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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SHORT TITLE: FATTY ACID COMPOSITION IN OBESE WOMEN AFTER WEIGHT REDUCTION WITH n-3 FAT

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 either the group with n-3 LCD or LCD with yogurt without 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

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 effect on insulin sensitivity (Kontrová *et al* 2007). Lower proportions 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

40 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

behavioural modification of lifestyle. The diet was prepared in the spa central kitchen and its energy content was calculated using the PC programme „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 a 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, 620mg/day was eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 20:6 n-3) acid. The yogurt was produced by Dairy Research Institute Milcom^{R}. The weight reducing regimen included daily light to moderate physical activity lasting about 60 minutes/day.

Body composition, laboratory analysis and psychobehavioural 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).

Psychobehavioural examination

Eating behaviour 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 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 the 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 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 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 (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.

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There is no conflict of interest.

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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	$^{*}p$	^{x}p	^{xx}pa
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
^{x}p significance of the difference in treatment effect between the groups
^{xx}pa significance of the difference in treatment effect between the groups , after adjustment for baseline weight

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 ± 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

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

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 ± 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