ASSOCIATION OF CHANGES IN MACRONUTRIENT AND CALCIUM INTAKES WITH BODY WEIGHT CHANGE IN OBESE SUBJECTS

K. Kabrnová, R. Braunerová, B. Aldhoon, P. Hlavatý, M. Wagenknecht, M. Kunešová, J. Pa ízková, V. Hainer

Obesity Management Centre, Endocrine Institute, Prague, Czech Republic

Background:

Association between the intake of high density food and body weight gain has been clearly demonstrated. The role of dietary calcium in body weight regulation was confirmed in experimental studies whereas inconsistent data on relationship between dietary calcium intake and body weight regulation in humans were reported.

Aims:

To evaluate association between body weight change and change in consumption of macronutrients, calcium and phosphorus in response to the weight reduction regime.

Methods:

Two hundred and eight obese subjects (Tab. 1) were followed during the comprehensive weight management in duration of 3-6 months. Energy and nutrient intake was assessed from one week dietary record utilizing PC programme Nutrition both at the beginning and at the end of weight reduction regime.

Statistical analysis: ANOVA was used for comparison of changes in all followed parameters in response to negative energy balance. Partial correlation coefficients were calculated to evaluate associations between body weight change and intake of nutrients.

Table 1. Characteristics of the cohort

n = 208	
BMI	40.0 ± 7.3
Body Weight	112.8 ± 25.6
Age	48.6 ± 13.0

Results:

Weight reduction programme lead to weight loss and reduction in intake of energy, macronutrients, and both calcium and phosphorus (Tab. 2). Change in body weight was positively related to the change in fat intake and phosphorus intake and negatively related to the change in protein intake and calcium intake (Tab. 3). On the other hand, no significant association between the change in carbohydrate intake and change in body weight was observed.

Table 2. Body weight, energy and nutrient intake at baseline and after weight

		Before	SD	After	SD	change	SD	P
Body Weight	kg	112.8	25.6	106.7	24.1	6.1	7.9	0.001
Energy	kJ/day	8192	3041	6183	2017	2009	2812	0.001
Carbohydrate	g/day	252.3	91.1	198.7	70.3	53.6	92.6	0.001
Fat	g/day	70.5	32.7	48.3	22.5	22.2	30.4	0.001
Protein	g/day	70.4	22.9	64.0	17.9	6.4	21.6	0.001
Phosphorus	mg/day	1222	413	1015	251	207	375.1	0.001
Calcium	mg/day	695	324	631	251	64	353	NS

Table 3. Partial correlation between weight change and change in intake of

	r	p
Carbohydrate	-0.023	0.750
Fat	0.220	0.002
Protein	-0.289	0.000
Phosphorus	0.195	0.006
Calcium	-0.210	0.003

Conclusion:

In contrast to the change in fat intake, changes in protein and calcium consumption are inversely related to body weight change in response to negative energy balance.

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Calcium Intake and the Outcome of Short-Term Weight Management

K. KABRNOVÁ - HLAVATÁ [1], V. HAINER [1], M. GOJOVÁ [2], P. HLAVATÝ [1], V. KOPSKÝ [1], J. NEDVÍDKOVÁ[1], M. KUNEŠOVÁ[1], J. PAŘÍZKOVÁ[1], M. WAGENKNECHT [1], M. HILL[1], J. DRBOHLAV [3]

[1]Institute of Endocrinology, Obesity Management Center, Prague, [2]Obesity Management Unit, Lipová Lázně and [3]Dairy Research Institute, Prague, Czech Republic

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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

Corresponding author

V. Hainer, Institute of Endocrinology, Obesity Management Centre, Národní třída 8, 116 94 Prague 1, Czech Republic. E-mail: vhainer@endo.cz

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

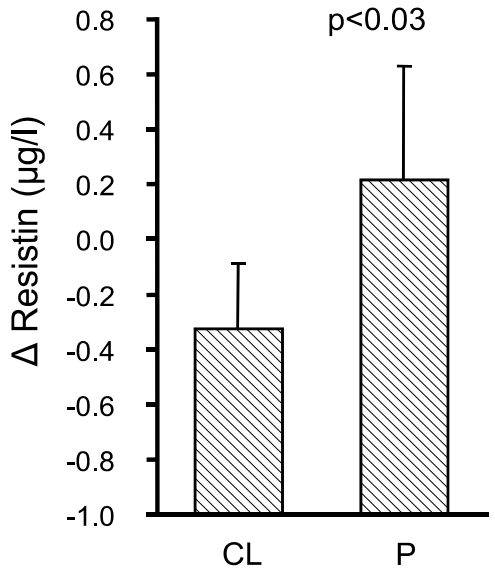

Fig. 1. Change of resistin level in response to WM in the placebo-treated group (group P) and joint calcium-treated group (group CL).

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.

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References

BARGER-LUX MJ, DAVIES KM, HEANEY RP, CHIN BK, RAFFERTY K: Calcium supplementation may attenuate accumulation of fat in young women. *J Bone Miner Res* **16**: 219S, 2001.

BARR SI: Increased dairy product or calcium intake: is body weight or composition affected in humans? *J Nutr* **133**: 245-248, 2003.

BECK AT, WARD CH, MENDELSON M, MOCK J, ERBAUGH J: An inventory for measuring depression. *Arch Gen Psychiatr* **4**: 561-571, 1961.

BOON N, KOPPES LL, SARIS WH, VAN MECHELEN W: The relation between calcium intake and body composition in a Dutch population: The Amsterdam Growth and Health Longitudinal Study. *Am J Epidemiol* **162**: 27-32, 2005.

BURSEY RG, SHARKEY T, MILLER GD: High calcium intake lowers weight in lean and fatty Zucker rats. *FASEB J* **3137**: 265, 1989.

CHAN GM, HOFFMEN K, MCMURRY M: Effects of dairy products on bone and body composition in pubertal girls. *J Pediatr* **126**: 551-556, 1995.

CHOI HK, WILLETT WC, STAMPFER MJ, RIMM E, HU FB: Dairy consumption and risk of type 2 diabetes mellitus in man: a prospective study. *Arch Intern Med* **165**: 997-1003, 2005.

DAS SK, ROBERTS SB, KEHAYIAS JJ, WANG J, HSU LK, SHIKORA SA, SALTZMAN E, M CCRORY MA: Body composition assessment in extreme obesity and after massive weight loss induced by gastric bypass surgery. *Am J Physiol* **284**: E1080-E1088, 2003.

DAVIES KM, HEANEY RP, RECKER RR, LAPPE JM, BURGER-LUX MJ, RAFFERTY K, HINDERS S: Calcium intake and body weight. *J Clin Endocrinol Metab* **85**: 4635-4638, 2000.

DRUCE M, BLOOM SR: The regulation of appetite. *Arch Dis Child* **91**: 183-187, 2006.

HEANEY RP, DAVIES KM, BARGER-LUX MJ: Calcium and weight: clinical studies. *J Am Coll Nutr* **21**: 152-155, 2002.

HEILBRONN LK, ROOD J, JANDEROVA L, ALBU JB, KELLEY DE, RAVUSSIN E, SMITH SR: Relationship between serum resistin concentrations and insulin resistance in nonobese, obese, and obese diabetic subjects. *J Clin Endocrinol Metab* **89**: 1844-1888, 2004.

JACOBSEN R, LORENZEN JK, TOUBRO S, KROG-MIKKELSEN I, ASTRUP A: Effect of short-term high dietary calcium intake on 24-h energy expenditure, fat oxidation, and fecal fat excretion. *Int J Obes* **29**: 292-301, 2005.

JACQMAIN M, DOUCET E, DESPRES JP, BOUCHARD C, TREMBLAY A: Calcium intake, body composition, and lipoprotein-lipid concentrations in adults. *Am J Clin Nutr* **77**: 1448-1452, 2003.

JEBB SA, SIERVO M, MURGATROYD PR, EVANS S, FRUHBEC K G, PRENTICE AM. Validity of the leg-to-leg bioimpedance to estimate changes in body fat during weight loss and regain in overweight women: a comparison with multi-compartment models. *Int J Obes* **31**: 756-762, 2007.

KABRNOVÁ K, BRAUNEROVÁ R, ALDHOON B, HLAVATÝ P, WAGENKNECHT M, KUNEŠOVÁ M, PAŘÍZKOVÁ J, HAINER V: Association of changes in macronutrient and calcium intakes with body weight change in obese subjects. *Int J Obes* **28** (Suppl 1): S138, 2004.

KUNEŠOVÁ M, BRAUNEROVÁ R, HLAVATÝ P, TVRZICKÁ E, STAŇKOVÁ B, ŠKRHA J, HILGERTOVÁ J, HILL M, KOPECKÝ J, WAGENKNECHT M, HAINER V, MATOULEK M, PAŘÍZKOVÁ J, ŽÁK A, SVAČINA S: 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. *Physiol Res* **55**: 63-72, 2006.

KYLE UG, BOSAEUS I, DE LORENZO AD, DEURENBERG P, ELIA M, MANUEL GOMEZ J, HEITMANN LB, KENT-SMITH L, MECHIOR JC, PIRLICH M, SCHARFETTER H, SCHOLS A, PICHARD C; ESPEN: Bioelectrical impedance analysis-part II: utilization in clinical practice. *Clin Nutr* **23**: 1430-1453, 2004.

LAYMAN DK, WALKER DA: Potential importance of leucine in treatment of obesity and the metabolic syndrome. *J Nutr* **136** (Suppl 1): 319-323, 2006.

LIN YC, LYLE RM, MCCABE LD, MCCABE GP, WEAVER CM, TEEGARDEN D: Dairy calcium is related to changes in body composition during a two-year exercise intervention in young women. *J Am Coll Nutr* **19**: 754-760, 2000.

LIU S, SONG Y, FORD ES, MANSON JE, BURING JE, RIDKER PM: Dietary calcium, vitamin D, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. *Diabetes Care* **28**: 2926-2932, 2005.

LU HL, WANG HW, WEN Y, ZHANG MX, LIN HH: Roles of adipocyte derived hormone adiponectin and resistin in insulin resistance of type 2 diabetes. *World J Gastroenterol* **12**: 1747-1751, 2006.

MELANSON EL, SHARP TA, SCHNEIDER J, DONAHOO WT, GRUNWALD GK, HILL JO: Relation between calcium intake and fat oxidation in adult humans. *Int J Obes Relat Metab Disord* **27**: 196-203, 2003.

MORRIS KL, ZEMEL MB: 1,25-dihydroxyvitamin D3 modulation of adipocyte glucocorticoid function. *Obes Res* **13**: 670-677, 2005.

PEREIRA MA, JACOBS DR, VAN HORN L, SLATTERY ML, KARTASHOV AI, LUDWIG DS: Dairy consumption, obesity, and the insulin resistance syndrome in young adults. The CARDIA Study. *J Am Med Assoc* **287**: 2081-2089, 2002.

PING-DELFOS WC, SOARES MJ, CUMMINGS NK: Acute suppression of spontaneous food intake following dairy calcium and vitamin D. *Asia Pac J Clin Nutr* **13** (Suppl): 82, 2004.

PITTAS AG, DAWSON-HUGHES B, LI T, VAN DAM RM, WILLET WC, MANSON JE, HU FB: Vitamin D and calcium intake in relation to type 2 diabetes in women. *Diabetes Care* **29**: 650-656, 2006.

RAJPATHAK SN, RIMM EB, ROSNER B, WILLET WC, HU FB: Calcium and dairy intakes in relation to long-term weight gain in US men. *Am J Clin Nutr* **83**: 559-566, 2006.

RECKER RR, HINDERS S, DAVIES KM, HEANEY RP, STEGMAN MR, LAPPE JM, KIMMEL DB: Correcting calcium nutritional deficiency prevents spine fractures in elderly women. *J Bone Miner Res* **11**: 1961-1966, 1996.

SHI H, DIRIENZO D, ZEMEL MB: Effects of dietary calcium on adipocyte lipid metabolism and body weight regulation in energy-restricted aP2-agouti transgenic mice. *FASEB J* **15**: 291-293, 2001.

SILHA JV, KRSEK M, SKRHA JV, SUCHARDA P, NYOMBA BL, MURPHY LJ: Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance. *Eur J Endocrinol* **149**: 331-335, 2003.

STUNKARD AJ, MESSICK S: The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *J Psychosom Res* **29**: 71-83, 1985.

THOMPSON WG, ROSTAD HOLDMAN N, JANZOW DJ, SLEZAK JM, MORRIS KL, ZEMEL MB: Effect of energy-reduced diets high in dairy products and fiber on weight loss in obese adults. *Obes Res* **13**: 1344-1353, 2005.

XUE B, GREENBERG AG, KRAEMER FB, ZEMEL MB: Mechanism of intracellular calcium inhibition of lipolysis in human adipocytes. *FASEB J* **17**: 2527-2529, 2001.

YU X, SUN C, LIU R, LI Y: The effect of dietary calcium on the expression of uncoupling protein 3 gene in skeletal muscle of rat fed with high fat diet. *Wei Sheng Yan Jiu* **32**: 204-207, 2003.

ZEMEL MB: Calcium modulation of adiposity. *Obes Res* **11**: 375-376, 2003.

ZEMEL MB: Role of calcium and dairy products in energy partitioning and weight management. *Am J Clin Nutr* **79**: 907-912, 2004.

ZEMEL MB, SHI H, GREER B, DIRIENZO D, ZEMEL PC: Regulation of adiposity by dietary calcium. *FASEB J* **14**: 1132-1138, 2000.

ZEMEL MB, THOMPSON W, MILSTEAD A, MORRIS K, CAMPBELL P: Calcium and dairy acceleration of weight and fat loss during energy restriction in obese adults. *Obes Res* **12**: 582-590, 2004.

ZEMEL MB, RICHARDS J, MATHIS S, MILSTEAD A, GEBH ARDT L, SILVA E: Dairy augmentation of total and central fat loss in obese subjects. *Int J Obes* **29**: 391-397, 2005a.

ZEMEL MB, RICHARDS J, MILSTEAD A, CAMPBELL P: Effects of calcium and dairy on body composition and weight loss in African-American adults. *Obes Res* **13**: 1218-1225, 2005b.

Serotonin and Norepinephrine Reuptake Inhibition and Eating Behavior

VOJTECH HAINER, KAROLINA KABRNOVA, BASHAR ALDHOON, MARIE KUNESOVA, AND MARTIN WAGENKNECHT

Institute of Endocrinology, Obesity Management Center, 11694 Prague 1, Czech Republic

ABSTRACT: **Brain neurotransmitters, serotonin and norepinephrine, play an important role in the central nervous control of energy balance and are involved in symptomatology related to both obesity and depression. Therefore both serotonin and norepinephrine neural pathways have been paid a special attention as targets for the antiobesity drugs, antidepressants, and drugs used in the treatment of eating disorders. Selective serotonin reuptake inhibitors (SSRI) have been used in the treatment of depression and eating disorders but have failed to achieve sustained weight loss in the treatment of obesity. Sibutramine, a serotonin and norepinephrine reuptake inhibitor, which induces satiety and prevents decline in metabolic rate associated with a hypocaloric diet, is currently the sole centrally acting drug indicated for the long-term treatment of obesity. Depression, dietary disinhibition (evaluated by the Eating Inventory [EI]), and stress are associated with the accumulation of abdominal fat and the development of metabolic syndrome and related diseases. Subjects with abdominal obesity demonstrate neuroendocrine abnormalities which result in disturbances in hypothalamo-pituitary-adrenal (HPA) function. Treatment with SSRI might interrupt the vicious circle which leads to endocrine abnormalities and the accumulation of abdominal fat. Obesity treatment with sibutramine results, not only in significant weight loss, but also in reduction of abdominal fat and in the improvement of health risks associated with metabolic syndrome (lipid profile, blood glucose, insulin, HbA1c, and uric acid), as well as in the decline in disinhibition score of the EI. In a 1-year sibutramine trial, only a decrease in the disinhibition score remained a significant correlate of weight loss among the psychobehavioral and nutritional factors which were taken into account.**

KEYWORDS: **serotonin; norepinephrine; eating behavior; dietary disinhibition; sibutramine; abdominal obesity; metabolic syndrome**

Address for correspondence: Dr. Vojtech Hainer, Institute of Endocrinology, Narodni 8, 116 94 Prague 1, Czech Republic. Voice: 00420-724211155; fax: 00420-224905325.
e-mail: vhainer@endo.cz

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CENTRALLY ACTING DRUGS THAT AFFECT SEROTONIN AND NOREPINEPHRINE PATHWAYS (TABLE 1)

Obesity reached epidemic proportions at the beginning of the new millennium in both developed and developing countries. Therefore intensive research has been focused on the development of new antiobesity agents in order to control the increasing epidemic of obesity. Central nervous control of eating consists of two separate neuroregulatory systems. Regulation of satiety and hunger is mediated by neurotransmitters and hormones, such as serotonin, norepinephrine, leptin, neuropeptide Y/agouti-related peptide, proopiomelanocortin/MSH/CART, orexins, ghrelin, peptide YY, glucagon-like peptide- 1, etc. On the other hand, in the regulation of hedonic responses (eating represents a source of pleasure and addiction) dopamine, endo-cannabinoids, opioids, and glutamate are involved. Drugs affecting energy balance through the central serotonin and norepinephrine pathways have been paid a special attention in the treatment of obesity. Amphetamines and phentermine act as releasing agents for norepinephrine and dopamine. Because of serious side effects associated with norepinephrine-induced tachycardia and hypertension and dopamine-induced addiction, amphetamines do not have any indication for the treatment of obesity. Phentermine exhibits less addiction properties than amphetamines, but still exerts adverse cardiovascular side effects. Therefore phentermine is indicated only for short-term use as an antiobesity agent in some countries and was withdrawn from the market in other countries. Fenfluramine and dexfenfluramine act as both serotonin-releasing agents and serotonin reuptake inhibitors and were used as antiobesity agents which induced a significant weight loss by suppression of the appetite.[1] They were withdrawn from the market because of the development of heart valve disease associated with stimulation of the heart serotonin 2b receptors. A new generation of selective serotonin (2C) agonists (ADP356, Ro 60-0175, Org 12962, VER-3323, BVT-933, YM348) have been developed that induce satiety and have been devoid of cardiotoxicity.[2] ADP356 is currently undergoing clinical trials. ADP356 administered for 1 month in a daily dose of 15 mg induced a highly significant weight loss in comparison with placebo. The drug was well tolerated and there were no apparent harmful side effects on the heart as assessed by echocardiograms. Selective serotonin reuptake inhibitors (SSRI) used as antidepressant agents (fluoxetine, sertraline, citalopram) induced weight loss during the short-term period, but failed to achieve sustained weight loss in the treatment of obesity. Sibutramine is currently the only centrally acting drug that is indicated for the long-term treatment of obesity. Sibutramine acts as a serotonin and norepinephrine reuptake inhibitor, which induces satiety and prevents a hypocaloric diet-induced decline in metabolic rate.[3] Venlafaxine, milnacipran, and duloxetine as serotonin and norepinephrine reuptake inhibitors represent a new treatment option for depression, anxiety, and stress urinary incontinence, as well as painful physical symptoms.[4,5] Their use in the treatment of obesity

has not been reported. However, even the long-term treatment of depression with serotonin–norepinephrine reuptake inhibitor, duloxetine, is not associated with substantial weight gain.

Norepinephrine reuptake inhibitor, GW320659, that possesses antidepressant activities is also investigated as an antiobesity agent and a drug for the management of attention-deficit/hyperactivity disorder. Reboxetine, a potent and selective norepinephrine reuptake inhibitor, has been approved for the treatment of major depression[6] as well as for the treatment of chronic pain in patients with depression.[7] In healthy volunteers, an administration of reboxetine stimulates cortisol secretion and this cortisol response is higher in males than in females.[8] Reboxetine has not been evaluated in obese patients. However, a case of significant weight loss in normal weight woman treated with reboxetine was described.[9]

ROLE OF PSYCHOLOGICAL FACTORS IN BODY WEIGHT REGULATION AND HEALTH RISKS OF OBESITY

Body weight, fat accumulation, and fat distribution, as well as the subsequent health risks are influenced by the interaction of genetic and environmental factors. This process is under a complex control of neurotransmitters and hormones which could affect health risks either directly or indirectly through their influence on body fat accumulation and/or body fat distribution. Psychological factors affecting eating behavior and physical activity are influenced not only by genes and environment, but also by neurotransmitters, hormones, fat accumulation, and by the health risks manifested by obesity-related diseases. Psychological factors might exert their influence on health risks either directly or indirectly by neurohormonal pathways or through their influence on fat accumulation.

DEPRESSION, OBESITY, AND ABDOMINAL FAT ACCUMULATION

Per Björntorp emphasized the following main characteristics of the "Civilization Syndrome":[10]

(1) High stress,
(2) poor coping,
(3) increased alcohol and tobacco consumption,
(4) overeating, and
(5) physical inactivity.

According to Bjornorp, the characteristics of the "Civilization Syndrome," together with depression, lead to the accumulation of abdominal fat, a major

TABLE 1. Centrally acting drugs affecting serotoninergic and adrenergic pathways

	Releasing agents			Reuptake inhibitors	
	5-HT	NE	DA	5-HT	NE
Dexamphetamine		√	√		
Phentermine		√	√		
Fenfluramine	√			√	
Dexfenfluramine	√			√	
ADP 356*	√				
Fluoxetine				√	
Sibutramine				√	√
Duloxetine				√	√
Milnacipran				√	√
Venlafaxine				√	√
Atomoxetine					√
Reboxetine					√

*Selective 5-HT (2C) agonist.
NOTE: 5-HT = serotonin; NE = norepinephrine; DA = dopamine.

feature of the metabolic syndrome.[10] Depression could be one of the causal factors resulting in abdominal obesity. Ahlberg *et al.* classified abdominal obesity according to the waist to hip ratio (WHR) and evaluated depression by three different depression inventories: Hamilton Depression Scale, Montgomery–Asberg Depression Rating Scale, and Beck Depression Inventory.[11] He found that individuals with the WHR ≥ 1 exhibited significantly higher depression scores in all three depression scales than individuals with the WHR < 1 (TABLE 2). Recent study in overweight premenopausal women demonstrated a significant positive association of depressive mood with visceral and not with subcutaneous adipose tissue measured by computed tomography at the level of vertebral body L4-L5.[12] Depression could be a consequence of obesity in females who negatively perceive weight discrimination and weight teasing. In contrast, obesity is not usually accompanied by depression in males, as men do not perceive obesity as a psychosocial obstacle. Major depression in adolescents predicted a greater body mass index in adult life. Adverse childhood experiences promote the development of both depression and obesity and their co-occurrence. A genetic susceptibility to both depression and obesity may be expressed by environmental influences.[13] However, it is unclear whether the co-occurrence of depression and obesity is functionally related. Brain serotonin is involved in the regulation of appetite, mood, and other neuroendocrine functions. Reduction of brain serotonin might result in hyperphagia, depression, and pertubation of the pituitary–adrenal axis, suggesting that there may be common pathophysiology elements between obesity and depression.[14] The role of the serotoninergic system in body weight regulation is further supported by the observed association between polymorphism of serotonin 2C receptor[15]

TABLE 2. Depression rating and abdominal obesity classified according to the WHR

Depression scale	WHR < 1.0	WHR ≥ 1.0	*P* value
Hamilton depression scale	1.2 ± 1.8	4.1 ± 4.1	<0.001
Montgomery–Asberg depression rating scale	0.9 ± 1.1	3.0 ± 4.0	0.007
Beck depression score	2.7 ± 2.7	4.9 ± 4.7	0.044

NOTE: According to Ahlberg *et al.*[11]

and body weight gain in response to treatment with antipsychotic drugs, as well as by the finding of differential expression of serotonin receptors among mice, prone or resistant to chronic high-fat diet-induced obesity.[16]

It is also important to note that stress and depression represent barriers to the initiation and maintenance of healthy behaviors. P.Rhode *et al.* found that that higher stress and depression scores at the termination of a 6-month weight loss intervention predicted an increase in the percentage of total fat intake at 9 and 12 months, a trend that continued at 18 months.[17] Energy-deficient diets exert changes in the serum cortisol level which predict changes in appetite and weight loss during the subsequent follow-up.[18] However, it should be taken into account that changes in serum cortisol levels induced by a negative energy balance are also significantly determined by hereditary factors. This was demonstrated in our study of monozygotic twins who underwent 1-month treatment with very-low-calorie diet (VLCD). Significant within-pair resemblance in VLCD-induced changes in serum cortisol level was demonstrated throughout the day.[19]

An apparent overlap of obesity and depression is being frequently observed. Both diseases frequently exhibit increased appetite, hypersomnia, psychomotor retardation, and fatigue or loss of energy. Furthermore, both abdominal obesity and major depression are associated with coronary heart disease (CHD) and represent an increased risk of mortality. Depressive symptoms constitute an independent risk factor for the development of CHD and total mortality.[20] Major depression was also recognized as an independent risk factor that accelerated the development of CHD in diabetic women.[21] On the other hand, depressive patients treated with specific serotonin reuptake inhibitors have a significantly lower risk of death or nonfatal myocardial infarction.[22] Depression is associated with hyperglycemia in patients with diabetes.[23] Serum concentration of adiponectin, an adipose tissue hormone which is involved in the prevention of the development of metabolic syndrome, is negatively related to a depression score evaluated by the Beck Depression Inventory.[24] Norepinephrine and serotonin as brain neurotransmitters are involved in symptomatology related to both obesity and depression. Brain norepinephrine deficiency might be related to lethargy, decreased alertness, decreased energy, and abnormal feeding, whereas deficiency of serotonin might be related to obsessive and compulsive symptoms, dysphoria, abnormal feeding, eating disorders, and binge eating.

ABDOMINAL OBESITY, PERTUBATION OF HYPOTHALAMO-PITUITARY-ADRENAL AXIS AND TREATMENT WITH SSRI

Subjects with abdominal obesity demonstrate neuroendocrine abnormalities resulting in disturbances in the hypothalamo-pituitary-adrenal (HPA) function. A normal HPA function is characterized by high variability and high morning cortisol levels and a clear postprandial response in the cortisol level after lunch ingestion, as well as by an appropriate suppression with dexamethasone.[25] Low morning serum cortisol levels[26–29] and a decreased dexamethasone-induced suppression of cortisol release[28] characterize a pertubation of HPA axis in abdominal obesity. Dysfunction of the HPA axis as a consequence of frequently repeated or chronic stressful stimuli is associated with abdominal obesity and increased risks for cardiovascular disease, type 2 diabetes, and stroke.[25] Treatment with the SSRI citalopram, induced an increase in morning serum cortisol concentrations, suggesting a change toward normalization of the pertubed HPA axis activity.[29] Improvement in plasticity of the HPA axis by treatment with citalopram was also demonstrated by the increased cortisol levels in response to stimulation with corticotropin-releasing hormone (CRH) and stress.[29] Rosmond and Björntorp suggested to use the SSRI antidepressants in order to interrupt the vicious circle of pertubed HPA axis leading to an increasing abdominal obesity and endocrine abnormalities that, in turn, lead to progressive accumulation of intra-abdominal fat.[30] Administration of dexfenfluramine (an antiobesity drug which was withdrawn from the market), acting both as serotonin reuptake inhibitor and serotonin-releasing agent, was shown to induce a specific reduction of abdominal fat stores when assessed by magnetic resonance imaging (MRI). In the trial of Marks *et al.*, treatment of obesity with dexfenfluramine for 3 months was accompanied by a significant reduction of the visceral fat area ($-21.0 \pm 4.0\%$ in the dexfenfluramine group versus $-6.7 \pm 2.2\%$ in the placebo group, $P < 0.01$), although there was no significant difference between placebo and dexfenfluramine groups with regard to a reduction in the subcutaneous fat area.[31] The role of the serotoninergic system in the development of abdominal obesity and metabolic syndrome was recently demonstrated by an association of the low central nervous system serotoninergic responsivity with the metabolic syndrome and physical inactivity.[32]

EATING DISORDERS, SEROTONINERGIC SYSTEM, HPA AXIS AND TREATMENT WITH SSRI

Eating disorders, such as anorexia nervosa and bulimia nervosa, are diseases characterized by aberrant patterns of feeding behavior and weight regulation, as well as disturbances in the attitudes toward the perception of body weight and body shape. Both disturbances in the HPA axis and alterations in the central serotoninergic mechanisms were observed in patients with bulimia nervosa.

About one-third of women with bulimia nervosa failed to suppress the salivary cortisol level after dexamethasone and bulimic nonsuppressors exhibited elevated basal salivary cortisol levels.[33] Lester *et al.* described that an exacerbation of bulimic symptoms was followed by an elevated cortisol secretion.[34] On the other hand, the blunted response of cortisol and prolactin to administration of the partial serotonin agonist, meta-chlorophenylpiperazine (m-CPP), was demonstrated in patients with bulimia nervosa in comparison to healthy subjects.[35] The blunting of neuroendocrine responses was most remarkable in bulimic women who reported in their history a self-destructiveness. Serotoninergic abnormalities in bulimia nervosa might be therefore most characteristic of individuals with self-destructive potential.[35] Studies using brain positron emission tomography with serotonin-specific radioligands revealed alterations of 5-HT1A and 5-HT2A receptors and the 5-HT transporter in eating disorders.[36] Alterations of these circuits may affect mood and impulse control as well as the motivating and hedonic aspects of feeding behavior. It has been suggested that reduced serotonin activity triggers some of the cognitive and mood disturbances associated with bulimia nervosa and therefore, the pharmacological treatment of bulimia nervosa is focused mainly on SSRI. SSRIs are effective in reducing binge eating, as well as purging episodes in patients with bulimia nervosa.[37] Elevated concentrations of 5-hydroxyindoleacetic acid in the cerebrospinal fluid observed after recovery suggest that altered serotonin activity in eating disorders is a trait-related characteristic.[38] Treatment with SSRI reduces the clinical symptoms of eating disorders independently of their antidepressant effects. However, effectiveness of SSRIs in the treatment of anorexia nervosa requires an adequate supply of nutrients, which are essential to the appropriate synthesis and function of serotonin. This is the reason why the treatment with SSRI fails in malnourished and severely underweight anorectic patients, but is effective after weight restoration and improvement of nutritional status.[38]

Binge-eating disorder (BED) is characterized by recurrent episodes of binge eating in the absence of compensatory behaviors to avoid weight gain, such as vomiting or laxative abuse, usually seen in bulimia nervosa.[39,40] Related characteristics include eating until uncomfortably full, eating alone, eating when not physically hungry, as well as consequent depressive and guilty feelings. BED is associated with depression and personality disorders. Almost 30% of obese patients manifest BED.[40] Stress is the most frequently reported trigger of binge eating. Cortisol response to a cold pressor stress test positively correlated with abdominal obesity in obese women with BED.[41] Hyperactivity of the HPA axis related to abdominal obesity persisted even after cognitive-behavioral treatment, suggesting that cortisol might play an essential role in the pathogenesis of this disorder.[41] SSRI, such as fluoxetine, fluvoxamine, sertraline, and citalopram, have been used in the treatment of BED, reducing binge-eating frequency and body weight over the short term.[42]

The *night eating syndrome* is an eating disorder characterized by morning anorexia, evening hyperphagia and insomnia, as well as night awakenings

accompanied by eating of small amounts of food with rapid return to sleep.[43] Stress is closely related to night eating. An abnormal eating behavior, as observed in night eaters, is associated with an activation of the HPA axis, resulting in higher diurnal salivary cortisol levels,[44] as well as with disturbances in the HPA axis manifested by an attenuated adrenocorticotropic hormone (ACTH) and cortisol response to CRH.[45] According to Stunkard, coincidence of the night eating syndrome with psychiatric disorders, especially depression, is very high.[44,46] The role of the serotoninergic system in the pathogenesis of the night eating syndrome was confirmed by the effective treatment with SSRI. Administration of sertraline to patients with the night eating syndrome significantly reduced evening hyperphagia, awakenings, and night eating episodes.[44,46]

SIBUTRAMINE IN THE TREATMENT OF OBESITY AND BED

Sibutramine is a combined reuptake inhibitor of both serotonin and norepinephrine which has a dual mode of action on energy balance. It reduces food intake by enhancing satiety and attenuates the weight loss-induced decline in energy expenditure.[47] Mechanism of action, that is, inhibition of the reuptake of serotonin and norepinephrine in the target tissues, clearly differentiates sibutramine (as well as the antidepressant duloxetine) from amphetamine and fenfluramine, which act as releasing agents.[3,4] Sibutramine has been shown to produce dose-dependent weight loss.[48] Weight loss achieved at week 4 was predictive of weight loss achieved at week 24. Clinical trials clearly demonstrated that two-thirds of patients taking sibutramine lose $\geq$5% of initial body weight.[47] Simultaneously with weight loss, patients treated with sibutramine demonstrate significant improvement in lipid profile, blood glucose, HbA1c, and uric acid and those with hypertension at base line exhibit a significant decline in blood pressure.[49,50] Sibutramine is an efficient antiobesity drug not only for weight loss but also for the long-term maintenance of weight loss. In the STORM study, an individualized weight management program achieved weight loss in 77% of obese patients and sustained weight loss in most patients continuing therapy for 2 years.[51] A 2-year weight management with sibutramine resulted in a significant decrease in waist circumference, as well as in improvement in lipid profile. Changes in concentrations of high-density lipoprotein (HDL) cholesterol, very-low-density lipoprotein (VLDL) cholesterol, and triglyceride observed in the STORM study exceeded those expected from the weight loss alone.[51] It appears, therefore, that there is an independent effect of sibutramine on the HDL cholesterol level, beyond weight loss, that requires further elucidation. Efficacy of pharmacotherapy for weight loss in adults with type 2 diabetes was evaluated in meta-analysis conducted by Norris.[52] Weight loss in patients with diabetes was modest and achieved 4.5 kg in response to a 26-week treatment with sibutramine, 5.8 kg in response to a 52-week treatment with SSRI fluoxetine, and 2.6 kg in response to a 26-week treatment

with lipase inhibitor, orlistat. Diminished drug treatment-induced weight loss in obese patients with diabetes might be caused by genetic and metabolic factors, as well as by the concomitant drug therapy. Glycosylated hemoglobin was also only modestly reduced with each drug included in the meta-analysis. A randomized trial of sibutramine in the management of obese, type 2 diabetic patients treated with metformin demonstrated that glycemic control improves with corresponding weight loss.[53] Patients who lost $\geq$10% initial body weight showed a mean 1.2% decrease in HbA1c. Because sibutramine treatment may lead to both increased blood pressure and heart rate, its use is contraindicated in patients with uncontrolled hypertension, dysrhythmias, and congestive heart failure.[54] Recent combined analysis of two placebo-controlled trials concludes that sibutramine treatment is unlikely to elicit a critical increase in blood pressure in hypertensive patients because a sibutramine-induced central nervous system blockade of norepinephrine reuptake attenuates the sympathetic outflow through activation of α-2 adrenoreceptors ("clonidine-like effect").[55]

It is important that intermittent treatment with sibutramine was equally effective as continuous treatment during a 48-week randomized, placebo-controlled trial.[56] The proportion of adverse events was lowest in the group receiving intermittent therapy. Sibutramine was also used in the treatment of adolescent obesity and its administration was associated with a higher weight loss in comparison with a placebo or behavioral therapy alone.[57] Sari *et al.* demonstrated that an addition of orlistat to sibutramine did not lead to a greater weight loss than the treatment with sibutramine alone.[58]

Long-term weight maintenance after weight loss in the STORM trial was significantly determined by a higher leisure-time activity index.[59] We confirmed the role of sibutramine treatment in increasing the habitual physical activity: the increase in reported daily walking time during the 4-month placebo-controlled period was significantly higher in the sibutramine-treated than in the placebo group.[60] Spontaneous locomotor activity was also significantly increased in experimental animals following the administration of sibutramine.[61]

As mentioned before, SSRI have exhibited efficacy in reducing the frequency of binge-eating episodes and ameliorating depressive symptomatology in BED, but they have not demonstrated the ability to achieve long-term weight reduction.[42] On the contrary, the sibutramine treatment of BED resulted not only in a decreased frequency of binge eating and an improvement of comorbid conditions as depressive symptoms, but also in the reduction of body weight.[62,63]

OBESITY TREATMENT WITH SIBUTRAMINE AND EATING BEHAVIOR

Weight loss in response to weight management is influenced by genetic, metabolic, neurohormonal, nutritional, and psychobehavioral factors. Weight loss in response to a short-term nutritionally well-defined weight reduction

program is determined by energy efficiency, substrate oxidation, sympathetic nervous system activity, and insulin sensitivity,[64] as well as by the levels of hormones involved in energy balance regulation.[65] Psychobehavioral factors, such as depression, anxiety, and eating behavior, do play an important role in the adherence to weight management and thus in weight loss maintenance. Depression represents a negative prognostic marker for weight reduction.[66,67]

Psychobehavioral predictors of weight loss have been evaluated during the pharmacotherapy of obesity which employed the combined administration of drugs (fenfluramine + phentermine, fenfluramine + mazindol), most of which have now been withdrawn from the market.[68] The Eating Inventory (EI), also known as the Three Factor Eating Questionnaire, has usually been used for evaluation of the eating behavior.[69] The EI assess three behavioral traits: (*a*) cognitive dietary restraint—deliberate control of intake, (*b*) disinhibition—measure of the loss of control over food intake which might be triggered, for example, by stress, depression, anxiety, and alcohol intake, and (*c*) perceived hunger—awareness of and susceptibility to hunger. In the Womble's study, base line values of dietary restraint and hunger predicted weight loss at 6 and 12 months of drug treatment.[68] High base line scoring on dietary restraint and hunger was associated with lower weight loss after a 6-month treatment, whereas only high hunger scoring at base line predicted lower weight loss at 12 months. A high restraint score at base line was also associated with a lower weight loss at 1 year after gastric banding.[70] In the STORM study, which evaluated a 2-year weight loss maintenance in response to sibutramine/placebo treatment, the baseline body weight, mode of treatment, and age, explained 9% of the variation in weight change over a 2-year period of follow-up.[71] In the same study gender, resting metabolic rate, smoking history, previous attempts to lose weight, and the age of the onset of obesity did not affect the weight change at a 2-year follow-up, however, psychobehavioral and nutritional parameters were not considered. In our study, obese women were followed for 12 months and treated either with sibutramine alone for the whole period of 12 months, or with a placebo and sibutramine (a placebo was administered over an initial 4 months and sibutramine was given in a subsequent period of 8 months).[60] The role of baseline BMI, psychobehavioral, and nutritional parameters on weight loss in patients treated with 10 mg sibutramine/day was evaluated at 4-month and 12-month follow-ups. Three factors of the EI, Beck depression score, energy, and macronutrient intake were assessed before the trial started, and at 4-month and at 12-month follow-ups. Baseline values for BMI and dietary restraint, together with the mode of treatment, predicted body weight change after a 4-month treatment ($r^2 = 30.8\%$). Higher initial BMI and lower baseline dietary restraint and treatment with sibutramine predicted a better outcome during a 4-month placebo-controlled period of the trial. Baseline values for BMI, depression score, restraint score, and energy intake were significant predictors of body weight change after a 12-month treatment and explained 43.8% of the variance in the BMI change (TABLE 3). It means that

TABLE 3. BMI after 12-month treatment

Method	Independent variables	Parameter	Standard error	*t*-statistics	*P* value
Backward stepwise regression (Fisher's statistics>3 was chosen as a selection criterion)	Constant	4.8	1.44	3.33	0.001
	Depression$^{0.11}$	−1.63	0.58	−2.79	0.007
$R = 0.662$	Restraint$^{0.90}$	−0.0552	0.0206	−2.67	0.010
$P < 0.0001$	Energy$^{0.32}$	−0.201	0.082	−2.46	0.017
	Protein$^{0.21}$	1.64	0.95	1.73	0.088
	− (BMI$^{-3.86}$)	683, 722	126, 175	5.42	0.000

Backward stepwise regression of the relations between the change in BMI after a 12-month treatment with sibutramine/placebo, and the mode of treatment, baseline BMI, and the psychobehavioral and nutritional characteristics.

NOTE: According to Hainer *et al.*[60]

patients with higher depression and dietary restraint scores at base line lost less weight at the 12-month follow-up. High dietary restraint at the pretreatment period might be associated with dieting and weight control before the trial started, and therefore these patients did not lose as much weight in response to treatment, compared to those with low restraint initially who increased their dietary restraint during the treatment. The impact of the changes in psychobehavioral and nutritional parameters seen after 4 and 12 months of treatment on BMI changes was also investigated. The BMI decrease over a 4-month treatment period was significantly associated with the mode of treatment and changes in dietary restraint and disinhibition, as well as with changes in protein and fat intake. These variables accounted for 56.6% of the variance in weight change after a 4-month treatment. Sibutramine administration, increases in dietary restraint and protein intake, and reductions in dietary disinhibition and fat intake resulted in a greater weight loss. However, among the changes in the psychobehavioral and nutritional parameters over the 12-month period of the sibutramine trial, only the change in the disinhibition score remained the sole significant factor related to the BMI decrease (TABLE 4). A significant relation between the decrease of the disinhibition score and the decrease of the BMI is shown in FIGURE 1. The association between weight loss and decline in the disinhibition score in response to the sibutramine treatment might be related to the previously described beneficial effects of sibutramine treatment on obesity-related health risks.[49–51] Our recent study using a quota sample of the Czech population demonstrated that the disinhibition score was closely related, not only to the BMI and waist circumference, but also to cardiovascular disease, hypertension, and hyper(dys)-lipidemia.[72] The revealed association between the factors of EI and these diseases remained significant even when the BMI and age were considered. The relationships between dietary disinhibition and diseases were especially pronounced in middle-aged persons. Prevalence of hypertension, cardiovascular disease, and hyper(dys)-lipidemia

TABLE 4. The relations between the BMI change after a 12-month treatment with either sibutramine alone or placebo/sibutramine, and changes in the psychobehavioral and nutritional parameters

Method	Independent variables	Parameter	Standard error	*t*-statistics	*P* value
Backward stepwise regression (Fisher's statistics>3 was chosen as a selection criterion) $R = 0.541$ $P < 0.0001$	Constant	1.019	0.086	11.91	0.000
	Δ Disinhibition	0.0911	0.0211	4.31	0.000

In the backward stepwise regression, a change in the disinhibition score remained the sole significant factor associated with the BMI change.

NOTE: According to Hainer *et al.*[60]

(%) in middle-aged males and females (33- to 44-years old) was significantly higher in subjects scoring high (upper quartile) than in those scoring low (lower quartile) on the disinhibition score (FIG. 2). There is no doubt that a decrease in the disinhibition score as a correlate of sibutramine-induced weight

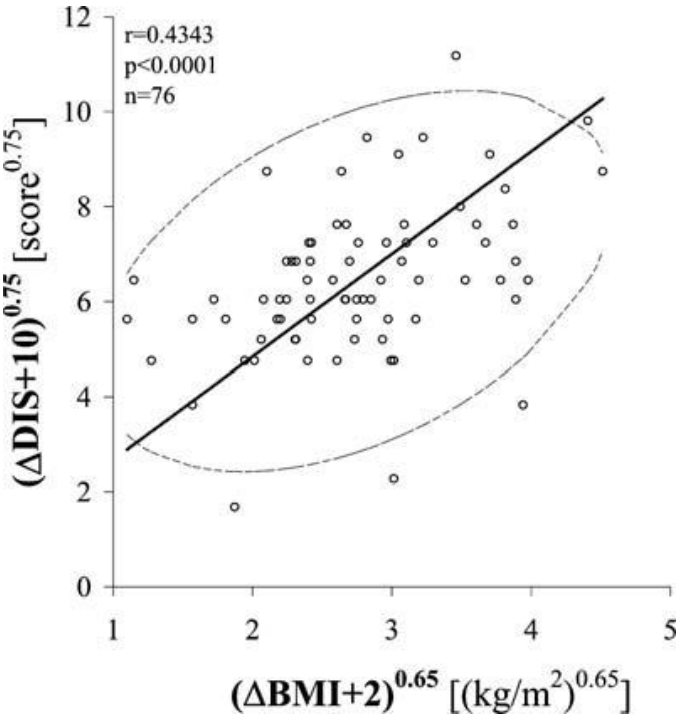

FIGURE 1. Relationship between the change of BMI and change of the disinhibition score at a 12-month follow-up in patients treated either with sibutramine alone for 12 months or with a placebo (initial 4 months) and sibutramine (subsequent 8 months) (unpublished figure according to Hainer *et al.*[60]).

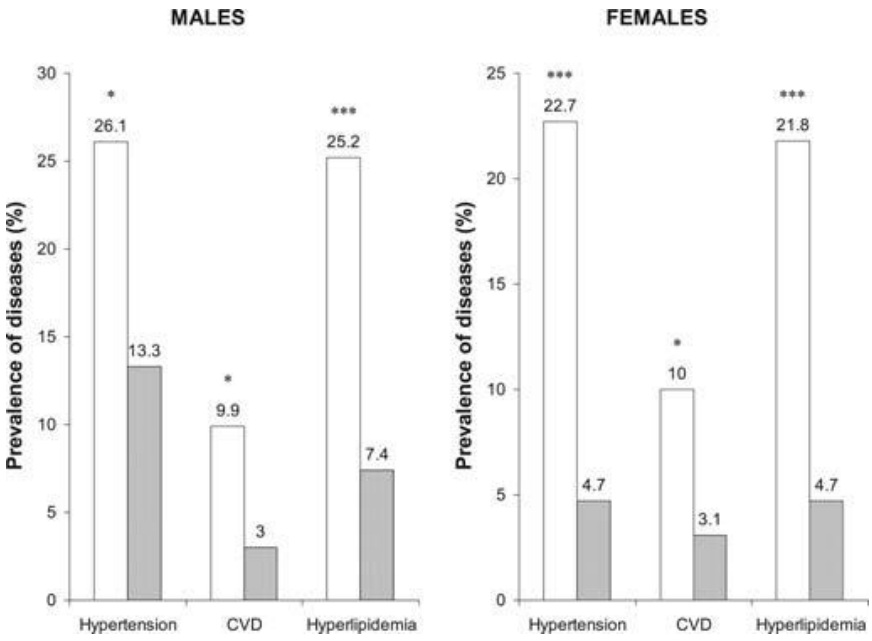

FIGURE 2. Prevalence of diseases (%) in middle-aged (33- 44-years old) males and females characterized by high and low disinhibition scores (upper versus lower quartile) (unpublished figure according to Hainer *et al.*[72]).

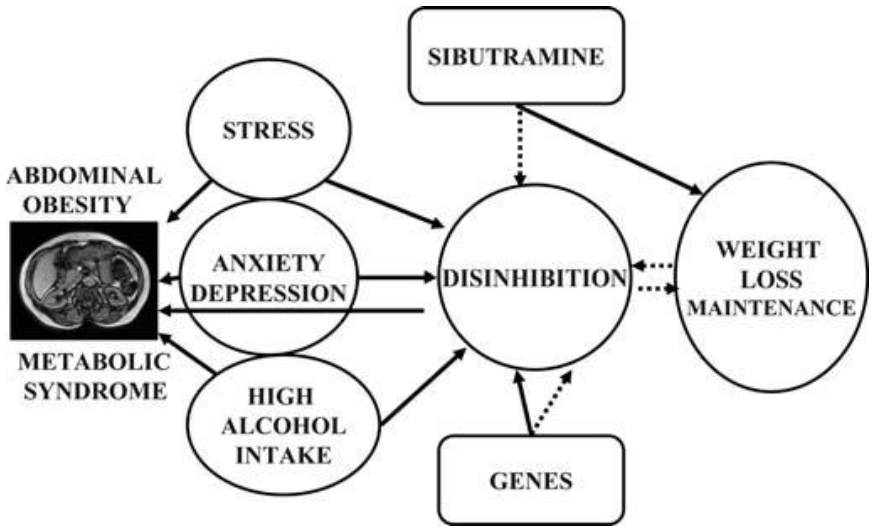

FIGURE 3. Dietary disinhibition is frequently triggered by stress, anxiety, and depression, but is also greatly influenced by genetic factors. Disinhibition is significantly related to abdominal obesity and to diseases associated with the metabolic syndrome. Demonstrated association of sibutramine-induced weight loss maintenance with changes in eating behavior characterized by a decrease in the disinhibition score might partly explain beneficial metabolic effects of sibutramine treatment.

loss does play an important role in the amelioration of health risks linked to the abdominal obesity and lifestyle-associated pathologies (FIG. 3). However, it should be mentioned that disinhibition as an eating behavior which reflects opportunistic eating is significantly genetically determined, whereas dietary restraint is most strongly influenced by environmental factors.[73–76]

In conclusion, among the drugs targeting central serotonin and norepinephrine pathways, only serotonin and norepinephrine reuptake inhibitor, sibutramine, has been approved as an efficient tool for both the long-term treatment of obesity and amelioration of obesity-related health risks. Other drugs affecting brain norepinephrine and serotonin pathways are mainly used in the treatment of depression and eating disorders. However, novel agents, possessing a high selectivity, are being investigated as potential antiobesity drugs, too.

REFERENCES

1. GUY-GRAND, B. *et al.* 1989. International trial of long-term dexfenfluramine in obesity. Lancet **8672:** 1142–1145.
2. HALFORD, J.C.G. *et al.* 2005. Serotonin (5-HT) drugs: effects on appetite expression and use for the treatment of obesity. Curr. Drug Targets **6:** 201–213.
3. HEAL, D.J. *et al.* 1998. Sibutramine: a novel anti-obesity drug: a review of the pharmacological evidence to differentiate it from d-amphetamine and d-fenfluramine. Int. J. Obes. Relat. Metab. Disord. **22:** 518–529.
4. RABASSEDA, X. 2004. Duloxetine: a new serotonin/noradrenaline reuptake inhibitor for the treatment of depression. Drugs Today **40:** 773–790.
5. STAHL, S.M. *et al.* 2005. SNRI : their pharmacology, clinical efficacy, and tolerability in comparison with other classes of antidepressants. CNS Spectr. **10:** 732–747.
6. MESSER, T. *et al.* 2005. Efficacy and tolerability of reboxetine in depressive patients treated in routine clinical practice. CNS Drugs **19:** 43–54.
7. KRELL, H.V. *et al.* 2005. Evaluation of reboxetine, a noradrenergic antidepressant, for the treatment of fibromyalgia and chronic low back pain. Psychosomatics **46:** 379–384.
8. TSE, W.S. & A.J. BOND. 2005. Sex differences in cortisol response to reboxetine. J. Psychopharmacol. **19:** 46–50.
9. LU, T.Y. *et al.* 2005. Profound weight loss associated with reboxetine use in a 44-year-old woman. Br. J. Clin. Pharmacol. **60:** 218–220.
10. BJÖRNTORP, P. 1993. Visceral obesity. A "civilization syndrome." Obes. Res. **1:** 206–222.
11. AHLBERG, A.C. *et al.* 2002. Depression and anxiety symptoms in relation to anthropometry and metabolism in men. Psychiatry Res. **112:** 101–110.
12. LEE, E.S. *et al.* 2005. Depressive mood and abdominal fat distribution in overweight premenopausal women. Obes. Res. **13:** 320–325.
13. STUNKARD, A.J. *et al.* 2003. Depression and obesity. Biol. Psychiatry **54:** 330–337.
14. ROSMOND, R. 2004. Obesity and depression: same disease, different names? Med. Hypotheses **62:** 976–979.

15. REYNOLDS, G.P. 2005. The role of 5-HT2C receptor polymorphisms in the pharmacogenetics of antipsychotic drug treatment. Prog. Neuropsychopharmacol. Biol. Psychiatry **29:** 1021–1028.
16. HUANG, X.F. 2004. Differential expression of 5-HT(2A) and 5-HT(2C) receptor mRNA in mice prone, or resistant, to chronic high-fat diet-induced obesity. Mol. Brain Res. **127:** 39–47.
17. RHODE, P. *et al.* 2005. The role of stress and depression in weight regain [abstract]. Obes. Res. **13**(Suppl): A3(11-OR).
18. DOUCET, E. *et al.* 2000. Appetite after weight loss by energy restriction and a low-fat diet-exercise follow-up. Int. J. Obes. Relat. Metab. Disord. **24:** 906–914.
19. HAINER, V. *et al.* 2001. The within-pair resemblance in serum levels of androgens, sex-hormone binding globulin and cortisol in female obese identical twins—effect of negative energy balance induced by very low-calorie diet. Horm. Metab. Res. **33:** 417–422.
20. ARIYO, A.A. *et al.* 2000. Depressive symptoms and risks of coronary heart disease and mortality in elderly Americans. Cardiovascular Health Study Collaborative Research Group. Circulation **102:** 1773–1779.
21. CLOUSE, R.F. *et al.* 2003. Depression and coronary heart disease in women with diabetes. Psychosom. Med. **65:** 376–383.
22. BERKMAN, L.F. *et al.* 2003. Effects of treating depression and low perceived social support on clinical events after myocardial infarction: the Enhancing Recovery in Coronary Heart Disease Patients (ENRICHD) randomized trial. JAMA **289:** 3106–3116.
23. LUSTMAN, P.J. 2000. Depression and poor glycemic control: a meta-analytic review of the literature. Diabetes Care **23:** 934–942.
24. WAGENKNECHT, M. *et al.* 2004. Associations of hormonal and psychobehavioral characteristics and their role in the long-term weight loss maintenance in obese patients [abstract]. Int. J. Obes. Relat. Metab. Disord. **28**(Suppl 1): S225.
25. ROSMOND, R. & P. BJÖRNTORP. 2000. The hypothalamic-pituitary-adrenal axis activity as a predictor of cardiovascular disease, type 2 diabetes and stroke. J. Intern. Med. **247:** 188–197.
26. MARIN, P. *et al.* 1992. Cortisol secretion in relation to body fat distribution in obese premenopausal women. Metabolism **41:** 882–886.
27. HAINER, V. *et al.* 2002. Serum cortisol and sex hormone binding globulin (SHBG) levels, body fat distribution and the role of genetic factors in obese females. Sb. Lek. **103:** 471–475.
28. LJUNG, T. *et al.* 1996. Inhibition of cortisol secretion by dexamethasone in relation to body fat distribution: a dose-response study. Obes. Res. **4:** 277–282.
29. LJUNG, T. *et al.* 2001. Treatment of abdominally obese men with a serotonin reuptake inhibitor: a pilot study. J. Intern. Med. **250:** 219–224.
30. ROSMOND, R. & P. BJÖRNTORP. 2000. The role of antidepressants in the treatment of abdominal obesity. Med. Hypotheses **54:** 990–994.
31. MARKS, S.J. *et al.* 1998. The metabolic effects of preferential reduction of visceral adipose tissue in abdominally obese men. Int. J. Obes. Relat. Metab. Disord. **22:** 893–898.
32. MULDOON, M.F. *et al.* 2004. Low central nervous system serotonergic responsivity is associated with the metabolic syndrome and physical inactivity. J. Clin. Endocrinol. Metab. **89:** 266–271.

33. NEUDECK, P. *et al.* 2001. Dexamethasone suppression test using saliva cortisol measurement in bulimia nervosa. Physiol. Behav. **72:** 93–98.
34. LESTER, N.A. *et al.* 2003. Symptom fluctuation in bulimia nervosa: relation to menstrual-cycle phase and cortisol levels. Psychol. Med. **33:** 51–60.
35. STEIGER, K. *et al.* 2001. Self-destructiveness and serotonin function in bulimia nervosa. Psychiatry Res. **103:** 15–26.
36. KAYE, W.H. *et al.* 2005. Serotonin alterations in anorexia and bulimia nervosa: new insights from imaging studies. Physiol. Behav. **85:** 73–81.
37. MILANO, W. *et al.* 2005. Treatment of bulimia nervosa with fluvoxamine: a randomized controlled trial. Adv. Ther. **22:** 278–283.
38. KAYE, W.H. 1998. Serotonin neuronal function and selective serotonin reuptake inhibitor treatment in anorexia and bulimia nervosa. Biol. Psychiatry **44:** 825–838.
39. DE ZWAAN, M. *et al.* 2001. Binge eating disorder and obesity. Int. J. Obes. Relat. Metab. Disord. **25**(Suppl 1): S51–S55.
40. SPITZER, R.L. 1993. Binge eating disorder: its further validation in a multisite study. Int. J. Eat. Disord. **13:** 137–153.
41. GLUCK, M.E. *et al.* 2004. Cortisol stress response is positively correlated with central obesity in obese women with binge eating disorder (BED) before and after cognitive-behavioral treatment. Ann. N. Y. Acad. Sci. **1032:** 202–207.
42. APPOLINARIO, J.C. & S.L. MCELROY. 2004. Pharmacological approaches in the treatment of binge eating disorder. Curr. Drug Targets **5:** 301–307.
43. STUNKARD, A.J. 1955. The night-eating syndrome: a pattern of food intake among certain obese patients. Am. J. Med. **19:** 78–86.
44. STUNKARD, A.J. 2004. The night eating syndrome: new developments [abstract]. Int. J. Obes. Relat. Metab. Disord. **28**(Suppl 1): S48.
45. BIRKETVEDT, G.S. *et al.* 2002. Hypothalamic-pituitary-adrenal axis in the night eating syndrome. Am. J. Physiol. Endocrinol. Metab. **282:** E366–E369.
46. STUNKARD, A.J. *et al.* 2005. The night eating syndrome: a progress report. Appetite **45:** 182–186.
47. FINER, N. 2002. Sibutramine: its mode of action and efficacy. Int. J. Obes. Relat. Metab. Disord. **26**(Suppl 4): S29–S33.
48. BRAY, G.A. *et al.* 1999. Sibutramine produces dose-related weight loss. Obes. Res. **7:** 189–198.
49. SCHOLZE, J. 2002. Treating obesity with sibutramine under practical conditions. Positive effects on the metabolic parameters and blood pressure. Dtsch. Med. Wochenschr. **127:** 606–610.
50. HAUNER, H. *et al.* 2004. Weight reduction by sibutramine in obese subjects in primary care medicine: the S.A.T. Study. Exp. Clin. Endocrinol. Diabetes **112:** 201–207.
51. JAMES, W.P. *et al.* 2000. Effect of sibutramine on weight maintenance after weight loss: a randomized trial. STORM Study Group. Sibutramine trial of obesity reduction and maintenance. Lancet **356:** 2119–2125.
52. NORRIS, S.L. 2004. Efficacy of pharmacotherapy for weight loss in adults with type 2 diabetes mellitus: a meta-analysis. Arch. Intern. Med. **164:** 1395–1404.
53. MCNULTY, S.J. *et al.* 2003. A randomized trial of sibutramine in the management of obese type 2 diabetic patients treated with metformin. Diabetes Care **26:** 125–131.
54. KIM, S.H. *et al.* 2003. Effect of sibutramine on weight loss and blood pressure: a meta-analysis of controlled trials. Obes. Res. **11:** 1116–1123.

55. JORDAN, J. *et al.* 2005. Influence of sibutramine on blood pressure: evidence from placebo-controlled trials. Int. J. Obes. Relat. Metab. Disord. **26:** 509–516.
56. WIRTH, A. & J. KRAUSE. 2001. Long-term weight loss with sibutramine: a randomized controlled trial. JAMA **286:** 1331–1339.
57. BERKOWITZ, R.I. *et al.* 2003. Behavior therapy and sibutramine for the treatment of adolescent obesity: a randomized controlled trial. JAMA **289:** 1805–1812.
58. SARI, R. *et al.* 2004. Comparison of efficacy of sibutramine or orlistat versus their combination in obese women. Endocr. Res. **30:** 159–167.
59. VAN BAAK, M.A. 2003. Leisure-time activity is an important determinant of long-term weight maintenance after weight loss in the Sibutramine Trial on Obesity Reduction and Maintenance (STORM trial). Am. J. Clin. Nutr. **78:** 209–214.
60. HAINER, V. *et al.* 2005. Psychobehavioral and nutritional predictors of weight loss in obese women treated with sibutramine. Int. J. Obes. Relat. Metab. Disord. **29:** 208–216.
61. KIM, E.J. *et al.* 2005. Safety pharmacology of sibutramine mesylate, an anti-obesity drug. Hum. Exp. Toxicol. **24:** 109–119.
62. APPOLINARIO, J.C. *et al.* 2003. A randomized, double-blind, placebo-controlled study of sibutramine in the treatment of binge-eating disorder. Arch. Gen. Psychiatry **60:** 1109–1116.
63. MILANO, W. *et al.* 2005. Use of a sibutramine, an inhibitor of the reuptake of serotonin and noradrenaline, in the treatment of binge eating disorder: a placebo-controlled study. Adv. Ther. **22:** 25–31.
64. FLATT, J.-P. & A. TREMBLAY. 1998. A substrate balances and weight maintenance. *In* Handbook of Obesity. G.A. Bray, C. Bouchard & W.P.T. James, Eds.: 528–532. Marcel Dekker. New York, NY.
65. HAINER, V. *et al.* 2005. Psychobehavioral and hormonal predictors of weight loss in response to a 3-week weight management program: effect of calcium intake [abstract]. Obes. Res. **13**(Suppl): A142.
66. CLARK, M.M. *et al.* 1996. Depression, smoking, activity level, and health status: pretreatment predictors of attrition in obesity treatment. Addict. Behav. **21:** 509–513.
67. DELAHANTY, L.M. *et al.* 2002. Psychological and behavioral correlates of baseline BMI in the Diabetes Prevention Program (DPP). Diabetes Care **25:** 1992–1998.
68. WOMBLE, L.G. *et al.* 2001. Psychological and behavioral predictors of weight loss during drug treatment for obesity. Int. J. Obes. Relat. Metab. Disord. **25:** 340–345.
69. STUNKARD, A.J. & S.M. MESSICK. 1985. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. J. Psychosom. Res. **29:** 71–83.
70. ADAMI, G.F. *et al.* 1996. Preoperative eating behavior and weight-loss following gastric banding for obesity. Obes. Surg. **6:** 244–246.
71. HANSEN, D.L. *et al.* 2001. Predictors of weight loss and maintenance during 2 years of treatment by sibutramine in obesity. Results from the European multi-centre STORM trial. Int. J. Obes. Relat. Metab. Disord. **25:** 496–501.
72. HAINER, V. *et al.* 2006. The Eating Inventory, body adiposity, and prevalence of diseases in Czech adults. Int. J. Obes. Relat. Metab. Disord. **30:** 830–836.
73. STEINLE, N.I. *et al.* 2002. Eating behavior in the Old Order Amish: analysis and a genome-wide linkage analysis. Am. J. Clin. Nutr. **75:** 1098–1106.

74. BOUCHARD, L. *et al*. 2004. Neuromedin β: a strong candidate gene linking eating behaviors and susceptibility to obesity. Am. J. Clin. Nutr. **80:** 1478–1486.
75. NAELE, B.M. *et al*. 2003. A twin study of dietary restraint, disinhibition and hunger: an examination of the eating inventory (Three Factor Eating Questionnaire). Twin Res. **6:** 471–478.
76. BOUTIN, P. *et al*. 2003. GAD2 on chromosome 10p12 is a candidate gene for human obesity. PloS Biol. **1:** 361–371.

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Hormonal and Psychobehavioral Predictors of Weight Loss in Response to a Short-Term Weight Reduction Program in Obese Women

V. HAINER [1], K. HLAVATÁ [1], M. GOJOVÁ [2], M. KUNEŠOVÁ [1], M. WAGENKNECHT [1], V. KOPSKÝ [1], J. PAŘÍZKOVÁ [1], M. HILL [1], J. NEDVÍDKOVÁ [1]

[1]Institute of Endocrinology, Prague, Czech Republic, [2]Obesity Management Unit, Lipová – lázně, Czech Republic

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Summary

Among the factors influencing weight loss and maintenance, psychobehavioral, nutritional, metabolic, hormonal and hereditary predictors play an important role. Psychobehavioral factors influence adherence to lifestyle changes and thus weight loss maintenance. The outcome of short-term weight reduction treatment is mainly affected by changes in energy and nutrient intake and physical activity and thus the impact of hormones can possibly be obscured. In order to reveal hormonal determinants of weight loss, a 4-week in-patient comprehensive weight reduction program was introduced in which food intake and physical activity were under the strict control. Women (n = 67, BMI: 32.4 ± 4.4 kg; age: 48.7 ± 12.2 years) who exhibited stable weight on a 7 MJ/day diet during the first week of weight management were given a hypocaloric diet yielding daily energy deficit 2.5 MJ over the subsequent 3-week period. This treatment resulted in a mean weight loss of 3.80 ± 1.64 kg. Correlation analysis revealed that baseline concentrations of several hormones were significantly associated either with a higher (free triiodothyronine, C-peptide, growth hormone, pancreatic polypeptide) or with a lower (insulin-like growth factor-I, cortisol, adiponectin, neuropeptide Y) reduction of anthropometric parameters in response to weight management. In a backward stepwise regression model age, initial BMI together with baseline levels of growth hormone, peptide YY, neuropetide Y and C-reactive protein predicted 49.8 % of the variability in weight loss. Psychobehavioral factors (items of the Eating Inventory, Beck Depression score) did not contribute to weight change induced by a well-controlled short-term weight reduction program.

Key words

Obesity • Weight loss predictors • Hormones • Eating Inventory • Beck Depression Inventory - Anthropometric indexes

Corresponding author

Vojtěch Hainer, Institute of Endocrinology, Národní třída 8, 116 94 Prague 1, Czech Republic. E-mail: vhainer@endo.cz

Introduction

Among the factors influencing weight loss and weight loss maintenance, hereditary, psychobehavioral, nutritional, metabolic and hormonal predictors play an important role. Twin and family studies revealed that weight loss is strongly controlled by genotype (Hainer *et al.* 2000a). Polymorphisms in obesity candidate genes affect the outcome of weight management (Moreno-Aliaga *et al.* 2005, Hainer *et al.* 2008). Energy and nutrient intake, as well as the level of physical activity, represent essential targets in weight management programs. It has been demonstrated that not only macronutrient intake but also intake of dietary calcium (Kabrnová-Hlavatá *et al.* 2007) and n-3 polyunsaturated fatty acids (Kunešová *et al.* 2006) might influence the outcome of weight management programs. Among the psychobehavioral factors level of depression, dietary disinhibition and their decrease in response to weight management significantly affect weight loss maintenance (Vogels *et al.* 2005). On the other hand, an increase in restraint score in response to weight management favorably influence weight loss maintenance. Metabolic predictors of weight loss and its maintenance include energy expenditure and substrate oxidation. Decreased energy expenditure and/or decreased ability to oxidize fat result in failure to maintain weight loss and in weight cycling (Ravussin 1995, Vogels *et al.* 2005, Hainer *et al.* 2000b). Special attention has been paid to hormonal predictors of weight loss and weight loss maintenance. A high baseline leptin/BMI ratio (Naslund *et al.* 2000),

inadequately high decreases in leptin levels in response to weight management (Geldszus *et al.* 1996, Filozof *et al.* 2000, Celi *et al.* 2003) and lower ghrelin levels at baseline might predict failure in weight management. Lower baseline peptide YY levels and their weight management-induced increases have been shown to be associated with the highest levels of weight loss (Roth *et al.* 2005, le Roux *et al.* 2006). Substantial weight loss was shown to be associated with significantly lower obestatin and a tendency to higher ghrelin concentrations at baseline (Reinehr *et al.* 2007).

The aim of the current study was to evaluate the role of psychobehavioral and hormonal factors as predictors of weight loss in response to a 3-week weight management in-patient program conducted in a group of overweight and obese women.

Methods

Sixty seven women (Body Mass Index /BMI/: 32.4±4.4 kg; age: 48.7±12.2 y) who exhibited stable weight on a 7 MJ/day diet during the 1st week of weight management obtained a hypocaloric diet providing 4.5 MJ/day (protein 26.0 %, fat 28.0 %, carbohydrate 46 %) over the subsequent 3-week period. Such a diet yielded an average daily energy deficit of 2.5 MJ when compared with the pretreatment week. The comprehensive weight management included a well-defined, low calorie diet, which was supervised by a dietitian, daily physical activity supervised by a physiatrist and cognitive behavioral modification of the lifestyle. Energy and nutrient content of the meals prepared in the spa kitchen during the entire period of study were calculated using the PC program „Nutrition“ which covers about 3000 food items and evaluates the intakes of energy, macronutrients and micronutrients. All subjects were advised to eat each entire meal served in four daily portions in the spa eating room. Subjects with endocrine disorders or type 2 diabetes were excluded from the study. The participants did not take any medication susceptible to affect body weight.

The following psychobehavioral and hormonal parameters were examined before and after 3-week weight management: psychobehavioral parameters: Beck Depression Inventory (BDI – Beck *et al.* 1961), Eating Inventory (EI - Stunkard and Messick 1985); hormonal parameters: thyrotropin (TSH), free triiodothyronine (fT3), free thyroxine (fT4), insulin, C-peptide, prolactin (PRL), growth hormone (GH), insulin-like growth factor I (IGF-I), cortisol, sex hormone binding globulin (SHBG), parathormone (PTH), ghrelin, leptin, peptide YY (PYY), neuropetide Y (NPY), pancreatic polypeptide (PP), adiponectin, resistin and inflammatory parameter: C-reactive protein (CRP). Fasting levels of hormones were determined by radioimmunoassay, ELISA or electrochemiluminiscence using commercial kits. Total plasma ghrelin, leptin, adiponectin, PYY, PP and NPY were determined using RIA kits and resistin by ELISA of Linco Research, Inc. (St. Charles, Missouri, U.S.A.), prolactin, SHGB, GH and IGF-1 by RIA kits of Immunotech, Inc. (Prague, Czech Republic) and plasma insulin, C-peptide, fT3, fT4, PRL and CRP levels were measured on Modular Analytics E170 (Roche Diagnostics, GmbH, Mannheim). All assays were run twice in duplicate. After blood withdrawal, anthropometric measurements (body weight, height, waist and hip circumference) were carried out according to WHO recommendations (WHO Expert Committee, 1995). BMI and waist to hip ratio were calculated. Body composition was assessed by bioimpedance (BIA Tanita BC-418MA).

The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Institute of Endocrinology in Prague. Before the study, each participant signed an informed consent form.

Statistical analysis

Data were presented as means ± SD. We used Wilcoxon's robust paired test to compare the data before and after weight management. Pearson's correlations of weight management-induced changes in anthropometric indexes (Δ WEIGHT (kg), Δ WEIGHT (%)Δ BMI (kg/m^2), Δ WAIST (cm)) with age and baseline values of selected anthropometric, psychobehavioral and hormonal parameters were calculated. Subsequently, backward stepwise multiple regression analysis was performed to obtain the combined independent predictors of weight loss. Baseline values of anthropometric, psycho-behavioral and hormonal parameters were used as independent variables. In regression analysis, Fisher's statistic > 4 was used as inclusion criterium for individual parameters. Differences were considered significant at $p<0.05$.

Results

The changes in anthropometric, psycho-

Table 1. Anthropometric, psychobehavioral and hormonal characteristics before and after weight loss

Variable	Before		After		Difference		
	mean	SD	mean	SD	mean	SD	$p <$
Weight (kg)	84.6	12.9	80.8	12.6	-3.8	1.6	0.000001
BMI (kg/m^2)	32.40	4.51	30.90	4.36	-1.50	0.63	0.000001
Waist (cm)	98.8	12.0	93.6	11.5	-5.2	2.3	0.000001
Hip (cm)	115	9.3	112	9.1	-3.4	1.6	0.000001
WHR	0.86	0.07	0.83	0.07	-0.03	0.02	0.000001
Fat (kg)	35.8	9.6	32.0	8.9	-3.8	2.9	0.000001
Fat (%)	41.7	5.7	39.0	6.2	-2.7	2.9	0.000001
FFM (kg)	48.8	4.7	48.5	5.4	-0.3	3.2	0.035526
TBW (%)	35.72	3.4	35.71	4.0	-0.01	1.9	NS
BDI - depression	10.4	6.4	7.6	6.5	-2.8	4.2	0.000003
EI - restraint	10.0	4.6	12.9	4.6	2.9	4.3	0.000004
EI – hunger	4.1	3.3	2.8	2.8	-1.2	2.7	0.000641
EI - disinhibition	6.6	3.0	4.9	2.7	-1.7	2.6	0.000008
CRP (mg/l)	5.0	4.3	4.4	4.8	-0.6	6.3	0.094821
TSH (mIU/l)	4.0	12.2	4.3	12.2	0.3	3.2	NS
fT_4 (pmol/l)	15.9	4.9	16.3	4.9	-0.4	3.1	NS
fT_3 (pmol/l)	4.96	1.93	5.04	1.67	0.08	0.84	NS
C peptide (nmol/l)	0.92	0.35	0.93	0.31	0.01	0.04	NS
Glucose (mmol/l)	5.1	1.6	4.8	1.3	-0.3	1.7	0.045540
Insulin (mIU/l)	8.37	4.49	7.83	4.85	-0.50	6.04	0.048028
IGF-I (μg/l)	221.0	96.4	231.0	100.0	9.9	74.2	0.080448
Prolactin (μg/l)	20.0	16.4	25.3	23.6	5.3	21.1	0.009322
GH (mIU/l)	3.16	6.43	3.45	4.91	0.29	7.01	NS
Cortisol (nmol/l)	924	531	888	273	-41	462	NS
SHBG (nmol/l)	61.5	44.6	79.1	54.1	14.9	26.9	0.000001
PTH (ng/l)	40.7	18.0	43.4	17.3	2.7	25.9	NS
Adiponectin (mg/l)	11.4	5.4	11.3	4.9	-0.1	2.6	NS
Ghrelin (ng/l)	1159	412	1169	423	10	213	NS
Leptin (μg/l)	21.2	9.1	15.4	7.2	-5.9	6.5	0.000001
PYY (ng/l)	196	85	213	98	17	61	NS
NPY (nmol/l)	102.0	52.7	84.0	41.4	-18.0	31.7	0.000020
PP (ng/l)	47.1	38.4	44.3	40.0	-2.8	22.4	NS
Resistin (μg/l)	2.4	0.8	2.2	0.8	-0.2	0.8	NS

BMI = body mass index, WHR = waist/hip ratio, FFM = fat free mass, TBW = total body water, BDI = Beck Depression Inventory, EI = Eating Inventory, CRP = C reactive protein, TSH = thyrotropin, fT4 = free thyroxine, fT3 = free triiodothyronine, IGF-I = insul in growth factor I, GH = growth hormone, SHBG = sex hormone binding globulin, PTH = parathormone, PYY = peptide YY, NPY = neuropeptide Y, PP = pancreatic polypeptide

behavioral and hormonal indexes are summarized in Table 1. Weight management resulted in significant decreases in all followed anthropometric and body composition parameters. Mean body weight decrease in response to 3-week weight managment was 3.80 ± 1.64 kg. Weight loss was accompanied by a significant increase in restraint score whereas Beck depression score, hunger score and disinhibition score exhibited a significant decrease. Among the hormones, insulin, leptin and NPY levels declined, while a significant rise was demonstrated in SHBG and PTH levels. No significant changes in the levels of the other determined hormones

Table 2. Pearson's correlations of weight management-induced changes in anthropometric indexes (Δ OWEIGHT (kg)Δ OWEIGHT (%), Δ BMI (kg/m^2), Δ O WAIST (cm)) with age and baseline values of selected anthropometric and hormonal parameters. Significant correlations are in bold.

	Δ WEIGHT (kg)	Δ WEIGHT (%)	Δ BMI (kg/m^2)	Δ WAIST (cm)
AGE (years)				
r	**-0.391**	**-0.309**	**-0.418**	-0.138
p	**0.001**	**0.011**	**0.000**	0.269
WEIGHT (kg)				
r	**-0.255**	0.079	-0.192	-0.151
p	**0.039**	0.529	0.122	0.225
BMI (kg/m^2)				
r	**-0.331**	-0.046	**-0.340**	-0.187
p	**0.007**	0.716	**0.005**	0.132
WAIST (cm)				
r	**-0.268**	-0.005	**-0.253**	-0.138
p	**0.030**	0.969	**0.041**	0.268
fT_3 (pmol/l)				
r	**-0.308**	**-0.276**	**-0.304**	**-0.325**
p	**0.012**	**0.025**	**0.013**	**0.008**
C-PEPTIDE (nmol/l)				
r	**-0.325**	**-0.277**	**-0.338**	-0.179
p	**0.008**	**0.025**	**0.006**	0.150
IGF-I (μg/l)				
r	**0.313**	**0.244**	**0.344**	0.131
p	**0.011**	**0.050**	**0.005**	0.300
GH (mIU/l)				
r	**-0.323**	**-0.350**	**-0.364**	-0.089
p	**0.009**	**0.004**	**0.003**	0.479
CORTISOL (nmol/l)				
r	**0.311**	**0.220**	**0.316**	**0.400**
p	**0.012**	**0.079**	**0.010**	**0.001**
ADIPONECTIN (mg/l)				
r	0.042	0.006	0.069	**0.308**
p	0.738	0.962	0.582	**0.012**
LEPTIN (μg/l)				
r	-0.229	-0.067	-0241	-0.224
p	0.064	0.593	0.051	0.070
NPY (nmol/l)				
r	0.212	**0.256**	0.225	0.192
p	0.087	**0.038**	0.070	0.122
PP (ng/l)				
r	**-0.258**	-0.207	**-0.255**	**-0.254**
p	**0.036**	0.096	**0.039**	**0.040**
CRP (mg/l)				
r	-0.242	-0.235	**-0.262**	-0.196
p	0.051	0.058	**0.033**	0.115

BMI = body mass index, fT3 = free triiodothyronine, IGF I = insulin-like growth factor I, GH = growth hormone, NPY = neuropeptide Y, PP = pancreatic polypeptide, CRP = C reactive protein

Table 3. Prediction of weight loss by age, BMI and baseline hormone levels as evaluated using the backward stepwise multiple regression (the final model)

Dependent variable: 100 x (Weight$_2$ - Weight$_1$) / Weight$_1$				
Independent variables	**Parameter**		**T-statistic**	**P-Value**
	Estimate	**Standard Error**		
Constant	6.80	3.92	1.74	0.0881
$Age^{1.75}$	-0.00086	0.00039	-2.16	0.0347
$-(BMI^{-0.75})$	-48.56	23.07	-2.11	0.0398
$-(GH_1^{-0.15})$	-2.75	0.875	-3.15	0.0026
$-(PYY_1^{-0.35})$	36.88	9.47	3.89	0.0003
$-(NPY_1^{-0.14})$	17.32	4.70	3.69	0.0005
$Log\ (CRP_1)$	-0.556	0.197	-2.82	0.0066

R^2=49.8 %, Fisher's statistic=9.26, p<0.0001, *Fisher's statistic > 4 was used as inclusion criterium for individual parameters, BMI = body mass index, GH = growth hormone, PYY = peptide YY, NPY = neuropeptide Y, CRP = C reactive protein

were demonstrated.

Correlations between the changes in anthropometric variables (Δ WEIGHT (kg), Δ WEIGHT (%), Δ BMI (kg/m^2), Δ WAIST (cm)) and baseline values of those anthropometric, psychobehavioral and hormonal parameters which achieved statistical significance are shown in Table 2. Change in body weight (expressed both as kg and percentage) and BMI correlated negatively with age and with the fasting baseline levels of fT3, C-peptide and GH, and also correlated positively with the fasting baseline levels of IGF-I and cortisol. Baseline BMI and baseline waist circumference exhibited significant negative correlations with change in body weight (kg) and BMI, whereas baseline body weight correlated with weight loss only if expressed in absolute values (kg). The change in waist circumference was negatively related to baseline levels of fT3 and PP, whereas positive correlations were revealed between the change in waist circumference and baseline levels of cortisol and adiponectin. Baseline PP levels negatively correlated with changes in both body weight and BMI whereas baseline NPY levels positively correlated only with percent change in body weight.

Baseline CRP was inversely related to changes in BMI. However, its association with body weight change achieved only borderline significance. The relations of baseline leptin levels to changes in the anthropometric indexes were not significant although borderline significance was reached for changes in body weight (kg), BMI and waist circumference. Baseline values of the Eating Inventory factors and Beck depression score did not correlate with the changes in the anthropometric measures.

Baseline values of parameters which appeared as significant predictors of weight loss in backward stepwise multiple regression are shown in Table 3 and Figure 1. Baseline levels of GH, PYY, NPY and CRP together with age and initial BMI predicted 49.8 % of variability in weight loss after the 3-week weight management.

Discussion

The magnitude of weight loss in response to a negative energy balance is determined by hereditary, psychobehavioral, nutritional, metabolic and hormonal factors. It has been shown in previous studies that long-term weight loss and weight loss maintenance is mainly influenced by psychobehavioral determinants, which affect lifestyle changes in eating behavior and physical activity. It is apparent that such a change in energy and nutrient intake and physical activity plays a crucial role in energy balance during weight management and thus the potential impact of hormonal determinants is obscured. In order to reveal potential hormonal determinants of weight loss in our study, a short-term weight reduction program was introduced in which both prescribed food intake and physical activity was under strict control during in-patient spa treatment. The prescribed diet yielded an average daily energy deficit of 2.5 MJ in comparison with the pretreatment week. Negative energy balance over the 3-week period resulted in a significant reduction of body weight (-3.8 ± 1.6 kg) and waist circumference (-5.2 ± 2.3 cm) accompanied by a significant improvement in all determined anthropometric and body composition

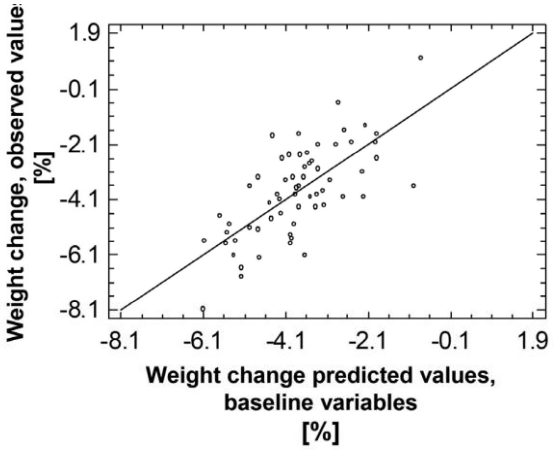

Fig. 1. Prediction of weight loss from age, BMI and baseline hormonal levels

indexes. Weight loss was mainly due to the loss of adipose tissue and cannot be attributed to the changes in body hydration as no changes in the content of total body water (TBW) were observed in response to the treatment. In agreement with previous studies the weight management program induced favorable changes in psychobehavioral characteristics (Wadden *et al.* 1987, Westerterp-Plantenga *et al.* 1998, Lejeune *et al.* 2003, Westenhoefer *et al.* 2004, Hainer *et al.* 2005, Vogels *et al.* 2005). A significant decrease in the Beck depression score, hunger and disinhibition scores along with a parallel significant increase in restraint score in response to short term weight management might contribute to a better outcome during the follow-up period.

In agreement with previous findings (Mingrone *et al.* 2002), significant decrease in serum insulin and a significant increase in serum SHBG concentrations were demonstrated as a consequence of weight loss. Low SHBG levels have been shown as a marker of insulin resistance and a strong independent risk factor for the development of type 2 diabetes in women (Lindstedt *et al.* 1991). Thus the weight management-induced increase in SHBG levels reflects improved insulin sensitivity, which resulted in a significant decline in the fasting blood glucose level. The hypothesis that increases in insulin sensitivity with weight loss are associated with subsequent weight regain were not confirmed in the study of Wing (1997).

We failed to show any significant changes in concentrations of plasma adiponectin, which has been noted as an important antiatherogenic, antidiabetic and an anti-inflammatory protein (Matsuzawa 2006). Discordant results have been published concerning the response of adiponectin to weight loss. After the weight loss either an increase (Behre *et al.* 2007) or no change (Xydakis *et al.* 2004) in adiponectin concentrations was demonstrated. These discrepancies might be due to the character of the treatment (duration of the treatment, magnitude of weight loss etc.). The observed significant decrease in leptin levels might be related to a reduction of body weight and fat stores (Pilcová *et al.* 2003) as well as to the weight management-induced negative energy balance which is associated with a decline in fasting insulin levels (Doucet *et al.* 2000). A significant decrease in the fasting level of NPY was demonstrated in response to weight loss. Previous studies failed to demonstrate any changes in plasma NPY levels in obese women after weight loss (Zahorska-Markiewicz *et al.* 2001, Nam *et al.* 2001, Moro *et al.* 1998) whereas a decrease in serum levels of NPY was observed in young obese men during the first phases of weight loss (Moro *et al.* 1998). Cerebrospinal fluid NPY levels were shown to decrease significantly in response to weight loss (Nam *et al.* 2001). This finding is in agreement with the experimental studies in obese rats who exhibited down regulation of hypothalamic NPY after weight loss induced by Roux-en-Y gastric bypass (Romanova *et al.* 2004). One could speculate that the decrease of orexigenic hormone NPY observed in our study might contribute to the simultaneous decline in hunger score after weight loss. However, no significant correlations of changes in the NPY level and hunger score were revealed.

Spontaneous prolactin release is considerably enhanced in obese women in proportion to the size of their visceral fat mass (Kok *et al.* 2004). We demonstrated a significant increase in prolactin level after weight loss. There have been contradictory results concerning the effect of weight loss on prolactin secretion. According to Kok *et al.* (2006) increased circadian prolactin secretion is blunted after weight loss in obese premenopausal women. On the other hand, short-term weight loss through a combination of dieting or dieting and exercise lead to higher plasma prolactin concentrations in lactating women (McCrory *et al.* 1999).

Hormones of the central nervous system, adipose tissue and gastrointestinal tract involved in the regulation of energy balance were investigated as predictors of weight loss. As shown in Table 2 the baseline values of several hormones influencing energy balance regulation were associated with a change in body weight (fT3, C-peptide, IGF1, GH, cortisol, PP and NPY), BMI (fT3,

C-peptide, IGF1, GH, cortisol and PP) and waist circumference (fT3, cortisol, adiponectin and PP). Negative correlations with changes in anthropometric parameters were demonstrated for baseline values of fT3, C-peptide, GH and PP, whereas positive correlations were shown for those of IGF1, cortisol, adiponectin and NPY. In our study the baseline leptin levels exhibited borderline, but in contrast to other studies (Naslund *et al.* 2000, Verdich *et al.* 2001, Savoye *et al.* 2002, Sartorio *et al.* 2003), negative association with BMI change. Lower weight loss was reported in obese patients with a high leptin/BMI ratio (Naslund *et al.* 2000) and with high leptin levels adjusted for fat mass (Verdich *et al.* 2001, Sartorio *et al.* 2003). However, we failed to find a significant association between baseline leptin levels and change in anthropometric measures even after adjustment of leptin levels for BMI and fat mass. Di Stefano *et al.* (2000) described in children and adolescents a significant correlation between baseline leptin level and subsequent reduction in the BMI Z-score in response to a weight reduction program lasting 2 years. Discrepancies concerning the baseline leptin levels as predictors of weight loss might be due to the different protocols used in the studies. Our study evaluated a short-term weight loss, whereas that of Verdich *et al.* (2001) employed 24-week weight management regimen and others assessed weight changes over 2-year (Naslund *et al.* 2000) or 2.5-year (Savoye *et al.* 2002) follow-up. However, an inappropriately large decrease in leptin levels in response to weight management might predispose to subsequent weight regain (Geldszus *et al.* 1996, Filozof *et al.* 2000, Celi *et al.* 2003) and weight cycling (Benini *et al.* 2001). In a long-term weight reducing regimen a fall in leptin levels reflects a reduction in fat stores (Reinehr *et al.* 2005). Excessive limitation of energy intake leads to an exaggerated reduction of leptin levels which markedly exceeds that which corresponds to the reduction of fat stores (Miyawaki 2002). In this case leptin levels reflect energy deficit and not a reduction in fat stores. It is generally agreed that leptinemia is an important and sensitive indicator of energy balance, not just a pure marker of fat stores.

Baseline levels of fT3 were inversely related to changes in body weight (expressed as kg and %), BMI and waist circumference. This means that higher levels of fT3 predicted greater reduction in the anthropometric indexes in response to weight management. No association between baseline concentrations of fT4 and changes in the anthropometric indexes was revealed. Our study supports the results obtained in euthyroid healthy Pima Indians after an average follow-up of 4 years which demonstrated that lower fT3, but not fT4 concentrations were independent predictors of sleeping metabolic rate, lipid oxidation and weight gain (Ortega *et al.* 2007). It is not surprising that fT3 as a predictor of sleeping metabolic rate and lipid oxidation predicted changes in body weight. We did not succeed in confirming the findings of Kozlowska and Rosolowska-Huszcz (2004), which reported that the ratio of both total and free thyroxine to TSH predicts weight loss.

Baseline levels of both GH and pancreatic polypeptide (PP) were inversely related to weight change. Secretions of both GH and PP are suppressed in obesity and reversed by weight loss (Scacchi *et al.* 1999, Reinehr *et al.* 2006). It is not surprising that the baseline GH level was associated with weight loss as GH increases lipid mobilization and energy expenditure. Low-dose recombinant human GH was successfully used as adjuvant therapy to lifestyle modifications in the management of obesity (Albert and Mooradian 2004). Although PP has been reported to reduce food intake in humans (Batterham *et al.* 2003, Jesudason *et al.* 2007), its clinical relevance in human obesity remains to be explained. On the other hand, baseline concentrations of orexogenic NPY were positively related to percent weight change (Table 2) and negatively to baseline levels of thermogenic fT3 ($r = -0.479$, $p = 0.000$) and anorexogenic PYY ($r = -0.307$, $p = 0.014$). PYY is a hormone secreted postprandially in the distal intestine and binds to Y2 receptors of the NPY neurons in the arcuate nucleus of the hypothalamus, which leads to inhibition of food intake. We failed to find a significant correlation of the baseline PYY level with changes in anthropometric parameters. However, PYY significantly contributed to the prediction of weight loss in the backward stepwise regression model. Our finding is in agreement with that of Roth *et al.* (2005) who demonstrated that a low baseline PYY as well as its increase in response to one-year weight management is related to successful weight loss in obese children. Postprandial increase in PPY level was shown to be associated with weight loss after bariatric surgery (le Roux *et al.* 2006).

Baseline insulin levels positively predicted weight loss in a six month weight loss trial carried out in overweight and obese women (Santosa *et al.* 2007). In our short-term study no significant associations of baseline insulin and C-peptide level with the changes in

anthropometric parameters were demonstrated. Similarly, we failed to show a relationship between the baseline ghrelin concentrations and weight loss although children with substantial weight loss have recently been reported to have a tendency to higher ghrelin concentrations at baseline (Reinehr *et al.* 2007).

Baseline cortisol levels were positively related to changes in anthropometric parameters. These baseline cortisol concentrations were inversely related to initial values of BMI ($r = -0.305$, $p = 0.013$), waist circumference ($r = -0.316$, $p = 0.010$) and to concentrations of fT3 ($r = -0.463$, $p = 0.000$). This means that individuals with lower BMI and waist circumference have higher fasting cortisol concentrations and lower concentrations of the thermogenic fT3 and are more prone to lower weight reduction in response to a negative energy balance and vice versa. Our previously reported negative correlation between the fasting cortisol level and waist circumference in a cohort of obese non-diabetic females (Hainer *et al.* 2002) supports the findings of Rosmond *et al.* (1998) concerning the association of abdominal obesity with low morning cortisol values and suppressed diurnal cortisol variability. The results of our current study demonstrate that obese individuals with low morning cortisol concentrations, i.e. those with anticipated perturbations of the hypothalamic-pituitary-adrenal axis, are able to reduce weight more than those who do not exhibit such a disturbance.

Baseline adiponectin levels were positively related to changes in waist circumference, i.e. individuals with high baseline adiponectin levels exhibited a lower decrease in waist circumference than those with low baseline adiponectin levels. This relationship might be explained by the fact that there is an inverse association between adiponectin concentration and waist circumference at baseline ($r = -0.248$, $p = 0.045$) which reflects the role of adiponectin in the development of abdominal obesity and metabolic syndrome (Matsuzawa 2006). Subjects with higher adiponectin levels exhibited lower waist circumference at the beginning and thus have a lower chance for further decrease in this parameter.

We also investigated the predictive role of CRP, a biomarker of inflammation, which is associated with the risk of cardiovascular disease (Dietrich and Jialal 2005). Previous findings clearly demonstrated that increasing body weight is positively associated with CRP levels and weight loss significantly decreases CRP (Dietrich and Jialal 2005). Minor weight loss in our study did not lead to significant changes in CRP concentrations. However, an inverse relationship between the baseline CRP level and BMI change was shown in correlation analysis whereas log CRP contributed to the prediction of weight change in backward stepwise regression. This means that individuals with higher baseline BMI, and thus higher baseline CRP levels, are able to lose more weight in response to a weight reduction program.

Conclusions

The study indicated that a short-term weight management program induced many favorable changes in anthropometric, psychobehavioral and hormonal indexes. Correlation analysis revealed that the baseline concentrations of several hormones involved in energy balance regulation were significantly associated with the reduction of anthropometric parameters in response to a well-controlled weight reduction program. In the backward stepwise regression model age, initial BMI together with baseline levels of growth hormone, peptide YY, neuropetide Y and C-reactive protein predicted 49.8 % of variability in weight loss. Psychobehavioral factors (items of the Eating Inventory, Beck Depression score) did not contribute to weight change in the weight reduction program with a well-controlled diet and exercise protocol.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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References

ALBERT SG, MOORADIAN AD: Low-dose recombinant human growth hormone as adjuvant therapy to lifestyle modifications in the management of obesity. *J Clin Endocrinol Metab* **89**: 695-701, 2004.

BATTERHAM RL, LE ROUX CW, COHEN MA, PARK A, ELLIS SM, PATTERSON M, FROST GS, GHATEI MA, BLOOM SR: Pancreatic polypeptide reduces appetite and food intake in humans. *J Clin Endocrinol Metab* **88**: 3989–3992, 2003.

BECK AT, WARD CH, MENDESON M, MOCK J, ERBAUGH J. An inventory for measuring depression. *Arch Gen Psychiat* **4**: 561-571, 1961.

BEHRE CJ, GUMMESSON A, JERNAS M, LYSTIG TC, FAGERBERG B, CARLSSON B, CARLSSON LM: Dissociation between adipose tissue expression and serum levels of adiponectin during and after diet-induced weight loss in obese subjects with and without the metabolic syndrome. *Metabolism* **56**: 1022-1028, 2007.

BENINI ZL, CAMILLONI MA, SCORDATO C, LEZZI G, SAVIA G, ORIANI G, BERTOLI S, BALZOLA F, LIUZZI A, PETRONI ML: Contribution of weight cycling to serum leptin in human obesity. *Int J Obes* **25**: 721-726, 2001.

CELI F, BINI V, PAPI F, CONTESSA G, SANTILLI E, FALORNI A: Leptin serum levels are involved in the relapse after weight excess reduction in obese children and adolescents. *Diabetes Nutr Metab* **16**: 306-311, 2003.

DIETRICH M, JIALAL I: The effect of weight loss on a stable biomarker of inflammation, C-reactive protein. *Nutr Rev* **63**: 22-28, 2005.

DI STEFANO G, BINI V, PAPI F, CELI F, CONTESSA G, BERIOLI MG, BACOSI ML, FALORNI A: Leptin serum concentrations predict the responsiveness of obese children and adolescents to weight excess reduction program. *Int J Obes* **24**: 1586-1591, 2000.

DOUCET E, ST-PIERRE S, ALMÉRAS N, MAURIÉGE P, DESPRÉS JP, RICHARD D, BOUCHARD C, TREMBLAY A; QUEBEC FAMILY STUDY: Fasting insulin levels influence plasma leptin levels independently from the contribution of adiposity: evidence from both a cross-sectional and an intervention study. *J Clin Endocrinol Metab* **85**: 4231-4237, 2000.

FILOZOF CM, MURUA C, SANCHEZ MP, BRAILOVSKY C, PERMAN M, GONZALES CD, RAVUSSIN E: Low plasma leptin concentration and low rates of fat oxidation in weight-stable post-obese subjects. *Obes Res* **8**: 205-210, 2000.

GELDSZUS R, MAYR B, HORN R, GEISTHOVEL F, V ON ZUR MUHLEN A, BRABANT G: Serum leptin and weight reduction in female obesity. *Eur J Endocrinol* **135**: 659-662, 1996.

HAINER V, KUNEŠOVÁ M, PA ŘÍZKOVÁ J, ŠTICH V, MIKULOVÁ R, SLABÁ S: Respiratory quotient in obesity: its association with an ability to retain weight loss and with parental obesity (In Czech). *Sb Lek* **101**: 99-104, 2000a.

HAINER V, STUNKARD AJ, KUNEŠOVÁ M, PA ŘÍZKOVÁ J, ŠTICH V, ALLISON D: Intrapair resemblance in very low calorie diet-induced weight loss in female obese identical twins. *Int J Obes* **24**: 1051-1057, 2000b.

HAINER V, KUNEŠOVÁ M, PA ŘÍZKOVÁ J, MIKULOVÁ R, STUNKARD A: Serum cortisol level and sex hormone binding globulin (SHBG) levels, body fat distribution and the role of genetic factors in obese females (In Czech). *Sb Lek* **103**: 471-475, 2002.

HAINER V, KABRNOVÁ K, GOJOVÁ M, KUNEŠOVÁ M, KLEPETÁŘ J, DRBOHLAV J, KOPSKÝ V, NEDVÍDKOVÁ J, PAŘÍZKOVÁ J, HILL M: Psychobehavioral and hormonal predictors of weight loss in response to a 3-week weight management program. *Obes Res* **13** (Suppl): A142, 2005.

HAINER V, ZAMRAZILOVÁ H, SPÁLOVÁ J, HAINEROVÁ I, KUNEŠOVÁ M, ALDHOON B, BENDLOVÁ B: Role of hereditary factors in weight loss and its maintenance. *Physiol Res* **57**(Suppl 1): S1-S15, 2008.

JESUDASON DR, MONTEIRO MP, MCGOWAN BM, NEARY NM, PARK AJ, PHILIPPOU E, SMALL CJ, FROST GS, GHATEI MA, BLOOM SR: Low-dose pancreatic polypeptide inhibits food intake in man. *Br J Nutr* **97**: 426-429, 2007.

KABRNOVÁ-HLAVATÁ K, HAINER V, GOJOVÁ M, HLAVATÝ P, KOPSKÝ V, NEDVÍDKOVÁ J, KUNEŠOVÁ M, PAŘÍZKOVÁ J, WAGENKNECHT M, HILL M, DRBOHLAV J: Calcium intake and the outcome of short-term weight management. *Physiol Res* 2007 (In press).

KOK P, ROELFSEMA F, FROLICH M, MEINDERS AF, PIJL H: Prolactin release is enhanced in proportion to excess visceral fat in obese women. *J Clin Endocrinol Metab* **89**: 4445-4449, 2004.

KOK P, ROELFSEMA F, LANGENDONK JG, D E WITT CC, FROLICH M, BURGGRAAF J, MEINDERS AE, PIJL H: Increased circadian prolactin release is blunted after body weight loss in obese premenopausal women. *Am J Physiol* **290**: E218-E224, 2006.

KOZLOWSKA L, ROSOLOWSKA-HUSZCZ D: Leptin, thyrotropin, and thyroid hormones in obese/overweight women before and after two levels of energy deficit. *Endocrine* **24**: 147-153, 2004.

KUNEŠOVÁ M, BRAUNEROVÁ R, HLAVATÝ P, TVRZICKÁ E, STA ŇKOVÁ B, ŠKRHA J, HILGERTOVÁ J, HILL M, KOPECKÝ J, WAGENKNECHT M, HAINER V, MATOULEK M, PA ŘÍZKOVÁ J, ŽÁK A, SVA ČINA S: 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 acids composition in severely obese women. *Physiol Res* **55**: 63-72, 2006.

LEJEUNE MP, V AN AGGEL-LEIJSSEN DP, V AN BAAK MA, WESTERTERP-PLANTENGA MS: Effects of dietary restraint vs. exercise during weight maintenance in obese men. *Eur J Clin Nutr* **57**: 1338-1344, 2003.

LE ROUX CW, AYLWIN SJ, BATTERHAM RL, BORG CM, COYLE F, PRASAD V, SHUREY S, GHATEI MA, PATEL AG, BLOOM SR: Gut hormone profiles following bariatric surgery favor an anorectic state, facilitate weight loss, and improve metabolic parameters. *Ann Surg* **243**: 109-114, 2006.

LINDSTEDT G, LUNDBERG PA, LAPIDUS L, LUNDGREN H, BENGTSSON C, BJORNTORP P: Low sex-hormone-binding globulin concentration as independent risk factor for development of NIDDM. 12-yr follow-up of population study of women in Gothenburg, Sweden. *Diabetes* **40**: 123-128, 1991.

MATSUZAWA Y: The metabolic syndrome and adipocytokines. *FEBS Lett* **580**: 2917-2921, 2006.

MCCRORY MA, NOMMSEN-RIVERS LA, MOLÉ PA, LONNERDAL B, DEWEY KG: Randomized trial of the short-term effects of dieting compared with dieting plus aerobic exercise on lactation performance. *Am J Clin Nutr* **69**: 959-967, 1999.

MINGRONE G, GRECO AV, GIANCATERINI A, SCARFONE A, CASTAGNETO M, PUGEAT M: Sex hormone-binding globulin levels and cardiovascular risk factors in morbidly obese subjects before and after weight reduction induced by diet or malabsorptive surgery. *Atherosclerosis* **161**: 455-462, 2002.

MIYAWAKI T, MASUZAKI H, OGAWA Y, HOSODA K, NISHIMURA H, AZUMA N, SUGAWARA A, MASUDA I, MURATA M, MATSUO T, HAYASHI T, INOUE G, YOSHIMASA Y, NAKAO K: Clinical implications of leptin and its potential humoral regulators in long-term low-calorie diet therapy for obese humans. *Eur J Clin Nutr* **56**: 593-600, 2002.

MORENO-ALIAGA MJ, SANTOS JL, MARTI A, MARTÍNEZ JA: Does weight loss prognosis depend on genetic make-up? *Obes Rev* **6**: 155-168, 2005.

MORO D, MAZZILLI G, GRUGNI G, GUZZALONI G, TEDESCHI S, MORABITO F: Leptin and neuropeptide Y serum levels in young obese during weight loss. *Minerva Endocrinol* **23**: 105-110, 1998.

NAM SY, KRATZSCH J, KIM KW, KIM KR, LIM SK, MARCUS C: Cerebrospinal fluid and plasma concentrations of leptin, NPY, and alpha-MSH in obese women and their relationship to negative energy balance. *J Clin Endocrinol Metab* **86**: 4849-4853, 2001.

NASLUND E, ANDERSSON I, DEGERBLAD M, KOGNER P, KRAL JG, ROSSNER S, HELLSTROM PM: Associations of leptin, insulin resistance and thyroid function with long-term weight loss in dieting men. *Intern Med* **248**: 299-308, 2000.

ORTEGA E, PANNACCIULLI N, BOGARDUS C, KRAKOFF J: Plasma concentrations of free triiodothyronine predict weight change in euthyroid persons. *Am J Clin Nutr* **85**: 440-445, 2007.

PILCOVÁ R, ŠULCOVÁ J, HILL M, BLÁHA P, LISÁ L: Leptin levels in obese children: effects of gender, weight reduction and androgens. *Physiol Res* **52**: 53-60, 2003.

RAVUSSIN E: Low resting metabolic rate as a risk factor for weight gain: role of the sympathetic nervous system. *Int J Obes* **19** (Suppl 7): S8-S9, 1995.

REINEHR T, KRATZSCH J, KIESS W, ANDLER W: Circulatin g soluble leptin receptor, leptin, and insulin resistance before and after weight loss in obese children. *Int J Obes* **29**: 1230-1235, 2005.

REINEHR T, ENRIORI PJ, HARZ K, COWLEY MA, ROTH CL: Pancreatic polypeptide in obese children before and after weight loss. *Int J Obes* **30**: 1476-1481, 2006.

REINEHR T, DE SOUSA G, ROTH CL: Obestatin and ghrelin levels in obese children and adolescents before and after reduction of overweight. *Clin Endocrinol* (Oxf) 2007 (In press).

ROMANOVA IV, RAMOS EJ, XU Y, QUINN R, CHEN C, GEORGE ZM, INUI A, DAS U, MEGUID MM: Neurobiologic changes in the hypothalamus associated with weight after gastric bypass. *J Am Coll Surg* **199**: 887-895, 2004.

ROSMOND R, DALLMAN MF, BJORNTORP P: Stress-related cortisol secretion in men: Relationships with abdominal obesity and endocrine, metabolic and hemodynamic abnormalities. *J Clin Endocrinol Metab* **83**: 1853-1859, 1998.

ROTH CL, ENRIORI PJ, HARZ K, WOELFLE J, COWLEY MA, REINEHR T: Peptide YY is a regulator of energy homeostasis in obese children before and after weight loss. *J Clin Endocrinol Metab* **90**: 6386-6391, 2005.

SANTOSA S, DEMONTY I, LICHTENSTEIN AH, CIAFLONE K, JONES PJ: An investigation of hormone and lipid associations after weight loss in women. *J Am Coll Nutr* **26**: 250-258, 2007.

SARTORIO A, AGOSTI F, RESNIK M, LAFORTUNA CL: E ffects of a 3-week integrated body weight reduction program on leptin levels and body composition in severe obese subjects. *J Endocrinol Invest* **26**: 250-256, 2003.

SAVOYE M, DZIURA J, CASTLE J, D I PIETRO L, TAMBORLANE WV, CAPRIO S: Importance of plasma leptin in predicitng future weight gain in obese children: a two-and-a half-year longitudinal study. *Int J Obes* **26**: 942-946, 2002.

SCACCHI M, PINCELLI AL, CAVAGNINI F: Growth hormone in obesity. *Int J Obes Relat Metab Disord* **23**: 260-271, 1999.

STUNKARD AJ, MESSICK SM: The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *J Psychosom Res* **29**: 71-83, 1985.

VERDICH C, TOUBRO S, BUEMANN B, HOLST JJ, BULOW J, SIMONSEN L, SONDERGAARD SB, CHRISTIANSEN NJ, ASTRUP A: Leptin levels are associated with fat oxidation and dietary-induced weight loss in obesity. *Obes Res* **9**: 452-461, 2001.

VOGELS N, DIEPVENS K, WESTERTERP-PLANTENGA MS: Predictors of long-term weight maintenance. *Obes Res* **13**: 2162-2168, 2005.

WADDEN TA, STUNKARD AJ, DAY SC, GOULD RA, RUBIN CJ: Less food, less hunger: reports of appetite and symptoms in a cotrolled study of a protein-sparing modified fast. *Int J Obes* **11**: 239-249, 1987.

WESTENHOEFER J, VON FALCK B, STELLFELDT A, FINTELMANN S. Behavioural correlates of successful weight reduction over 3 y. Results from the Lean Habits Study. *Int J Obes* **28**: 334-335, 2004.

WESTERTERP-PLANTENGA MS, KEMPEN KP, SARIS WHM: Determinants of weight maintenance in women after diet-induced weight reduction. *Int J Obes* **22**: 1-6, 1998.

WHO EXPERT COMMITTEE Physical status: The use and interpretation of anthropometry. *WHO Technical Series Report* No. 854, Geneva, 1995, pp 452.

WING RR: Insulin sensitivity as a predictor of weight regain. *Obes Res* **5**: 24-29, 1997.

XYDAKIS AM, CASE CC, JONES PH, HOOGEVEEN RC, LIU MY, SMITH EO, NELSON KW, BALLANTYNE CM: Adiponectin, inflammation, and the expression of the metabolic syndrome in obese individuals: the impact of rapid weight loss through caloric restriction. *J Clin Endocrinol Metab* **89**: 2697-2703, 2004.

ZAHORSKA-MARKIEWICZ B, OBUCHOWICZ E, WALUGA M, TKACZ E, HERMAN ZS: Neuropeptide Y in obese women during treatment with adrenergic modulation drugs. *Med Sci Monit* **7**: 403-408, 2001.

PSYCHOBEHAVIORAL AND HORMONAL PREDICTORS OF WEIGHT CHANGE IN RESPONSE TO A 6-MONTH WEIGHT MANAGEMENT PROGRAM

Kabrnova K.[1], Hainer V.[1], Gojova M.[2], Nedvidkova J.[1], Kopsky V.[1], Kunesova M.[1], Parizkova J.[1], Hlavaty P.[1], Wagenknecht M.[1], Hill M.[1], Drbohlav J.[3]

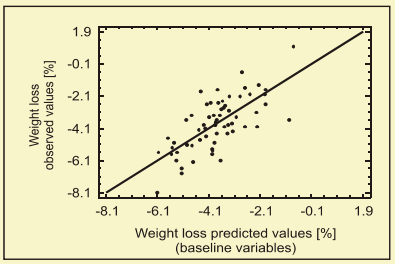
Endocrine Institute, Obesity Management Centre, Prague,Czech Republic [1]
Obesity Management Unit, Lipova Lazne, Czech Republic [2]
Dairy Research Institute, Prague, Czech Republic [3]

BACKGROUND

Our previous study of 67 overweight/obese women demonstrated that weight loss in response to a 3-week weight management (WTM) with precisely defined dietary intake is influenced by age, initial BMI and the baseline levels of GH, PYY, NPY and CRP. These factors explained 49.8% of weight change (WTC) variability whereas factors of the Eating Inventory (EI) and Beck Depression Inventory (BDI) at baseline were not significantly related to WTC.

Prediction of weight loss from age, BMI and baseline hormonal levels

Backward stepwise multiple regression (the final model)*				
Dependent variable: Weight loss (%)				
Parameter	Estimate	Error	T-statistic	P-value
Constant	6.80	3.92	1.74	0.0881
$Age^{1.75}$	-0.0086	0.00039	-2.16	0.0347
$-(BMI^{-0.75})$	-48.56	23.07	-2.11	0.0398
$-(GH_t^{-0.15})$	-2.75	0.875	-3.15	0.0026
$-(PYY_t^{-0.35})$	36.88	9.47	3.89	0.0003
$-(NPY_t^{-0.14})$	17.32	4.70	3.69	0.0005
$Log(CRP_t)$	-0.556	0.197	-2.82	0.0066
R^2 = 49.8%, Fisher's statistic = 9.26, p<0.0001				
*Fisher's statistic > 4 was used as inclusion criterium for individual parameters				



METHODS

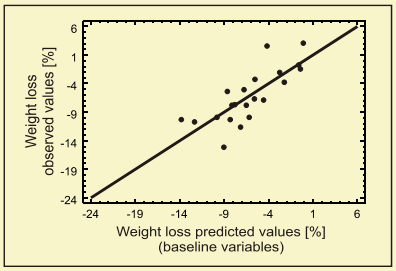
24 women (BMI 32.6+5.1 kg/m2; age 49.9+11.5 yr) who underwent an initial 4-week in-patient treatment with a hypocaloric diet under the strict control of dietary intake were followed during the subsequent 5 months of lifestyle modification. Diet yielding a 2.5 MJ daily energy deficit was individually prescribed. The following parameters were examined before and after a 6-month WTM: body weight and composition; factors of EI and BDI; hormones: TSH, fT3, fT4, insulin, C-peptide, PRL, GH, IGF-1, cortisol, SHBG, PTH, ghrelin, leptin, PYY, NPY, PP, adiponectin, resistin; CRP. Statistical analysis: ANOVA, backward stepwise multiple regression.

RESULTS

WTM-induced weight loss (-6.5±5.6 kg; - 7.3±5.8%, p<0.01) resulted in significant decreases of BMI, waist and hip circumferences, skinfolds´ thicknesses, disinhibition and hunger score of the EI, and levels of LDL-C, triglyceride, HbA_1c, C-peptide, ghrelin, leptin, PYY and NPY. On the other hand, significant increases in restraint score of the EI and HDL-C and adiponectin levels were observed in response to WTM. At a 6-month follow-up, initial BMI and baseline values of dietary restraint and disinhibition of the EI explained 60.8% of WTC variability. Changes in leptin and HDL-C concentrations were significant correlates of WTC at 6-months, explaining 42.2% of WTC variability.

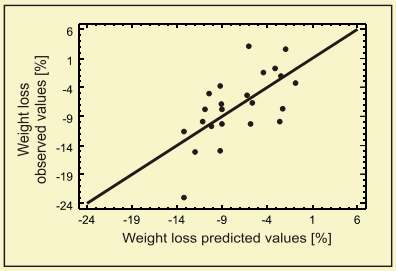
Prediction of weight loss by initial BMI and baseline values of dietary restraint and disinhibition.

Multiple regression analysis				
Dependent variable: Weight loss (%)				
Parameter	Estimate	Error	T-statistic	P-Value
Constant	-0.294808	0.0782985	-3.76519	0.0015
$-BMI^{-0.6}$	-1.96682	0.656365	-2.99654	0.0081
Disinhibition	0.0070879	0.0027099	2.6155	0.0181
Restraint	-0.005587	0.0015578	-3.58666	0.0023
R^2=60.6%				



Prediction of weight loss from WTM - induced changes in leptin and HDL cholesterol level.

Multiple regression analysis				
Dependent variable: Weight loss (%)				
Parameter	Estimate	Error	T-statistic	P-Value
Constant	-0.095102	0.0605634	-1.57029	0.132
$(leptin+25)^{0.6}$	0.0188925	0.0073814	2.5595	0.0187
$(HDL+0.2)^{0.6}$	-0.149713	0.0646696	-2.31504	0.0314
R^2=42.1%				



CONCLUSION

Baseline values of BMI, dietary restraint and disinhibition of the eating inventory predict weight change in response to a 6-month lifestyle weight management.

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Neuromedin Beta: P73T Polymorphism in Overweight and Obese Subjects

J. SPÁLOVÁ[1], H. ZAMRAZILOVÁ[1], J. VČELÁK[1], M. VAŇKOVÁ[1], P. LUKÁŠOVÁ[1], M. HILL[1], K. HLAVATÁ [1], P. ŠRÁMKOVÁ[2], M. FRIED[2], B. ALDHOON[1,3], M. KUNEŠOVÁ[1], B. BENDLOVÁ[1], V. HAINER[1]

[1]Institute of Endocrinology, [2]Clinical Centre ISCARE I.V.F., [3] Department of Cardiology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

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Summary

Neuromedin beta (*NMB*) is a member of the bombesin-like peptide family expressed in brain, gastrointestinal tract, pancreas, adrenals and adipose tissue. The aim of our study was to compare the frequency of P73T polymorphism in overweight and obese patients (37 men: age 50.6±11.7 years, BMI 41.1±7.8 kg/m^2; 255 women: age 49.0±11.9 years, BMI 37.9±6.8 kg/m2) with that of healthy normal weight subjects (51 men: age 28.2±7.1 years, BMI 22.3±2.0 kg/m^2; 104 women: age 29.1±9.1 years, BMI 21.5±1.9 kg/m^2) and to investigate the polymorphism's influence on anthropometric, nutritional and psychobehavioral parameters in overweight/obese patients both at the baseline examination and at a control visit carried out 2.5 years later, regardless of the patient´s compliance with the weight reduction program. No significant differences in the genotype distribution were demonstrated between normal weight and overweight/obese subjects. Male T allele non-carriers compared to T allele carriers had higher energy (p=0.009), protein (p=0.018) and fat (p=0.002) intakes and hunger score (p=0.015) at the beginning of treatment. Male T allele non-carriers had a more favorable response to weight management at the follow-up, as they exhibited a significant reduction in waist circumference, energy intake and depression score as well as a significant increase in dietary restraint. No significant differences between carriers and non-carriers were demonstrated in women at the baseline examination. Both female T allele carriers and non-carriers demonstrated similar significant changes in nutritional parameters and in restraint score at the follow-up. Nevertheless, only female non-carriers showed a significant decrease in the hunger score.

Key words

Neuromedin beta • Obesity • Gene polymorphism • Eating behavior • Nutrient intake • Weight loss

Corresponding author

Hana Zamrazilová, Institute of Endocrinology, Národní třída 8, 116 94 Prague 1, Czech Republic. E-mail: hzamrazilova@endo.cz

Introduction

Neuromedin beta (*NMB*) is a member of the bombesin-like peptide family, a subfamily of ranatensins. These peptides are initially released from the gastrointestinal tract in response to food ingestion and bridge the gut and brain, through neurocrine means, to inhibit further food intake. Northern blot analysis revealed two *NMB* gene transcripts of 750-850 bases in human brain and gastrointestinal tissues with high expression levels in the hypothalamus, stomach, colon and low levels in cerebellum, pancreas, adrenals and adipose tissue (Ohki-Hamazaki 2000). *NMB* was identified in the hypothalamus (Krane *et al.* 1988), where afferent signals reflecting the nutritional state and efferent pathways that control feeding behavior and energy expenditure are integrated (Oeffner *et al.* 2000). *NMB* exerts its effect by binding to *NMB* receptor, a G-protein coupled receptor (Ohki-Hamazaki 2000).

As shown in Figure 1, human *NMB* is encoded by a 121-amino acid precursor consisting of an N-terminal hydrophobic signal sequence followed by the prohormone *NMB*-32 and then a carboxy-terminal extension peptide (Oeffner *et al.* 2000). The gene for this precursor is localized on chromosome 15 (15q22.3-q23) and is comprised of three exons. This chromosome region contains a gene for the Bardet-Biedl syndrome (BBS) type 4 (Bruford *et al.* 1997). BBS is a rare disease described in the early 1920s (Biedl 1922) which is associated with severe obesity and congenital abnormalities such as retinal dystrophy, polydactyly,

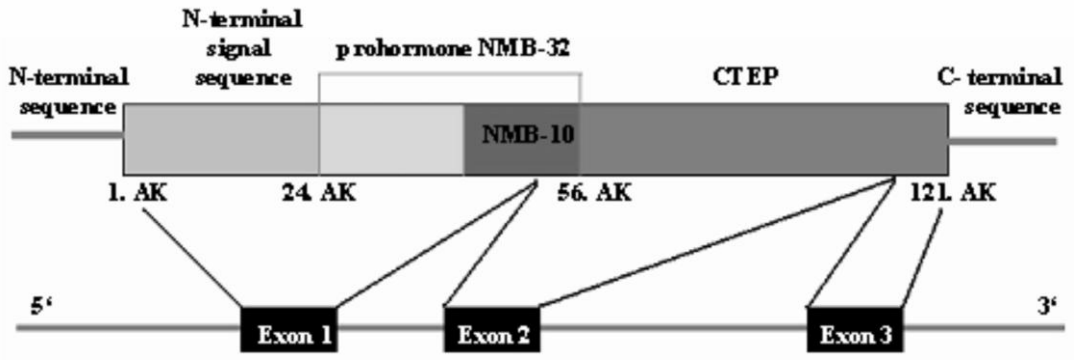

Fig. 1. The structure of human neuromedin beta gene (CTEP – Carboxy-Terminal Extension Peptide)

renal anomalies and hypogonadism in males. BBS is characterized by an autosomal recessive mode of inheritance (Beales *et al.* 1997).

It has been shown that weight loss maintenance is significantly influenced by psychobehavioral factors such as eating behavior assessed by the Eating Inventory and the level of depression evaluated by the Beck Depression Inventory (Hainer *et al.* 2005, Vogels *et al.* 2005, Kabrnová-Hlavatá *et al.* 2008). Eating Inventory evaluates three factors: dietary restraint, dietary disinhibition and hunger (Stunkard and Messick 1985). Dietary restraint is a conscious behavior aimed at limiting food intake. Disinhibition characterizes eating behavior of a person prone to non-compliance with a weight loss regimen and to overeating in response to stress, increased alcohol intake, anxiety, depression etc. Hunger score quantifies perceived hunger. Different contributions of hereditary components in determination of the factors of the Eating Inventory have been demonstrated in the Québec family study (Provencher *et al.* 2003). The heritability of disinhibition and susceptibility to hunger was found to be 19 % and 32 %, respectively, whereas the heritability of cognitive restraint was not statistically significant. Bouchard *et al.* (2004) found a significant association between the missense polymorphism P73T (or C253A) in exon 2 of the *NMB* and levels of disinhibition and susceptibility to hunger, increased body weight, body mass index (BMI), waist circumference and fat mass. The primary aim of our study was to compare the frequences of *NMB* P73T polymorphisms in overweight and obese patients with that of healthy normal weight subjects. The second aim of our study was to investigate the influence of P73T polymorphism on selected anthropometric, nutritional and psychobehavioral parameters in overweight/obese patients both at their baseline visit to the Obesity Management Centre and at the follow-up visit carried out 2.5 years later regardless of compliance with the weight reduction program.

Methods

Subjects

The overweight and obese patients (37 men: age 50.6±11.7 years, BMI 41.1±7.8 kg/m^2; 255 women: age 49.0±11.9 years, BMI 37.9±6.8 kg/m^2) were examined in the Obesity Management Centre of the Institute of Endocrinology in Prague and in the Obesity Management Unit of the Clinical Centre ISCARE IVF in Prague. Frequency of the P73T polymorphism was compared with healthy normal weight subjects without family history of morbid obesity or diabetes type 2 (51 men: age 28.2±7.1 years, BMI 22.3±2.0 kg/m^2; 104 women: age 29.1±9.1 years, BMI 21.5±1.9 kg/m^2). Essential characteristics of the overweight and obese patients are presented in Table 1.

Our study was carried out over a 3-year period. The comprehensive weight management program included low energy diet (recommended daily energy deficit cca 2.5 MJ), increased physical activity (recommended 30 minutes of aerobic exercise per day such as walking, cycling etc.) and behavioral lifestyle modification provided by a psychologist. All patients underwent a control examination 2.5 years after the baseline visit without regard to their adherence to the weight reduction program or their attendance in regular check-ups. Even the patients who dropped out from the study (in 39 % patients less than three visits per year were recorded) were able to participate at the control visit.

Anthropometric parameters (body weight, height, waist and hip circumferences) were measured according to the WHO recommendations (WHO Expert Committee 1995). Body composition (fat mass %, fat free

Table 1. Anthropometric, nutritional and psychobehavioral characteristics of the overweight/obese subjects at baseline and follow-up visits (lower quartile; **median**; upper quartile)

	MEN			**WOMEN**		
	The beginning of the treatment	**The control visit after 2.5 years**	**Statistics[a] (p)**	**The beginning of the treatment**	**The control visit after 2.5 years**	**Statistics[a] (p)**
number	**37**		--	**255**		--
BMI (kg/m^2)	35.9; **39.4**; 47.1	33.9; **38.6**; 46.1	0.331	33.1; **36.8**; 42.1	32.4; **36.2**; 41.2	**0.002**
Body weight (kg)	109.2; **126.8**; 152.8	104.9; **121.0**; 146.0	0.361	89.6; **100.2**; 113.1	87.1; **97.4**; 110.8	**0.003**
Waist circumference (cm)	119.4; **126.5**; 135.8	110.3; **124.0**; 133.0	**0.044**	99.0; **108.0**; 117.0	98.0; **107.0**; 117.0	0.113
Fat mass (%)	30.2; **35.0**; 37.3	30.4; **33.4**; 37.5	0.673	41.7; **44.5**; 48.1	41.6; **44.7**; 47.8	0.960
Fat free mass (%)	62.7; **65.0**; 69.8	62.5; **66.6**; 69.6	0.673	51.9; **55.5**; 58.3	52.2; **55.3**; 58.4	0.960
Energy intake (kJ/day)	7028; **8455**; 12120	5813; **6584**; 9633	0.088	5919; **7321**; 9017	5271; **6144**; 7343	**0.000**
Protein intake (g/day)	59.7; **79.2**; 91.8	57.6; **66.3**; 88.6	0.256	56.3; **65.6**; 79.0	52.4; **62.3**; 70.8	**0.000**
Carbohydrate intake (g/day)	185.5; **259.8**; 405.8	115.4; **206.7**; 258.0	0.108	168.7; **211.4**; 272.1	145.9; **181.2**; 220.5	**0.000**
Fat intake (g/day)	57.9; **77.9**; 107.6	49.2; **54.7**; 76.8	0.218	49.5; **63.4**; 85.1	40.6; **52.1**; 62.7	**0.000**
EI – restraint score	3.3; **7.0**; 12.3	9.0; **12.0**; 14.0	**0.034**	6.0; **10.0**; 14.0	10.0; **14.0**; 16.8	**0.000**
EI –disinhibition score	7.0; **4.0**; 9.0	3.5; **5.0**; 7.0	0.776	3.0; **6.0**; 9.0	3.0; **6.0**; 9.0	0.830
EI – hunger score	2.3; **4.5**; 8.0	1.0; **6.0**; 6.0	0.455	2.0; **4.0**; 7.0	1.0; **3.0**; 6.0	**0.000**
BDI – depression score	4.0; **9.0**; 16.8	3.0; **11.5**; 15.5	0.337	6.0; **11.0**; 16.0	5.8; **9.0**; 17.0	**0.029**

[a]Wilcoxon test, BMI – body mass index, EI – Eating Inventory, BDI – Beck Depression Inventory

mass %) was assessed using a bipedal – bimanual bioimpedance analyser – TANITA BC-418MA.

Daily energy and macronutrients (protein, carbohydrate, fat) intakes were evaluated from a one-week dietary record, which was performed at the baseline and follow-up visits. A computerized version of the Czech Nutrition File „Vyziva“, which includes almost 3000 food items, was used for dietary intake assessment.

A validated Czech version of the Eating Inventory (Stunkard and Messick 1985) was used to measure dietary restraint, disinhibition and susceptibility to hunger. This Three Factor Eating Questionnaire was used in our previous studies involving obese patients (Hainer *et al.* 2005) and in a quota sample of Czech adults (Hainer *et al.* 2006). The depression score was measured by the Beck Depression Inventory (Beck *et al.* 1961).

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethic Committee of the Institute of Endocrinology. All subjects signed informed consent before the study initiation.

Genotyping

Genomic DNA was isolated from peripherial blood leukocytes using the QIAamp® DNA Blood Kit (QIAGEN, Germany). Genomic DNA was amplified by polymerase chain reaction (PCR; T-Gradient Cycler, Biometra, Germany). Reactions were performed in a final volume of 12 μl. Primers were designed in accordance with Oeffner *et al.* (2000). In order to find variants of the P73T polymorphism (PP, PT, TT) the sequencing method was used (ALFExpress II, Amersham Pharmacia Biotech, USA). For screening of P73T polymorphism the Single Strand Conformation Polymorphism (SSCP) method was employed, using control genotypes in every run (ALFExpress II, Amersham Pharmacia Biotech, USA). Allele determinations were performed independently by two experienced persons. Detailed procedures of the methods used are available from the corresponding author.

Statistical analysis

Genotype frequencies were tested by the χ^2-test. The differences between groups were evaluated using the robust Mann-Whitney test, while the differences between the beginning of the treatment and the control visit at the 2.5-year follow-up were assessed by Wilcoxon's paired robust test. The PC programs NCSS 2004, QC.Expert 2.7 and Microsoft Excel 2007 were used for statistical analysis.

Results

Table 1 summarizes essential anthropometric, nutritional and psychobehavioral characteristics of the overweight/obese subjects at the baseline and follow-up visits. The cohort of men was made up of less individuals, most likely due to the fact that Czech men tend to not be as motivated as women to participate in a long term life style modification program. In men a significant reduction in waist circumference was revealed at the follow-up visit. In both genders an observed significant increase in dietary restraint score could have contributed to a better control of energy intake. A minor but significant decrease in body weight and BMI in women at the follow-up visit might reflect a significant decrease in energy and nutrient intakes as well as a decline in hunger and depression scores.

Table 2 shows the frequencies of genotypes (PP, PT, TT) in overweight and obese population and in healthy normal weight persons. No significant differences in the frequencies of genotypes were demonstrated between the two groups studied (χ^2=2.08, p=0.353).

Due to the low frequency of TT homozygotes, overweight and obese patients were divided into two groups – T allele non-carriers (homozygotes PP) and T allele carriers (heterozygotes PT together with homozygotes TT). Table 3 shows anthropometric, psychobehavioral and nutritional parameters before the treatment and after the 2.5-year follow-up period in overweight and obese men classified as T allele carriers or non-carriers. Male T allele non-carriers had higher energy intake (p=0.009), protein intake (p=0.018), fat intake (p=0.002) and hunger score (p=0.015) at the beginning of treatment in comparison with male T allele carriers. After the follow-up period, significant decreases in waist circumference (p=0.021), energy intake (p=0.038), carbohydrate intake (p=0.038), dietary restraint (p=0.021) and score of depression (p=0.032) were observed in male T allele non-carriers. Changes in energy and carbohydrate intakes over the follow-up period significantly differed between male T allele carriers and non-carriers (p=0.043, p=0.034 respectively). T allele carriers increased and T allele non-carriers decreased energy intake and carbohydrate consumption. Maximum weight loss observed at follow-up was higher in T allele non-carriers than in T allele carriers (median: 15.1 vs. 11.5 kg). However, the difference in weight loss between the two groups was not statistically significant (p=0.365).

Table 2. The frequencies of genotypes in overweight/obese patients and in normal weight subjects

	PP		PT		TT	
	overweight and obese n (%)	normal weight n (%)	overweight and obese n (%)	normal weight n (%)	overweight and obese n (%)	normal weight n (%)
men	17 (45.9)	28 (54.9)	18 (48.6)	20 (39.2)	2 (5.4)	3 (5.9)
women	128 (50.2)	59 (56.7)	108 (42.4)	36 (34.6)	19 (7.5)	9 (8.7)
total	145 (49.7)	87 (56.1)	126 (43.2)	56 (36.1)	21 (7.2)	12 (7.7)

χ^2=2.08, p=0.353 (for total numbers)

Table 4 shows anthropometric, psychobehavioral and nutritional parameters before the treatment and after the 2.5-year follow-up period in overweight and obese women who were classified as T-allele carriers or non-carriers. At the beginning of the treatment no significant differences between carriers and non-carriers were demonstrated in women. In neither men nor women did P73T polymorphism affect the maximum weight loss achieved (median: 9.9 vs. 10.0 kg, p=0.434). Both female T allele carriers and non-carriers exhibited significant decreases in energy, protein, carbohydrate and fat intakes and increases in restraint scores. However, a decrease in BMI and body weight was revealed in T allele carriers only. In contrast to T allele carriers, female non-carriers significantly decreased in the hunger score. Nevertheless, changes in anthropometric, psychobehavioral and nutritional parameters at the follow-up were not significant for either female T allele carriers or non-carriers.

Discussion

NMB is a member of the bombesin-like peptides family. These peptides have many biological effects that may be related to eating behaviors and obesity (Bouchard *et al.* 2004). Monitoring P73T polymorphism in *NMB* revealed many associations with dietary disinhibition, susceptibility to hunger and fat mass change over time (Bouchard *et al.* 2004). Recent results from the Québec Family Study confirmed a significant contribution of *NMB* polymorphism to the adiposity changes in adult subjects with a wide range of adiposity (BMI range from 17.5 to 55.6 kg/m^2) who were followed over a period of 6-10 years (Bouchard *et al.* 2007). It was therefore considered possible that *NMB* could play a role in the regulation of eating behavior and thus might affect body weight.

Several gene polymorphisms have been implicated in the determination of weight loss and weight loss maintenance (Hainer *et al.* 2008). As weight loss maintenance remains an essential target in weight loss strategies (Wadden *et al.* 2004), the aim of our study was to evaluate the influence of P73T *NMB* polymorphism on anthropometric, nutritional and psychobehavioral indexes in obese patients at baseline examination and at a 2.5-year follow-up visit.

The frequency of P73T genotypes in our cohort was not different between healthy normal weight control group and overweight/obese subjects. Frequency of T allele in overweight/obese patients and in healthy control group was similar (28.8 % vs. 25.8 %). This our finding confirms that of Oeffner *et al.* (2000), who monitored obese German children and adolescents, and also did not detect an association of the T allele to body weight. In their study frequencies of this allele in severely obese (29.03 %) were similar to those observed in underweight subjects (26.60 %). However, they revealed a significant association between the G401A polymorphism in the *NMB* gene and body weight (Oeffner *et al.* 2000). Bouchard *et al.* (2004) who studied a randomized sample of the Québec population did not find significant differences in P73T genotypes with regard to body weight, BMI, waist circumference, fat mass (kg), restraint score and intakes of macronutrients. However, TT homozygotes exhibited significantly higher level of disinhibition and hunger when compared to P allele carriers. TT homozygotes gained significantly more fat over a 6-year follow-up than PP homozygotes.

Our study demonstrated the influence of P73T polymorphism on anthropometric, nutritional and psychobehavioral parameters, esp. in men. At the beginning of the treatment male T allele non-carriers exhibited higher susceptibility to hunger (p=0.015) and higher dietary disinhibition (of borderline significance

Table 3. Baseline anthropometric, nutritional and psychobehavioral characteristics of overweight/obese men (classified as T-alelle carriers or non-carriers) and their changes at follow-up (lower quartile; **median**; upper quartile)

MEN	The beginning of the treatment			The follow-up change				
	T allele non-carriers	T allele carriers	Statistics[a] (p)	T allele non-carriers	Statistics[b] (p)	T allele carriers	Statistics[b] (p)	Statistics[a] (p)
BMI (kg/m^2)	36.2; **39.1;** 46.9	35.8; **39.6;** 47.7	0.855	-2.3; **-1.0;** 0.8	0.102	-1.3; **0.3;** 1.6	0.779	0.247
Body weight (kg)	109.0; **123.0;** 152.0	111.0; **131.0;** 154.0	0.626	-7.8; **-2.8;** 2.3	0.113	-4.2; **0.9;** 4.3	0.779	0.300
Waist circumference (cm)	119.0; **125.0;** 132.0	121.0; **127.0;** 137.0	0.490	-5.0; **-4.0;** -2.0	**0.021**	-3.0; **-2.0;** 4.0	0.491	0.153
Fat mass (%)	33.0; **35.8;** 37.2	30.2; **31.9;** 37.3	0.622	-8.0; **-0.7;** -0.2	0.091	-1.0; **1.0;** 3.6	0.286	0.071
Fat free mass (%)	63.0; **64.0;** 67.0	63.0; **68.0;** 70.0	0.622	0.2; **0.7;** 8.0	0.091	-3.6; **-1.0;** 1.0	0.286	0.071
Energy intake (kJ/day)	8379; **11540;** 14469	5955; **7460;** 10151	**0.009**	-5536; **-3079;** -1216	**0.038**	-718; **211;** 1101	0.779	**0.043**
Protein intake (g/day)	77.4; **90.4;** 107.8	55.3; **63.5;** 85.0	**0.018**	-36.5; **-12.8;** -7.7	0.139	-4.1; **0.5;** 7.7	0.674	0.102
Carbohydrate intake (g/day)	222.0; **322.0;** 410.0	168.0; **231.0;** 272.0	0.067	-204.0; **-76.0;** -64.0	**0.038**	-13.0; **2.0;** 49.0	0.674	**0.034**
Fat intake (g/day)	81.0; **107.0;** 129.0	50.0; **62.0;** 78.0	**0.002**	-40.2; **-33.9;** 5.1	0.139	-11.2; **2.7;** 12.2	0.889	0.149
EI – restraint score	2.5; **6.5;** 9.0	3.8; **7.0;** 14.3	0.391	4.0; **5.0;** 8.0	**0.021**	-3.0; **1.0;** 5.0	0.593	0.132
EI – disinhibition score	5.3; **8.0;** 10.0	3.0; **4.0;** 9.0	0.060	-3.0; **-1.0;** 0.0	0.233	-1.0; **1.0;** 3.0	0.257	0.101
EI – hunger score	5.3; **7.5;** 9.0	1.8; **3.0;** 4.8	**0.015**	-4.0; **-1.0;** 0.0	0.134	-2.0; **1.0;** 2.0	0.717	0.195
BDI – depression score	4.3; **15.0;** 17.0	3.0; **7.5;** 11.5	0.318	-9.0; **-2.0;** -1.0	**0.032**	-4.0; **2.0;** 9.0	0.594	0.157

[a]Mann-Whitney test, [b]Wilcoxon test, BMI – body mass index, EI – Eating Inventory, BDI – Beck Depression Inventory

Table 4. Baseline anthropometric, nutritional and psychobehavioral characteristics of overweight/obese women (classified as T-alelle carriers or non-carriers) and their changes at follow-up (lower quartile; **median**; upper quartile)

WOMEN	The beginning of the treatment			The follow-up change				
	T allele non-carriers	T allele carriers	Statistics[a] (p)	T allele non-carriers	Statistics[b] (p)	T allele carriers	Statistics[b] (p)	Statistics[a] (p)
BMI (kg/m^2)	32.4; **36.4;** 41.1	33.9; **37.1;** 42.6	0.153	-1.7; **-0.1;** 0.9	0.118	2.7; **-0.5;** 1.0	**0.008**	0.360
Body weight (kg)	86.0; **99.0;** 112	92.0; **101.0;** 115.0	0.163	-4.4; **-0.4;** 2.8	0.126	-7.2; **-1.3;** 2.5	**0.009**	0.651
Waist circumference (cm)	99.0; **108.0;** 115.0	101.0; **110.0;** 118.0	0.374	-5.0; **0.5;** 4.5	0.562	-5.5; **0.0;** 3.0	0.111	0.446
Fat mass (%)	41.2; **44.2;** 47.9	42.3; **44.7;** 48.2	0.548	-1.8; **-0.2;** 2.1	0.574	-2.2; **-0.5;** 2.1	0.728	0.398
Fat free mass (%)	52.0; **56.0;** 59.0	52.0; **55.0;** 58.0	0.548	-2.1; **0.2;** 1.8	0.574	-2.1; **0.5;** 2.2	0.728	0.398
Energy intake (kJ/day)	5948; **7203;** 9090	5904; **7438;** 8936	0.877	-3099; **-927;** 300	**0.000**	-2661; **-836;** 237	**0.000**	0.988
Protein intake (g/day)	54.0; **65.0;** 78.2	57.7; **66.8;** 79.0	0.645	-18.1; **-6.3;** 4.4	**0.006**	-19.8; **-3.5;** 5.5	**0.007**	0.858
Carbohydrate intake (g/day)	167.0; **211.0;** 264.0	171.0; **212.0;** 277.0	0.884	-71.0; **-21.0;** 9.0	**0.000**	-73.0; **-30.0;** 12.0	**0.000**	0.630
Fat intake (g/day)	49.0; **63.0;** 85.0	51.0; **64.0;** 86.0	0.905	-29.9; **-8.6;** 2.3	**0.000**	-30.0; **-11.0;** 4.0	**0.000**	0.821
EI – restraint score	5.0; **9.0;** 14.0	7.0; **11.0;** 14.0	0.132	-1.0; **3.0;** 7.0	**0.000**	0.0; **3.0;** 6.0	**0.000**	0.930
EI – disinhibition score	3.0; **6.0;** 10.0	3.0; **6.0;** 9.0	0.807	-2.0; **0.0;** 1.0	0.991	-1.0; **0.0;** 2.0	0.753	0.833
EI – hunger score	2.0; **4.0;** 7.5	2.0; **4.0;** 7.0	0.356	-2.0; **-1.0;** 0.0	**0.000**	-2.0; **-1.0;** 1.0	0.113	0.111
BDI – depression score	6.0; **11.0;** 17.5	6.0; **10.0;** 16.0	0.818	-5.0; **-1.0;** 2.0	0.072	-4.0; **0.0;** 2.0	0.179	0.667

[a]Mann-Whitney test, [b]Wilcoxon test, BMI – body mass index, EI – Eating Inventory, BDI – Beck Depression Inventory

p=0.06) compared to T allele carriers. Higher baseline energy (p=0.009), protein (p=0.018) and fat (p=0.002) intakes in T allele non-carriers probably reflected the higher hunger score. On the other hand, Bouchard *et al.* (2004) found that TT homozygotes had significant higher susceptibility to hunger than P allele carriers. It should be taken into account that Bouchard *et al.* (2004), in contrast to our study, evaluated the items of the Eating Inventory in the whole cohort comprised of both genders while we conducted our evaluation in men and women separately. This our approach seems reasonable as significant gender differences in the items of the Eating Inventory have been demonstrated; restraint score being higher in women whereas disinhibition score and hunger score are higher in men (Hainer *et al.* 2006). However, if we evaluated overall changes at follow-up period we might support some of the conclusions of the study of Bouchard *et al.* (2004). T allele carriers seem to be disadvantaged in several aspects against non-carriers. In our cohort of obese males T allele non-carriers but not T allele carriers exhibited significant decreases in waist circumference and energy intake after the follow-up period. Observed concomitant increase in dietary restraint score and decrease in depression score in T allele non-carriers might contribute to reduction of energy intake and abdominal obesity assessed by waist circumference. The Québec Family Study, which followed subjects over a 6-year period, demonstrated the highest increase of body weight, BMI, body fat (expressed both as kg and %) and waist circumference in TT homozygotes when compared to other genotypes. In agreement with the results of Bouchard *et al.* (2004) it could be presumed that carrying the T allele is a disadvantage in terms of reduction of abdominal obesity and energy intake, however only in men.

In women, the baseline values for anthropometric measures, energy and macronutrient intakes, items of the Eating Inventory and Beck depression score did not differ between the T allele carriers and non-carriers. No significant differences in the maximum weight loss nor in the changes of anthropometric measures over follow-up period were shown between the female T allele carriers and non-carriers. However, a decrease in body weight and BMI was significant only in females who were T allele carriers and a decrease in hunger score achieved statistical significance in female T allele non-carriers. Inconsistency in the results obtained in men and women might be due to gender specific role of genes in the phenotypic manifestation of genotype. Such a gender specific role of genes as determinants of obesity development has recently been shown for perilipin gene (Qi *et al.* 2004) and for β_2- and β_3-adrenergic receptor genes (Ukkola *et al.* 2000, Corella *et al.* 2001).

Several differences in the character of the cohort (ethnicity, age, BMI) and design (duration of follow-up) of our study and in that of Bouchard *et al.* (2004) might explain different outcomes. We followed Czech overweight/obese subjects over 2.5-year period, whereas Bouchard *et al.* (2004) monitored a sample of genetically homogenous Québec adults over 6 years. Our subjects were on average 6.5 years older than those in the Bouchard´s cohort (49.2 vs. 42.7 years) (Bouchard *et al.* 2004). It has been demonstrated that the effect of genes, which contributes to adiposity changes is stronger in the younger than in older subjects (Bouchard *et al.* 2007). The role of gender and age was confirmed in investigations into the influence of the PPARGC1A G482S polymorphism on the risk of obesity (Ridderstrĺle *et al.* 2006).

Our pilot study demonstrated some gender specific associations of P73T polymorphism of *NMB* with eating behavior and weight changes at 2.5-year follow-up. The results should be confirmed on large groups of men.

Abbreviations

BBS Bardet-Biedl syndrome
BDI Beck Depression Inventory
BMI body mass index
DNA deoxyribonucleic acid
EI Eating Inventory
NMB neuromedin beta

Conflict of Interest

There is no conflict of interest.

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References

BEALES PL, WARNER AM, HITMAN GA, THAKKER R, FLINTER FA: Bardet-Biedl syndrome: a molecular and phenotypic study of 18 families. *J Med Genet* **34**: 92-98, 1997.

BECK AT, WARD CH, MENDELSON M, MOCK J, ERBAUGH J: An inventory for measuring depression. *Arch Gen Psychiatry* **4**: 561-571, 1961.

BIEDL A: A pair of sisters with adiposo-genital dystrophy (in German). *Dtsch Med Wochenschr* **48**: 1630, 1922.

BOUCHARD L, DRAPEAU V, PROVENCHER V, LEMIEUX S, CHAGNON Y, RICE T, RAO DC, VOHL MC, TREMBLAY A, BOUCHARD C, PÉRUSSE L: Neuromedin ß: a strong candidate gene linking eating behaviors and susceptibility to obesity. *Am J Clin Nutr* **80**: 1478-1486, 2004.

BOUCHARD L, TREMBLAY A, BOUCHARD C, PÉRUSSE L: Contribution of several candidate genes polymorphisms in the determination of adiposity changes: results from the Québec Family Study. *Int J Obes* **31**: 891-899, 2007.

BRUFORD EA, RIISE R, TEAGUE PW, PORTER K, THOMSON KL, MOORE AT, JAY M, WARBURG M, SCHINZEL A, TOMMERUP N, TORNQVIST K, ROSENBERG T, PATTON M, MANSFIELD DC, WRIGHT AF: Linkage mapping in 29 Bardet-Biedl syndrome families confirms loci in chromosomal regions 11q13, 15q22.3-q23, and 16q21. *Genomics* **41**: 93-99, 1997.

CORELLA D, GUILLÉN M, PORTOLÉS O, SORLÍ JV, ALONSO V, FOLCH J, SÁIZ C: Gender specific associations of the Trp64Arg mutation in the beta3-adrenergic receptor gene with obesity-related phenotypes in a Mediterranean population: interaction with a common lipoprotein lipase gene variation. *J Intern Med* **250**: 348-360, 2001.

HAINER V, KUNEŠOVÁ M, BELLISLE F, HILL M, BRA UNEROVÁ R, WAGENKNECHT M: Psychobehavioral and nutritional predictors of weight loss in obese women treated with sibutramine. *Int J Obes* **29**: 208-216, 2005.

HAINER V, KUNEŠOVÁ M, BELLISLE F, PA ŘÍZKOVÁ J, BRAUNEROVÁ R, WAGENKNECHT M, LAJKA J, HILL M, STUNKARD A: The Eating Inventory, body adiposity and prevalence of diseases in a quota sample of Czech adults. *Int J Obes* **30**: 830-836, 2006.

HAINER V, ZAMRAZILOVÁ H, SPÁLOVÁ J, HAINEROVÁ I, KUNEŠOVÁ M, ALDHOON B, BENDLOVÁ B: Role of hereditary factors in weight loss and its maintenance. *Physiol Res,* Suppl 1, 2008.

KRANE IM, NAYLOR SL, HELIN-DAVIS D, CHIN WW, SP INDEL ER: Molecular cloning of cDNAs encoding the human bombesin-like peptide neuromedin B. Chromosomal localization and comparison to cDNAs encoding its amphibian homolog ranatensin. *J Biol Chem* **263**: 13317-13323, 1988.

KABRNOVÁ-HLAVATÁ K, HAINER V, GOJOVÁ M, HLAVATÝ P, KOPSKÝ V, NEDVÍDKOVÁ J, KUNEŠOVÁ M, PAŘÍZKOVÁ J, WAGENKNECHT M, HILL M, DRBOHLAV J: Calcium intake and the outcome of short-term weight management. *Physiol Res* 2008 (In press)

OEFFNER F, BORNHOLDT D, ZIEGLER A, HINNEY A, GÖRG T, GERBER G, GOLDSCHMIDT HP, SIEGFRIED W, WRIGHT A, HEBEBRAND J, GRZESCHIK KH: Significant association between a silent polymorphism in the neuromedin B gene and body weight in German children and adolescents. *Acta Diabetol* **37**: 93-101, 2000.

OHKI-HAMAZAKI H: Neuromedin beta. *Prog Neurobiol* **62**: 297-312, 2000.

PROVENCHER V, PÉRUSSE L, DRAPEAU V, TREMBLAY A, DEPRÉS J, LEMIEUX S: Familial resemblence in eating behaviors in men and women from the Québec Family Study. *J Am Diet Assoc* **102** (suppl 2): A-35, 2003.

QI L, SHEN H, LARSON I, SCHAEFER EJ, GREENBERG AS, TREGOUET DA, CORELLA D, ORDOVAS JM: Gender-specific association of a perilipin gene haplotype with obesity risk in a white population. *Obes Res* **12**: 1758–1765, 2004.

RIDDERSTRĹLE M, JOHANSSON LE, RASTAM L, LINDBLAD U: Increased risk of obesity associated with the variant allele of the PPARGC1A Gly482Ser polymorphism in physically inactive elderly men. *Diabetologia* **49**: 496-500, 2006.

STUNKARD AJ, MESSICK S: The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *J Psychosom Res* **29**: 71-83, 1985.

UKKOLA O, RANKINEN T, WEISNAGEL SJ, SUN G, PÉRUSSE L, CHAGNON YC, DESPRÉS JP, BOUCHARD C: Interactions among the alpha2-, beta2-, and beta3-adrenergic receptor genes and obesity-related phenotypes in the Quebec Family Study. *Metabolism* **49**: 1063-1070, 2000.

VOGELS N, DIEPVENS K, WESTERTERP-PLANTENGA MS: Predictors of long-term weight maintenance. *Obes Res* **13**: 2162-2168, 2005.

WADDEN TA, BUTRYN ML, BYRNE KJ: Efficacy of life style modification for long-term weight control. *Obes Res* **12** (Suppl): 151S-162S, 2004.

WHO EXPERT COMMITTEE Physical status: The use and interpretation of anthropometry. Geneva: *WHO Technical Series* Report No. 854, Geneva, 1995, pp 452.

Physiol. Res. 57 (Suppl. 1): S57-S65, 2008

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

P. HLAVATÝ [1], M. KUNEŠOVÁ[1], M. GOJOVÁ[2], E. TVRZICKÁ[3], M. VECKA[3], P. ROUBAL[4], M. HILL[1], K. HLAVATÁ [1], P. KALOUSKOVÁ[1], V. HAINER[1], A. ŽÁK[3], J. DRBOHLAV[4]

[1]Obesity Management Centre, Institute of Endocrinology, Prague, [2]Priessnitzovy léčebné lázně, Jeseník, [3]Fourth Department of Internal Medicine, First Medical Faculty, Charles University, Prague, [4]Milcom Prague, Prague, Czech Republic

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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

Corresponding author

Marie Kunešová, Institute of Endocrinology, Národní třída 8, 116 94 Prague 1, Czech Republic. E-mail: mkunesova@endo.cz

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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References

AILHAUD G, MASSIERA F, WEILL P, LEGRAND P, ALESSANDRI JM, GUESNET P: Temporal changes in dietary fats: role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity. *Prog Lipid Res* **45**: 203-236, 2006.

BARRETT PHR, WATTS GF: Kinetic studies on lipoprotein metabolism in the metabolic syndrome including the effects of nutritional interventions. *Curr Opin Lipidol* **14**: 61-68, 2003.

BECK AT, WARD CH, MENDELSON M, MOCK J, ERBGAUGH J. An inventory for measuring depression. *Arch Gen Psychiatry* **4**: 561-571, 1961.

CLIFTON PM, NOAKES M, KEOGH JB: Very low-fat (12 %) and high monounsaturated fat (35 %) diets do not differentially affect abdominal fat loss in overweight, nondiabetic women. *J Nutr* **134**: 1741-1745, 2004.

COUET C, DELARUE J, RITZ P, ANTOINE JM, LAMISSE F: Effect of dietary fish oil on body fat mass and basal fat oxidation in healthy adults. *Int J Obes* **21**: 637-643, 1997.

CZECH STATISTICAL OFFICE: http://www.czso.cz/csu/2006 edicniplan.nsf/engt/34004EF06E/$File/3004rr_01.xls

FLACHS P, HORÁKOVÁ O, BRAUNER P, ROSSMEISL M, PECINA P, FRANSSEN-VAN HAL N, R ŮŽIČKOVÁ J, ŠPONAROVÁ J, DRAHOTA Z, VL ČEK C, KEIJER J, HOUŠTĚK J, KOPECKÝ J: Polyunsaturated fatty acids of marine origin upregulate mitochondrial biogenesis and induce beta-oxidation in white fat. *Diabetologia* **48**: 2365-2375, 2005.

GARAULET M, PÉREZ-LLAMAS F, PÉREZ-AYALA M, MARTÍNEZ P, SÁNCHEZ D E MEDINA F, TEBAR FJ, ZAMORA S: Site-specific differences in the fatty acid composition of abdominal adipose tissue in an obese population from a Mediterranean area: relation with dietary fatty acids, plasma lipid profile, serum insulin, and central obesity. *Amer J Clin Nutr* **74**: 585-591, 2001.

HAINER V, KUNEŠOVÁ M, BELLISLE F, HILL M, BRA UNEROVÁ R, WAGENKNECHT M: Psychobehavioral and nutritional predictors of weight loss in obese women treated with sibutramine. *Int J Obes* **29**: 208-216, 2005.

HIRAI A, HAMAZAKI T, TERANO T, NISHIKAWA T, TAMU RA Y, SAJIKI J: Eicosape ntaenoic acid and platelet function in Japanese (letter). *Lancet* **2**: 1132-1133, 1980.

HUBER J, LOFFLER M, BILBAN M, REIMERS M, KADL A, TODORIC J, ZEYDA M, GEYEREGGER R, SCHREIER M, WEICHHART T, LEI TINGER N, WALDHAUSL W, STULNIG TM: Prevention of high-fat diet-induced adipose tissue remodeling in obese diabetic mice by n-3 polyunsaturated fatty acids. *Int J Obes Relat Metab Disord* **31**: 1004-1013, 2007.

KARLSSON M, MĹRILD S, BRANDBERG J, LÖNN L, FRIBERG P, STRANDVIK B: Serum phospholipid fatty acids, adipose tissue, and metabolic markers in obese adolescents. *Obesity* **14**: 1931-1939, 2006.

KONTROVÁ K, ZÍDKOVÁ J, BARTOŠ B, SKOP V, SAJDOK J, KAZDOVÁ L, MIKULÍK K, MLEJNEK P, ZÍDEK V, PRAVENEC M: CD36 regulates fatty acid composition and sensitivity to insulin in 3T3-L1 adipocytes. *Physiol. Res.* **56**: 493-496, 2007.

KREBS JD, BROWNING LM, MCLEAN NK, ROTHWELL JL, MISHRA GD, MOORE CS, JEBB SA: Additive benefits of long-chain n-3 polyunsaturated fatty acids and weight-loss in the management of cardiovascular disease risk in overweight hyperinsulinaemic women. *Int J Obes Relat Metab Disord* **30**: 1535-1544, 2006.

KRIKETOS AD, ROBERTSON RM, SHARP TA, DROUGAS H, REED GW, STORLIEN LH, HILL JO: Role of weight loss and polyunsaturated fatty acids in improving metabolic fitness in moderately obese, moderately hypertensive subjects. *J Hypertens* **19**: 1745-54, 2001.

KUNEŠOVÁ M, PHINNEY S, HAINER V, TVRZICKÁ E, ŠTICH V, PA ŘÍZKOVÁ J, ŽÁK A, STUNKARD A: The responses of serum and adipose fatty acids to a one-year weight reduction regimen in female obese monozygotic twins. *Ann N Y Acad Sci* **967**: 311-323, 2002.

KUNEŠOVÁ M, BRAUNEROVÁ R, HLAVATÝ P, TVRZICKÁ E, STA ŇKOVÁ B, ŠKRHA J, HILGERTOVÁ J, HILL M, KOPECKÝ J, WAGENKNECHT M, HAINER V, MATOULEK M, PA ŘÍZKOVÁ J, ŽÁK A, SVAČINA S: 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. *Physiol Res* **55**: 63-72, 2006.

LOHMAN T, ROCHE A, MARTOREL R: (eds.) *Standardization of Anthropometric Measurements*. Human Kinetics Publishers, Champaign, IL. 1989.

MARSH JB, TOPPING DL, NESTEL PJ. Comparative effects of dietary fish oil and carbohydrate on plasma lipids and hepatic activities of phosphatidate phosphohydrolase, diacylglycerol acyltransferase and neutral lipase activities in the rat. *Biochim Biophys Acta* **922**: 239-243, 1987.

MCKENNEY JM, SICA D: Role of prescription omega-3 fatty acids in the treatment of hypertriglyceridemia. *Pharmacotherapy* **27**: 715-728, 2007.

METCALF RG, JAMES MJ, MANTZIORIS E, CLELAND LG: A practical approach to increasing intakes of n-3 polyunsaturated fatty acids: use of novel foods enriched with n-3 fats. *Eur J Clin Nutr* **57**: 1605-1612, 2003.

MORI TA, BAO DQ, BURKE V, PUDDEY IB, WATTS GF, BEILIN LJ: Dietary fish as a major component of a weight-loss diet: effect on serum lipids, glucose, and insulin metabolism in overweight hypertensive subjects. *Am J Clin Nutr* **70**: 817-825, 1999.

PACKIANATHAN I, SHEIKH M, BONIFACE D, FINER N: Predictors of program adherence and weight loss in women in an obesity program using meal replacements. *Diabetes Obes Metab* **7**: 439-447, 2005.

PHINNEY SD, TANG AB, JOHNSON SB, HOLMAN RT: Reduced adipose 18:3w3 with weight loss by very low calorie dieting. *Lipids* **25**: 798-806, l990.

PHINNEY SD, DAVIS PG, JOHNSON SB, HOLMAN RT: Obesity and weight loss alter serum polyunsaturated lipids in humans. *Am J Clin Nutr* **53**: 831-838, 1991.

RŮŽIČKOVÁ J, ROSSMEISL M, PRAŽÁK T, FLACHS P, ŠPONAROVÁ J, BRYHN M, KOPECKÝ J: Omega-3 polyunsaturated fatty acids of marine origin reduce dietary obesity in mice by affecting cellularity of adipose tissue. *Lipids* **39**: 1177-1185, 2004.

SANDERS TAB, LEWIS F, SLAUGHTER S, GRIFFIN BA, GRIFFIN M, DAVIES I, MILLWARD DJ, COOPER JA, MILLER GJ: Effect of varying the ratio of n-6 to n-3 fatty acids by increasing the dietary intake of α-linolenic acid, eicosapentaenoic and docosahexaenoic acid, or both on fibrinogen and clotting factors VII and XII in persons aged 45–70 y: the OPTILIP Study *Am J Clin Nutr* **84**: 513-522, 2006.

STUNKARD AJ, MESSICK S: The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *J Psychosom Res* **29**: 71-83, 1985.

SURETTE ME, WHELAN J, BROUGHTON KS, KINSELLA JE: Evidence for the mechanisms of the hypotriglyceridemic effect of n-3 polyunsaturated fatty acids. *Biochim Biophys Acta* **1126**: 199-205, 1992.

SZAPARY PO, RADER DJ. Pharmacological management of high triglycerides and low high density lipoprotein cholesterol. *Curr Opin Pharmacol* **1**: 113-120, 2001.

THORSDOTTIR I, TOMASSON H, GUNNARSDOTTIR I, GISLADOTTIR E, KIELY M, PARRA MD, BANDARRA NM, SCHAAFSMA G, MARTINÉZ JA: Randomized trial of weight-loss-diets for young adults varying in fish and fish oil content. *Int J Obes* **31**: 1560-1566, 2007.

TVRZICKÁ E, VECKA M, STAŇKOVÁ B, ŽÁK A: Analysis of fatty acids in plasma lipoproteins by gas chromatography-flame ionization detection. Quantitative aspects. *Anal Chim Acta* **465:** 337-350, 2002.

UKROPEC J, RESELAND JE, GAŠPERIKOVÁ D, DEMČÁKOVÁ E, MADSEN L, BERGE RK, RUSTAN AC, KLIMEŠ I, DREVON CA, ŠEBÖKOVÁ E: The hypotriglyceridemic effect of dietary n-3 FA is associated with increased beta-oxidation and reduced leptin expression. *Lipids* **38**: 1023-1029, 2003.

YOUNG GS, CONQUER JA, THOMAS R: Effect of randomized supplementation with high dose olive, flax or fish oil on serum phospholipid fatty acid levels in adults with attention deficit hyperactivity disorder. *Reprod Nutr Dev* **45**: 549–558, 2005.

ZOCK PL, MENSINK RP, HARRYVAN J, D E VRIES JH, KATAN MB: Fatty acids in serum cholesteryl esters as quantitative biomarkers of dietary intake in humans *Am J Epidemiol* **145**: 1114-1122, 1997.

Lifestyle intervention discloses an association of the Eating Inventory-51 factors with cardiometabolic health risks

Irena Aldhoon Hainerová[2], Hana Zamrazilová[1], Karolína Hlavatá[1], Milena Gojová[3], Marie Kunešová[1], Martin Hill[1], Jara Nedvídková[1], France Bellisle[4], Vojtěch Hainer[1]

[1]Institute of Endocrinology, Prague, Czech Republic; [2]Department of Paediatrics and Centre for Research of Diabetes, Metabolism and Nutrition, Third Faculty of Medicine, Charles University, Prague, Czech Republic; [3]Obesity Management Unit, Lázně Lipová, Czech Republic; [4]Department of social and preventive medicine, Laval University, Québec City, Canada

Running title: **Eating Inventory and cardiometabolic risk factors**

Corresponding author:
Irena Aldhoon Hainerová, M.D., Ph.D.
Department of Paediatrics and Centre for Research of Diabetes,
Metabolism and Nutrition
Third Faculty of Medicine, Charles University,
Šrobárova 50
100 34 Prague 10
Czech Republic
E-mail: ihainer@hotmail.com
Tel: +420 267 162 561
Fax: +420 272 736 326

Abstract

Factors of the Eating Inventory-51 (EI) were revealed as significant predictors of diseases characterizing the metabolic syndrome. Associations of EI factors (restraint – EI-R, disinhibition – EI-D, hunger – EI-H) with cardiometabolic risk parameters (blood pressure, lipid profile, fasting blood glucose (FBG), C peptide (CP), insulin) and hormones (leptin, adiponectin, resistin, peptide YY, pancreatic polypeptide, neuropeptide Y (NPY), ghrelin) were analysed both before and after an in-patient weight reduction programme. 67 women (age: 48.7 ± 12.2 y; BMI: 32.4 ± 4.4 kg/m^2), who exhibited stable weight on a 7 MJ/day diet during the 1st week, obtained a 4.5 MJ/day diet over the subsequent 3-week period. The weight reduction programme led to mean weight loss of 3.80 ± 1.64 kg. No significant relations of the EI factors with lipid, glucose and hormonal profiles were observed before the weight reduction. After weight loss, EI-R negatively correlated with total cholesterol (TC), FBG, CP, insulin and NPY. EI-H was positively related to insulin and NPY. EI-D correlated positively with TC, LDL cholesterol, triglyceride, NPY while negatively with adiponectin. We assume that an implementation of a standard dietary and lifestyle pattern for 3 weeks revealed significant associations between factors of the EI and metabolic risks in overweight/obese women.

Key words: obesity; Eating Inventory; metabolic risks; hormones

Introduction

The Eating Inventory (EI) assesses three dimensions of human eating attitudes: cognitive restraint (EI-R), disinhibition (EI-D) and hunger (EI-H).[1] The EI-R score describes the tendency to control food intake. The EI-D score refers to dysregulation of eating in response to emotional or cognitive cues. The EI-H score examines the subjective feeling of general hunger. It has been shown that a weight management leads to favourable changes in psycho-behavioural characteristics.[2,3,4,5,6,7,8] The cognitive EI-R score increases during an energy restriction programme, while the EI-D and EI-H scores decrease.

It is well recognized that human obesity, mainly central obesity with enlarged abdominal fat stores, is frequently accompanied by cardiometabolic health risks.[9] Relationships between the EI scores and morbidity have only been investigated in a few studies. Factors of the EI were identified as significant predictors of diseases characterizing the metabolic syndrome.[10] A study conducted in a quota sample of Czech adults showed an association of EI-R and EI-H scores with hypertension, diabetes, cardiovascular diseases and hyperlipidemia even after adjustment for body mass index (BMI) and age.[10] Further, EI-D score was positively and EI-R score negatively associated with BMI and waist circumference. Finally, high EI-D score was significantly associated with higher prevalence of hyperlipidemia and hypertension in both genders. In a study of severely obese subjects, lower dietary EI-R and higher EI-D and EI-H scores were associated with a poor health-related quality of life, greater depression and psychosocial dysfunction.[11] Straub et al. reported that type 2 diabetics with high EI-D score were prone to poorer glycaemic control.[12] On the other hand, in nonobese postmenopausal women dietary EI-R score did not correlate with any of the studied physiological, metabolic and health characteristics.[13] Hays et al. did not find any association of dietary EI-R with morbidity and only moderately increased risk for hypercholesterolemia with higher EI-D scores.[14] It was also demonstrated that EI-D had been associated with higher BMI, poorer success at weight loss, poor psychological health and lower self-esteem,[15] and a lower level of psychological well-being.[16] It was suggested that restrained eating has consequences on metabolic functions.[17]

It is well known that the cardiometabolic health risks are greatly influenced by the current diet and physical activity. Eating attitudes especially dietary disinhibition have been shown to be significantly genetically determined.[18,19] It is apparent that individually different diet and lifestyle patterns may mask associations between the eating attitudes and cardiometabolic health risks. We therefore hypothesized that the standardization of dietary and physical activity pattern may disclose relationships between the eating attitudes and health risks, which

were under baseline conditions hidden by the lifestyle influences. The aim of the study was to investigate potential association of the EI factors with cardiometabolic risk factors and selected hormones both before and after weight reduction.

Methods

Study subjects were recruited among women who participated in the 4-week lifestyle obesity management in the Lipová spa. All these patients had been referred to the spa treatment by obesity specialists. Sixty-seven women (age: 48.7 ± 12.2 y; BMI: 32.4 ± 4.4 kg/m^2) who exhibited stable weight on a 7 MJ/day diet during the 1st week of in-patient weight management programme obtained a hypocaloric diet providing 4.5 MJ/day (protein 26.0 %, fat 28.0 %, carbohydrate 46 %) over the subsequent 3-week period. The detailed programme, which also included supervised physical activity and cognitive behaviour therapy, has previously been described.[8] Subjects with endocrine disorders, type 2 diabetes or on current medication influencing body weight were excluded from the study. The following psychobehavioural and laboratory parameters were measured twice, once before and once after a 3-week weight management: psychobehavioural parameters - EI; biochemical parameters - fasting blood glucose (FBG), total cholesterol (TC), HDL-cholesterol, LDL-cholesterol, triglyceride and C-reactive protein (CRP); hormonal parameters - insulin, C peptide (CP), ghrelin, leptin, peptide YY (PYY), neuropetide Y (NPY), pancreatic polypeptide (PP), adiponectin and resistin. Before blood withdrawal, blood pressure was measured and anthropometric measurements (body weight, height, waist and hip circumferences) were carried out according to the WHO recommendations.[20] BMI and waist to hip ratio were calculated. Most patients were normotensive, only seven women presented hypertension, which was well controlled by antihypertensive drug treatment. Body composition was assessed by bioimpedance (BIA Tanita BC-418MA). The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Institute of Endocrinology in Prague. Before the study, each participant signed an informed consent form.

Data are presented as means ± SD. We used Wilcoxon's robust paired test to compare the data obtained before and after weight management. Spearman rank correlation coefficient was calculated to examine the association between psychobehavioural parameters and selected anthropometric, hormonal and biochemical parameters before and after weight management were calculated. Differences were considered significant at $p < 0.05$. Statgraphics Plus v. 5.1

from Manugistics (Rockville, MD, USA) and NCSS 2002 from Number Cruncher Statistical Systems (Kaysville, UT, USA) were used for data analysis.

Results and discussion

The changes in anthropometric, psychobehavioural, biochemical and hormonal characteristics are presented in Table 1. Weight management resulted in significant decreases in all studied anthropometric and body composition parameters. In response to 3-week weight management, the mean body weight decrease was 3.80 ± 1.64 kg. Weight loss was accompanied by a significant increase in EI-R whereas EI-H and EI-D exhibited a significant decrease. This finding is in accordance with previously published studies.[2,3,4,5,6,7,8] FBG, insulin, leptin and NPY levels significantly declined. No significant changes in the levels of the other determined hormones and lipid profile parameters were demonstrated. We assume that a lack of significant changes in lipid profile was due to the short duration of the weight reduction programme. Still there was a tendency toward improvement of these parameters.

Correlations between psychobehavioural factors, biochemical and hormonal parameters before and after the weight reduction, which achieved statistical significance (including BMI and waist circumference), are shown in Table 2. Baseline values of the EI factors did not correlate with hormonal or biochemical parameters at baseline. However, after weight loss, several correlations between EI factors and metabolic risk factors were revealed. EI-R significantly inversely correlated with TC, FBG, insulin, CP and NPY. EI- H was positively related to insulin and NPY. EI-D correlated positively with TC, LDL cholesterol, triglyceride, NPY concentrations and negatively with adiponectin levels. A correlation of EI-D with lipid profile is in line with findings of Hays[14] and Hainer[10]. Both before and after weight loss, EI-H negatively correlated with EI-R and positively with EI-D.

In contrast to the studies of Hainer[10] and Bryant[15], we did not find any significant association between the EI factors, and BMI and other anthropometric parameters except for borderline correlation of waist circumference with EI-R and EI-D. We suppose that the lack of such a correlation is due to the sample size and small BMI range of our cohort. The study of Hainer et al. was performed on a large quota sample of the Czech population with a broad BMI range and thus the association between EI factors and BMI could be demonstrated.[10]

The strength of our study is the strictly controlled in-patient weight management programme. However, this challenging aim led to limited recruitment of overweight/obese patients. The limited number of participants did not allow us to perform analyses concerning a prediction of outcome from the baseline data. In conclusion, the standardization of dietary and physical

activity pattern revealed associations between factors of the EI with several metabolic risk factors and hormones after a 3-week weight reduction programme.

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Conflict of interest

The authors declare no conflict of interest.

References

1 Stunkard AJ, Messick S. The Three-Factor Eating Questionnaire to measure dietary restraint, disinhibition and hunger. *J Psychosom Res* 1985; **29**: 71-83.

2 Bjorvell H, Aly A, Langius A, Nordstrom G. Indicators of changes in weight and eating behaviour in severely obese patients treated in a nursing behavioural program. *Int J Obes* 1994; **18**: 521-525.

3 Karlsson J, Hallgren P, Kral JG, Lindross AK, Sjostrom L, Sullivan M. Predictors and effects of long-term dieting on mental well-being and weight loss in obese women. *Appetite* 1994; **23**: 15-26.

4 Pekkarinen T, Takala I, Mustajoki P. Two year maintenance of weight loss after VLCD and behavioural therapy for obesity: Correlation to the scores of questionnaires measuring eating behaviour. *Int J Obes* 1996; **20**: 332-337.

5 Foster GD, Wadden TA, Swain RM, Stunkard AJ, Platte P, Vogt RA. The Eating Inventory in obese women: clinical correlates and relationship to weight loss. *Int J Obes Relat Metab Disord* 1998; **22**: 778-785.

6 Westerterp-Plantenga MS, Kempen KP, Saris WHM. Determinants of weight maintenance in women after diet-induced weight reduction. *Int J Obes Relat Metab Disord* 1998; **22**: 1–6.

7 Hainer V, Kunesova M, Bellisle F, Hill M, Braunerova R, Wagenknecht M. Psychobehavioral and nutritional predictors of weight loss in obese women treated with sibutramine. *Int J Obes (Lond)* 2005; **29**: 208-216.

8 Hainer V, Hlavatá K, Gojová M, Kunešová M, Wagenknecht M, Kopský V *et al.* Hormonal and psychobehavioral predictors of weight loss in response to a short-term weight reduction program in obese women. *Physiol Res* 2008; **57**(Suppl 1): S17-27.

9 Reaven GM. The metabolic syndrome: time to get off the merry-go-round? *J Intern Med* 2011; 269: 127-136.

10 Hainer V, Kunesova M, Bellisle F, Parizkova J, Braunerova R, Wagenknecht M, Lajka J, Hill M, Stunkard A. The Eating Inventory, body adiposity and prevalence of diseases in a quota sample of Czech adults. *Int J Obes (Lond)* 2006; **30**: 830-836.

11 Karlsson J, Sjöström L, Sullivan M. Swedish obese subjects (SOS)--an intervention study of obesity. Two-year follow-up of health-related quality of life (HRQL) and eating behavior after gastric surgery for severe obesity. *Int J Obes Relat Metab Disord* 1998; **22**: 113-126.

12 Straub RH, Lamparter-Lang R, Palitzsch KD, Schölmerich J. Association between eating behaviour and current glycaemic control, body mass or autonomic nervous function in long-term type I and type II diabetic patients. *Eur J Clin Invest* 1996; **26**: 564-568.

13 Bathalon GP, Hays NP, Meydani SN, Dawson-Hughes B, Schaefer EJ, Lipman R *et al.* Metabolic, psychological, and health correlates of dietary restraint in healthy postmenopausal women. *J Gerontol A Biol Sci Med Sci* 2001; **56**: 206-211.

14 Hays NP, Bathalon GP, Roubenoff R, Lipman R, Roberts SB. The association of eating behavior with risk for morbidity in older women. *J Gerontol A Biol Sci Med Sci* 2002; **57**: 128-133.

15 Bryant EJ, King NA, Blundell JE. Disinhibition: its effects on appetite and weight regulation. *Obes Rev* 2008; **9**: 409-419.

16 Provencher V, Bégin C, Piché ME, Bergeron J, Corneau L, Weisnagel SJ *et al.* Disinhibition, as assessed by the Three-Factor Eating Questionnaire, is inversely related to psychological well-being in postmenopausal women. *Int J Obes (Lond)* 2007; **31**: 315-320.

17 Wurmser H, Platte P, Laessle RG, Pirke KM. Resting metabolic rate and glucose induced thermogenesis in female in restrained eaters. In: Tuschen B, Florin I (eds). *Current Research in Eating Disorders*, Verlag für Psychotherapie, Münster, Germany, 1995, pp 36–47.

18 Steinle NI, Hsueh WC, Snitker S, Pollin TI, Sakul H, St Jean PL, Bell CJ, Mitchell BD, Shuldiner AR. Eating behavior in the Old Order Amish: heritability analysis and a genome-wide linkage analysis. *Am J Clin Nutr* 2002; **75**: 1098-1106.

19 Bouchard L, Drapeau V, Provencher V, Lemieux S, Chagnon Y, Rice T, Rao DC, Vohl MC, Tremblay A, Bouchard C, Pérusse L. Neuromedin beta: a strong candidate gene linking eating behaviors and susceptibility to obesity. *Am J Clin Nutr* 2004; **80**: 1478-1486.

20 WHO Expert Committee Physical status: The use and interpretation of anthropometry. *WHO Technical Series Report* 1995; **854**: 452.

Table 1 Anthropometric, psychobehavioural, biochemical and hormonal characteristics of participants (n = 69; mean ± SD)

Variable	Before	After	p-value
Weight (kg)	84.60 ± 12.91	80.78 ± 12.57	**0.000**
BMI (kg/m^2)	32.39 ± 4.51	30.92 ± 4.36	**0.000**
Waist circumference (cm)	98.83 ± 12.01	93.64 ± 11.52	**0.000**
Hip circumference (cm)	115.49 ± 9.28	112.12 ± 9.05	**0.000**
WHR	0.86 ± 0.07	0.83 ± 0.07	**0.000**
Total fat (%)	41.65 ± 5.74	38.98 ± 6.17	**0.000**
BP systolic (Hg mm)	124.03 ± 14.80	121.97 ± 14.57	0,340
BP diastolic (Hg mm)	77.65 ± 10.0	77.5 ± 7.91	0,990
EI - restraint score	10.03 ± 4.57	12.94 ± 4.60	**0.000**
EI – disinhibition score	6.61 ± 3.00	4.86 ± 2.72	**0.000**
EI - hunger score	4.08 ± 3.31	2.83 ± 2.75	**0.001**
T-C (mmol/l)	5.92 ± 7.95	4.87 ± 0.84	0.170
HDL-C (mmol/l)	1.42 ± 0.76	1.37 ± 0.34	0.906
LDL-C (mmol/l)	3.60 ± 1.03	3.49 ± 1.05	0.174
Triglycerides (nmol/l)	1.40 ± 0.90	1.25 ± 0.53	0.072
C-peptide (nmol/)	0.92 ± 0.35	0.93 ± 0.31	0.578
Blood glucose (mmol/l)	5.12 ± 1.58	4.83 ± 1.26	**0.046**
Insulin (mIU/l)	8.37 ± 4.49	7.83 ± 4.85	**0.048**
Adiponectin (mg/l)	11.36 ± 5.35	11.30 ± 4.88	0.484
CRP (mg/l)	5.04 ± 4.29	4.45 ± 4.76	0.095
Ghrelin (ng/l)	1158.96 ± 411.94	1168.56 ± 423.21	0.141
Leptin (ug/l)	21.21 ± 9.05	15.36 ± 7.18	**0.000**
PYY (ng/l)	196.28 ± 85.39	212.57 ± 98.02	0.126
NPY (nmol/l)	101.84 ± 52.71	84.14 ± 41.40	**0.000**
Resistin (ug/l)	2.58 ± 0.72	2.46 ± 0.78	0.308
PP (ng/l)	47.14 ± 38.35	44.31 ± 39.95	0.501

BP, blood pressure; BMI, body mass index; CRP, C-reactive protein; EI, Eating Inventory; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NPY, neuropeptide Y; PP, pancreatic polypeptide; PYY, peptide YY; T-C, total cholesterol; WHR, waist to hip ratio.

Table 2 Spearman's correlation of the Eating Inventory factors with selected anthropometric, biochemical and hormonal parameters before and after weight loss in a cohort of overweight/obese women (n = 69)

		Before			**After**		
		Eating Inventory			Eating Inventory		
Variable		restraint score	disinhibition score	hunger score	restraint score	disinhibition score	hunger score
BMI (kg/m^2)	r	-0.146	0.193	0.103	-0.108	0.164	0.158
	p	0.241	0.120	0.411	0.387	0.188	0.204
Waist circumference (cm)	r	-0.235	0.131	0.164	-0.212	0.207	0.167
	p	0.057	0.296	0.189	0.087	0.096	0.181
EI - restraint score	r	1.000	-0.191	**-0.260**	1.000	-0.095	**-0.323**
	p	---	0.125	**0.035**	---	0.446	**0.008**
EI - disinhibition score	r	-0.191	1.000	**0.558**	-0.095	1.000	**0.455**
	p	0.125	---	**0.000**	0.446	---	**0.000**
EI - hunger score	r	**-0.260**	**0.558**	1.000	**-0.323**	**0.455**	1.000
	p	**0.035**	**0.000**	---	**0.008**	**0.000**	---
T-C (mmol/l)	r	0.098	-0.023	-0.030	**-0.256**	**0.284**	0.128
	p	0.435	0.854	0.813	**0.038**	**0.021**	0.304
LDL-C (mmol/l)	r	0.128	-0.076	-0.084	-0.201	**0.257**	0.039
	p	0.306	0.546	0.501	0.106	**0.037**	0.755
Triglycerides (nmol/l)	r	0.098	-0.023	-0.030	-0.087	**0.265**	0.163
	p	0.435	0.854	0.813	0.487	**0.032**	0.191
C-peptide (nmol/l)	r	-0.073	-0.060	0.082	**-0.268**	0.155	0.205
	p	0.562	0.633	0.511	**0.030**	0.213	0.099
Blood glucose (mmol/l)	r	0.103	-0.108	-0.046	**-0.327**	0.164	0.126
	p	0.412	0.390	0.715	**0.007**	0.189	0.314
Insulin (mU/l)	r	0.040	-0.021	0.059	**-0.336**	0.163	**0.256**
	p	0.748	0.868	0.637	**0.006**	0.190	**0.038**
Adiponectin (mg/l)	r	0.080	-0.121	-0.020	0.057	**-0.335**	-0.060
	p	0.521	0.332	0.871	0.650	**0.006**	0.630
NPY (nmol/l)	r	0.010	-0.085	0.036	**-0.241**	**0.274**	**0.289**
	p	0.938	0.498	0.776	**0.051**	**0.026**	**0.019**

BMI, Body Mass Index; EI, Eating Inventory; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NPY, neuropeptide Y; T-C, total cholesterol. Significant correlations are in bold.