

Association of Plasma Lipids Fatty Acid Composition With Metabolic Profile of Czech Adolescents

P. HLA VATY^{1,2}, E. TVRZICKA³, B. STANKOVA³, H. ZAMRAZILOVA¹,
B. SEDLACKOVA¹, L. DUSATKOVA¹, V. HAINER¹, M. KUNESOVA¹

¹Institute of Endocrinology, Prague, Czech Republic, ²OB Clinic, Prague, Czech Republic, ³Fourth Department of Internal Medicine, First Faculty of Medicine, Charles University in Prague and General University Hospital, Prague, Czech Republic

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Summary

Obesity in childhood increases the risk of obesity in adulthood and is predictive for the development of metabolic disorders. The fatty acid composition is associated with obesity and obesity-associated disorders. We investigated the relationship between serum fatty acids composition, adiposity, lipids profile, parameters of glucose metabolism and leptin. The study subjects were 380 adolescents aged 15.0-17.9 years. The study's variables included anthropometric measurements, levels of serum lipids and hormonal parameters. Individual fatty acids were determined in plasma by gas-liquid chromatography. Palmitoleic acid (16:1n-7, PA) significantly positively correlated with percentage of body fat. Saturated fatty acids in phospholipids (PL) positively correlated with BMI and percentage of body fat. PA content in all lipids classes positively correlated with total cholesterol (TC), HDL cholesterol, triglycerides (TG) levels. Stearoyl-CoA desaturase (SCD) activity positively correlated with percentage of body fat and positive correlations of SCD and PA level with leptin were found. Plasma PA content and SCD are associated with adiposity and leptin in obese adolescents. No significant correlation between PA level and insulin resistance was found. Palmitoleate positively correlated with TC, HDL cholesterol, TG and LDL cholesterol levels.

Key words

Obesity • Adolescents • Fatty acids • Palmitoleic acid • Stearoyl-CoA desaturase • Leptin

Corresponding author

P. Hlavaty, Obesity Management Center, Institute of Endocrinology, Narodni 8, CZ-116 94 Prague, Czech Republic.
Fax: +420224905325. E-mail: phlavaty@endo.cz

Introduction

Obesity is characterized by an imbalance between energy intake and expenditure, resulting in an increase of body fat storage. Visceral obesity plays an important role in the development of metabolic syndrome (Moller and Kaufman 2005).

Fatty acid composition in adipose tissue is affected by a dietary intake of fatty acids and processing of fat (lipolysis and endogenous lipogenesis). The fatty acid composition in serum phospholipids (PL) influences several important physiological functions related to the development of the metabolic syndrome components – obesity, insulin sensitivity and the risk of type 2 diabetes (Huang *et al.* 2010, Pelikanova *et al.* 2001, Vessby *et al.* 1994, Zak *et al.* 2007). Some studies have also shown a relationship between individual saturated fatty acids (SAFA), insulin sensitivity (Iggman *et al.* 2010) and adiposity indexes (Kunesova *et al.* 2002a,b).

Palmitoleic acid (16:1n-7, PA) is an important product of the endogenous lipogenesis and is one of the main monounsaturated fatty acids (MUFA). In rats, levels of PA and oleic acid (18:1n-9) were increased after a sucrose diet. Moreover, plasma glucose, insulin, and triglyceride (TG) levels were also significantly higher (Fukuchi *et al.* 2004).

The results from studies suggest that PA in serum cholesterol esters (CE) may be a metabolic indicator reflecting endogenous lipogenesis (Kunesova *et al.* 2002a,b). Conversely, the level of PA in serum free fatty acids is a strong predictor of insulin sensitivity in individuals with an increased risk of type 2 diabetes

(Stefan *et al.* 2010).

PA is a product of desaturation of palmitic acid (16:0) by stearoyl-CoA desaturase (SCD). SCD enzymes are involved in the *de novo* synthesis of MUFA from SAFA (Miyazaki and Ntambi 2003). SCD activity and PA level may be positively associated with obesity and elevated accumulation of abdominal fat in humans (Attie *et al.* 2002, Paillard *et al.* 2008).

Overall, results indicate that changes in the MUFA composition and activity of SCD may significantly contribute to the development of obesity and metabolic syndrome. The aim of the presented study was to investigate the relationship between serum lipids fatty acids composition, adiposity, lipids profile, parameters of glucose metabolism and leptin in Czech adolescents.

Subjects and Methods

Subjects

The participants in this study were from the Childhood Obesity Prevalence And Treatment (COPAT) project. The analyses were performed on 380 Czech adolescents aged 15.0-17.9 years from a general population including all body weight categories. The basic and anthropometric characteristics of the study cohort are shown in Table 1. The mean age was 16.4±0.9 years and the mean BMI was 22.34±4.0 kg/m².

A detailed design of the project has already been described (Aldhoon-Hainerova *et al.* 2014). All subjects and their parent(s) provided written informed consent to the participation in the study. The study protocol was approved by the Ethical Committee of the Institute of Endocrinology in Prague.

Table 1. Basic and anthropometrical characteristics of the study cohort.

| | |
|---------------------------------|-----------|
| <i>n</i> | 380 |
| <i>Age (years)</i> | 16.4±0.9 |
| <i>Weight (kg)</i> | 66.2±14.4 |
| <i>BMI (kg/m²)</i> | 22.3±4.0 |
| <i>Waist circumference (cm)</i> | 80.6±10.2 |
| <i>Hip circumference (cm)</i> | 94.7±8.4 |
| <i>Fat mass (%)</i> | 21.4±7.3 |
| <i>Fat mass (kg)</i> | 14.6±7.5 |
| <i>Lean body mass (kg)</i> | 51.5±9.8 |

Anthropometry and body composition

The percentage of body fat was assessed by a bioelectrical impedance method (Tanita BC-418 MA, Tanita Corporation, Tokyo, Japan). Body mass index was calculated by dividing mass in kilograms by the square of height in meters (kg/m²).

Biochemical and hormonal parameters

Blood samples were collected from the subjects after they had fasted overnight. Plasma leptin was measured by using an immunoradiometric assay kit (DRG Diagnostics, Germany). Serum total cholesterol (TC), HDL and LDL cholesterol and TG and insulin were measured by an automatic biochemical analyzer (Cobas 6000, Roche Diagnostics GmbH, Germany), insulin resistance was evaluated by the HOMA-IR that was obtained by the following formula: fasting plasma insulin (microunits per liter) x fasting glucose (millimoles per liter)/22.5 (Matthews *et al.* 1985).

Lipid extraction and fatty acid composition

Total lipid was extracted from 1 ml of plasma by the method of Folch and coworkers (Folch *et al.* 1957) using dichlormethane instead of chloroform (Carlson 1985). Individual lipid classes (CE, TG and PL), were separated by TLC with the mobile phase heptane-diethylether-acetic acid (80:20:1, v/v/v). Separated lipid classes were transmethylated to FAME with 1M sodium methoxide in dry methanol under nitrogen atmosphere in darkness without previous separation from the layer material. Samples of TG and PL reacted for 60 min at ambient temperature, those of CE 20 min at 80 °C. The reaction mixture was neutralized with 1M acetic acid, methyl esters were extracted twice into hexane and passed through the column (5x20 mm) of anhydrous sodium sulphate. The combined extracts were dried under nitrogen, dissolved in an appropriate volume of isooctane and stored at -20 °C until analyzed.

Gas chromatography was performed with a Trace GC gas chromatograph combined with AS2000 autosampler (Thermo Finnigan, USA). Chromatograph was equipped with a capillarysplit/splitless injector and flame ionization detector (FID).

Analysis of FAME was performed on the fused-silica capillary column coated with 0.25 µm chemically bonded stationary phase Select FAME (100 m, 0.25 mm I.D., Agilent Technologies, The Netherlands). The oven temperature was programmed from 80 °C to 120 °C at 4°/min, to 270 °C at 2°/min,

Table 2. Spearman's correlation coefficients between fatty acid composition and anthropometrical parameters.

| Fatty acids | Triglycerides | | | | Phospholipids | | | | Cholesterol esters | | | |
|-------------|--------------------------|--------------|---------------|---------------------|--------------------------|--------------|---------------|---------------------|--------------------------|--------------|---------------|---------------------|
| | BMI (kg/m ²) | Fat mass (%) | Fat mass (kg) | Lean body mass (kg) | BMI (kg/m ²) | Fat mass (%) | Fat mass (kg) | Lean body mass (kg) | BMI (kg/m ²) | Fat mass (%) | Fat mass (kg) | Lean body mass (kg) |
| 12:0 | 0.079 | 0.131 * | 0.126 * | -0.007 | -0.041 | 0.011 | -0.015 | -0.053 | 0.012 | 0.019 | 0.008 | 0.014 |
| 14:0 | 0.073 | 0.105 * | 0.113 * | 0.022 | 0.023 | 0.095 | 0.071 | -0.090 | 0.110 * | 0.117 * | 0.129 * | 0.043 |
| 16:0 | 0.126 * | 0.173 ** | 0.182 *** | 0.025 | 0.094 | 0.172 ** | 0.153 ** | -0.066 | 0.010 | -0.052 | -0.026 | 0.077 |
| 18:0 | -0.010 | -0.085 | -0.031 | 0.113 * | 0.083 | -0.082 | -0.004 | 0.187 *** | 0.012 | -0.079 | -0.030 | 0.126 * |
| 20:0 | -0.017 | -0.081 | -0.063 | 0.086 | -0.090 | -0.143 ** | -0.115 * | 0.093 | -0.027 | -0.045 | -0.027 | 0.033 |
| 14:1n5 | 0.044 | 0.152 ** | 0.123 * | -0.100 | 0.011 | 0.019 | 0.027 | 0.019 | 0.046 | 0.068 | 0.059 | 0.013 |
| 16:1n7c | 0.057 | 0.243 *** | 0.178 *** | -0.188 *** | 0.110 * | 0.234 *** | 0.193 *** | -0.125 * | 0.184 *** | 0.271 *** | 0.263 *** | -0.038 |
| 18:1n9c | -0.012 | -0.122 * | -0.102 | 0.069 | -0.080 | -0.147 ** | -0.128 * | 0.057 | 0.019 | -0.132 * | -0.069 | 0.162 ** |
| 20:1n9 | 0.030 | -0.115 * | -0.073 | 0.117 * | -0.200 *** | -0.205 *** | -0.240 *** | -0.032 | -0.048 | -0.018 | -0.045 | -0.052 |
| 18:2n6 | -0.137 ** | -0.123 * | -0.136 * | -0.037 | -0.314 *** | -0.145 ** | -0.242 *** | -0.226 *** | -0.167 ** | -0.041 | -0.102 * | -0.173 *** |
| 20:2n6 | -0.073 | -0.237 *** | -0.180 *** | 0.140 ** | 0.001 | -0.023 | -0.018 | 0.014 | 0.003 | 0.023 | 0.017 | -0.002 |
| 18:3n6 | -0.032 | -0.152 ** | -0.098 | 0.109 * | 0.095 | -0.060 | 0.009 | 0.173 ** | 0.192 *** | 0.056 | 0.122 * | 0.142 ** |
| 20:3n6 | 0.002 | -0.180 *** | -0.108 * | 0.166 ** | 0.196 *** | 0.038 | 0.127 * | 0.212 *** | 0.287 *** | 0.125 * | 0.210 *** | 0.202 *** |
| 20:4n6 | -0.018 | -0.034 | -0.022 | 0.008 | 0.211 *** | 0.062 | 0.131 * | 0.179 *** | 0.155 ** | 0.116 * | 0.148 ** | 0.059 |
| 22:4n6 | 0.016 | -0.128 * | -0.053 | 0.157 ** | -0.042 | -0.226 *** | -0.134 * | 0.226 *** | -0.022 | 0.020 | 0.001 | -0.027 |
| 22:5n6 | 0.000 | 0.002 | -0.008 | -0.050 | -0.101 | -0.036 | -0.055 | -0.065 | 0.019 | 0.044 | 0.034 | -0.009 |
| 18:3n3 | 0.065 | -0.009 | 0.027 | 0.084 | -0.052 | 0.094 | 0.034 | -0.135 * | 0.089 | 0.149 ** | 0.137 ** | -0.023 |
| 20:5n3 | 0.100 | -0.009 | 0.043 | 0.138 ** | 0.221 *** | 0.080 | 0.145 ** | 0.174 *** | 0.019 | 0.051 | 0.045 | -0.013 |
| 22:5n3 | -0.007 | -0.143 ** | -0.082 | 0.153 ** | 0.062 | -0.137 ** | -0.047 | 0.239 *** | 0.017 | 0.070 | 0.041 | -0.035 |
| 22:6n3 | 0.079 | 0.057 | 0.074 | 0.023 | 0.066 | 0.148 ** | 0.112 * | -0.080 | 0.041 | 0.064 | 0.046 | -0.040 |
| SAFA | 0.100 | 0.148 ** | 0.157 ** | 0.028 | 0.199 *** | 0.186 *** | 0.221 *** | 0.087 | 0.019 | -0.044 | -0.012 | 0.094 |
| MUFA | 0.030 | -0.031 | -0.030 | 0.010 | -0.069 | -0.101 | -0.098 | 0.021 | 0.080 | -0.015 | 0.033 | 0.125 * |
| n-6 PUFA | -0.138 ** | -0.141 ** | -0.148 ** | -0.021 | -0.158 ** | -0.135 * | -0.163 ** | -0.069 | -0.071 | 0.026 | -0.020 | -0.132 * |
| n-3 PUFA | 0.050 | -0.030 | 0.006 | 0.085 | 0.126 * | 0.107 * | 0.116 * | 0.043 | 0.067 | 0.131 * | 0.113 * | -0.036 |

* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Table 3. Spearman's correlation coefficients between fatty acid composition and lipid metabolic parameters.

| Fatty acids | Triglycerides | | | | Phospholipids | | | | Cholesterol esters | | | |
|-------------|-------------------|-----------------|-----------------|---------------|-------------------|-----------------|-----------------|---------------|--------------------|-----------------|-----------------|---------------|
| | Total cholesterol | HDL cholesterol | LDL cholesterol | Triglycerides | Total cholesterol | HDL cholesterol | LDL cholesterol | Triglycerides | Total cholesterol | HDL cholesterol | LDL cholesterol | Triglycerides |
| 12:0 | 0.132 * | 0.082 | 0.106 * | 0.191 *** | 0.041 | 0.008 | 0.022 | 0.043 | -0.053 | 0.006 | -0.085 | 0.056 |
| 14:0 | 0.172 ** | 0.084 | 0.120 * | 0.319 *** | 0.155 ** | 0.110 * | 0.065 | 0.221 *** | 0.153 ** | 0.060 | 0.116 * | 0.223 *** |
| 16:0 | 0.232 *** | 0.043 | 0.172 ** | 0.338 *** | 0.129 * | 0.049 | 0.079 | 0.220 *** | -0.162 ** | -0.069 | -0.122 * | -0.062 |
| 18:0 | -0.070 | -0.067 | -0.058 | -0.135 * | -0.027 | -0.244 *** | 0.066 | 0.002 | -0.085 | -0.213 *** | -0.032 | 0.041 |
| 20:0 | -0.095 | -0.083 | -0.085 | -0.103 | -0.163 ** | -0.190 *** | -0.099 | -0.106 * | -0.049 | -0.126 * | -0.037 | 0.078 |
| 14:1n5 | 0.186 *** | 0.127 * | 0.143 ** | 0.283 *** | -0.040 | -0.030 | -0.054 | 0.016 | 0.058 | 0.025 | 0.012 | 0.155 ** |
| 16:1n7c | 0.298 *** | 0.194 *** | 0.238 *** | 0.337 *** | 0.168 ** | 0.104 * | 0.091 | 0.259 *** | 0.270 *** | 0.120 * | 0.206 *** | 0.285 *** |
| 18:1n9c | -0.194 *** | -0.073 | -0.137 ** | -0.216 *** | -0.113 * | -0.034 | -0.101 | -0.022 | -0.161 ** | -0.176 *** | -0.101 | -0.075 |
| 20:1n9 | -0.058 | 0.000 | -0.038 | -0.114 * | -0.134 * | -0.091 | -0.085 | -0.116 * | -0.059 | -0.059 | -0.078 | -0.010 |
| 18:2n6 | -0.123 * | -0.062 | -0.091 | -0.160 ** | -0.123 * | 0.184 *** | -0.162 ** | -0.298 *** | 0.016 | 0.123 * | -0.005 | -0.100 |
| 20:2n6 | -0.066 | -0.032 | -0.036 | -0.061 | 0.166 ** | 0.010 | 0.178 *** | 0.163 ** | -0.052 | -0.041 | -0.080 | 0.090 |
| 18:3n6 | -0.173 ** | -0.102 | -0.133 * | -0.092 | -0.068 | -0.116 * | -0.091 | 0.214 *** | 0.120 * | -0.032 | 0.098 | 0.178 *** |
| 20:3n6 | -0.077 | -0.050 | -0.054 | -0.036 | 0.190 *** | -0.108 * | 0.224 *** | 0.323 *** | 0.210 *** | -0.122 * | 0.224 *** | 0.290 *** |
| 20:4n6 | -0.082 | 0.031 | -0.083 | -0.282 *** | -0.024 | -0.116 * | 0.017 | -0.044 | 0.130 * | -0.024 | 0.126 * | 0.089 |
| 22:4n6 | -0.136 ** | -0.052 | -0.117 * | -0.220 *** | -0.150 ** | -0.179 *** | -0.077 | -0.055 | 0.058 | 0.022 | 0.009 | 0.037 |
| 22:5n6 | 0.041 | 0.126 * | 0.037 | -0.129 * | 0.108 * | 0.043 | 0.077 | 0.161 ** | 0.049 | -0.023 | 0.030 | 0.033 |
| 18:3n3 | -0.062 | -0.100 | -0.013 | 0.045 | 0.013 | 0.074 | -0.044 | 0.060 | 0.149 ** | 0.041 | 0.117 * | 0.171 *** |
| 20:5n3 | -0.078 | -0.064 | -0.046 | -0.118 * | -0.023 | -0.080 | -0.027 | 0.088 | 0.057 | 0.026 | -0.015 | 0.022 |
| 22:5n3 | -0.195 *** | -0.087 | -0.121 * | -0.251 *** | -0.152 ** | -0.176 *** | -0.073 | -0.060 | 0.083 | -0.003 | 0.042 | 0.100 |
| 22:6n3 | 0.024 | 0.043 | 0.061 | -0.120 * | 0.118 * | 0.017 | 0.114 * | 0.129 * | 0.104 * | 0.042 | 0.066 | 0.067 |
| SAFA | 0.214 *** | 0.043 | 0.156 ** | 0.307 *** | 0.172 ** | -0.126 * | 0.180 *** | 0.290 *** | -0.140 ** | -0.083 | -0.102 * | -0.025 |
| MUFA | -0.113 * | -0.017 | -0.072 | -0.124 * | -0.098 | -0.045 | -0.086 | 0.023 | -0.061 | -0.114 * | -0.031 | 0.040 |
| n-6 PUFA | -0.142 ** | -0.070 | -0.109 * | -0.190 *** | -0.099 | 0.099 | -0.093 | -0.278 *** | 0.109 * | 0.118 * | 0.081 | -0.036 |
| n-3 PUFA | -0.098 | -0.090 | -0.037 | -0.090 | 0.048 | -0.041 | 0.047 | 0.114 * | 0.148 ** | 0.054 | 0.096 | 0.136 ** |

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 4. Spearman's correlation coefficients between fatty acid composition and glucose metabolic parameters and leptin.

| Fatty acids | Triglycerides | | | | Phospholipids | | | | Cholesterol esters | | | |
|-------------|---------------|-----------|-----------|------------|---------------|-----------|----------|------------|--------------------|---------|---------|------------|
| | C peptide | Insulin | HOMA-IR | Leptin | C peptide | Insulin | HOMA-IR | Leptin | C peptide | Insulin | HOMA-IR | Leptin |
| 12:0 | 0.165 ** | 0.162 ** | 0.171 ** | 0.155 ** | 0.063 | 0.044 | 0.078 | 0.109 * | -0.059 | -0.074 | -0.052 | 0.019 |
| 14:0 | 0.227 *** | 0.255 *** | 0.254 *** | 0.196 *** | 0.155 ** | 0.142 ** | 0.157 ** | 0.218 *** | 0.120 * | 0.093 | 0.062 | 0.175 *** |
| 16:0 | 0.143 ** | 0.164 ** | 0.161 ** | 0.172 ** | -0.041 | -0.059 | -0.078 | 0.163 ** | -0.008 | -0.009 | 0.026 | -0.095 |
| 18:0 | 0.116 * | 0.114 * | 0.131 * | -0.068 | 0.054 | 0.097 | 0.110 * | -0.111 * | 0.066 | 0.048 | 0.069 | -0.164 ** |
| 20:0 | 0.043 | 0.004 | 0.051 | -0.039 | -0.042 | 0.013 | -0.003 | -0.133 * | -0.031 | 0.016 | 0.035 | -0.059 |
| 14:1n5 | 0.125 * | 0.163 ** | 0.159 ** | 0.245 *** | 0.069 | 0.052 | 0.079 | 0.067 | 0.013 | -0.015 | 0.002 | 0.085 |
| 16:1n7c | 0.006 | 0.029 | 0.003 | 0.262 *** | 0.053 | 0.039 | 0.023 | 0.259 *** | 0.101 | 0.084 | 0.073 | 0.276 *** |
| 18:1n9c | -0.076 | -0.099 | -0.110 * | -0.226 *** | -0.053 | -0.035 | -0.023 | -0.177 *** | -0.008 | -0.004 | 0.003 | -0.199 *** |
| 20:1n9 | -0.058 | -0.033 | -0.035 | -0.097 | -0.149 ** | -0.151 ** | -0.116 * | -0.209 *** | 0.064 | -0.008 | 0.039 | -0.009 |
| 18:2n6 | -0.111 * | -0.132 * | -0.137 ** | -0.108 * | 0.044 | -0.029 | 0.008 | -0.080 | -0.041 | -0.029 | -0.015 | 0.037 |
| 20:2n6 | -0.020 | 0.048 | 0.032 | -0.138 ** | 0.039 | 0.054 | 0.031 | 0.096 | 0.053 | 0.059 | 0.082 | 0.007 |
| 18:3n6 | -0.026 | 0.001 | 0.011 | -0.161 ** | 0.108 * | 0.124 * | 0.140 ** | -0.079 | 0.026 | 0.064 | 0.032 | 0.070 |
| 20:3n6 | -0.120 * | -0.055 | -0.071 | -0.136 * | 0.088 | 0.152 ** | 0.108 * | 0.084 | 0.089 | 0.100 | 0.045 | 0.130 * |
| 20:4n6 | -0.101 | -0.092 | -0.093 | -0.047 | -0.060 | -0.008 | -0.031 | -0.025 | 0.004 | 0.009 | -0.040 | 0.102 * |
| 22:4n6 | -0.063 | -0.010 | 0.003 | -0.124 * | -0.098 | -0.010 | -0.031 | -0.261 *** | -0.008 | -0.024 | 0.017 | -0.024 |
| 22:5n6 | -0.152 ** | -0.086 | -0.124 * | 0.037 | -0.075 | 0.007 | -0.040 | 0.030 | -0.039 | -0.062 | -0.075 | 0.008 |
| 18:3n3 | 0.008 | 0.042 | 0.025 | 0.042 | 0.126 * | 0.109 * | 0.134 * | 0.161 ** | 0.028 | 0.030 | -0.012 | 0.209 *** |
| 20:5n3 | -0.045 | -0.022 | -0.010 | -0.028 | 0.064 | 0.063 | 0.058 | 0.054 | 0.018 | -0.022 | 0.011 | 0.042 |
| 22:5n3 | -0.130 * | -0.125 * | -0.121 * | -0.176 *** | -0.096 | -0.058 | -0.052 | -0.209 *** | 0.060 | 0.031 | 0.025 | 0.049 |
| 22:6n3 | -0.082 | -0.036 | -0.049 | 0.068 | -0.052 | 0.033 | -0.025 | 0.157 ** | -0.003 | -0.015 | -0.007 | 0.044 |
| SAFA | 0.193 *** | 0.203 *** | 0.203 *** | 0.181 *** | 0.046 | 0.041 | 0.039 | 0.173 ** | 0.009 | 0.006 | 0.036 | -0.094 |
| MUFA | -0.083 | -0.089 | -0.108 * | -0.129 * | -0.069 | -0.057 | -0.037 | -0.135 ** | 0.037 | 0.024 | 0.038 | -0.079 |
| n-6 PUFA | -0.129 * | -0.145 ** | -0.147 ** | -0.122 * | 0.053 | 0.003 | 0.015 | -0.110 * | -0.031 | -0.013 | -0.040 | 0.094 |
| n-3 PUFA | -0.047 | -0.013 | -0.027 | 0.010 | -0.019 | 0.041 | -0.008 | 0.100 | 0.022 | 0.003 | -0.004 | 0.154 ** |

* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

then isothermal 25 min for PL and TG, and 55 min for CE. The injector and detector temperatures were 250 and 270 °C, respectively. Hydrogen carrier gas was maintained at a head pressure of 70 kPa and split flow 15 ml/min, splitless time 0.25 min.

Integration software Clarity (Data Apex Ltd. Prague, Czech Republic) was used for data acquisition and handling.

SCD activity can be estimated from the desaturation indices. The product-to-precursor ratio (palmitoleic/palmitic, 16:1n-7/16:0) was used to estimate the activity of stearoyl-CoA desaturase (SCD) (Attie *et al.* 2002).

Statistical analyses

All data are expressed as mean \pm standard error of the mean (SEM). The Spearman's correlation coefficient was used to assess the correlation between 2 variables. For all statistical analyses, P values below 0.05 were used to indicate the statistical significance. The statistical software was Statgraphics Centurion XVI (Statistical Graphics Corp., Rockville, MD, USA).

Results

Spearman's correlation coefficients between fatty acid composition and anthropometrical parameters are shown in Table 2. PA in plasma TG, PL and CE significantly positively correlated with percentage of body fat. Negative correlation between omega-6 fatty acids content in plasma TG and PL, and BMI and percentage of body fat were observed while omega-3 fatty acids in PL positively correlated with BMI and percentage of body fat. Linoleic acid (18:2n-6) in all lipid classes had a significant negative correlation with BMI; with percentage of fat mass only in TG and PL. Dihomo- γ -linoleic acid (20:3n-6, DGLA) in PL positively correlated with BMI and lean body mass. SAFA in PL positively correlated with BMI and percentage of body fat.

Spearman's correlation coefficients between fatty acid composition and lipid metabolic parameters are shown in Table 3. Myristic (14:0) and palmitic (16:0) acids positively correlated with serum TC and TG. PA content in all lipids classes positively correlated with TC, HDL cholesterol, TG levels and LDL cholesterol (except for PA in PL). DGLA in PL and CE positively correlated with TC, HDL and LDL cholesterol and TG.

Spearman's correlation coefficients between

fatty acid composition and glucose metabolic parameters and leptin are shown in Table 4. SAFA in TG positively correlated with C peptide, insulin, HOMA-IR and leptin. PA in all lipid classes positively correlated with leptin.

SCD activity expressed as the ratio 16:1n-7/16:0 had a positive correlation with percentage of body fat and with leptin; correlation with 18:1n-9/18:0 ratio was not significant. However, only the ratio 18:1n-9/18:0 had a significant negative correlation with HOMA-IR (Table 5). We found no statistical difference in SCD activity between adolescents with normal BMI (defined as BMI between the 25th and 75th percentile, according to the age and gender) and obese adolescents with BMI above the 95th percentile (data not shown).

Table 5. Spearman's correlation coefficients between stearoyl-CoA desaturase and anthropometric and metabolic parameters.

| | 16:1n-7/16:0 | | 18:1n-9/18:0 | |
|--------------------------|--------------|----------|--------------|----------|
| | <i>r</i> | <i>p</i> | <i>r</i> | <i>p</i> |
| <i>Body fat mass (%)</i> | 0.272 | <0.0001 | 0.001 | NS |
| <i>Leptin</i> | 0.294 | <0.0001 | -0.040 | NS |
| <i>HOMA-IR</i> | 0.048 | NS | -0.152 | <0.01 |

NS – non-significant.

Discussion

Our results show significant relation of plasma PA content with higher percentage of body fat mass. Several studies in obese adults and children already reported similar findings (Gong *et al.* 2011, Kunesova *et al.* 2002b). In the study of obese and non-obese children, Okada *et al.* (2005) found significant relation between plasma PA content and visceral adiposity in obese children.

The fatty acid composition of lipids in serum and muscle is influenced by a diet, a degree of physical activity, and genetic disposition (Vessby 2000). Specific dietary fatty acids may influence the development of diabetes by modifying the PL composition of cell membranes (Boden 1996). The evidence suggests that replacing saturated fats and trans fatty acids with polyunsaturated fatty acids (PUFA) and/or MUFA fats has beneficial effects on insulin sensitivity and is likely to reduce risk of type 2 diabetes (Riserus *et al.* 2009). A decrease in unsaturated fatty acids was correlated with insulin resistance in childhood obesity (Toledo *et al.*

2014). Obese children also showed higher values of DGLA compared to healthy controls in all lipid classes (Decsi *et al.* 1996).

PA is mostly absent in common diet and is primarily biosynthesized from palmitic acid (16:0) by the stearoyl-CoA desaturase (SCD) in liver and adipose tissue (Paillard *et al.* 2008). In a genetic mouse model, Cao *et al.* (2008) suggested that PA released from adipose tissue may serve as a lipokine that mediates the communication between the adipose tissue and other tissues. Stefan *et al.* (2010) described that the level of PA in serum free fatty acids is a strong predictor of insulin sensitivity in individuals with increased risk of type 2 diabetes. In our study, no significant correlation between PA level and insulin resistance as assessed by HOMA-IR was found.

SCD is an essential and a highly regulated enzyme involved in *de novo* synthesis of MUFA from SAFA. SCD preferentially converts palmitic acid (16:0) and stearic acid (18:0) into PA (16:1) and oleic acid (18:1) (Enoch *et al.* 1976). These products are key substrates for synthesis of TG, CE, PL and other kinds of lipids (Paton and Ntambi 2009).

SCD expression is regulated by many dietary and hormonal factors (Ntambi and Miyazaki 2004). High carbohydrate intake and insulin increase SCD gene expression. However, intake of n-3 and n-6 PUFA and leptin inhibit SCD transcription in the liver (Mauvoisin and Mounier 2011). Results from animal and human studies suggest that SCD activity may play an important role in the development of obesity. SCD1-deficient mice have reduced body adiposity and increased insulin sensitivity, and are resistant to diet-induced obesity (Enser 1979, Ntambi *et al.* 2002). SCD1-deficient mice also have reduced lipid synthesis and enhanced lipid oxidation. Obese people manifest abnormally high activities of SCD in skeletal muscle (Hulver *et al.* 2005). Okada *et al.* (2005) showed that SCD activity correlates positively with leptin concentrations but not with serum insulin in obese children. It has also been shown that SCD1 deficiency improves adiposity in leptin-deficient ob/ob mice (Cohen *et al.* 2002). The results of the study observing dietary and genetic factors influencing fatty acid composition in obese female identical twins suggest that PA may be a metabolic indicator under strong genetic control in humans reflecting endogenous lipogenesis (Kunesova *et al.* 2002a). In our study, SCD activity together with PA level positively correlated with percentage of body fat. This outcome is in an agreement

with previous findings in humans that described an association between SCD activity and PA level on one hand and hypertriglyceridemia, abdominal adiposity, and obesity on the other (Attie *et al.* 2002, Paillard *et al.* 2008).

Palmitic acid (16:0) and DGLA are both associated with an unfavorable lipid profile. Dietary palmitic acid is generally regarded as a cholesterol increasing factor, but the results of certain studies have shown only a minor cholesterol raising effect (Hayes and Khosla 1996). Nevertheless, not all of long chained SAFA have the same effect on raising cholesterol level. Myristic acid (14:0) is one of the fatty acids, which increases cholesterol level the most. On the other hand, stearic acid, similarly as oleic and linoleic acid are associated with a favorable lipid profile. PA in plasma PL is independently associated with more favorable HDL cholesterol, total to HDL cholesterol ratio, and fibrinogen, which supports the potential metabolic benefits seen in animal experiments. Conversely, palmitoleate was associated with a higher BMI, greater waist circumference, and elevated TG (Mozaffarian *et al.* 2010). The effect of PA on TC and LDL cholesterol resembles more the SAFA than MUFA. Our results confirm this finding. However, in addition to increasing levels of TC and LDL cholesterol, also the increase of HDL cholesterol occurs.

In summary, our findings suggest that plasma PA content together with SCD activity is associated with adiposity in obese adolescents. No significant correlation between PA level and insulin resistance was found. PA content in all lipids classes positively correlated with TC, HDL cholesterol, LDL cholesterol levels and TG.

Conflict of Interest

There is no conflict of interest.

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Abbreviations

BMI, body mass index; CE, cholesterol esters; DGLA, dihomog- γ -linoleic acid; MUFA, monounsaturated fatty acids; PA, palmitoleic acid; PL, phospholipids; PUFA, polyunsaturated fatty acids; SAFA, saturated fatty acids; SCD, stearoyl-CoA desaturase; SEM, standard error of the mean; TC, total cholesterol; TG, triglycerides.

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