)	16 (28.1)
<i>MS_{lip}</i>	53 (48.6)	34 (59.6)
<i>Glucose (mmol/l)</i>	6.24 ± 2.22 ^{a**}	5.44 ± 0.96
<i>Insulin (mU/l)</i>	11.75/7.17 ^b	9.40/5.83
<i>HOMA-IR (ratio)</i>	3.03/2.30 ^{b*}	2.07/1.94
<i>TC (mmol/l)</i>	6.51 ± 1.53	6.26 ± 1.21
<i>TG (mmol/l)</i>	2.86/3.09 ^b	2.43/1.60
<i>HDL-C (mmol/l)</i>	1.25 ± 0.34	1.30 ± 0.36
<i>NEFA (mmol/l)</i>	0.69/0.73 ^{b***}	0.44/0.40
<i>Apo B (g/l)</i>	1.36 ± 0.38	1.36 ± 0.26
<i>CD-LDL (μmol/l)</i>	70.9/34.9 ^{b*}	60.9/19.7

^a – mean ± SD (mol%); ^b – median/IQR; ^c – number of subjects (%) in individual phenotypes of MS. Statistical analysis: Student's t-test, or Wilcoxon test (in cases of non-Gaussian distribution of data); p – values were adjusted for multiple comparisons using Benjamini-Hochberg corrections: * P<0.05, ** P<0.01, *** P<0.001. ^d – Fisher's exact test: * P<0.05. Abbreviations: see Table 2. Characterization of metabolic syndrome phenotypes according to combination of metabolic syndrome components: MS_{gly} – increased waist, elevated BP (or hypertension), increased fasting serum glycemia; MS_{lip} – increased waist, elevated TG and/or decreased HDL-C, elevated BP (or hypertension) (alternatively); MS_{glylip} – increased waist, elevated TG and/or decreased HDL-C, increased fasting serum glycemia, elevated BP (alternatively).

cluster 1 (MS1) and 57 patients (34.3 %) were classified as cluster 2 (MS2). Conversely, in the control group, 71 probands (37.8 %) were classified as cluster 1 (CON1) and 117 subjects (62.2 %) were classified as cluster 2 (CON2). These results indicated a non-random distribution of subjects in both groups into cluster 1 and cluster 2 ($\chi^2=26.35$; P<0.001). This means that MS was present mainly in cluster 1, while the CON probands presented mainly in cluster 2.

The basic clinical and biochemical characteristics of patients with MS according to cluster analysis are shown in Table 4. Patients in cluster 1 had a more detrimental metabolic profile in comparison with patients in cluster 2. There were no statistically significant differences between both clusters in sex ratio, age, BMI, fat mass, systolic and diastolic blood pressure, total cholesterol, HDL-C, triglycerides, and apoB concentrations. Between both clusters of MS, there was a non-random distribution of patients with different phenotypes of MS. In cluster 1, in comparison with cluster 2, the phenotype of MS with fasting hyperglycemia and dyslipidemia (MS_{glyclip}) was more prevalent ($P<0.05$). The patients with MS in cluster 1 (MS1) were characterized by increased waist circumference and HOMA-IR (both $P<0.05$). Moreover, they had increased glucose ($P<0.01$), NEFA ($P<0.001$), and CD-LDL concentrations ($P<0.05$). There were no statistically significant differences in the concentration of hs-CRP (data not shown).

The plasma FA patterns, including derived parameters, of MS patients categorized according to cluster analysis are shown in Table 5. Apart from FAs used in the cluster analysis (see Table 5), the levels of palmitic (16:0), palmitoleic (16:1n-7), oleic (18:1n-9), vaccenic (18:1n-7), γ -linolenic (18:3n-6), eicosapentaenoic (20:5n-3), and arachidonic (20:4n-6) acids were increased in cluster 1. In addition, patients in cluster 1 were characterized by higher activities of D9D for palmitoleic acid (D9D16), D9D for oleic acid (D9D18), and D6D. Contrary to the comparison of the whole MS with CON, there was no significant difference between cluster 1 and cluster 2 in the D5D activity.

In comparison of controls in cluster 1 (CON1) with those in cluster 2 (CON2) significantly increased BMI (27.5 ± 4.5 vs 25.9 ± 3.7 kg/m², $P<0.01$), and waist circumference (95.5 ± 12.2 vs 88.9 ± 10.6 cm, $P<0.01$) were found. After ANCOVA adjustment (with BMI and waist circumference as covariates), probands in CON1 had, in comparison with CON2, increased concentrations of palmitoleic (16:1n-7) ($0.60/0.29$ vs $0.49/0.18$, median/IQR, mol%, $P<0.01$), oleic (18:1n-9) (10.14 ± 2.30 vs 9.57 ± 1.91 , $P<0.01$), and arachidonic (20:4n-6) (11.66 ± 1.91 vs 10.46 ± 1.62 , $P<0.01$) acids. In addition, higher contents of Σ SFA (44.48 ± 1.68 vs 43.10 ± 1.43 , $P<0.001$), Σ MFA (12.59 ± 2.68 vs 11.74 ± 2.20 , $P<0.001$), Σ PUFA n-3 (5.76 ± 1.50 vs 4.88 ± 1.42 , $P<0.01$), as well as a lower content of Σ PUFA n-6 (36.69 ± 3.36 vs 39.64 ± 2.89 , $P<0.001$) were found. Similarly, probands in

Table 5. Plasma phosphatidylcholine fatty acid composition of metabolic syndrome according to cluster analysis.

Fatty acid ^c	Cluster 1	Cluster 2
Number of persons	109	57
14:0 ^h	0.28 ± 0.08^a	0.28 ± 0.08
16:0	$29.89\pm1.87^{a**}$	29.29 ± 1.34
16:1n-9	0.11 ± 0.03^a	0.10 ± 0.02
16:1n-7	$0.63/0.28^{b***}$	$0.48/0.18$
18:0 ^h	14.59 ± 1.34^a	14.16 ± 1.12
18:1n-9	10.50 ± 1.56^a	9.20 ± 1.38
18:1n-7	$1.62\pm0.38^{a***}$	1.45 ± 0.30
18:2n-6 ^h	20.17 ± 2.07^a	25.31 ± 1.88
18:3n-6	$0.09/0.05^{b*}$	$0.07/0.04$
18:3n-3	$0.20/0.08^b$	$0.19/0.08$
20:1n-9	0.14 ± 0.05^a	0.13 ± 0.03
20:2n-6	0.42 ± 0.13^a	0.40 ± 0.10
20:3n-6 ^h	3.45 ± 0.70^a	3.16 ± 0.68
20:4n-6	$11.34\pm2.03^{a**}$	10.34 ± 1.95
20:5n-3	$1.09/0.49^{b***}$	$0.80/0.28$
22:4n-6	0.32 ± 0.08^a	0.29 ± 0.06
22:5n-6	0.21 ± 0.06^a	0.18 ± 0.05
22:5n-3 ^h	0.94 ± 0.19^a	0.82 ± 0.13
22:6n-3 ^h	3.76 ± 1.18^a	3.20 ± 0.85
Σ SFA	$44.85\pm1.84^{a***}$	43.79 ± 1.04
Σ MFA	$13.08\pm1.96^{a***}$	11.40 ± 1.74
Σ PUFA n-6	$35.84\pm2.79^{a***}$	39.70 ± 2.28
Σ PUFA n-3	$6.19\pm1.95^{a***}$	5.07 ± 1.06
D9D16 ^d	$0.021/0.01^{b***}$	$0.016/0.005$
D9D18 ^e	$0.726\pm0.120^{a**}$	0.655 ± 0.118
D6Dn6 ^f	$0.005/0.003^{b***}$	$0.003/0.002$
D5Dn6 ^g	3.45 ± 1.06^a	3.46 ± 1.13

^a – mean \pm SD (mol%); ^b – median/IQR; ^c – shorthand notation of fatty acids: number of carbon atoms: number of double bonds n – number of carbon atom from the methyl end to the nearest double bond. Only relevant fatty acids are presented. ^d – D9D16, delta 9 desaturase for palmitoleic acid (16:1n-7/16:0); ^e – D9D18, delta 9 desaturase for oleic (18:1n-9/18:0); ^f – D6D, delta 6 desaturase (18:3n-6/18:2n-6); ^g – D5D, delta 5 desaturase (20:4n-6/20:3n-6); statistical analysis: Wilcoxon test; p – values were adjusted for multiple comparisons using Benjamini-Hochberg corrections: * $P<0.05$, ** $P<0.01$, *** $P<0.001$. ^h – Fatty acids used in cluster analysis separating MS patients into cluster 1 and cluster 2: dihomo- γ -linolenic (20:3n-6), stearic (18:0), myristic (14:0), docosahexaenoic (22:6n-3), docosapentaenoic (22:5n-3), and linoleic (18:2n-6) acids. Therefore, the respective P values for these FAs are not indicated. IQR – interquartile range; Σ SFA – total content (the sum) of saturated fatty acids; Σ MFA – the sum of monounsaturated fatty acids; Σ n-6 PUFA – the sum of polyunsaturated fatty acids n-6 family; Σ n-3 PUFA – the sum of polyunsaturated fatty acids n-3 family.

CON1 presented increased activities of D9D for palmitoleic, (0.020/0.010 vs 0.017/0.06, P<0.01), and D9D for oleic (0.729/0.164 vs 0.708/0.141, P<0.05) acids, along with increased activity of D6D (0.004/0.003 vs 0.003/0.002, P<0.001). The differences in FA profiles between cluster 1 and cluster 2 in CON group were similar to those observed for MS 1 and MS 2 cluster.

The control persons did not differ from the patients with MS in the daily energy intake, the energy content of proteins, fats as well as carbohydrates. No differences were observed in the intakes of dietary FA – saturated FA, monounsaturated FA and PUFA (as sum of both n-3 and n-6 PUFA). Similarly, there were not statistically significant differences of macronutrients consumed in diets between the probands in cluster 1 and those in cluster 2 of MS (data not shown).

Discussion

The metabolic syndrome is a heterogeneous clustering of metabolic and non-metabolic abnormalities that are associated with various degrees of cardiovascular risk. Most clinical studies use either the diagnostic criteria according to ATP-III (NCEP Expert Panel, 2001), or those according to IDF (Alberti *et al.* 2006), which require the presence of central (abdominal) obesity and minimally two of four further risk factors (elevated glucose, triglycerides and arterial blood pressure, low HDL-C levels). Obesity is a key factor in the development of MS; increased body fat percentage unfavorably influences insulin resistance, oxidative stress and chronic systemic inflammation, as well as prothrombotic and proatherogenic metabolic traits (Phillips *et al.* 2013). In comparison with BMI, the values of waist circumference correlate more tightly with hemodynamic (blood pressure, endothelial dysfunction), hemostatic (coagulation, fibrinolysis), and metabolic parameters (plasma lipids, glucose, uric acid, insulin sensitivity) involved in MS (Savva *et al.* 2013). Visceral fat displays a high degree of lipolysis; it is almost exclusively perfused by the portal vein which delivers triglycerides, fatty acids and adipokines directly into the liver; this fact significantly influences hepatic metabolic processes (Carey 1998, Miranda *et al.* 2005).

Both definitions of MS, according to ATP-III and IDF, allow considerable inter-individual differences concerning the presence of the constitutive components (risk factors). In the German study PROCAM, for example, 72.9 % of participants with MS had elevated

blood pressure, 32 % dyslipidemia with dysglycemia, and 20 % dyslipidemia without dysglycemia (Assmann and Seedorf 2009). In our study, dyslipidemia (dyslipidemia with and without dysglycemia) was almost twice more frequent in comparison with the PROCAM study. On the other hand, only 51.8 % of patients in our MS group had elevated blood pressure (hypertension, respectively).

The MS_{glylip} phenotype (MS with dyslipidemia and dysglycemia) is associated with high plasma concentrations of NEFA that point to insulin resistance of the adipose tissue. One Finnish prospective study has shown that increased NEFA levels predict the development of hyperglycemia and type 2 diabetes mellitus (Mahendran *et al.* 2013). Furthermore, elevated NEFA can contribute to low-grade systemic inflammation. In this study, we observed higher concentrations of CD-LDL in the MS group. The levels of CD-LDL partially reflect systemic oxidative stress and/or the presence of minimally modified LDL particles (Ahotupa *et al.* 1996). In our previous study, subjects with MS had low values of the antioxidative potential, mainly due to decreased activities of paraoxonase 1 (Vávrová *et al.* 2013).

To extend upon the investigations of visceral fat and NEFA concentrations, several recent clinical studies have concentrated on plasma fatty acid patterns. The spectrum of plasma FAs in MS depends both on the quality of dietary fat and on the metabolic processes (e.g. endogenous synthesis, activity of desaturases) that are partly influenced by the genotype (Hodson *et al.* 2008).

In this study, we performed a cluster analysis of FA profiles in plasma phosphatidylcholine for the participants of the MS and CON groups. Cluster analysis is a method for statistical data exploration that groups a set of probands in such a way that probands in the same group (called a cluster) are more similar to each other than to those in other groups (clusters). In our MS group, this analysis rendered two clusters independent of clinical and biochemical parameters, that were characterized by six FAs: dihomo-γ-linolenic (20:3n-6), stearic (18:0), myristic (14:0), docosahexaenoic (22:6n-3), docosapentaenoic (22:5n-3), and linoleic (18:2n-6) acids. In comparison with cluster 2, cluster 1 displayed a higher consistency and a more adverse risk profile (waist circumference, HOMA insulin resistance index, concentrations of NEFA, glucose and CD-LDL). To our knowledge, this is the first phenotyping of MS based on cluster analysis of plasma FA.

The FA profiles in cluster 1 differed from those in cluster 2 in higher proportions of ΣSFA, ΣMFA and ΣPUFA n-3, while ΣPUFA n-6 were decreased. Enhanced proportion of ΣSFA was particularly due to palmitic (16:0) and stearic (18:0) acids, that of ΣMFA to palmitoleic (16:1n-7), oleic (18:1n-9) and vaccenic (18:1n-7) acids; lower ΣPUFA n-6 were caused by the drop in concentrations of linoleic acid (18:2n-6). The patients in cluster 1 had higher activities of D9D for palmitoleic acid, D9D for oleic acid and D6D. Elevated activities of D6D were connected with higher concentrations of γ-linolenic (18:3n-6) and dihomo-γ-linolenic (20:3n-6) acids. Our previous study has shown significant positive correlations between the number of MS components and the concentrations of palmitic, palmitoleic, stearic and dihomo-γ-linolenic acids, as well as negative correlations for linoleic acid. ΣSFA and activities of both D9D for palmitoleic acid and D6D correlated positively, ΣPUFA n-6 and activities of D5D negatively with the number of MS components (Žák *et al.* 2007). In several other studies, SFA, MFA, D9D and D6D correlated positively with cardiovascular risk factors (BMI, waist circumference, HOMA-IR, glycemia, triglycerides, blood pressure), while negative correlations were proved with both PUFA n-3 and n-6 series (Lee *et al.* 2008, Sethom *et al.* 2011, Mayneris-Perxachs *et al.* 2014). In a long-term prospective study, serum FA composition and activities of desaturases predicted the development of MS in middle-aged men (Warensjö *et al.* 2005).

The proportions of MFA, especially palmitoleic, oleic and vaccenic acids are indicators of *de novo* synthesis of FAs; higher concentrations of MFA were shown to predict an elevated risk of sudden cardiac arrest (Wu 2011). Increased level of palmitoleic acid was associated with enhanced lipogenesis induced by carbohydrates (Aarsland *et al.* 1997), as well as with hyperglycemia in Indian women in Peru (Lindgärde *et al.* 2006). Middle-aged Chinese with higher concentrations of palmitoleic acid in erythrocyte phospholipids had higher levels of several MS components and decreased concentrations of adiponectin (Zong *et al.* 2012).

We did not prove decreased concentrations of n-3 PUFA, which are – according to several authors – associated with the progression of MS (Warensjö *et al.* 2006, Lee *et al.* 2008, Chien *et al.* 2011, Mahendran *et al.* 2013). On the contrary, a prospective Finnish study failed to prove any association between the incidence of MS and n-3 PUFA, while relative proportions of n-6 PUFA

correlated negatively (Vanhala *et al.* 2012). In our study, elevated ΣPUFA n-3 in cluster 1 can be explained by a more pronounced decrease in ΣPUFA n-6, expressed in relative concentrations. A similar phenomenon was described for FA composition in patients with anorexia nervosa (Žák *et al.* 2005).

Decreased plasma concentrations of n-6 PUFA (mainly due to low concentrations of linoleic acid) as a hallmark of MS were observed in obese adults (Vessby 2003), children (Decsi *et al.* 2000), and postinfarction middle-aged men (Leskinen *et al.* 2005). Low concentrations of linoleic acid were found to be associated with the progression of MS (Laaksonen *et al.* 2002, Warensjö *et al.* 2006). The decrease in linoleic acid content can be explained by a higher degree of oxidative stress, lipoperoxidation, and synthesis of proinflammatory eicosanoids (Hardwick *et al.* 2013).

Patients with MS were shown to have increased activities of the enzyme stearoyl-CoA desaturase-1 (SCD1), synonym delta 9-desaturase (D9D). The preferred substrates for this enzyme are stearoyl-CoA and palmitoyl-CoA, which are converted to oleoyl-CoA (18:1n-9) and palmitoleyl-CoA (16:1n-7), respectively. The human diet contains only very small amounts of palmitoleic acid, whereas oleic acid is usually present in abundance. In our study, activities of D9D for palmitoleic acid (D9D16) and D9D for oleic acid (D9D18) were significantly higher in cluster 1 than in cluster 2; high activities of D9D18 suggest a low dietary intake of fats rich in oleic acid. Increased activities of D6D are associated with hyperinsulinemia and higher BMI (Decsi *et al.* 2000, Vessby 2003), while decreased activities of D5D, specific for MS, are independent of BMI and physical activity (Warensjö *et al.* 2005). In a group of Japanese men, low D5D activities predicted the development of abdominal obesity (Kawashima *et al.* 2009).

We did not prove statistically significant differences in dietary habits between MS and CON subjects, as well as between MS patients in cluster 1 and in cluster 2. It is known that dietary assessment methods have many limitations. Among them, the most important are deficient data (e.g. finite food list, no quantification or imprecise estimation of portion size, absence of dietary details, underreporting of data), and the measurement error. The accuracy for individual dietary component reaches maximally 70 to 80 % (Thompson and Subar 2013).

The limitations of our study are a relatively

small number of participants and the cross sectional design. Estimated activities of desaturases are calculated as product/precursor ratio, reflecting both the metabolism of FAs and their dietary intake. For technical reasons, we did not estimate *trans*-FAs that are supposed to play an important role in the etiopathology of MS (Lottenberg *et al.* 2012). Among the strengths of our study is the fact that the participants with MS were not treated with lipid-lowering drugs, dietary supplements containing n-3 PUFA/n-6 PUFA, or antioxidants. The implementation of the cluster analysis of plasma FAs seems to be a promising approach to identify distinct phenotypes of MS.

Conclusions

By means of the cluster analysis of plasma FAs, MS can be categorized into two clusters, independent of clinical and biochemical parameters. These clusters differ in the biochemical abnormalities associated with insulin

resistance, lipolysis, and oxidative stress, as well as in the degree of the risk for type 2 diabetes mellitus and cardiovascular disease. The results of this study warrant further research concerning dietary FA intake and genetic factors that influence FA metabolism. Improved understanding of fatty acid patterns in the pathogenesis of metabolic syndrome could lead to novel approaches in its prevention and treatment.

Conflict of Interest

There is no conflict of interest.

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