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Change in Fatty Acid Composition of Serum Lipids in Obese Females After Short-Term Weight-reducing Regimen with the Addition of n-3 Long Chain Polyunsaturated Fatty Acids in Comparison to Controls

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Summary

Short-term weight-reducing regimens were shown to influence fatty acid composition of serum lipids unfavorably. Adding long chain n-3 polyunsaturated fatty acids (n-3 LC PUFA) to a low-calorie diet (LCD) could avoid these changes. The aim of this study was to examine the effect of a short-term in-patient weight-reducing regimen including LCD with yogurt enriched by low doses of n-3 PUFA (n-3 LCD). The enriched yogurt contained 790 mg of fish oil, predominantly eicosapentaenoic (20:5n-3; EPA) and docosahexaenoic (22:6n-3; DHA). Forty obese women were randomly assigned to the group consuming LCD and joghurt either with or without n-3 enrichment. Following the 3-week diet in the n-3 LCD group a significantly higher increase in the proportion of n-3 LC PUFA (sum of n-3 FA, EPA and DHA) in serum lipids was confirmed. In phospholipids (PL) a significant difference in the sum of n-6 fatty acids was found, a decrease in the n-3 LCD group and an increase in LCD group. Significantly higher increase in the PL palmitate (16:0) was shown in the LCD group. The results suggest that low doses of n-3 fatty acid enrichment can help to avoid unfavorable changes in fatty acid composition in serum lipids after a short-term weight-reducing regimen.

Key words

Obesity treatment • Fatty acid composition • Weight reduction n-3 fatty acids • EPA • DHA

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Introduction

Composition of dietary fat is one of the nutritional factors, which has been shown in the last years to influence the outcome of weight-reducing regimens in human (Kriketos *et al.* 2001, Clifton *et al.* 2004, Huber *et al.* 2007, Krebs *et al.* 2006, Kunešová *et al.* 2006, Thorsdottir *et al.* 2007) as well as in animal experiments (Růžičková *et al.* 2004, Flachs *et al.* 2005). Increased beta-oxidation was shown in rodents (Ukropec et al 2003) and in human (Kunešová *et al.* 2006, Couet *et al.* 1997). Long chain fatty acids act at the preadipocyte stage during adipogenesis and stimulate the formation of adipocytes. Long chain fatty acids behave as ligands of PPAR alpha, beta/delta and gamma. Arachidonic acid (20:4n-6, AA) is the predominant precursor of eicosanoids and leucotriens participating in this process. Under isoenergetic conditions *in vivo* experiments have shown that diet enriched by linoleic acid (18:2n-6, LA) enhances fat mass and alpha-linolenic acid (18:3n-3, LNA), counteracting this effect. A critical role is played by AA and prostacyclin receptors in excessive adipose tissue development in the gestation/lactation period. Epidemiological studies in infants found the same results as animal experiments. So, n-6 and n-3 fatty acids differ in their effect on development of adipose tissue (for review see Ailhaud *et al.* 2006). Fatty acid composition of membranes was shown to be influenced by many factors as CD36 fatty acid transporter with subsequent

Table 1. Characteristics of obese women before treatment and the effect of the weight-reducing regimen (n-3 LCD versus LCD)

	LCD with n-3				LCD						
	Baseline		After 21 days		Baseline		After 21 days				
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*p	xp	xxpa
Age (years)	55.2	13.2			59	10.2			NS		
Height (cm)	163	6.98			163	6.74			NS		
Weight (kg)	87.6	9.5	85.2	9.54	96.3	13.9	92	13.8	0.03	0.0003	<0.0001
BMI	33.1	2.83	32.1	2.9	36.2	4.11	34.6	4.14	NS	0.001	<0.0001
Percentage FM	42.2	4.07	41.6	3.67	45	3.9	41.7	3.9	NS	0.0001	<0.0001
Fat mass (kg)	37.2	6.91	35.6	5.2	43.5	9.04	37.1	2.59	0.02	0.005	<0.008
Fat free mass (kg)	50.4	4.52	49.6	4.57	52.9	5.99	55.7	3.88	NS	NS	NS
Waist (cm)	99.9	10.5	97.8	10.1	111	11.2	107	11	0.02	NS	NS
Hip (cm)	117	8.94	115	7.66	122	11.7	119	12.2	NS	NS	NS

FM - fat mass, FFM - fat free mass, *p significance of the difference in baseline levels between the groups, xp significance of the difference in treatment effect between the groups, xxpa significance of the difference in treatment effect between the groups, after adjustment for baseline weight

Table 2. Effect of the treatment on blood lipids, markers of glucose metabolism and inflammation

	LCD with n-3 FA				LCD						
	Baseline		After 21 days		Baseline		After 21 days				
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*p	xp	xxpa
FBG (mmol/l)	5.39	1.24	4.84	1.42	5.86	0.9	5.07	0.65	NS	NS	NS
CP (nmol/l)	1.02	0.29	1.03	0.26	1.33	0.42	1.25	0.38	0.02	NS	NS
Insulin (mIU/l)	12.8	6.85	10.1	4.01	14.9	5.41	12.8	5.69	NS	NS	NS
TC (mmol/l)	5.58	1.01	5.07	0.77	5.63	0.87	5.2	0.88	NS	NS	NS
HDL-C (mmol/l)	1.47	0.4	1.49	0.32	1.39	0.33	1.28	0.3	NS	0.01	0.04
LDL-C (mmol/l)	3.98	0.9	3.71	0.66	4.11	0.92	3.1	0.74	NS	0.001	0.001
TG (mmol/l)	1.40	0.46	1.38	0.47	1.97	1.02	1.46	0.81	0.05	0.01	0.001
CRP (mg/l)	2.78	1.9	2.8	2.85	6.46	7.06	4.52	5.51	NS	NS	NS

*p significance of the difference in baseline levels between the groups, xp significance of the difference in treatment effect between the groups,xxpa significance of the difference in treatment effect between the groups, after adjustment for baseline weight

effect on insulin sensitivity (Kontrová et al 2007). Lower proportion of n-3 long chain polyunsaturated fatty acids (n-3 LC PUFA) in serum phospholipids content was confirmed in obese adolescents (Karlsson *et al.* 2006). In adults, central obesity was positively associated with high quantities of 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).

These findings should be reflected also in changes in human dietary habits. In the Czech Republic there is low consumption of fish and fish products resulting in low n-3 long chain polyunsaturated fatty acids (n-3 LC PUFA) intake (5.8 kg of fish and fish products/person/year, Czech Statistical Office 2005).

Inclusion of fish oils in a weight-reducing diet has been shown to have positive effect on health risks associated with obesity (Mori et al 1999). Short-term weight-reducing regimens influence fatty acid composition of serum and adipose tissue lipids unfavorably (Phinney *et al.* 1990, 1991, Kunešová *et al.*, 2002).

The aim of our study was to examine the effect of the usage of yogurt enriched with n-3 fatty acids during a weight-reducing regimen in moderately obese women.

Methods

Subjects

Forty moderately obese women were randomly assigned to a low calorie diet including yogurt containing n-3 PUFA supplement (n-3 LCD, n=20) or yogurt without n-3 supplementation (LCD, n=20) during their weight-reducing regimen in the Spa Obesity Unit in spring 2004. Characteristics of the study subjects at the baseline are given in Tables 1 and 2. The women were mostly postmenopausal and the number of premenopausal women was similar in both groups. Subjects with diabetes, uncompensated thyroid dysfunction and subjects treated with hormonal contraceptives or hormonal replacement therapy, diuretics or other drugs affecting water balance were excluded from the study.

The study was approved by the Medical Ethical Committee of the Institute of Endocrinology.

Design of the study

The weight-reducing regimen consisted of a baseline weight stabilization period followed by an in-patient weight-reducing period. The regimen included a defined low calorie diet (LCD), daily light to moderate physical activity supervised by a physiatrist and cognitive behavioral modification of lifestyle. The diet was prepared in the spa central kitchen and its energy content was calculated using the PC program „Nutrition“. This software has nearly 3000 food items, and its evaluation includes energy intake, macronutrient and micronutrient content. Patients consumed a weight maintenance diet during their initial 3 days of the in-patient stay. Then the LCD was started with 5500 kJ/day (protein 22.7 %, fat 28.7 %, carbohydrate 48.6 %). The energy deficit was 2500 kJ/day compared to both the calculated energy expenditure and the diet during the weight maintenance period. The patients were assigned to LCD either including yogurt supplemented with n-3 highly unsaturated fatty acids (n-3 LCD) or without this supplement (LCD). Supplemented yogurt contained 790 mg/day of n-3 PUFA, from which, 620 mg/day was eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 20:6 n-3) acid. The yogurt was produced by Dairy Research Institute Milcom®. The weight-reducing regimen included daily light to moderate physical activity lasting about 60 min/day.

Body composition, laboratory analysis and psychobehavioral examination were investigated before the intervention and after 21 days of weight-reducing regimen.

Biochemical analysis

Blood samples were drawn in the morning after 12 hour overnight fasting. Biochemical parameters measured included total cholesterol (TC), HDL-cholesterol (HDL-C), LDL cholesterol (LDL-C), triglycerides (TG), fasting blood glucose (FBG), fasting serum insulin (insulin), C-peptide (CP) and C-reactive protein (CRP). Laboratory analyses were performed by routine laboratory methods.

Fatty acid composition

The measurement of fatty acid composition of serum lipids was performed by gas chromatography after separation of individual serum lipid fractions (serum phospholipids – PL, triglycerides – TG and cholesterol esters - CE) by thin-layer chromatography on silica gel (Tvrzická *et al.* 2002).

Body composition and regional tissue distribution

Anthropometric estimation of body fat was performed by measurement of the following skinfolds: subscapular, suprailiac, triceps and biceps. Waist and hip circumference were measured following the standardized procedure recommended at the Airlie Conference (Lohman *et al.* 1989). Body fat content was estimated by bioelectrical impedance measurement (Tanita BC 418 MA, Tanita Inc., Japan).

Psychobehavioral examination

Eating behavior was evaluated by the 3-item Eating Inventory (Stunkard and Messick 1985) and for the evaluation of depression score the Beck Depression Inventory (Beck *et al.* 1961) was used.

Statistical methods

Data are expressed as means ± SD. The Mann-Whitney robust test was used for testing the differences between groups, while the Wilcoxon test was applied for evaluation of treatment effect. The differences between individual groups or subgroups were evaluated using ANOVA and least significant difference multiple comparisons.

Results

The characteristics of the group and the results

Table 3a. Fatty acid composition in serum lipids before and after treatment - Phospholipids

Phospholipids	LCD with n-3 FA n=20		LCD n=19	
Fatty acid	**Baseline**	**Day 21**	**Baseline**	**Day 21**
12:0	0.05±0.05	0.05±0.03	0.03±0.01	0.03±0.01
14:0	0.60±0.72	0.53±0.57	0.22±0.06	0.18±0.04***
14:1n-5	0.03±0.05	0.03±0.04	0.01±0.01	0.01±0.00
16:0	29.97±1.60	29.59±1.93	29.95±1.08	31.14±1.12***++
16:1n-9	0.17±0.16	0.19±0.19	0.09±0.01	0.09±0.01
16:1n-7c	1.12±1.14	1.18±1.26	0.51±0.11	0.49±0.09
18:0	11.44±4.44	11.15±4.47*	14.14±1.15	12.59±1.15***+++
18:1n-9	15.28±12.30	14.69±12.16	9.05±0.69	9.05±0.73
18:1n-7	1.70±0.48	1.77±0.51	1.46±0.18	1.61±0.17***
18:2n-6	21.68±4.85	21.08±3.60	21.89±3.12	22.39±3.08+
18:3n-6	0.10±0.08	0.11±0.12	0.07±0.03	0.06±0.03
18:3n-3	0.29±0.26	0.28±0.27	0.16±0.06	0.14±0.04
20:0	0.03±0.01	0.03±0.01	0.03±0.00	0.03±0.00*
20:1n-9	0.14±0.04	0.13±0.03*	0.12±0.02	0.12±0.01+
20:2n-6	0.42±0.11	0.37±0.08***	0.44±0.12	0.37±0.06***
20:3n-6	2.66±1.33	2.47±1.23*	3.19±0.67	2.75±0.70**
20:4n-6	9.17±4.56	9.46±4.57	12.18±1.63	12.81±1.92*
20:5n-3	0.79±0.55	1.44±0.66***	1.17±0.46	0.78±0.22**+++
22:4n-6	0.25±0.08	0.23±0.06**	0.26±0.05	0.26±0.04
22:5n-6	0.18±0.08	0.16±0.06*	0.16±0.05	0.16±0.05+
22:5n-3	0.66±0.22	0.79±0.24***	0.78±0.14	0.76±0.14+++
22:6n-3	3.24±1.71	4.25±1.87***	4.10±0.81	4.17±0.82+++
Saturated	42.10±3.99	41.35±5.17	44.37±0.84	43.97±0.95*
Monounsaturated	18.46±14.07	18.00±14.06	11.23±0.78	11.37±0.78
PUFA n-6	34.46±8.96	33.89±7.16	38.19±1.62	38.81±1.92+++
PUFA n-3	4.98±2.10	6.76±2.43***	6.21±1.17	5.85±1.01+++

Values are expressed as mean ± S.E.M. (in mol %), *p<0.05 **p<0.01 ***p<0.001 in comparison with baseline value, +p<0.05 ++p<0.01 +++p<0.001 in comparison with change in the LCD group

of the weight-reducing regimen are shown in Table 1. Significantly higher initial weight, fat mass and waist circumference was found in the control group which was the most likely cause of the higher weight, BMI, per cent of fat and fat mass loss in the LCD group after the weight-reducing regimen.

In n-3 LCD an increase in HDL-cholesterol was found, while the LCD group showed a decrease, nevertheless, in LDL cholesterol a significantly higher decrease was found in the LCD group. Basal triglycerides (TG) were significantly higher and their decrease was higher after weight reduction in the LCD group (see Table 2). Differences in the changes in glucose metabolism were not found (fasting glucose, fasting insulin and C-peptide). Changes in characteristics of food intake and Beck depression score were not significantly different (data not shown).

In all examined lipids fractions the increase in n-3 fatty acids in the treated group in comparison with controls was found (Table 3a-c). The increase in n-3 fatty acid proportion in the n-3 LCD group was accompanied by a significant decrease of n-6 fatty acid proportion in serum phospholipids (PL) in comparison with LCD group. On the contrary, a significant increase in proportion of arachidonic acid (20:4n-6) and palmitic acid (16:0) in the LCD group PL was shown. Stearic acid (18:0) in PL decreased in both groups, the decline was significantly higher in LCD group. The changes in fatty

Table 3b. Fatty acid composition in serum lipids before and after treatment – Triglycerides

Triglycerides	LCD with n-3 FA n=20		LCD n=19	
Fatty acid	**Baseline**	**Day 21**	**Baseline**	**Day 21**
12:0	0.17±0.10	0.13±0.09*	0.18±0.15	0.12±0.04*
14:0	1.76±0.53	1.66±0.52	1.63±0.44	1.29±0.25***+
14:1n-5	0.11±0.07	0.10±0.04	0.09±0.04	0.07±0.02*
16:0	28.29±2.71	27.38±3.32*	27.74±2.06	27.46±1.19
16:1n-9	0.60±0.10	0.65±0.13**	0.64±0.10	0.60±0.11++
16:1n-7c	3.84±0.92	4.06±0.96	3.73±0.87	3.53±0.72+
18:0	3.10±0.53	2.86±0.74*	3.01±0.55	2.71±0.44**
18:1n-9	39.21±2.63	38.10±3.48	40.31±2.72	40.79±2.06
18:1n-7	2.75±0.36	2.72±0.36	2.73±0.31	2.76±0.25
18:2n-6	15.87±2.73	17.24±3.75**	15.66±2.13	16.62±2.69*
18:3n-6	0.26±0.11	0.31±0.17	0.25±0.11	0.25±0.07
18:3n-3	0.74±0.25	0.84±0.27*	0.85±0.25	0.81±0.24
20:0	0.03±0.01	0.03±0.01	0.03±0.01	0.03±0.01
20:1n-9	0.21±0.04	0.19±0.03*	0.22±0.04	0.20±0.02*
20:2n-6	0.23±0.04	0.21±0.06	0.21±0.05	0.20±0.06
20:3n-6	0.27±0.05	0.26±0.07	0.23±0.04	0.21±0.03*
20:4n-6	1.25±0.26	1.26±0.24	1.22±0.34	1.20±0.20
20:5n-3	0.16±0.12	0.29±0.10**	0.18±0.09	0.13±0.04*+++
22:4n-6	0.14±0.03	0.14±0.03	0.13±0.03	0.13±0.02
22:5n-6	0.09±0.02	0.09±0.02	0.08±0.02	0.08±0.01
22:5n-3	0.28±0.10	0.39±0.09**	0.27±0.07	0.27±0.07+++
22:6n-3	0.63±0.36	1.08±0.41**	0.62±0.25	0.55±0.23+++
Saturated	33.36±3.33	32.05±4.16*	32.58±2.48	31.62±1.42*
Monounsaturated	46.73±2.88	45.83±4.10	47.71±2.57	47.94±2.50
PUFA n-6	18.10±2.91	19.51±3.90**	17.78±2.13	18.68±2.62*
PUFA n-3	1.81±0.70	2.60±0.77**	1.92±0.55	1.75±0.48+++

Values are expressed as mean ± SE (in mol %), *p<0.05 **p<0.01 ***p<0.001 in comparison with baseline value, +p<0.05 ++p<0.01 +++p<0.001 in comparison with change in the LCD group

acid composition were found in phospholipids in the highest rate, while the changes in fatty acid composition in serum triglycerides and cholesteryl esters were not so striking, and the least change was found in cholesteryl esters.

Discussion

The higher initial weight, BMI and body fat lead to significantly higher weight, BMI and body fat loss in the control group following the 3 week in-patient weight-reducing regimen as shown previously (Hainer *et al.* 2005, Packianathan *et al.* 2005). On the other hand, we found significant changes in fatty acid composition of serum lipids after the calorie restricted diet containing yogurt supplemented with low doses of n-3 fatty acids of fish origin (n-3LCD). A significant increase in EPA (20:5n-3), DHA (22:6n-3) and the sum of n-3 fatty acids in the n-3 LCD group in contrast with the control group consuming LCD with yogurt without supplementation was confirmed.

The increase in HDL cholesterol caused by the consumption of fish oil was noted by Barret and Watts (2003). We confirmed the positive effect of n-3 supplementation on HDL-C in our study. We did not find a hypotriglyceridaemic effect of fish oil supplementation (Marsh *et al.* 1987, Sanders *et al.* 2006, Surette *et al.* 1992) probably as a result of a significantly higher initial

Table 3c. Fatty acid composition in serum lipids before and after treatment - Cholesterol esters

Cholesterol esters	LCD with n-3 FA n=20		LCD n=19	
Fatty acid	**Baseline**	**Day 21**	**Baseline**	**Day 21**
12:0	0.11±0.05	0.11±0.07	0.13±0.07	0.13±0.06
14:0	0.77±0.22	0.71±0.18	0.61±0.17	0.47±0.12***
14:1n-5	0.06±0.04	0.05±0.02	0.06±0.04	0.06±0.05
16:0	10.93±0.84	10.74±0.89	10.70±0.89	10.36±1.29
16:1n-9	0.42±0.09	0.38±0.07**	0.40±0.10	0.36±0.06*
16:1n-7c	3.36±1.00	3.26±0.81	3.15±0.89	2.87±0.73*
18:0	0.56±0.10	0.50±0.11*	0.55±0.08	0.47±0.10**
18:1n-9	17.67±1.78	17.21±1.69	17.53±1.42	17.06±1.80
18:1n-7	1.04±0.12	1.10±0.19*	1.03±0.13	1.11±0.16**
18:2n-6	55.89±5.62	55.45±5.48	55.36±4.72	55.51±4.22
18:3n-6	0.76±0.40	0.77±0.37	0.88±0.50	0.73±0.32**
18:3n-3	0.51±0.10	0.50±0.09	0.47±0.14	0.44±0.09
20:0	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01
20:1n-9	0.04±0.02	0.04±0.02	0.04±0.02	0.04±0.02
20:2n-6	0.06±0.02	0.06±0.04	0.07±0.02	0.06±0.02
20:3n-6	0.68±0.14	0.64±0.13*	0.69±0.12	0.63±0.14**
20:4n-6	6.36±2.43	7.24±2.07*	7.31±2.33	8.67±2.53**
20:5n-3	0.42±0.44	0.73±0.46**	0.56±0.47	0.49±0.31+++
22:4n-6	0.02±0.01	0.03±0.04	0.02±0.01	0.02±0.01
22:5n-6	0.02±0.01	0.01±0.01	0.02±0.01	0.02±0.01
22:5n-3	0.03±0.02	0.04±0.01	0.04±0.01	0.05±0.04
22:6n-3	0.29±0.21	0.42±0.23**	0.35±0.24	0.44±0.20*
Saturated	12.37±0.98	12.08±1.04	12.00±1.00	11.44±1.35**
Monounsaturated	22.59±2.61	22.03±2.46	22.23±2.22	21.50±2.41
PUFA n-6	63.79±3.77	64.20±3.59	64.35±3.08	65.64±3.39*
PUFA n-3	1.25±0.68	1.69±0.70**	1.42±0.76	1.42±0.54++

Values are expressed as mean ± S.E.M. (in mol %), *p<0.05, **p<0.01, ***p<0.001 in comparison with baseline value, +p<0.05, ++p<0.01, +++p<0.001 in comparison with change in the LCD group

triglyceride level in the control group and due to higher effect of low calorie diet and higher weight loss on triglyceride level in comparison with the effect of low dose of n-3 fatty acids. The recommended dose for treatment of hypertriglyceridaemia is approximately 2-4g/day (McKenney and Sicca 2007), a much higher dose than the one used in the study. The greater decrease of LDL-C in the control group can be caused by similar reasons and concurrently is in accordance with others (Szapary and Rader 2001).

In the Japanese population and in the Inuit of Greenland high consumption of fish and fish products results in low ratios of n-6 AA to n-3 EPA with the Japanese showing AA/EPA ratios of approximately 1.7 and the Greenland Inuit showing ratios of less than 1.0 (Hirai *et al.* 1980). Young *et al.* (2005) gave high dose of oils 60g/day; fish oil (39 g EPA and DHA), flax oil (36g alpha-linolenic acid 18:3n-3) and olive oil (less than 0.6g of n-3 fatty acids) to subjects with attention deficit/hyperactivity disorder. They found a significant effect on serum phospholipid fatty acid composition with a significant increase of n-3 fatty acid proportion reflecting oil composition. A significant decrease in the AA/EPA ratio in the fish oil supplemented group was shown. Unfavorable changes have been shown in fatty acid composition of serum lipids after short-term weight loss (Phinney *et al.* 1990, 1991, Kunešová *et al.*, 2002). In our study we found that adding a low dose of long

chain fish oil supplement to a typical foodstuff such as yogurt increased the proportion of EPA and DHA in serum lipids (phospholipids, triglycerides, cholesteryl esters) during a low calorie diet in obese women. The AA/EPA ratio in phospholipids decreased from 11.6 to 6.5 in the treated subjects and increased from 10.4 to 16.4 in controls.

The role of the use of novel foods enriched with n-3 LC PUFA was confirmed in a study which showed an increase in the proportion of EPA and DHA in plasma and also mononuclear and platelet phospholipids as a result of consuming foodstuffs naturally containing n-3 PUFA and items fortified with fish oil (margarine spread, milk, sausages etc.) in healthy males (Metcalf *et al.* 2003). The changes in fatty acid composition were greatest in phospholipids while the changes in fatty acid composition in serum triglycerides and cholesteryl esters were less pronounced. Our results confirm that plasma phospholipids are sensitive markers of the fatty acid composition of food and they also reflect the fatty acid composition of membranes. In contrast, cholesteryl esters reflect longer-term intake (Zock *et al.* 1997).

In conclusion the results of the study show that low dose supplementation of n-3 polyunsaturated fatty acids in yogurt in a low calorie diet increase the proportion of n-3 PUFA in serum lipids and prevent unfavorable changes in serum fatty acid composition following a short term low calorie diet.

Conflict of Interest

There is no conflict of interest.

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The Influence of n-3 Polyunsaturated Fatty Acids and Very Low Calorie Diet during a Short-term Weight Reducing Regimen on Weight Loss and Serum Fatty Acid Composition in Severely Obese Women

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Summary

Polyunsaturated fatty acids of n-3 series (n-3 PUFA) were shown to increase basal fat oxidation in humans. The aim of the study was to compare the effect of n-3 PUFA added to a very low calorie diet (VLCD), with VLCD only during three-week inpatient weight reduction. Twenty severely obese women were randomly assigned to VLCD with n-3 PUFA or with placebo. Fatty acids in serum lipid fractions were quantified by gas chromatography. Differences between the groups were determined using ANOVA. Higher weight (7.55±1.77 vs. 6.07±2.16 kg, NS), BMI (2.82± 0.62 vs. 2.22±0.74, $p<0.05$) and hip circumference losses (4.8±1.81 vs. 2.5±2.51cm, $p<0.05$) were found in the n-3 group as compared to the control group. Significantly higher increase in beta-hydroxybutyrate was found in the n-3 group showing higher ketogenesis and possible higher fatty acid oxidation. The increase in beta-hydroxybutyrate significantly correlated with the increase in serum phospholipid arachidonic acid (20:4n-6; $r = 0.91$, $p<0.001$). In the n-3 group significantly higher increase was found in n-3 PUFA (eicosapentaenoic acid, 20:5n-3, docosahexaenoic acid, 22:6n-3) in trigycerides and phospholipids. The significant decrease of palmitoleic acid (16:1n-7) and vaccenic acid (18:1n-7) in triglycerides probably reflected lower lipogenesis. A significant negative correlation between BMI change and phospholipid docosahexaenoic acid change was found ($r = -0.595$, $p<0.008$). The results suggest that long chain n-3 PUFA enhance weight loss in obese females treated by VLCD. Docosahexaenoate (22:6n-3) seems to be the active component.

Key words

Obesity treatment • Fatty acid composition • Very low calorie diet • Beta-hydroxybutyrate • PUFA

Introduction

Caloric restriction and weight loss are responsible for a modification of gene expression in adipose tissue while the proportion of dietary fat does not seem to be decisive (Arvidson *et al.* 2004, Viguerie *et al.* 2005). Composition of dietary fat has been shown to modify the effect of weight reducing diets (Kriketos *et al.* 2001, Clifton*et al.* 2004). Reducing the energy intake tends to dilute the changes in membrane phospholipid fatty acid composition reflecting dietary fat composition in rats (Cha and Jones 2000).

In moderately obese women we have previously found a significant increase of phospholipid palmitic acid (16:0) and significant decrease of linoleic acid (18:2n-6) and dihomo-gamma-linolenic acid (20:3n-6) after very low calorie diet (VLCD) (Kunešová *et al.* 2002a). In humans adipose alpha-linolenic acid was permanently reduced during VLCD (Phinney *et al.* 1990). In rats, linoleic and alpha-linolenic acids (18:3n-3) were shown to be rapidly beta-oxidized even under conditions of extreme dietary linoleate deficiency (Cunnane *et al.* 1998). Moreover, enhanced recycling of carbon from linoleic and alpha-linolenic acid to palmitic acid was suggested (Cunnane *et al.* 2003). The conversion of linoleic and alpha-linolenic acids to long chain polyunsaturated acids was shown to be a quantitatively minor route of utilization in healthy women (McCloy *et al.* 2004). The composition of fatty acids in serum lipids and adipose tissue triglycerides reflects the composition of fatty acids in dietary fats. Fatty acid composition in skeletal muscle triglycerides and phospholipids correlates with parameters of insulin resistance and with the risk of type 2 diabetes (Vessby *et al.* 1994a). Positive association of arachidonic acid (20:4n-6, AA) in serum and muscle phospholipids with insulin sensitivity was shown (Pelikánová *et al.* 1989, Borkman *et al.* 1993). Inverse association between insulin sensitivity and serum dihomo-gamma-linolenic acid (20:3n-6, DHGLA) found by Vessby *et al.* (1994b) and Lovejoy *et al.* (2001) reflects higher activity of delta-5 desaturation of DHGLA to AA (Felton *et al* 2004). High ratios of linoleic to arachidonic acid concentrations have been observed in subjects with insulin resistance (Berry 2001).

Long chain n-3 PUFA enhance lipid oxidation in healthy humans (Delarue *et al.* 1996, Couet *et al.* 1997). In animals, addition of n-3 fatty acids led to a preferential loss of epididymal fat (Raclot *et al.* 1997) associated with decreased cellularity (Růžičková *et al.* 2004). We have previously reported a strong genetic influence on the composition of serum and adipose tissue lipids under basal conditions in monozygotic twins (Kunešová *et al.* 2002b), after a short-term weight reduction regimen and after one-year of weight stabilization (Kunešová *et al.* 2002a). After the weight loss the consistent intra-pair resemblances for n-3 fatty acids were found despite dietary stress induced by a very low calorie diet, indicating that the conservation and distribution of this family of essential fatty acids is subject to considerable genetic variance in humans. The resemblances were not influenced by differences in the diet. This was shown when comparing twins concordant and discordant in fat intake.

The present study shows the effect of a short-term inpatient weight-reducing regimen consisting of a very low calorie diet (VLCD) and long chain n-3 PUFA as compared to weight-reducing regimen with VLCD only in severely obese women.

Methods

Subjects

Twenty severely obese women were randomly assigned to a very low calorie diet with n-3 PUFA supplement (n-3 VLCD) or with saline solution (VLCD). There were no significant differences between groups in basal BMI (n-3 VLCD 40.60±4.05; VLCD 45.14±6.9 kg/m^2) and age (n-3 VLCD 54.27±5.36; VLCD 49.78± 12.35 years). The study was approved by the Charles University Medical Ethical Committee.

Design of the study

The regimen consisted of a one-week eucaloric outpatient baseline stabilization period followed by three weeks of inpatient weight reducing period. During the baseline week and last 3 days of the inpatient stay the subjects did not change their weight. Weight reducing regimen consisted of VLCD Redita^R (Promil Nový Bydžov, Czech Republic) providing 2200kJ/day, comprised of 40 g protein, 70 g carbohydrates and 9 g fat, with addition of n-3 highly unsaturated fatty acids 2.8 g/day (Omega 3 Forte^R, SVUS Pharma, Hradec Kralove, CzR), (n-3 VLCD) or VLCD with saline solution (VLCD group). Supplement with n-3 consisted of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in ratio 2:1, vitamin E 0.9 mg/500 mg capsule was added to prevent fatty acid peroxidation. The reduction regimen included also daily light to moderate

physical activity lasting about 60 min/day. Compliance to the diet was tested by daily semiquantitative measurement of urine ketone bodies and adherence to the physical training by pedometers (Hainer *et al.* 2000). All these procedures were undertaken in the Third Department of Internal Medicine, Charles University in Prague. At the beginning of the weight reducing regimen and on the day 3, 7, and 21 of fasting blood was withdrawn for determination of beta-hydroxybutyrate, free fatty acids, total cholesterol, HDL-cholesterol, triglycerides, fasting blood glucose, fasting insulin, superoxide dismutase, malonyldialdehyde, C and E vitamins. Laboratory analyses were performed by routine laboratory methods. Serum beta-hydroxybutyrate was assessed fluorimetrically (Olsen 1971) and non-esterified free fatty acids were determined photometrically (Duncombe 1964).

At baseline and on day 21, serum lipid fraction of fatty acid analysis was carried out (serum phospholipids – PL, triglycerides – TG and cholesteryl esters – CE); at the same time abdominal adipose tissue was also obtained by needle biopsy. Analysis of the results from adipose tissue samples will be reported elsewhere. Proteins of acute phase fibrinogen, C-reactive protein, α_1 antitrypsin, orosomukoidα_2 macroglobulin, transferrin and prealbumin were also assessed by routine laboratory methods.

Body composition and regional tissue distribution

Anthropometric estimation of body fat was performed by measurement of ten skinfolds according to Pařízková (1977) and of four skinfolds according to Durnin and Wommersley (1974). Waist and hip circumference and sagittal abdominal diameter at the level L4/5 were measured following the standardized procedure recommended at the Airlie Conference (Lohman *et al.* 1989). Body fat content was estimated by bioelectrical impedance measurement (Tanita TBF 105, Japan).

Fatty acid composition

Fatty acid composition of serum lipids was performed by gas chromatography after separation of individual serum fractions by thin-layer chromatography on silica gel. Detailed description of the method is given elsewhere (Tvrzická *et al.* 2002).

Statistical methods

Relationships between one dependent variable and the set of independent variables were evaluated using a stepwise backward multiple regression. Prior to the analysis, the original dependent variables were transformed by power transformation using a normal probability plot for finding the best transformations. The minimum value of the mean squared error of the linear regression fit between theoretical fractiles of the Gaussian distribution and experimental fractiles indicated the optimum transformation parameter. Severe non-homogeneities as detected using the aforementioned plot were not included in the finding of the optimum transformation parameters. Nevertheless, these outliers were included in further processing. The optimum transformation of the dependent variable was searched using the minimum skewness of Studentized residuals of the multiple regression model as an indicator. Further regression diagnostics was performed as described elsewhere (Meloun *et al.* 2002, 2004). The relationships between two variables were estimated using Pearson's correlations. Respecting a non-Gaussian distribution and a non-constant variance in some variables, these were transformed prior to testing as described above. For transformation strategy see also our previous study (Meloun *et al.*2000). The effects of status (patients, controls) and stage of the treatment (before treatment, after treatment) and a between-factor interaction were evaluated using a repeated measures ANOVA model. Again, respecting the non-Gaussian data distribution in some variables, such data underwent power transformations. The transformation strategy as well as the residual analysis was analogous as in the regression.

The results tested by the aforesaid ANOVA model were checked by non-parametric tests of the differences between the beginning and end of the treatment. The significances of the differences were evaluated using Wilcoxon's paired test. To estimate how the differences diverged between controls and patients, the Mann-Whitney test of their means was applied.

Data are expressed as means ± S.E.M. P=0.05 was taken as the threshold of statistical significance.

Results

Baseline characteristics of the subjects before the treatment and the effect of the treatment are presented in Table 1. Weight loss (7.55±1.77 vs. 6.07±2.16 kg, $p<0.10$) and BMI decrease (2.82±0.62 vs. 2.22±0.74 kg/m^2, $p<0.05$) were higher in the n-3 PUFA supplemented group. The decrease of hip circumference

was significantly higher after n-3 VLCD (4.8±1.81 cm) than in the VLCD (2.5±2.51 cm, p<0.05) group. Higher increase in beta-hydroxybutyrate was found in n-3 group (p<0.01, Fig. 1A). A tendency to higher decrease in fibrinogen was observed in n-3 group (p<0.09, Fig. 1B).

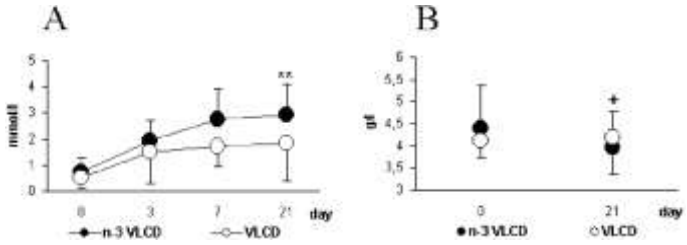

Fig. 1. A. Change in serum beta-hydroxybutyrate during the weight-reducing regimen ** p<0.01. **B**. Change in serum fibrinogen during the weight-reducing regimen + NS (p<0.09), Values are expressed as mean ± S.D.

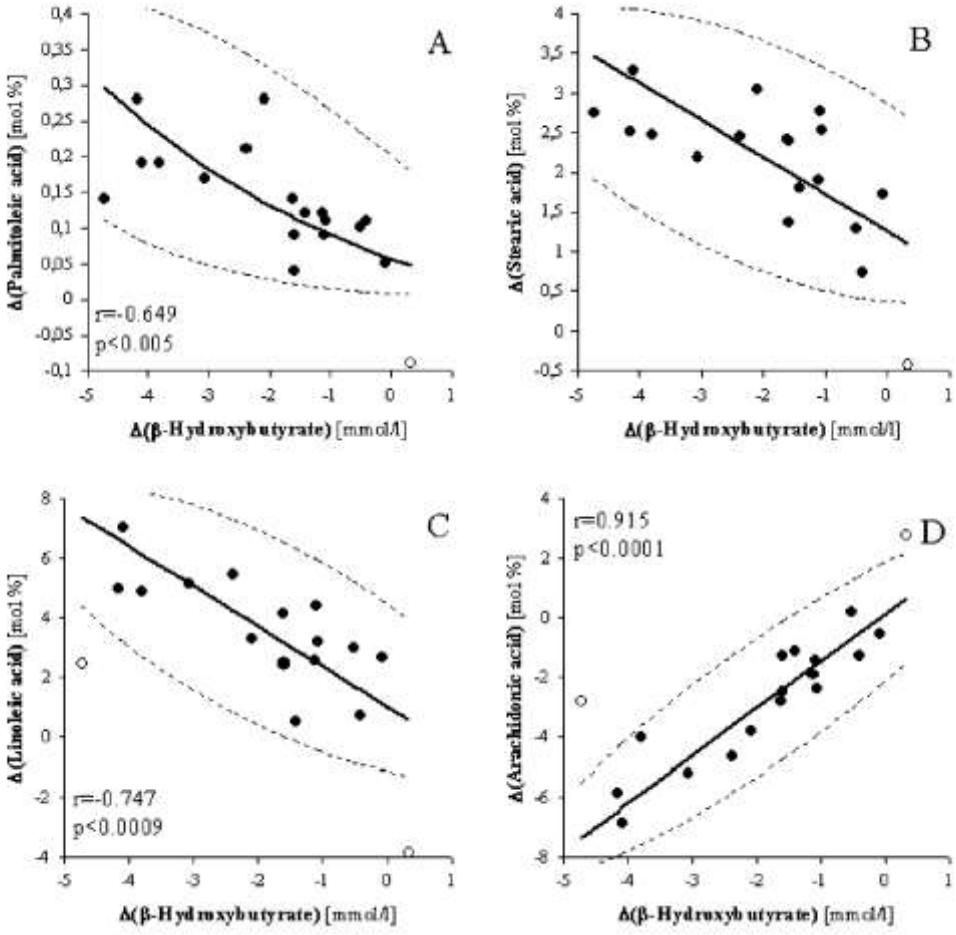

Fig. 2. Correlation of the change in beta-hydroxybutyrate and change in palmitoleic acid (16:1n-7) (**A**), change in stearic acid (18:0) (**B**), change in linoleic acid (18:2n-6) (**C**), change in arachidonic acid (20:4n-6) (**D**). Pearson's correlations between the change of beta-hydroxybutyrate and change in proportion of individual fatty acids in serum phospholipids. The full and empty circles indicate the individual subjects included in the analysis and outliers, respectively. The full and dashed curves indicate the retransformed principal axes and their 95 % confidence ellipsoids (the area in which the 95 % of the experimental points should be theoretically found provided that no outliers are present), respectively. Respecting the non-Gaussian data distribution and the non-constant variance the data were transformed using a power transformation prior analysis (for details see Statistical data treatment). The symbols r and p represent the correlation coefficients and their statistical significances when correlating the transformed data.

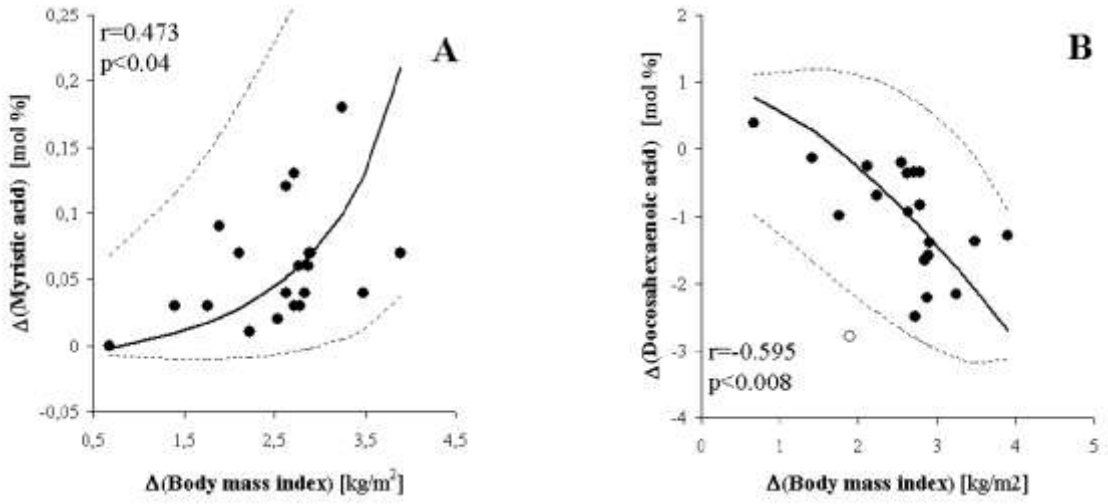

Fig. 3. Pearson's correlations between the change of BMI and change in proportion of myristic acid (14:0) (**A**) and docosahexaenoic acid (20:6n-3) (**B**). For comments see Fig. 2.

Table 1. Characteristics of the group before the treatment and the effect of the weight-reducing regimen

Variable	N3 VLCD (n=11)	VLCD (n=9)
Initial values		
Age (years)	54.27±5.36	49.78 ± 12.35
Weight (kg)	108.2±8.7	123.2 ±23.7
BMI (kg/m²)	40.60 ±4.05	45.14 ±6.9
Waist (cm)	116.2±10.0	119.4±11.6
Hip (cm)	136.0±7.8	140.4±13.1
Effect of treatment		
Weight loss (kg)	7.55±1.77	6.07±2.16 **
BMI loss (kg/m²)	2.82±0.62	2.22±0.74 *
Waist (cm)	5.5±1.71	3.3±3.41
Hip (cm)	4.8±1.81	2.5±2.51 *

* p<0.05 ** NS (p<0.1), Values are expressed as mean ± S.D.

Table 2 shows the composition of fatty acids in serum phospholipids (PL) and triglycerides (TG) before treatment and after the treatment in the n-3 VLCD and VLCD groups. An increase in eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic acids (DHA, 22:6n-3) in both PL and TG was found. In TG a significantly higher decrease of palmitoleic (16:1n-7, POA) and vaccenic (18:1n-7) acids and significantly higher increase of oleic acid (18:1n-9) was observed. Palmitoleate was shown as a marker of lipogenesis previously (Fukuda *et al.* 1999, Kunešová *et al.* 2002b). A highly significant negative correlation between changes in levels of beta-hydroxybutyrate and in serum POA proportions in PL ($p = –0.83$, $p<0.0001$), TG and CE ($r = –0.69$, $p<0.005$, $r = –0.49$, $p<0.05$) respectively, could indicate a higher level of hepatic beta-oxidation. However, it could also suggest a lower lipogenesis. Negative correlation between initial BMI and change in POA was found ($r = –0.45$, $p<0.05$).

A higher decrease of TG POA was shown in the n-3 VLCD treated group. A higher hepatic beta-oxidation in response to n-3 supplement could be inferred by a higher increase of beta-hydroxybutyrate in n-3 treated group. Significantly higher increase in TG oleic acid (18:1n-9, OA) in n-3 VLCD was shown. A strong positive correlation was observed between the change of PL, TG and CE arachidonic acid and the change of beta-hydroxybutyrate ($r = 0.92$, $p<0.001$; $r = 0.68$, $p<0.005$; $r = 0.66$, $p<0.005$, respectively); significant negative correlation of beta-hydroxybutyrate change with change of palmitic (16:0), stearic (18:0) and linoleic (18:2n-6) acids in phospholipids was also shown (Fig. 2). The increase in PL DHA correlated significantly with BMI decrease ($r = –0.595$, $p = 0.008$) and there was a positive correlation of myristate change (14:0) with BMI change (Fig. 3). Serum triglyceride change correlated positively with TG palmitate change and a negative correlation with TG docosahexenoic acid (22:6n-3) change was found (data not shown). The changes in serum triglycerides, serum non-esterified fatty acids and fasting blood glucose levels were not significantly different between n-3 VLCD and VLCD groups (data not shown).

Table 2. Fatty acid composition in serum lipids before and after the treatment

Phospholipids	n-3 VLCD (n=11)		VLCD (n=9)	
Fatty acid	Baseline	21 Day	Baseline	Day 21
12:0	0.01±0.002	0.009±0.002	0.004±0.002	0.004±0.002
14:0	0.21±0.02	0.13±0.002	0.16±0.009	0.122±0.009
16:0	31.71±0.34	34.79±0.30	31.16±0.51	33.73±0.73
16:1n-9	0.14±0.008	0.19±0.008	0.14±0.007	0.13±0.001
16:1n-7	0.68±0.03	0.53±0.04	0.57±0.03	0.49±0.04
18:0	12.57±0.38	10.29±0.32	13.18±0.34	11.23±0.53
18:1n-9	8.86±0.31	8.71±0.38	9.22±0.33	9.48±0.18
18:1n-7	1.57±0.07	1.73±0.05	1.68±0.05	1.84±0.005
18:2n-6	20.86±0.05	16.68±0.73	20.11±0.57	18.67±0.69
18:3n-6	0.06±0.005	0.03±0.003	0.05±0.004	0.04±0.007
18:3n-3	0.13±0.008	0.09±0.005	0.12±0.008	0.10±0.011
20:0	0.04±0.002	0.03±0.009	0.03±0.002	0.03±0.001
20:1	0.11±0.007	0.11±0.006	0.12±0.006	0.12±0.006
20:2n-6	0.28±0.02	0.18±0.008	0.26±0.02	0.20±0.01
20:3n-6	3.67±0.22	1.95±0.06	3.73±0.20	2.1. ±0.17
20:4n-6	12.46±0.47	15.68±0.94	13.58±0.74	15.18±1.01
20:5n-3	1.05±0.10	1.68±0.15***	0.77±0.05	0.63±0.03
22:5n-3	0.925±0.06	1.04±0.07	0.88±0.04	0.904±0.04
22:6n-3	4.17±0.27	5.76±0.22*	4.07±0.21	4.52±0.27
Triglycerides	**n-3 VLCD (n = 11)**		**VLCD (n = 9)**	
Fatty acid	**Baseline**	**21 Day**	**Baseline**	**Day 21**
12:0	0.08±0.01	0.04±0.004	0.05±0.007	0.03±0.006
14:0	1.25±0.08	0.72±0.05	0.97±0.06	0.60±0.07
16:0	28.46±0.14	27.35±0.73	26.57±0.31	26.17±0.38
16:1n-9	0.76±0.04	0.61±0.04	0.72±0.03	0.56±0.04
16:1n-7	4.33±0.12	3.06±0.20*	3.64±0.21	3.06±0.28
18:0	2.59±0.07	2.33±0.09	2.51±0.19	2.08±0.11
18:1n-9	38.93±0.79	39.88±0.42*	41.39±0.49	41.84±0.79
18:1n-7	2.63±0.06	2.54±0.84*	2.86±0.07	2.79±0.09
18:2n-6	16.24±0.75	17.14±0.82	16.39±0.51	17.19±0.82
18:3n-6	0.18±0.04	0.15±0.01	0.19±0.01	0.17±0.02
18:3n-3	0.73±0.05	0.69±0.05	0.83±0.34	0.75±0.03
20:0	0.02±0.001	0.02±0.002	0.02±0.007	0.02±0.005
20:1	0.18±0.008	0.14±0.003	0.19±0.01	0.17±0.01
20:2n-6	0.12±0.007	0.09±0.004	0.11±0.005	0.09±0.004
20:3n-6	0.34±0.05	0.20±0.001	0.29±0.02	0.20±0.02
20:4n-6	1.58±0.21	2.13±0.23	1.60±0.12	2.10±0.18
20:5n-3	0.21±0.04	0.49±0.07**	0.17±0.01	0.19±0.03
22:5n-3	0.37±0.03	0.52±0.04	0.38±0.03	0.47±0.03
22:6n-3	0.76±0.08	1.22±0.10$^{\#}$	0.72±0.06	0.93±0.10

$^{\#}$ p<0.1 *p<0.05 **p<0.01 in comparison with change in the control group, Values are expressed as means ±S.E.M. (in mol %)

Discussion

The main finding of the study is that in severely obese females, n-3 PUFA supplementation added to VLCD significantly enhanced BMI loss and reduction of hip circumference. The secondary finding is a significantly higher elevation of serum beta-hydroxybutyrate in n-3 PUFA supplemented group. Both of these effects occurred despite the fact that the baseline mean BMI for the subjects in the n-3 VLCD group tended to be lower than that for the saline VLCD group, whereas one would expect to observe greater weight loss and higher ketones in heavier subjects given a fixed energy of VLCD.

Similar increase in ketogenesis was found in epileptic patients after using polyunsaturated fatty acids instead of saturated fats during ketogenic diet which lead to a higher increase in beta-hydroxybutyrate (and also in insulin sensitivity) (Fuehrlein*et al.* 2004). In the above study, the composition of PUFA in the diet was not given. A higher ketosis after diet containing n-3 PUFA in comparison with saturated fats is consistent with the results found in animals (Storlien *et al*. 1987, Likhodii *et al.* 2000). In epileptic children treated by ketogenic diet arachidonate and docosahexaenoate in plasma free fatty acids increased simultaneously with beta-hydroxy-butyrate elevation (Fraser *et al*. 2003). The increase in ketogenesis is probably caused by a higher fatty acid oxidation after n-3 PUFA supplementation. In rats, higher beta-oxidation was shown as a mediator of the hypotriglyceridemic effect of the n-3 PUFA (Ukropec *et al.* 2003). Stimulation of fatty acid oxidation in the liver is caused by the activation of peroxisome proliferator activated receptor alpha (Delarue *et al.* 2004)

Significant correlation of beta-hydroxybutyrate change with a change in arachidonate content in all serum lipid classes, especially in phospholipids, was demonstrated for the first time. If there is a causal relationship or simultaneous association should be studied further. Compliance to the supplementation was high as indicated by the increase in proportion of EPA and DHA in serum phospholipids and triglycerides.

The enhanced level of arachidonic acid after both treatments could be caused by its higher release from adipose tissue. On the other hand, a lower metabolism of AA after fasting and refeeding was shown by Qu *et al.* (1998), who demonstrated significant reduction in total hepatic microsomal AA metabolism in rat liver, concurrently with weight loss and increased beta-hydroxybutyrate levels. In addition to cyclooxygenases and lipooxygenases, cytochrome P450 monooxygenases metabolize AA to compounds which have an important role in the regulation of cellular processes. Fasting has been reported to increase AA accumulation in hepatic neutral and phospholipid pools (Larsson-Backstrom *et al.* 1990). In humans, Phinney *et al.* (1991) found that arachidonate increased during VLCD, and decreased after finishing the ketogenic diet. Concurrently with AA increase fibrinogen decrease tended to be higher in n-3 VLCD group confirming the previously shown antiinflammatory effect of n-3 PUFA (Browning 2003).

Polyunsaturated fatty acids regulate lipogenic gene expression in different tissues. Clarke and Jump (1994) have demonstrated that dietary fat composition directly affects fatty acid synthase gene expression. As described in rats, a higher decrease of palmitoleic acid in serum triglycerides confirms indirectly the supression of lipogenic genes including fatty acid synthase (Fukuda *et al.* 1999).

Previous studies in rodents indicated that n-3 PUFA enriched diets prevented accumulation of fat in the abdomen (Raclot *et al.* 1997, Růžičková *et al.* 2004). The effect of n-3 PUFA (EPA, DHA and mixed fat) on lipogenic genes expression was shown in retroperitoneal fat of rats (Raclot *et al.* 1997). The replacement of 3 % (wt/wt) of obesity-promoting HF composite diet with EPA/DHA reduced weight gain and reduced the accumulation of epididymal, but not of subcutaneous fat in mice, simultaneously with a depression of tissue cellularity and favorable changes in glucose homeostasis gene activity (Růžičková *et al.* 2004). The influence of the type of dietary fat on the composition of phospholipids in cellular membrane and possibly also function becomes increasingly important with positive energy balance (Cha and Jones 2000). The effect of n-3 PUFA addition to the diet of obese subjects could be more expressed under conditions of lower energy deficit.

Conclusion

The addition of n-3 PUFA of fish origin to a very low calorie diet results in a greater BMI loss and hip circumference reduction in severely obese women during an inpatient short-term weight-reducing regimen. Higher increase in beta-hydroxybutyrate was shown in the n-3 group probably due to a higher beta-oxidation of fatty

acids. Significant correlation of BMI loss with change in phospholipid docosahexaenoic acid level suggests a causal relationship.

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Reprint requests

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Calcium Intake and the Outcome of Short-Term Weight Management

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Summary

Experimental and epidemiological studies suggest that calcium intake is inversely related to weight gain. Calcium of dairy origin has been shown to be more effective in promoting weight loss. However, clinical studies yielded controversial results concerning the role of calcium intake in weight change. The aim of this study was to ascertain whether the addition of calcium can affect the outcome of 3-week weight management (WM) with a hypocaloric diet characterized by a decreased calcium intake. Overweight/obese women (n=67; BMI 32.2±4.1 kg/m^2; age 49.1±12.1 years) underwent a 4-week comprehensive WM program. WM included a 7 MJ/day diet resulting in a stable weight during the first week and a 4.5 MJ/day diet with mean daily calcium intake 350 mg during the second to fourth week. Participants were divided into three age- and BMI-matched groups who received placebo or calcium (500 mg/day). Calcium was administered either as carbonate or calcium of dairy origin (Lactoval). There was no significant difference in weight loss in response to WM between the placebo-treated and calcium-treated groups. However, addition of calcium to the diet resulted in a lower hunger score in the Eating Inventory as well as a decrease in plasma resistin levels. Body composition measured by bioimpedance demonstrated that added calcium leads to preservation of fat-free mass. Nevertheless, a greater loss of fat-free mass in the placebo group might be partly due to a greater loss of water.

Key words

Obesity • Calcium intake • Weight change • Fat-free mass • Hunger • Resistin

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Introduction

Recent studies demonstrated that body weight loss in response to the weight management is influenced not only by the energy intake and macronutrient composition of the diet but also by intake of other nutritional factors, i. e. calcium and n-3 fatty acids (Zemel *et al.* 2004, Kunešová *et al.* 2006. Experimental studies have demonstrated that calcium intake lowers body weight in rats and mice (Bursey *et al.* 1989, Shi *et al.* 2001). Several observational studies have shown an inverse relationship between dietary calcium intake or intake of dairy products and body weight (Lin *et al.* 2000, Davies *et al.* 2000, Zemel *et al.* 2000, Barger-Lux *et al.* 2001, Jacqmain *et al.* 2003, Zemel *et al.* 2004). Davies *et al.* (2000) analyzed four observational, two cross-sectional and two longitudinal studies and confirmed a significant negative association between calcium intake and body weight. According to this analysis, differences in calcium intake could explain 3 % of the variance in body weight. CARDIA study revealed a negative relationship between calcium intake or dairy consumption and obesity and insulin resistance syndrome (Pereira *et al.* 2002). Recently, Liu *et al.* (2005) reported that the intake of calcium and dairy products may be associated with lower prevalence of the metabolic syndrome in middle-aged and older women. In observational studies on the role of calcium intake the confounding factors, such as dairy protein, should be taken into account. In our recent study in 208 obese individuals (BMI 40.0±7.7 kg/m^2) (Kabrnová *et al.* 2004) body weight changes over

a 3- to 6-month weight management (WM) were negatively related to changes in the intake of both dietary calcium ($r = –0.210$, $p=0.003$) and protein ($r = –0.289$, $p<0.001$).

A few of the intervention studies conducted on the role of calcium intake on body weight and body composition yielded conflicting results (Chan *et al.* 1995, Thompson *et al.* 2005, Boon *et al.* 2005, Rajpathak *et al.* 2006,). It has also been demonstrated that calcium from dairy products is more effective in reducing body weight than calcium from supplements (Zemel *et al.* 2000, 2004).

Different mechanisms were proposed to mediate the effects of dietary calcium on body weight changes. Formation of fecal fatty acid complexes to reduce fat absorption may represent an important mechanism through which calcium affects body weight regulation (Jacobsen *et al.* 2005). Elevation of fecal fat excretion in response to increased calcium intake should be considered especially in individuals with an excessive fat intake. Intracellular Ca^{2+} is a key regulator of lipid metabolism. Its elevated intracellular concentrations stimulate the expression and activity of lipogenic enzymes and reduce lipolysis with a subsequent increased accumulation of fat in adipocytes (Zemel *et al.* 2000). Dietary calcium-induced suppression of 1.25-dihydroxy-vitamin D diminishes the entry of Ca^{2+} into adipocytes and as a consequence also diminishes fat storage in adipocytes (Xue *et al.* 2001, Zemel 2003). Calcium might affect energy balance by stimulating an expression of uncoupling proteins in adipocytes (UCP2) and skeletal muscles (UCP3) (Yu *et al.* 2003, Zemel *et al.* 2000, Zemel 2003). Calcium also affects fat oxidation. Melanson *et al.* (2003) demonstrated that calcium intake correlated positively with 24-h fat oxidation, during both sleep and moderate physical activity.

Food intake, energy balance and body weight as well as insulin sensitivity are regulated by complex neurohormonal signals (Druce and Bloom 2006). Adherence to the WM program is greatly influenced by psychobehavioral factors, among which the eating behavior plays a crucial role. However, an association between calcium intake and hormonal and psychobehavioral factors has not yet been assessed. The aim of the present study was to evaluate whether a calcium supplement (500 mg/day) would influence a change in body weight and body composition in response to a 3-week WM program with strictly defined and supervised caloric intake. The impact of calcium intake on metabolic, hormonal and psychobehavioral parameters was also evaluated.

Subjects and Methods

Sixty-seven overweight/obese women (BMI 32.2±4.1 kg/m^2; age: 49.1±12.1 years) participated in a 4-week WM program in the Spa Obesity Unit. Subjects with diabetes, uncompensated thyroid dysfunction and those treated with drugs affecting water balance (diuretics, hormonal contraceptive and replacement therapy etc.) were excluded from the study.

The comprehensive WM included a precisely defined low energy diet, daily physical activity supervised by a physiatrist and cognitive behavioral modification of lifestyle. Energy and nutrient content of meals prepared in the hospital kitchen during the entire period of study was calculated by the PC program „Nutrition“. This software covers almost 3000 food items and evaluates the intake of energy, macronutrients and micronutrients. All subjects were advised to eat all of the meals served in four daily portions in the spa dining room. Mean daily energy intake before initiation of the spa treatment was calculated to be about 7 MJ. Therefore, the patients were provided a 7 MJ/day diet during the first week of WM. Only those patients who exhibited a stable weight during the first week entered the trial and received a hypocaloric diet providing 4.5 MJ/day (protein 25.3 %, fat 28.7 %, carbohydrate 46.0 %) with a low calcium supply (350 mg/day) over the subsequent 3-week period. This diet yielded a 2.5 MJ deficit in comparison with the pretreatment week.

Participants were divided into three age- and BMI-matched groups who received either a placebo (P group, n=21) or calcium (500 mg/day), either as carbonate (C group, n=25) or calcium of dairy origin Lactoval (L group, n=21). Lactoval was prepared from milk and contained calcium as phosphate (70 %), lactate (10 %) and citrate (20 %). Tablets were analyzed in the Dairy Research Institute and their calcium content corresponded to the declared values. A dietitian distributed placebo or calcium tablets to the patients in three daily doses. The dietitian also checked that the patients took the tablets immediately after receiving them.

The studied women were predominantly perimenopausal. The number of women in menopause was comparable in the groups.

Anthropometric, biochemical, hormonal and

psychobehavioral investigations were conducted before and after a 3-week WM. Body weight and body composition were analyzed by a bipedal-bimanual Body Composition Analyzer Tanita BC-418MA (Tanita Inc., Tokyo, Japan). Anthropometric measurements included body weight, height, waist and hip circumference, subscapular, triceps, biceps and suprailiac skinfolds.

Eating behavior was evaluated by the Eating Inventory (Stunkard and Messick 1985) which assesses three behavioral traits: 1) dietary restraint – deliberate control of intake, 2) disinhibition – measure a loss of control over food intake (for example in response to stress, anxiety, depression and alcohol intake), 3) perceived hunger –- awareness of and susceptibility to hunger. Beck Depression Inventory (Beck *et al.* 1961) was used to evaluate the level of depression.

Blood samples for biochemical and hormonal investigations were taken in the morning after a 12-h overnight fast. Biochemical indexes (blood glucose, glycosylated hemoglobin, uric acid, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, C reactive protein) were assessed by standard laboratory procedures. Hormonal levels (TSH, fT3, fT4, insulin, C peptide, prolactin, growth hormone, total ghrelin, IGF-1, cortisol, sex hormone binding globulin /SHBG/, leptin, ghrelin, peptide YY, neuropeptide Y, adiponectin, resistin) were analyzed by radioimmunoassay.

Protocol of the study was reviewed and accepted by the Ethics Committee of the Institute of Endocrinology. All patients were informed about the study design and signed an informed consent form concerning their participation in the study.

Statistical analysis

Results are expressed as means ± S.D. Differences in parameters before and after WM as well as differences between the groups were assessed using the Kruskal-Wallis robust analysis of variance (ANOVA) followed by Kruskal-Wallis multiple comparisons. Differences between groups were assessed by the Mann-Whitney test. Changes were evaluated by Wilcoxon's paired test.

Results

The baseline data were similar in all three groups for body mass index (C group: 32.39±4.35 kg/m^2, L group: 32.42±4.22 kg/m^2, P group: 32.36±4.86 kg/m^2), fat stores (C group: 41.3±4.9 %; L group: 41.7±6.4 %; P group: 42.0±5.7 %) and plasma leptin levels (C group: 21.7±8.4 ng/ml; L group: 21.5±9.3 ng/ml; P group: 20.3±9.1 ng/ml) which under stable weight conditions reflect body fat stores. An average weight loss of 3.8±1.6 kg was achieved in response to WM. Table 1 summarizes significant changes in anthropometric, psychobehavioral and hormonal parameters in the whole cohort. Decreases in body weight, BMI, body circumferences and skinfolds were demonstrated. In response to WM, the Beck depression score, hunger scores and disinhibition scores decreased, whereas the restraint score increased. Significant decreases in serum leptin and NPY levels were shown. A significant decline in fasting blood glucose and insulin concentrations was demonstrated together with a significant rise in SHBG level, whereas no significant changes in serum adiponectin (–0.06±2.69 mg/l) and resistin (–0.18±0.78 μg/l) levels were observed.

No significant differences were observed in any anthropometric, body composition, psychobehavioral, biochemical and hormonal parameters when the three groups differing in the calcium intake were compared both before and after the weight reduction. Only selected data from 52 measured parameters are shown in Table 2.

As shown in Table 3, significant decreases in body weight, BMI and fat mass were observed in both the calcium-treated groups and in the group receiving placebo. No significant differences in the decreases in anthropometric indexes and fat mass were demonstrated between the three groups of patients. On the other hand, a significant decline in FFM was shown in the group treated with placebo (–1.46±3.36 kg, p=0.006), while both groups provided with additional calcium did not exhibit any significant changes in FFM. Hunger score decreased significantly in both groups treated with calcium (calcium carbonate group: –1.54±2.59, p=0.010; Lactoval group: –1.76±2.98, p=0.017) whereas a decline of hunger score in the placebo-treated group was not significant (0.38±2.56).

Figure 1 demonstrates a significant difference in the change of resistin level in response to WM between the placebo-treated group and the joint cohort including both calcium-treated groups. In contrast to the WM-induced increase in resistin levels in the placebo-treated group, a decrease in mean resistin level was demonstrated in the joint calcium-treated group.

Discussion

The main finding of the present study is that the

Table 1. Anthropometric, psychobehavioral and hormonal indexes and their changes in response to weight management in the whole cohort.

	All subjects						
	Before		After		Difference		Significance
	mean	S.D.	mean	S.D.	mean	S.D.	p
Weight (kg)	84.60	12.81	80.78	12.48	-3.82	1.63	0.000001
BMI (kg/m^2)	32.39	4.48	30.92	4.33	-1.47	0.63	0.000001
Waist (cm)	98.83	11.92	93.64	11.43	-5.19	2.28	0.000001
Hip (cm)	115.49	9.20	112.12	8.97	-3.37	1.61	0.000001
WHR	0.86	0.07	0.83	0.07	-0.02	0.02	0.000001
Fat (kg)	35.81	9.52	32.01	8.83	-3.80	2.83	0.000001
Fat (%)	41.65	5.69	38.98	6.12	-2.67	2.89	0.000001
FFM (kg)	48.79	4.68	48.48	5.32	-0.31	3.18	0.035526
Beck	10.38	6.37	7.56	6.43	-2.82	4.16	0.000003
Restraint	10.03	4.54	12.94	4.57	2.91	4.30	0.000004
Hunger	4.08	3.28	2.83	2.73	-1.24	2.71	0.000641
Disinhibition	6.61	2.97	4.86	2.70	-1.74	2.55	0.000008
Glucose (mmol/l)	5.13	1.56	4.83	1.24	-0.29	1.70	0.05
Insulin (mIU/l)	8.36	4.45	7.83	4.81	-0.53	5.99	0.05
SHBG (nmol/l)	61.49	44.21	79.08	53.73	14.89	26.72	0.000001
Leptin (µg/l)	21.21	8.98	15.36	7.13	-5.85	6.45	0.000001
NPY (pmol/l)	101.8	52.31	84.14	41.08	-17.7	31.49	0.000003

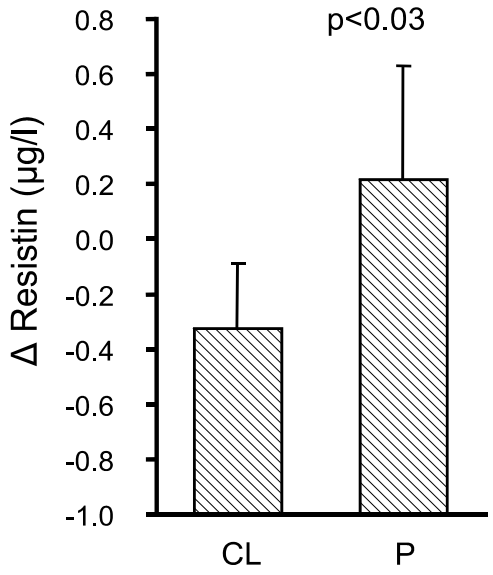

Fig. 1. Change of resistin level in response to WM in the placebo-treated group (group P) and joint calcium-treated group (group CL).

administration of a calcium supplement in a daily dose of 500 mg does not result in an increased weight loss during short-term weight management. When discussing the results, we should consider both advantages and limitations of our study. In all previous studies, calcium has been supplemented as calcium carbonate, calcium citrate or calcium citrate-malate, although calcium phosphate represents a major source of calcium in dairy products. No previous studies employed the administration of calcium of dairy origin or calcium phosphate. In our study, patients were given calcium tablets prepared from the milk which also contained calcium as phosphate, lactate and citrate. However, we failed to see any significant difference in body weight loss between the groups supplemented with 500 mg calcium provided as calcium carbonate or as calcium of dairy origin. The second advantage of our study was that we had an opportunity to maintain all subjects on the same diet providing a daily energy deficit of 2.5 MJ with an average daily calculated calcium intake of 350 mg. In previously published interventional studies the quantity of calcium obtained from each daily meal had not been evaluated and only the role of supplemented calcium or high dairy product consumption had been considered.

Zemel (2004) reported on weight loss reached over 24 weeks in obese subjects assigned to three different calorie-restricted diets prescribing a daily

Table 2. Comparison of selected anthropometric, psychobehavioral and hormonal characteristics in placebo-treated group (group P) and in groups treated with calcium carbonate (group C) or with calcium of dairy origin (Lactoval, group L) before and after weight reduction. No significant differences in measured parameters were observed between the groups both before and after weight reduction.

Variable	Before							After						
	Group C		Group L		Group P		ANOVA	Group C		Group L		Group P		ANOVA
	mean	S.D.	mean	S.D.	mean	S.D.		mean	S.D.	mean	S.D.	mean	S.D.	
Weight (kg)	84.95	11.75	83.43	11.65	85.37	14.83	NS	81.61	11.56	79.57	11.49	81.03	14.22	NS
BMI (kg/m^2)	32.39	4.35	32.42	4.22	32.36	4.86	NS	31.11	4.19	30.90	4.10	30.72	4.68	NS
Fat (kg)	35.57	9.12	35.38	9.39	36.51	10.03	NS	31.93	7.68	31.38	9.42	32.72	9.38	NS
Fat (%)	41.30	4.98	41.70	6.39	42.01	5.69	NS	38.69	4.54	38.71	7.58	39.58	6.03	NS
FFM (kg)	49.37	3.61	48.08	4.29	48.85	5.90	NS	49.69	5.38	48.19	4.95	47.40	5.33	NS
Beck	10.75	5.76	8.29	3.43	12.05	8.41	NS	7.42	5.35	5.72	3.94	9.57	8.61	NS
Restraint	10.38	3.83	9.81	4.03	9.86	5.62	NS	13.54	4.36	12.71	3.76	12.48	5.39	NS
Hunger	4.54	3.04	4.24	2.99	3.38	3.68	NS	3.00	2.84	2.47	2.06	3.00	3.13	NS
Disinhibition	6.79	3.04	6.95	2.90	6.05	2.90	NS	5.42	2.96	4.81	2.95	4.29	1.88	NS
Leptin (μg/l)	21.74	8.47	21.47	9.31	20.34	9.14	NS	17.51	7.67	15.13	7.51	13.13	5.01	NS
NPY (pmol/l)	108.30	54.23	99.07	42.45	97.17	57.96	NS	92.09	38.87	84.26	41.20	74.92	41.51	NS
ADN (mg/l)	10.66	3.09	13.07	7.35	9.99	4.22	NS	11.09	3.70	12.35	6.27	10.39	4.02	NS
Resistin (μg/l)	2.68	0.89	2.33	0.65	2.15	0.65	NS	2.08	0.54	2.28	0.80	2.37	0.99	NS

ADN - adiponectin

Table 3. Changes in selected anthropometric and psychobehavioral characteristics in placebo-treated group (group P) and in groups treated with calcium carbonate (group C) or with calcium of dairy origin (Lactoval, group L). Kruskal-Wallis ANOVA as well as Kruskal-Wallis multiple comparisons found no significant differences in changes between the groups.

	Group C			Group L			Group P		
Variable	Mean	S.D.	P	Mean	S.D.	P	mean	S.D.	p
Weight (kg)	–3.34	1.79	0.00002	–3.87	1.62	0.00006	–4.34	1.37	0.00006
BMI (kg/m^2)	–1.29	0.69	0.00002	–1.51	0.65	0.00006	–1.64	0.49	0.00006
Fat (kg)	–3.64	3.83	0.00002	–4.00	2.73	0.00006	–3.80	1.45	0.00006
FFM (kg)	0.32	3.49	0.64738	0.11	2.45	0.53124	–1.46	3.36	0.00630
Restraint	3.17	3.47	0.00068	2.90	4.15	0.00767	2.62	5.44	0.05544
Hunger	–1.54	2.59	0.01013	–1.76	2.98	0.01680	–0.38	2.56	0.44642
Disinhibition	–1.38	2.73	0.04447	–2.14	2.61	0.00421	–1.76	2.39	0.00482

energy deficit of 500 kcal: low dairy, high dairy and calcium-supplemented low dairy. Accelerated weight and fat loss in response to energy restriction was observed in high dairy consumers and calcium-supplemented groups in comparison with low dairy low-calcium consumers (Zemel 2004). It could be objected that our study was conducted over a short period of time and that the amount of supplemented calcium was not high enough to affect the weight loss. The duration of our intervention, only three weeks, was rather short. We were unable to extend the duration of the supervised in-patient stay above the usual 4-wk period of the spa treatment. The lack of evidence for the role of calcium in promoting weight loss in our study might be due to a rather high daily energy deficit which could surpass the effects of calcium over a short-time period of WM. In many interventional studies, patients received $\geq$1000 mg calcium/day. Our calcium-supplemented patients received a total daily dose of 850

mg calcium on the average which was shown to be sufficient for potentiating weight reduction as well as for its beneficial effects on body composition. According to Thompson *et al.* (2005) diets higher than 800 mg of calcium in dairy products or higher in fiber and lower in glycemic index do not enhance weight reduction beyond what is seen with caloric restriction alone. The Amsterdam Growth and Health Longitudinal Study which followed a cohort of men and women from age 13 years in 1977 to age 36 years in 2000 suggested a threshold of approximately 800 mg/day above which calcium intake has no additional beneficial effect on body composition (Boon *et al.* 2005). Barr (2003) in a Medline research project between 1966–2001 identified 17 randomized trials of calcium supplementation in subjects without caloric restriction. In most studies, no differences in body weight or body composition were detected between the calcium and placebo-treated or untreated groups. Recker *et al.* (1996) in a 4-year study detected a significant difference in body weight change. Postmenopausal women receiving 1.2 g calcium/day lost 0.35 kg/year more than did the control group.

Body composition in this study was evaluated by the bioimpedance method which is greatly influenced by the hydration of the examined subjects. Therefore patients treated with drugs which could affect the water balance were not included in the study. Some studies raised questions about the use of bioelectrical impedance (BIA) for evaluation of body composition in obese subjects as well as its changes in response to the weight management (Kyle *et al*. 2004). However, Jebb *et al.* (2007) declares that BIA is a useful method for measuring body composition changes during clinical weight management programs. BIA was also classified as a useful tool for body composition assessment in extremely obese subjects before and after massive weight loss induced by gastric bypass surgery (Das *et al.* 2003). In our study, a significant decrease in fat-free mass (FFM) was demonstrated in the placebo-treated group, whereas loss of FFM was not significant in both calcium-treated groups. It cannot be excluded that the higher loss of body weight in the placebo group (–4.34 kg) compared to –3.58 kg in both calcium-treated groups was partly due to differences in water balance. However, there are no data available about the association of low calcium intake with the loss of water.

On the other hand, the protective effect of calcium intake on FFM was reported by Heaney *et al.* (2002) in a 3-year calcium intervention trial in young women given either 1500 mg calcium per day or a placebo. The group as a whole had gained weight in a 3-year follow-up; there was no significant difference in weight gain between the calcium-supplemented and control groups. However, the weight gain in the calcium supplemented women consisted primarily in an increase in fat-free mass, while the placebo-treated women accumulated twice as much body fat. High dairy calcium diets in three weight loss studies conducted by Zemel *et al.* (2004, 2005a, 2005b) induced not only higher fat loss but also markedly reduced the loss of FFM compared with the low dairy calcium diet. Preservation of FFM by dairy products could be attributed to the high content of branched chain amino acids in proteins of dairy origin. Branched chain amino acids, especially leucine, play a key role the regulation of muscle protein synthesis (Layman and Walker 2006). It is difficult to find an explanation how dietary calcium *per se* might influence preservation of FFM during the weight loss studies. Calcium-induced suppression of 1,25-dihydroxyvitamin D levels has been proposed as a mechanism leading to reduction of visceral adiposity as 1,25-dihydroxyvitamin D has been shown to stimulate 11β-hydroxysteroid dehydrogenase-1 (Morris and Zemel 2005). However, it seems unlikely that such a calcium-induced inhibition of local cortisol production in adipose tissue could play any role in the modulation of overall body protein catabolism.

In addition, a significant decline in the hunger score of the Eating Inventory was demonstrated in calcium-treated groups, but not in the placebo-treated group. This change in perception of hunger could contribute to a better long-term outcome of weight management in calcium-treated patients. However, change in the hunger score does not necessarily implicate change in energy intake which was the same for all participants in our study. The observed decline in the hunger score cannot be attributed to changes in fasting concentrations of hormones involved in food intake regulation. No significant differences in profile of these hormones were seen between the calcium- and placebo-treated individuals. However, we did not examine postprandial hormonal responses which could affect both satiety and hunger feelings. Ping-Delfos *et al.* (2004) reported that a high dairy calcium and vitamin D diet did not affect subjective sensations of hunger and satiety in the immediate postprandial period, but spontaneous food intake over the subsequent 24 h period was significantly reduced.

Previous studies demonstrated that a diet

characterized by a higher calcium intake or higher dairy intake, especially low-fat dairy intake, may lower the risk of type 2 diabetes (Choi *et al.* 2005, Pittas *et al.* 2006). A combined daily intake of more than 1200 mg calcium and 800 IU vitamin D was associated with a 33 % lower risk of type 2 diabetes with RR of 0.67 (0.49-0.90) compared with an intake of less than 600 mg calcium and 400 IU vitamin D (Pittas *et al.* 2006). Among the mechanisms involved in lowering the risk of type 2 diabetes, hormonal mechanisms affecting insulin sensitivity should be considered (Silha *et al.* 2003, Heilbronn *et al.* 2004, Lu *et al.* 2006). A significant difference in the change of resistin levels in response to a negative energy balance was shown in our study between calcium-treated and placebo-treated individuals. Calcium mediated differences in resistin response to weight management might play a role in reducing the risk of type 2 diabetes and metabolic syndrome.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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IV

TT VARIANT OF FATTY-ACID BINDING PROTEIN TYPE 2 POLYMORPHISM TENDS TO LOWER BMI IN OBESE WOMEN, A PILOT STUDY

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INTRODUCTION

Fatty acid-binding protein 2 (FABP2) plays a role in intestinal absorption of fatty acid and in intracelular metabolism of fatty acids. The polymorphism of codon 54 results in substitution of threonin (Thr) for alanin (Ala). Genetic variation of FABP2 may influence prevalence of obesity and insulin sensitivity. Aim of this study was to determine relationship between Ala54Thr polymorphism of FABP2 and adipose tissue distribution, BMI, insulin resistance and parameters of lipid metabolism.

PATIENTS AND METHODS

117 obese women (age 45.3±11.0; BMI 36.1±6.5) were examined. The allelic variant of FABP2 was determined by polymerase chain reaction, followed by restriction fragment-length polymorphism analysis. Intraabdominal adipose tissue area was measured by CT. Food intake and eating behavior were evaluated by PC program Nutrition and by eating inventory (Stunkard and Messick, 1985), respectively. Fasting blood glucose, fasting insulin and C-peptide, blood lipids and apoprotein A and B levels were assessed in plasma samples. Data were analyzed by ANOVA after adjustment for age and BMI.

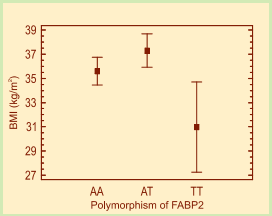
RESULTS

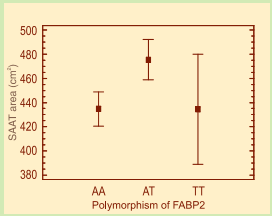
Prevalence of AA, AT and TT polymorphism was 52.1%, 41,1% and 6.9%. Significant differences were found in BMI and in the distribution of subcutaneous abdominal adipose tissue (SAAT). SAAT was subdivided into superficial and deep compartments by use of the fascia superficialis. In distribution of viscera abdominall adipose tissue (VAAT) was no significant difference. We found no significant differences in food intake between groups.

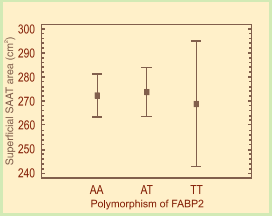
Variable	AA	AT	TT	p<...
n	61 (52.1%)	48 (41.0%)	8 (6.9%)	
Age (year)	47.0±10.0	44.8±11.4	35.8±12.3	p<0.084
BMI (kg/m^2)	35.5±6.9	37.6±5.8	31.8±5.2	p<0.065
W/H ratio	0.85±0.06	0.86±0.06	0.83±0.05	p<0.762
RMR (kJ/day)	6402±1144	6735±849	6207±1019	p<0.956
RQ	0.79±0.06	0.78±0.05	0.80±0.05	p<0.444
Body fat (%)	46.5±8.6	47.5±9.9	42.5±7.6	p<0.272

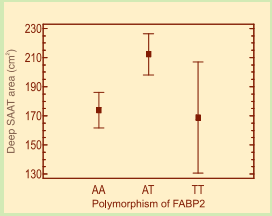
Variable	AA	AT	TT	p<...
SAAT (cm^2)	422±140	502±126	361±95	p<0.035
Superficial SAAT (cm^2)	265±81	286±76	238±78	p<0.951
Deep SAAT (cm^2)	174±72	215±86	117±35	p<0.016
VAAT (cm^2)	186±75	195±79	113±48	p<0.867

Variable	AA	AT	TT
TC	5.50±0.87	5.39±0.86	4.60±0.82
HDL-C	1.32±0.31	1.20±0.26	1.21±0,10
TAG	1.90±1.11	1.80±0.82	1.70±1.47
ApoA	1.46±0.30	1.36±0.33	1.41±0.27
ApoB	1.04±0.29	1.15±0.62	0.85±0.18
Glucose	5.29±1.86	5.66±2.05	5.09±1.22
IRI	13.05±11.70	11.74±6.36	12.85±15.85
C-peptide	1.05±0.46	1.06±0.41	0.80±0.36









CONCLUSION

Results of this study indicated that TT variant of FABP2 polymorphism could result in a lower weight gain and could influence adipose tissue distribution.

V

ASSOCIATION OF THE INTESTINAL FATTY ACID-BINDING PROTEIN 2 POLYMORPHISM AND DISTRIBUTION OF ABDOMINAL ADIPOSE TISSUE IN OBESE WOMEN

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BACKGROUND

Genetic variants in the intestinal fatty acid binding protein 2 (FABP2) gene may be associated with different types of fat distribution. Aim of this study was to determine relations between FABP2 Ala54Thr polymorphism and distribution of adipose tissue, anthropometrics, lipids parameters and levels of hormones.

PATIENTS AND METHODS

We examined a group of 118 obese women (age 45.1±11.4; BMI 35.6±6.2). The allelic variant of FABP2 was determined by PCR, followed by restriction fragment-length polymorphism analysis. Abdominal adipose tissue area was examined by computer tomography. Anthropometric parameters were measured according to standardized procedures. Parameters of lipid metabolism and hormonal levels were assessed in fasting plasma samples. Data were analyzed by ANOVA.

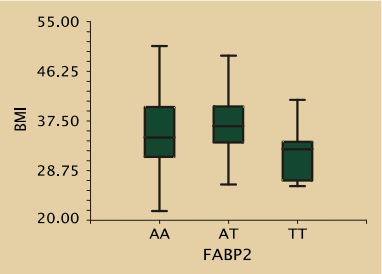
variable	AA	AT	TT	p
n	59 (50%)	51 (43.2%)	8 (6.8%)	NS
Age	46.3±10.6	10.0±11.8	35.7±12.3	NS
BMI	35.0±6.4	37.1±5.8	31.8±5.2	0.05471
Waist	103.2±14.1	107.9±12.8	94.0±9.6	NS
Hip	121.0±13.2	126.0±13.1	115.3±9.1	NS
Body fat %	46.3±8.4	47.4±10.0	42.3±6.9	NS

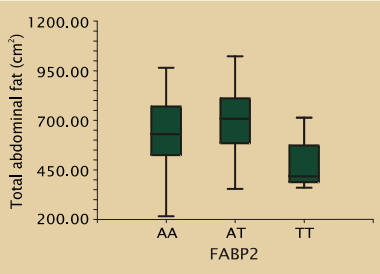
variable	AA	AT	TT	p
Total abdominal fat (cm^2)	620.5±187.4	692.9±161.1	475.5±123.8	0.00402
SAAT (cm^2)	432.4±136.1	500.1±125.7	361.4±94.7	0.00358
VAAT (cm^2)	188.0±73.9	192.7±79.2	114.1±44.0	0.02819

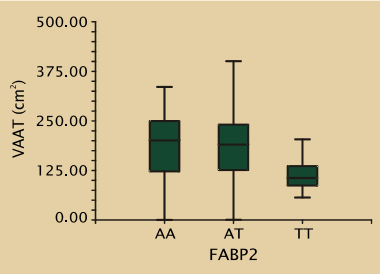
variable	AA	AT	TT
TC	5.5±0.9	5.3±0.8	4.6±0.8
HDL-C	1.3±0.3	1.2±0.2	1.2±0.1
TAG	1.9±1.1	1.8±0.9	1.7±1.5
ApoA	1.4±0.4	1.4±0.3	1.4±0.3
ApoB	1.0±0.3	1.1±0.2	0.8±0.2
Glucose	5.3±1.9	5.7±2.1	5.1±1.2
Insulin	13.2±11.9	11.4±6.2	12.8±15.9
C-peptide	1.1±0.5	1.1±0.4	0.8±0.4
STH	2.1±4.3	2.5±4.4	6.3±10.9
Testosterone	1.4±0.7	1.6±1.0	1.5±1.4
Cortisol	306.9±148.5	349.3±142.0	342.0±164.2
SHBG	56.2±38.7	43.8±25.3	47.0±22.1
DHEA	6.1±4.0	7.3±6.0	6.2±3.4
DHEAS	4.6±2.5	5.5±3.4	6.5±4.5

RESULTS

Genotype frequencies for FABP2 polymorphism were 50.0% for AA, 43.2% for AT and 6.8% for TT. We found significantly lower BMI in carriers of TT alleles (p=0.05). Women with TT polymorphism had also significantly lower mass of total and subcutaneous abdominal adipose tissue (SAAT) (p<0.01) and visceral abdominal adipose tissue (VAAT) (p<0.05). There were no differences in parameters of lipid metabolism and levels of hormones.







CONCLUSION

The results suggest that TT polymorphism of FABP2 in obese women could decrease accumulation of adipose tissue in both subcutaneous and intraabdominal area in comparison with wild type homozygotes and heterozygotes.

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