Fatty acid composition of adipose tissue triglycerides after weight loss and weight maintenance. The Diogenes Study.

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Short title: Fatty acids in adipose triglycerides after weight management

Abstract:

Background: Fatty acid composition of adipose tissue changes with weight loss. Palmitoleic acid as a possible marker of endogenous lipogenesis or its functions as a lipokine are under debate.

Objective: To assess the predictive role of adipose triglycerides fatty acids in weight maintenance in participants of the DIOGENES dietary intervention study.

Design: After an 8-week low calorie diet (LCD) subjects with > 8% weight loss were randomized to 5 *ad libitum* weight maintenance diets for 6 months: low protein (P)/low glycaemic index (GI) (LP/LGI), low P/high GI (LP/HGI), high P/low GI (HP/LGI), high P/high GI (HP/HGI), and a control diet.

Methods: Fatty acid composition in adipose tissue triglycerides was determined by gas chromatography in 195 subjects before the LCD (baseline), after LCD and weight maintenance.

Results: Weight change after the maintenance phase was positively correlated with baseline adipose palmitoleic (16:1n-7), myristoleic (14:1n-5) and *trans*-palmitoleic acid (16:1n-7t). Negative correlation was found with baseline oleic acid (18:1n-9).

Conclusion: Lower baseline monounsaturated fatty acids (14:1n-5, 16:1n-7 and trans 16:1n-7) in adipose tissue triglycerides predict better weight maintenance. Lower oleic acid predicts lower weight decrease. These findings suggest a specific role of monounsaturated fatty acids in weight management and as weight change predictors.

Key words: diet, palmitoleic acid, fatty acids, adipose tissue, obesity management

Introduction:

The fatty acid composition of adipose tissue reflects dietary fatty acid intake and also endogenous processing of fat, ie lipolysis and endogenous lipogenesis. Associations between obesity, diabetes mellitus and insulin sensitivity with specific patterns of fatty acid composition of serum phospholipids (Pelikánová *et al.* 2001, Huang *et al.* 0010, Zák *et al.* 2007), muscle membrane phospholipids (Borkman *et al.* 1993, Baur *et al.* 1999), skeletal muscle triglycerides (Manco *et al.* 2000), erythrocyte phospholipids (Ntali *et al.* 2011) and adipose tissue triglycerides (Iggman *et al.* 2010) have been reported. Endothelial dysfunction in type 2 diabetics has also been related to fatty acid composition; saturated fatty acids were related to endothelial dysfunction, while polyunsaturated fatty acids (PUFA) showed protective effects (Perassolo *et al.* 2008). A higher proportion of saturated fatty acids in serum phospholipids seemed to be related to insulin resistance while higher levels of polyunsaturated fatty acids are related to increased insulin sensitivity (Pelikánová *et al.* 2001). Recent studies brought evidence for varying relationships of individual saturated fatty acids to insulin sensitivity and obesity (Iggman *et al.* 2010, Sampath and Ntambi 2005).

Previously, we found that palmitoleic acid (16:1n-7) in serum cholesteryl esters and adipose tissue triglycerides correlated with multiple measures of adiposity and adipose tissue distribution (Kunešová *et al.* 2002). Palmitoleic acid is the product of desaturation of palmitic acid (16:0) by stearoyl CoA desaturase 1 (delta-9 desaturase, SCD1). In monozygotic twins, before and after weight loss followed by one year weight maintenance, highly significant intrapair resemblance of palmitoleic acid percentage in all serum lipid classes (cholesteryl esters, phospholipids and triglycerides) and in adipose tissue triglycerides were found independently of dietary fat intake. This result suggests palmitoleic acid as a metabolic indicator under genetic control reflecting endogenous lipogenesis (Kunešová *et al.* 2002a, Kunešová *et al.* 2002b). In experimental studies palmitoleic acid was suggested as a possible

”lipokine” that allows adipose tissue to communicate with distant organs (Cao *et al.* 2008). However, this lipokine function was not confirmed by other authors, who showed that adipose tissue palmitoleic acid corresponds to endogenous lipogenesis (Gong *et al.*2011, Hertzel *et al.*2006). On the contrary, circulating palmitoleic acid in serum free fatty acids strongly and independently predicted insulin sensitivity in subjects at increased risk of type 2 diabetes (Stefan *et al.*2010). Interestingly, *trans*-palmitoleic acid in serum phospholipids, which represents fatty acid mostly received from exogenous sources, was associated with slightly lower adiposity and independently associated with significantly lower insulin resistance (Mozaffarian *et al.*2010).

To investigate the role of protein and glycaemic index of carbohydrates in the weight maintenance after weight loss, the multicentre Pan European DIOGENES dietary intervention study was performed. Overweight and obese subjects started an eight weeks weight loss period using a low calorie formula diet (LCD 3.2 MJ per day). Only subjects with at least 8% loss of initial weight were eligible to start the weight maintenance phase lasting 6 months. For this period the subjects were randomized to five groups with different dietary protein content and glycaemic index. Energy intake during weight maintenance period was *ad libitum*. The DIOGENES dietary intervention is part of the European integrated project on Diet, Obesity and genes (DIOGENES, www.diogenes-eu.org) (Saris and Harper 2005). The study was performed in 8 European centres and the results of the 6 months weight maintenance period were published recently (Larsen *et al.*2010).

The aim of this part of the DIOGENES project was to assess the fatty acid composition of adipose tissue triglycerides at baseline, after 8 weeks of LCD and after 6 months of weight maintenance and to evaluate their change during this period. The second aim was to evaluate the baseline levels of adipose tissue fatty acids as possible predictors of weight change,

change in body fat distribution and body composition during the weight loss and maintenance period.

Material and methods:

Subjects and study design:

Subjects examined in this study were participants of the Diogenes project in which adipose tissue biopsies were performed before the start of the study, after the LCD period and after the 6-month weight maintenance period. Fatty acid composition of AT triglycerides was analyzed and necessary clinical data were available. This subgroup of the total Diogenes cohort was selected from 8 centres depending on the availability of the fat biopsies for fatty acid analysis. Baseline characteristics of the subjects and effect of the low calorie diet and weight maintenance period are given in Table 1.

The study protocol, methods, procedures and data processing have been described previously (Larsen *et al.*2010, Larsen *et al.* 2009, www.diogenes-eu.org) as well as the dietary intervention (Moore *et al.*2009). Briefly, the subjects were screened and a baseline examination was performed (clinical investigation day 1, CID 1). The subjects then started the weight loss phase following a LCD 3.2 MJ/day (Modifast, Nutrition et Santé, France) supplemented with up to 400g/d of vegetables. At the end of the LCD period, they were examined again (CID2). Subjects who reached a weight loss ≥ 8% of their initial body weight were randomized to one of the following diets: low protein, low glycaemic index (LPLGI), low protein high glycaemic index (LPHGI), high protein low glycaemic index (HPLGI), high protein high glycaemic index (HPHGI) and control diet (C) given the relevant national dietary guidelines. They were instructed to follow the *ad libitum* randomized diet for six months. Following the 6 months weight maintenance period the subjects were examined again (CID 3). Fasting blood samples were drawn at each of the 3 CIDs for the analysis of blood

metabolites. Samples of subcutaneous adipose tissue were obtained from the periumbilical area by needle aspiration under local anaesthesia following an overnight fast at each of the time points. All procedures were standardized between study centres across Europe, and biopsy samples were stored at -80^{0}C until analysis. The lipid fraction was extracted from the fat cake during RNA extraction by using the RNeasy total RNA Mini kit (Qiagen) (Márquez-Quiñones *et al.*2010). The values of the anthropometric and laboratory parameters are marked 1, 2 and 3 according to CID in which they were obtained.

Fatty acid composition:

Analysis of the fatty acid composition of the lipid fraction consisting of adipose tissue triglycerides was performed by gas chromatography. Total lipid was transmethylated to fatty acid methyl esters (FAME) with 1M sodium methoxide in dry methanol under nitrogen atmosphere in darkness (60 min at laboratory temperature). The reaction mixture was neutralized with 1M acetic acid, FAME were extracted twice into hexane and passed through a 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 (Thermo Finnigan, USA) gas chromatograph equipped with a capillary split/splitless injector and flame-ionization detector (FID), combined with AS 2000 autosampler (Thermo Finnigan). Analyses of FAME were performed on fused-silica capillary columns coated with chemically bonded stationary phases Select FAME (100 m x 0.32 mm I.D.) (Varian, the Netherlands). The oven temperature was programmed from 80°C to 260°C at 2°/min, then isothermal 25 min. The injector and detector temperatures were 250 and 270 °C, respectively. Hydrogen carrier gas was maintained at a head pressure of 70 kPa and total flow 25 ml/min. Integration software Clarity for Windows® (Data Apex® Ltd., Praha) was used for data acquisition and handling. Ratios

16:1n-7/16:0 and 18:1n-9/18:0 were used as measures of SCD activity because they reflect stearoyl CoA gene expression (SCD, delta-9 desaturase, Sjögren *et al.* 2007).

Statistical methods:

The data were evaluated by repeated measures ANOVA with Bonferroni correction.

To eliminate skewed data distribution and heteroscedasticity, the original data was transformed to a Gaussian distribution by a Box-Cox transformation before further processing using the statistical software Statgraphics Centurion, version XVI from Statpoint Inc. (Herndon, Virginia, USA).

The relationships between change of anthropometric characteristics (matrix **Y**) and their initial values and initial values of fatty acids and further laboratory data (matrix **X**) were simultaneously evaluated using multivariate regression with reduction of dimensionality, known as bidirectional orthogonal projections to latent structures (O2PLS). The data transformed by Box-Cox transformations underwent processing by O2PLS method (Trygg *et al.* 2007, Trygg and Wold 2002, Hill *et al.* 2010). In contrast to ordinary multivariate regression or multiple regression, O2PLS is effective in coping with the problem of severe multicollinearity within the X and within the Y. The aforementioned model enabled us to find the variables with high predictive value for description of relationships between **X** and **Y** and to find a structure of these relationships.

We have tested the relevance of individual variables for the model using a criterion Variable Importance (VIP). Only the variables that showed significant relevance for the first and/or the second predictive component were included in the model. Similarly, the relevant number of predictive components was tested using a criterion Prediction Error Sum of Squares (PRESS). The statistical software SIMCA-P+ Version 12.0.0.0 from UmetricsAB (Umeå, Sweden) was used for data analysis. The software enabled us to find the number of the relevant components

utilizing the prediction error sum of squares and also allowed the detection of multivariate non-homogeneities and testing the multivariate normal distribution and homoscedasticity (homogeneity of variance).

Relationships between two variables were evaluated by Spearman's correlations.

Due to significant heterogeneity in weight loss between the eight centers - in the Bulgarian group during the weight maintenance period significant higher weight loss was found in comparison with the other seven centers ($p<0.001$)- we excluded this center from analysis in this study.

Ethics

The study was approved by the local ethics committees in the respective countries. The protocol was in accordance with the Declaration of Helsinki (Declaration of Helsinki 2009), all study participants signed an informed consent document after verbal and written instructions and according to local legislation.

Results:

Changes in percentage of individual fatty acids in adipose tissue triglycerides are shown in table 2. In comparison with baseline values, decreases in saturated (myristic 14:0 and and palmitic 16:0), and monounsaturated fatty acids (myrimyristoleic 14:1n-5 and palmitoleic acid 16:1n-7) were found after LCD and weight maintenance period and in *trans*-linoleic acids (18:2 n-6tt,ct and tc) after weight maintenance. Significant increases were found in oleic acid (18:1n-9) and stearic (18:0) acid after weight maintenance, in oleic acid also after LCD. Increases were shown in n-6 PUFA´s - linoleic (18:2n-6cc), dihomo-gamma-linoleic (20:3n-6), arachidonic acid (20:4n-6) and docosatetraenoic acid (22:4n-6), and in n-3 PUFA´s

eicosapentaenoic (EPA, 22:5n-3) after LCD and weight maintenance and in docosahexaenoic (DHA, 22:6n-3) after weight maintenance only.

When evaluating percentages of adipose tissue fatty acids at baseline (CID1) as predictors of weight change after the weight management (CID3-CID1) we found that only myristoleic (14:1n-5), palmitoleic (16:1n-7) and *trans*-palmitoleic (16:1n-7 t) acids were significantly positively correlated with weight loss, ie the lower the initial percentage the greater was the weight loss. Percentage of oleic acid (18:1n-9) correlated negatively with weight loss as well as previously shown initial weight and BMI and waist (Handjieva-Darlenska *et al.* 2010) (Tab.3, Fig 1). The variability of the weight loss (dependent variable) explained by independent variables (initial fatty acids and anthropometrical traits) was 13.0% (10.9% after cross-validation).

Spearman rank correlations show significant positive association of weight and waist changes after weight maintenance with basal sum of saturated fatty acids, negative correlation with basal sum of monounsaturated fatty acids and SCD activity, expressed as ratio 18:1n-9/18:0, the correlation with 16:1n-7/16:0 was not significant. Trans fatty acids correlated positively with weight change only (Table 4).

The effect of protein quantity in the diet and of glycaemic index of the diet will be evaluated elsewhere.

Discussion:

In the DIOGENES group of subjects we found significant positive correlations between total weight loss with percentage of myristoleic (14:1n-5), palmitoleic (16:1n-7) and *trans*-palmitoleic acid (16:1n-7t) in adipose tissue triglycerides at baseline. This outcome in

agreement with previous findings suggests that palmitoleic acid levels in adipose tissue reflect endogenous lipogenesis (Kunesova *et al.* 2002a, Gong *et al.*2011, Hertzel *et al.*2006). Also myristoleic acid (product of desaturation of myristic acid in humans), a minor fatty acid in adipose tissue, seems to be an indicator of endogenous lipogenesis (Lands 1995). This observation is in agreement with a study in 1926 subjects in which a positive association between adipose tissue palmitoleic acid concentrations and adipose tissue desaturation indices with obesity was shown (Gong *et al.*2011). In elderly men, a negative correlation of insulin sensitivity with dietary and adipose tissue palmitic and palmitoleic acid and some PUFA´s (20:3n-6, 20:4n-6, 22:4n-6, 22:5n-3 and 22:6 n-3) was shown. In this group, insulin sensitivity positively correlated with lauric (12:0), myristic (14:0), margaric (17:0) and stearic (18:0) acids and essential fatty acids linoleic acid (18:2n-6) and alpha linolenic acid (18:3n-3). Most associations were diminished or disappeared in lean individuals, indicating a role of obesity (Iggman *et al.* 2010). A high proportion of palmitoleic acid in serum cholesteryl esters independently predicted high plasma glucose concentrations after 5 year follow-up in Amerindian women (Lindgärde *et al.* 2006).

The lipogenic capacity of human adipose tissue is lower than in rats (Letexier *et al.* 2003) and de novo lipogenesis is highly regulated, for review see (Strable and Ntambi 2010). In mice with modified FABP expression a relation between palmitoleate and de novo lipogenesis and SCD activity has been shown. In adipose tissue of adipose-FABP null mice increased de novo lipogenesis was associated with enhanced levels of palmitic acid and palmitoleic acid and increased fat mass (Hertzel *et al.*2006). In mice with FABP (aP2 and mal1) mutations characterized by resistance to diet-induced obesity and metabolic syndrome significantly enhanced insulin receptor signaling, enhanced muscle AMP-activated kinase (AMP-K) activity, and reduced liver SCD activity were found (Maeda *et al.*2005). These studies show

close relationship of palmitate and palmitoleate levels with endogenous lipogenesis in mice´ adipose tissue and in liver.

As shown in recent reviews (Flowers and Ntambi 2008, Paton and Ntambi 2008), scd1-deficient mice have reduced lipid synthesis and enhanced lipid oxidation, thermogenesis and insulin sensitivity in various tissues including liver, muscle and adipose tissue. SCD 1 is required for protection against dietary unsaturated fat deficiency, leptin deficiency-induced diabetes, and palmitate-induced lipotoxic insults in muscle and pancreatic beta-cells. In obesity, starvation and exercise increased muscle SCD1.

In humans, an association of delta-9 desaturase activity with adiposity and plasma lipid profile was suggested in a group of healthy adolescent women; this supports the assumption that delta-9 activity independently reflects higher body mass index and higher circulatory triglyceride levels (Zhou *et al.* 2009). We did not find a decrease in SCD1 activity calculated as ratios 16:1n-7/16:0 and 18:1n-9/18:0 in adipose tissue. This was in contrast to a study including a lifestyle intervention that found a significant decrease in total and saturated fat intake, a decrease in BMI and HOMA insulin resistance associated with a decrease in delta-9 desaturase (Corpeleijn *et al.* 02006).

The relationship between fatty acids reflecting endogenous lipogenesis and weight (a marker of total adipose tissue) was not related to waist circumference (a marker of abdominal fat). This could be related to the recently found greater enrichment of palmitoleic acid (16:1n-7) associated with higher expression of SCD1 and with higher content of SCD1-derived fatty acids in gluteofemoral adipose tissue (Pinnick *et al.*2012).

In our study, we found that basal *trans*-palmitoleic acid (trans 16:1n-7) was a negative predictor of weight loss. This is in contrast to a study showing that *trans*-palmitoleic acid correlates with slightly lower adiposity (Mozaffarian *et al.*2010). The different result could be

due to a weak association between dietary triglyceride fatty acids, which are predominantly saturated, and composition of fatty acids in adipose tissue triglycerides (Hodson *et al.* 2008). At the end of the study, we found a significant decrease in proportion of the most saturated and monounsaturated fatty acids with exception of stearic acid (18:0) and oleic acid. Concurrently, we found a negative correlation of baseline oleic acid (18:1n-9) with total weight loss (the higher baseline oleic acid the higher weight loss). Oleic acid was shown to control the expression of SCD1 at the transcriptional level leading to a decrease in SCD1 mRNA content in human aortic smooth muscle cells (Minville-Walz *et al.*2012). Oleic acid may exert a similar effect in adipose tissue. Stearic acid was shown to generate a lower lipemic response in comparison with palmitic and myristic acid, and also is a poor substrate for TG synthesis (Sampath and Ntambi 2005). Stearic acid exerts neutral effect also on cholesterol metabolism in comparison with saturated fatty acids with 12-16 carbons. These results are in agreement with a positive relationship of stearic acid to insulin sensitivity and negative to BMI (Iggman *et al.* 2010, Roberts *et al.* 2009). A lower decrease of stearic acid may also reflect a higher decrease in SCD activity. This precedes induction of other lipogenic genes and transcription factors as sterol regulatory element binding protein 1c (SREBP 1c), a key regulator of lipogenic gene transcription, carbohydrate response element binding protein (ChREBP) (Strable and Ntambi 2010, Clark *et al.*2002, Biddinger *et al.*2005) and peroxisome proliferator-activated receptor-gamma coactivator-1beta (PGC-1beta, Sampath *et al.* 2007). Insulin effect is mediated through SREBP 1c. Leptin (Hodson *et al.* 2008), glucagon (Lefevre *et al.* 1999) and AT II receptor blocker (Yokozawa *et al.* 2009) decrease SCD1 mRNA activity. The relationship of stearic acid content with insulin sensitivity (positive) and BMI (negative) could be mediated by adipocyte size, smaller adipocytes are more insulin sensitive. Roberts et al. have shown a strong positive relationship of adipose TG myristic and stearic acid with adipocyte size and insulin sensitivity (HOMA model) in humans. Lipogenic gene

expression was shown to be inversely correlated with adipocyte cell size (Roberts *et al.* 2009). Different regulation was proposed for adipose and hepatic de novo lipogenesis (Roberts *et al.* 2009). This is supported by a study showing up-regulation of SCD1 and enhancement of SCD activity in the liver of mice treated by rosiglitazone possibly in association with insulin sensitizing effect of thiazolidinediones (Kuda *et al.* 2009).

The consistent increase was found in this study in the percentage of n-6 PUFA and to a lesser extent increases in long chain n-3 PUFA [(eicosapentaenoic acid (20:5 n-3), docosapentaenoic (22:5n-3) and docosahaxaenoic acid (22:6n-3)] which most likely reflects changes in dietary fat composition during the weight maintenance phase (Hlavaty *et al.* 2008).

Adipose tissue distribution was shown to be related to fatty acid composition. Specifically, central obesity was positively associated with n-6 polyunsaturated fatty acids and inversely associated with monounsaturated fatty acids and n-3 polyunsaturated fatty acids in adipose tissue (Garaulet *et al.* 2001, Phinney *et al.*1994). We found a negative correlation of monounsaturated fatty acids and surprisingly also of SCD1 activity expressed as 18:1n-9/18:0, questioning usage of this ratio as reflection of SCD activity due to supposed higher role of exogenous sources of C18 acids, and a positive correlation of saturated fatty acids with change in weight and waist circumference.

In conclusion, higher baseline proportion of palmitoleic, myristoleic and *trans* palmitoleic acids in adipose triglycerides predict less successful weight maintenance. Conversely, oleic acid percentage negatively predicts weight change. During the long term weight maintenance diet a favorable change in fatty acid composition including a decrease in most saturated and monounsaturated (14:1n-5, 16:1n-7, 16:1n-7t) fatty acids was found concurrently with an increase in n-6 and to a lesser extent also in n-3 polyunsaturated fatty acids.

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Figure Legends

Fig 1. Relationships between change in weight (matrix Y) and basal anthropometry and adipose triglyceride fatty acid composition (matrix X)

1 initial values 3-1 difference between weight maintenance and initial value

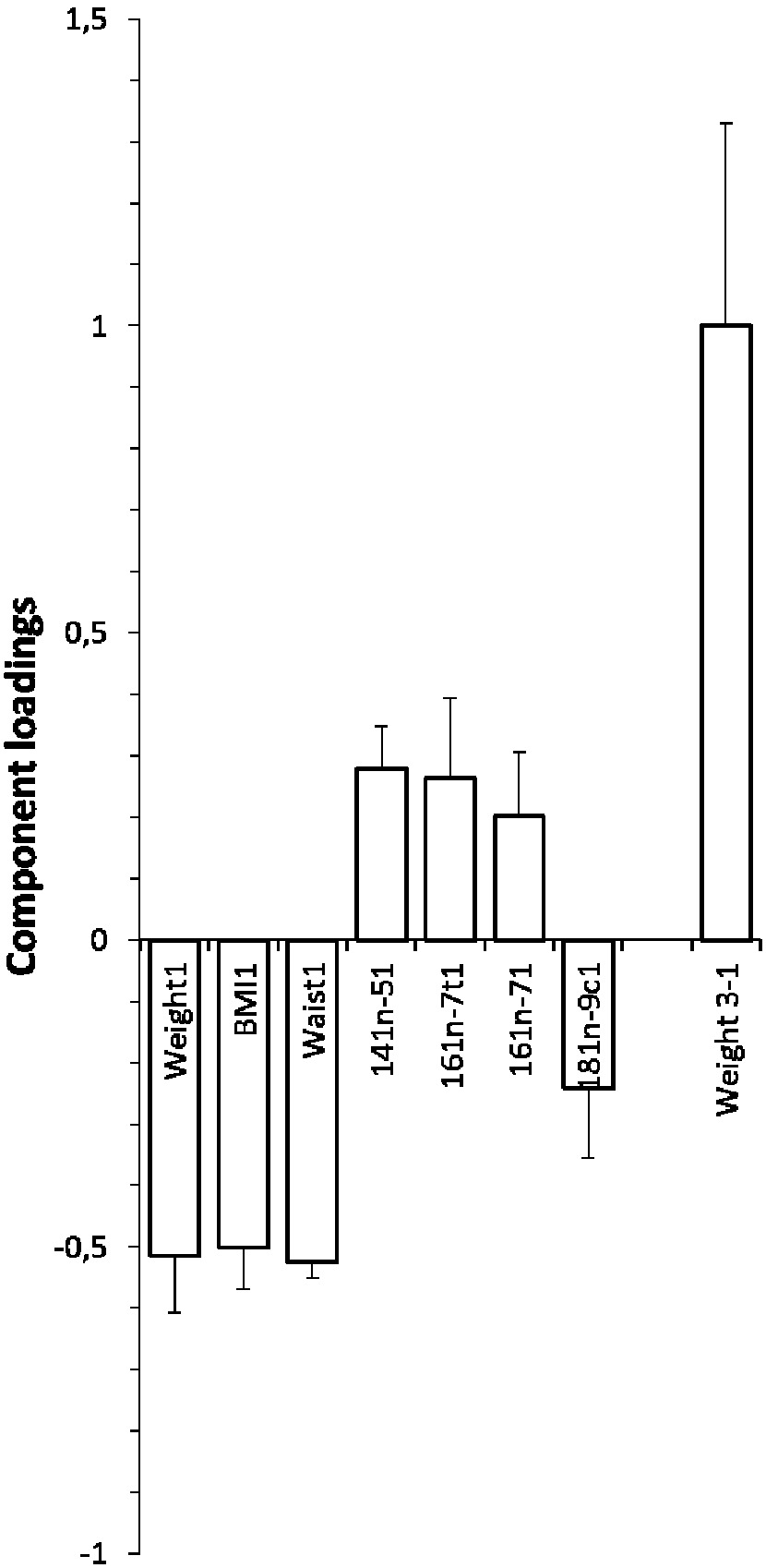
Component loadings
1,5
1
0,5
0
-0,5
-1
Weight1
BMI1
Waist1
141n-51
161n-7t1
161n-71
181n-9c1
Weight 3-1

Table 1
Characteristics of subjects before the treatment and after LCD and weight maintenance phase (n=195) Mean ±SD

Variable	Before	LCD	Weight maintenance
Age	42.05±5.61		
Weight (kg)	96.67±15.53	85.67±13.79*	86.99±14.47*
Height (m)	1.69±0.09		
BMI	33.57±4.41	29.84±4.06*	30.22±4.25*+
Waist (cm)	105.46±12.06	95.70±11.79*	96.55±11.47*
Hip (cm)	115.71±10.17	108.16±9.68*	108.88±9.88*
SAD (cm)	24.61±3.73	21.44±3.33*	21.70±6.45*
SBP (mm Hg)	123.27±13.74	116.16±13.44*	120.63±13.59*+
DBP (mm Hg)	75.63±10.39	71.78±10.12*	73.22±10.78*+
FM (kg)	38.19±10.64	30.12±10.50*	30.50±10.52*
FM (%)	39.44±8.25	34.87±10.08*	34.85±9.44*

* $p<0.05$ in comparison with basal level
+ $p<0.05$ in comparison with LCD level

Table 2
Percentage of fatty acids in adipose triglycerides during the weight management

Variable	*Before*	*LCD*	*Weight maintenance*
12:0	0.29±0.01	0.25±0.009*	0.30±0.01+
14:0	2.57±0.04	2.36±0.04*	2.44±0.05*+
14:1n5	0.26±0.007	0.23±0.006*	0.24±0.007*+
16:0	23.26±0.15	22.73±0.15*	22.58±0.15*
16:1n-7c	4.57±0.09	4.23±0.09*	4.25±0.09*
16:1n-7t	0.032±0.001	0.028±0.001*	0.027±0.001*+
16:1n-9	0.66±0.007	0.70±0.006*	0.67±0.007*+
18:0	3.79±0.06	3.85±0.05	3.98±0.06*
18:1n-7c	1.91±0.02	1.89±0.03	1.86±0.02
18:1n-9c	46.14±0.22	47.15±0.22*	46.82±0.23*+
18:1n-9sum t	1.03±0.03	1.04±0.02	1.03±0.02
18:2n-6cc	12.45±0.15	12.44±0.16	12.66±0.15*+
18:2n-6tt	0.013±0.0007	0.011±0.0005*	0.011±0.0005*
18:2n-6tc	0.10±0.005	0.092±0.005*	0.097±0.006*
18:2n-6ct	0.047±0.002	0.038±0.002*	0.036±0.002*
18:3n-6alc	0.049±0.002	0.05±0.001	0.05±0.002+
18:3n-3alc	0.47±0.02	0.41±0.02*	0.42±0.02+
20:0	0.35±0.02	0.31±0.02	0.34±0.02
20:1n-9c	0.63±0.008	0.69±0.009*	0.70±0.008*
20:2n-6cc	0.19±0.003	0.21±0.004*	0.20±0.003*
20:3n-9alc	0.024±0.0009	0.026±0.0009	0.026±0.001
20:3n-6alc	0.24±0.006	0.27±0.006*	0.26±0.006*+
20:4n-6alc	0.41±0.007	0.44±0.008*	0.44±0.008*
20:5n-3alc	0.06±0.002	0.05±0.002	0.06±0.003
22:4n-6alc	0.14±0.004	0.16±0.004*	0.16±0.003*
22:5n-6alc	0.035±0.0002	0.036±0.001	0.029±0.001*+
22:5n-3alc	0.15±0.004	0.17±0.004*	0.17±0.004*
22:6n-3alc	0.11±0.003	0.11±0.004	0.13±0.004*+
SFA	30.12±0.22	29.39±0.21*	29.51±0.22*
MFA	54.18±0.22	54.91±0.23*	54.55±0.23+
TFA	1.23±0.03	1.21±0.02	1.20±0.03
PUFAn6	13.51±0.15	13.61±0.02	13.8±0.16*+
PUFAn3	0.93±0.02	0.86±0.02*	0.91±0.02+
16:1n-7/16:0	0.20±0.004	0.19±0.004	0.19±0.003
18:1n-9c/18:0	12.76±0.23	12.82±0.24	12.28±0.22

mean±SE
* $p<0.05$ in comparison with basal level
+ $p<0.05$ in comparison with LCD level

Table 3 Relationships between weight change (weight3 - weight1), (explained variable) and baseline fatty acid composition and anthropometrical traits (explanatory variables) evaluated using multivariate regression with reduction of dimensionality (model of Orthogonal Projections to Latent Structures, OPLS).

		Component of weight loss				
		Explained variability = 13% (10.9%)				
	Variable	Parameter [a]	95% CI [b]	99% CI	Parameter /95% CI [b]	R [c]
	Weight1	-0.514	0.093	0.147	-5.54	-0.816 **
	BMI1	-0.501	0.068	0.107	-7.41	-0.795 **
	Waist1	-0.525	0.027	0.043	-19.37	-0.833 **
X	FA141n51	0.278	0.069	0.109	4.05	0.442 **
	FA161n7t1	0.264	0.131	0.207	2.01	0.418 **
	FA161n71	0.202	0.103	0.163	1.96	0.321 **
	FA181n9c1	-0.241	0.114	0.181	-2.11	-0.383 **
Y	Weight3 - Weight1	1.000	0.330	0.522	3.03	0.361 **

a…component loadings for the predictive components expressed as regression coefficients; b…confidence interval; c…component loadings for the predictive components expressed as correlation coefficients of individual variables with the predictive components

Table 4

Spearman rank correlations between basal percentage of fatty acids in adipose triglycerides and change in weight and waist after weight maintenance period

Fatty acid	*Weight 3 – weight 2*		*Waist 3- waist 2*	
Basal values	r	P	r	P
SFA	0.22	0.003	0.28	0.001
MFA	-0.22	0.002	-0.29	0.001
TFA	0.21	0.003	0.09	NS
16:1n-7/16:0	-0.001	NS	-0.04	NS
18:1n-9c/18:0	-0.16	0.02	-0.16	0.02

Values 2 after low calorie diet
Values 3 after weight maintenance