The Fatty Acid Composition of Serum Phospholipids in Adolescents is Associated With Body Composition in Early Adulthoods: An Eight-Year Follow-Up Study

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Summary

The fatty acid composition is associated with obesity. Omega 3 polyunsaturated fatty acid (PUFA) could have a beneficial role in the prevention and treatment of many disorders, including cardiometabolic diseases. A cohort of 84 men and 131 women were examined in adolescence and after 8 years. Body weight (BW) and fat mass (FM) were measured. The composition of fatty acids (FAs) of serum phospholipids was assessed using gas chromatography. Statistics: PLS method. Aim: to determine the relationships between FAs in adolescence and FM (explanatory variable 1, EV1) and BW (explanatory variable 2, EV2) in adulthood. In the predictive models, a cluster of FAs in boys explained 47.2 % of EV1 and a cluster of 6 FAs in girls explained 32.3 % of EV1 measured in adulthood. FAs measured in adolescents explained 23.7 % of EV2 in early adults regardless of gender. A significant negative association was found between 18:1n-9c and EV1 in males and EV2 in both genders. We found a significant negative association between 18:2n-6 and 20:0 and both EV1 and EV2. In all analyses, we found a significant negative association of 20:1n-9 and 18:3n-3 with EV1-2 in both genders. A significant positive association was found in 20:3n-6 with EV1 and EV2 in males. 20:4n-6 was positively associated with EV1 in females and EV2 in both genders. A positive association between FM and very long chain n- 6 PUFAs was also observed. It is concluded that serum MUFAs and essential PUFAs in adolescence are associated with lower BW and FM in adulthood.

Key words

Fatty acid composition • Overweight and obesity • Fat mass • Serum phospholipids • Adolescents • Early adults

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Introduction

More than half of obese children will remain obese in adulthood and early onset obesity has more serious health complications in later life. Abdominal obesity is associated with a higher risk of hypertension, type 2 diabetes mellitus, and dyslipidemia [1]. Visceral fat is characterized by high lipolytic activity.

Fatty acid intake affects the cardiovascular system [2], immune system, and nervous system [3], and regulates the inflammatory signaling pathway in adipose tissue [4]. A diet with a higher consumption of PUFAs and monounsaturated fatty acids (MUFAs) and reduced intake of saturated fatty acids (SFAs) has a positive effect on glucose metabolism [5]. The intake of PUFAs reduces the risks of developing insulin resistance, dyslipidemia and, inflammation [5].

Omega 6 and omega 3 PUFAs are precursors of eicosanoids [6,7]. Essential fatty acids (linoleic acid, LA 18:2n-6 and alpha-linolenic acid, ALA 18:3n-3) have

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to be obtained from the diet [8]. A high omega-6 fatty acid intake and a high omega-6/omega-3 ratio have been shown to be associated with weight gain [9]. An unbalance in the omega 6/omega 3 ratio in favor of omega 6 increases the risk of metabolic diseases [10-13]. The study of Zeman *et al.* also showed that body fat correlated with fatty acid composition [14].

Several studies have examined the effects of different types of fatty acids intake on weight development, but the results have generally been inconsistent. In a longitudinal study Hastert *et al.* suggested that PUFA had an association with baseline BMI, but no association was found between fatty acid composition and change of BMI [15]. Flannagan *et al.* found a protective effect of omega 3 to BMI gain [16]. In a group of obese children (n=63, aged 9.0±0.2 years, BMI Z-score 3.1±0.2), children in the 3th tercile had lower proportions of α-linolenic acid (-40 %) and eicosapentaenoic acid and docosahexaenoic acid (-15 %) in erythrocytes, adjusted for age (Tanner stage and race compared with the first tercile). The status of lower omega-3 dietary long-chain polyunsaturated fatty acids in obese children with greater adiposity was consistent with suboptimal intakes of omega-3 long chain PUFA and fish in the diet (when compared to obese children in the 3th tercile vs. the 1st tercile) [17].

In this paper, we present data obtained from adolescents examined in the COPAT (Childhood Obesity Prevalence And Treatment) project in 2009-2010 and then repeatedly investigated after 8 years. The aim of our study was to determine the relationships between the fatty acid composition in adolescence and body weight and body composition in early adulthood.

Material and Methods

Subjects

Samples obtained for the Childhood Obesity Prevalence And Treatment (COPAT) project were used. Details of the design of that study have been published previously [18]. In the first round, which was organized in 2009-2010 by the Institute of Endocrinology, more than 2000 adolescents (age 13-18 years) were examined in institutes across the Czech Republic. Eight years later, 215 subjects (10.3 % of the first round), 131 women and 84 men were re-examined. This low participation can be explained by the different organization of the second round of the project, when all examinations took place at the Institute of Endocrinology in Prague. Long distances from participant's residences to Prague led to frequent refusals to participate in the second round. Outdated contact information also contributed to reduced success in reaching patients. Moreover, a certain percentage of dropouts cannot be avoided in longitudinal studies [19].

Clinical characterization, anthropometry and body composition

The following parameters were assessed: age; anthropometric parameters and indexes (weight, height, waist circumference, percentage of body fat mass (FM), and BMI=weight (kg)/height (m)^2).

Bioelectrical impedance devices were used for the body fat mass measurements: a Tanita BC 418 MA (Tanita Corporation, Tokyo, Japan) in the first round, and a Tanita SC-240 (Tanita Corporation, Tokyo, Japan) in the followed up. A comparative study showed that the data obtained with these devices were well correlated. Characteristics of the studied subjects are summarized in Table 1. The BMI z-scores were calculated using the Czech reference population [20]. For statistical analysis, early adult subjects were classified as overweight (OW, including obesity BMI≥25 kg/m^2) or normal weight (BMI<25 kg/m^2).

Table 1. Cohort characteristics.

	1. round		2. round	
	Women	**Men**	**Women**	**Men**
	Median (95 % CI)	Median (95 % CI)	Median (95 % CI)	Median (95 % CI)
Age (years)	14.96 (11.52-18.49)	15.24 (12.52-18)	23.50 (20-27.5)	24.00 (20.5-26.8)
BMI (SDS)	0.30 (0.17-0.46)	0.36 (0.22-0.53)		
BMI (kg/m^2)	22.18 (21.36-23.00)	23.03 (21.97-24.09)	23.80 (19.73-27.88)	25.37 (19.95-30.79)
FM (%)*	25.65 (12.9-52.8)	16.60 (9.8-43.4)	28.1 (10.9-53.5)	17.40 (7.3-40.3)

Fatty acids

The composition of fatty acids (FAs) in serum lipids was assessed by gas chromatography (GC). The blood samples were tested for the set of 22 FAs in phospholipids (Table S1), and ratios between certain FA groups (omega 6/omega 3, PUFA/SFA) were calculated. GC identifies and quantifies the fatty acid content of lipids from tissues, cells, and plasma/serum, yielding results with high accuracy and high reproducibility [21]. Chromatographic analyses were performed with a Trace-GC gas chromatograph combined with an AS 2000 autosampler (Thermo-Finnigan, USA), equipped with a capillary split/splitless injector and a flame ionization detector Integration software Clarity version 2.4.1.57 (Data Apex Ltd. Prague, Czech Rep.) was used for data acquisition and handling. The results are expressed as mol% [22].

Statistical analysis

To interpret the relationships of the fatty acid composition of serum phospholipids in adolescents to their weight and fat mass in adulthood, the method of multivariate regression with reduction of dimensionality—orthogonal projections to latent structures (OPLS) was used [23]. For this analysis, subjects were divided into two groups: overweight including obesity (BMI≥25 kg/m^2) and normal weight (BMI<25 kg/m^2). Statistical analyses monitored the relationships between weight status (overweight / non – overweight) in adulthood and fatty acids in adolescence (in serum phospholipids) were evaluated regardless of gender, because gender did not correlate significantly with the variable weight status (OW/non-OW). The percentage of body fat mass was calculated separately for boys and girls. OPLS is capable of coping with the problem of severe multicollinearity (high intercorrelations) in a matrix of predictors, while ordinary multiple regression fails to evaluate such data. The multicollinearity in OPLS is favorable as it enhances the predictivity of the model. In our OPLS models, fat mass was chosen as a single dependent variable, while the fatty acid levels were independent variables. As fatty acids are mutually highly intercorrelated the application of ordinary multiple regression (based on evaluating relationships between a dependent variable and each independent variable while the remaining independent variable are mathematically set to constant values) is not appropriate. In OPLS, the variability in independent (explaining) variables was separated into two kinds of independent components. The first one contained the variability in explanatory variables, which was shared with the patient's status (OW/non-OW). The second one included the orthogonal components explaining the variability shared within the highly intercorrelated explanatory variables. The OPLS identified the best explanatory variables as well as their best combination to evaluate the relationship between obesity and fatty acids. After standardization of the variables, the OPLS model can be expressed as follows:

$$\mathbf{X} = \mathbf{T}_p \mathbf{P}_p^T + \mathbf{T}_0 \mathbf{P}_0^T + \mathbf{E}$$
$$\mathbf{Y} = \mathbf{T}_p \mathbf{P}_p^T + \mathbf{F}$$

where **X** is the matrix with explaining the variables and subjects, **Y** is the vector of the dependent variable and subjects; $\mathbf{T}_p$ is the vector of component scores from the single predictive component and subjects extracted from **Y**; $\mathbf{T}_0$ is the vector of component scores from the single orthogonal component and subjects extracted from **X**; $\mathbf{P}_p$ is the vector of component loadings for the predictive component extracted from **Y**; $\mathbf{P}_0$ is the vector of component loadings for the orthogonal component extracted from **X** and explanatory variables; and **E** and **F** are the error terms.

The relevant explanatory variables were chosen using variable importance (**VIP**) statistics. The statistical software SIMCA-P v.12.0 from Umetrics AB (Umeå, Sweden), which was used for the OPLS analysis, enabled the detection of multivariate non-homogeneities, as well as testing the multivariate normal distribution and homoscedasticity (constant variance) [24,25]. The ordinary multiple regression without a reduction of dimensionality identified relevant explanatory variables for which correlation with the dependent variable was independent of the remaining explanatory variables.

Ethics statement

Both rounds of the COPAT project were approved by the Ethical Committee of the Institute of Endocrinology in Prague and were undertaken in accordance with the Helsinki Declaration II. All participants and and/or their parent(s)/guardian(s) signed an informed consent before the initiation of study procedures.

Results

We investigated associations between the percentage of fat mass (FM) in early adulthood and fatty acid (FA) composition in adolescents. Also, we examined the relationships between the weight group (overweight and normal weight) and the composition of FAs.

A cluster of 8 FAs (negative: sum of mono, sum of omega 3, 18:1n-9c, 20:1n-9, 18:3n-3; 22:6n-3; positive: 16:1n-7c, 20:3n-6) in adolescent boys and 6 FAs (negative: 14:0, 20:1n-9, 18:2n-6, 18:3n-3, 20:0; positive: 20:4n-6) in adolescent girls were identified as the best predictors of body fat in their adulthood (the explained variability was 47.2 % for men and 32.3 % for women). A predictive model for being overweight in adulthood regardless of gender showed a significant power although rather weaker, with an explained variability of obesity of 23.7 %. In this model 7 FAs (negative: 18:1n-9c, 20:1n-9, 18:2n-6, 18:3n-3, 20:0; positive: 20:3n-6, 20:4n-6) were significantly associated with obesity. Essential PUFAs (LA, ALA) had a negative association with fat mass and obesity while omega 6 (other than LA) had a positive relationship with obesity. We also found that, omega 3 PUFAs in adolescence were negatively associated with fat mass in early adulthood, which in turn, aids in weight loss and weight management. More information is available in Tables 2-3 A/B. No significant associations between anthropometric parameters and omega6/omega 3 ratios were found.

Discussion

In human and other organisms, fatty acids (FAs) occur as free fatty acids or part of lipids in their esterified form. LA and ALA are essential fatty acids that must be derived from food [26]. Essential FAs and their metabolites affect lipoprotein levels, membrane fluidity, the function of many enzymes and receptors, and they are precursors of prostaglandins, and eicosanoids. These FAs are important for the proper function of the CNS, retina, liver, kidneys, adrenal glands, and gonads. The positive effects of omega 3 include decreased TG levels in the blood, a decrease in blood pressure, anti-inflammatory action, and a vasodilatory effect [27]. Previous studies have shown associations of body weight and body composition with fatty acids [10,14,15,16,28,29]. Our results confirm that an adequate intake of essential fatty acids is a protective factor in the risk of obesity. In our cohort, we found positive associations with gamma linoleic acid, dihomo-gamma linoleic acid, arachidonic acid, and negative associations with gondoic acid, alpha-linolenic acid and linoleic acid with % of FM and obesity. A negative association was found between FM and alpha-linoleic acid in the females. Our previous study demonstrated that saturated fatty acids in phospholipids (PL) positively correlated with BMI and percentage of body fat [30]. Typical western diets have an unbalanced omega6/omega 3 ratio [26], and Torres-Castillo *et al.* showed a positive association between higher omega 6/omega 3 ratios and obesity. However, we failed to confirm a significant relationship between metabolic/anthropometric parameters and the omega 6/omega 3 ratio [10]. As the study Bauman-Fortin *et al* points out, there can be a significant interaction between ethnic group and omega 6/omega 3 ratios in relation to BMI [31].

Mittendorfer *et al.* demonstrated that total body fat mass is an important determinant of the rate of free FA release into plasma in both men and women [32]. As expected, we found a significant association between the percentage of fat mass (FM) and fatty acid composition. In both males and females, FM was negatively associated with linoleic acid, alpha-linolenic acid, and gondoic acid. Consumption of linoleic acid is vital to proper health [33,34]. In cross-sectional and 10-year prospective data from the Young Finns Study [35], PUFAs (OR: 0.70), including linoleic (OR: 0.67) and docosahexaenoic acids (OR: 0.75), were inversely related with obesity, whereas γ-linolenic acid (OR: 1.32) was positively associated with obesity. Jandacek *et al*. showed that the intake of monounsaturated fatty acids had a positive effect on increasing HDL levels and decreasing TG levels in plasma [34]. Delgado *et al.* observed an inverse relationship between omega 9 and cardiovascular risks [36]. A longitudinal study showed that PUFA composition could affect fat mass over time [16,37].

Limitations and Strengths

In the first round, we examined a representative sample of both genders (2095 subjects: 1130 girls and 965 boys) but at follow-up, only 215 subject participated (131 women and 84 men). Subjects were young adults without comorbidities. The measurements were performed by trained personnel according to the same protocol. Gender dimorphisms in association with FM and FA could be caused by differences in body composition between men and women [32].

It should be taken into account that many factors contribute to fatty acid composition, including genetic predisposition, environmental factors, and metabolic health. For further research, it would be crucial to have more complex longitudinal studies.

Table 2. Relationships between being overweight (including obesity, BMI≥25 kg/m^2) in adulthood and fatty acids in adolescence (serum phospholipids) in both gender as evaluated by the OPLS model and multiple regression.

			OPLS, Predictive component				Multiple regression		
		Variable	Component loading	*t*-statistics	R^a		Regression coefficient	*t*-statistics	
Relevant predictors (matrix **X**)	MONO	PL_18:1n-9c	-0.37	-6.57	0.572	**	-0.117	-4.87	**
		PL_20:1n-9	-0.372	-5.69	0.576	**	-0.131	-4.17	**
	Essential PUFA	PL_18:2n-6	-0.402	-8.83	0.622	**	-0.121	-5.09	**
		PL_18:3n-3	-0.424	-6.67	0.656	**	-0.151	-5.24	**
	SFA	PL_20:0	-0.402	-8.86	0.622	**	-0.117	-6.85	**
	Omega 6	PL_20:3n-6	0.23	8.02	0.357	**	0.057	4.64	**
		PL_20:4n-6	0.422	10.65	0.654	**	0.143	6.05	**
(matrix **Y**)		Overweight	1	6.47	0.504	**			
Explained variability			25.4 % (23.7 % after cross-validation)						

R^a – Component loadings expressed as a correlation coefficient with a predictive component, * p<0.05, ** p<0.01.

Table 3. Relationships between % fat mass in adulthood and fatty acids in adolescence (serum phospholipids) as evaluated by the OPLS model and multiple regression. A: females, B: males.

(A)

			OPLS, Predictive component				Multiple regression		
		Variable	Component loading	*t*-statistics	R^a		Regression coefficient	*t*-statistics	
Relevant predictors (matrix **X**)	SFA	PL_14:0	-0.280	-2.41	-0.416	*	-0.134	-3.71	**
		PL_20:0	-0.499	-7.04	-0.742	**	-0.201	-5.84	**
	MONO	PL_20:1n-9	-0.393	-4.74	-0.584	**	-0.148	-4.14	**
	Essential PUFA	PL_18:2n-6	-0.413	-5.39	-0.614	**	-0.134	-3.29	**
		PL_18:3n-3	-0.443	-8.5	-0.658	**	-0.162	-5.62	**
	Omega 6	PL_20:4n-6	0.405	4.41	0.603	**	0.178	6.44	**
(matrix **Y**)		% fat mass	1.000	10.18	0.587	**			
Explained variability			34.5 % (32.3 % after cross-validation)						

(B)

			OPLS, Predictive component				Multiple regression		
		Variable	Component loading	*t*-statistics	R^a		Regression coefficient	*t*-statistics	
Relevant predictors (matrix **X**)		PL_mono	-0.301	-1.7	-0.397		-0.141	-2.02	*
		PL_w3	-0.445	-3.72	-0.586	**	-0.28	-5.37	**
	MONO	PL_18:1n-9c	-0.326	-1.85	-0.43		-0.159	-2.03	*
		PL_20:1n-9	-0.411	-2.81	-0.541	*	-0.227	-5.33	**
	Essential PUFA	PL_18:3n-3	-0.386	-6.53	-0.508	**	-0.199	-3.21	**
	Omega 3	PL_22:6n-3	-0.387	-4.16	-0.509	**	-0.227	-5.29	**
	MONO	PL_16:1n-7c	0.273	1.98	0.36	*	0.158	2.72	*
	Omega 6	PL_20:3n-6	0.254	2.14	0.335	*	0.164	3.56	**
(matrix **Y**)		% fat mass	1.000	9.05	0.740	**			
Explained variability			54.7 % (47.2 % after cross-validation)						

R^a – Component loadings expressed as a correlation coefficient with a predictive component, * p<0.05, ** p<0.01.

Conclusions

A positive association between omega 6 PUFAs in serum phospholipids in adolescence and excess fat later in life was observed. Dihomo-gamma-linolenic acid and arachidonic acid were significantly positively associated with being overweight. We found a significant negative associations between two FAs (linoleic acid, eicosanoic acid) and both fat mass in women and body weight. Linoleic acid, eicosanoic acid, and oleic acid had negative relationships with fat mass ($p \leq 0.01$). In all analyses, we found negative associations of body weight and % FM with gondoic acid and alpha linolenic acid ($p \leq 0.01$). Essential PUFAs (LA, ALA) exhibited negative associations with fat mass and obesity while omega 6 (other than LA) showed a positive relationship with obesity. On the other hand, MUFA and omega 3 PUFAs had a protective effect against weight gain. These results support previous findings that omega 3 FA intake reduces the risk of developing excess weight and could provide a protective benefit in cardiometabolic health.

Conflict of Interest

There is no conflict of interest.

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