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CONTENTS

1. SPECIFIC AIMS OF THE WORK

i) To assess the role of genetic factors in the control of serum levels of steroid hormones (i.e. hormonal markers of body fat distribution) both before and after the treatment of obesity by VLCD and to evaluate the changes in the hormones in response to the weight reduction;

ii) To assess the role of parental obesity in determination of RQ and to determine the role of RQ in susceptibility to weight regain;

iii) To evaluate the effect of starvation on metabolic characteristics of epididymal and subcutaneous adipose tissue in mice and to examine whether AMPK and UCP2 could be involved in the different responses of these fat depots to starvation;

iv) To assess the effect of n-3 PUFA on body weight reduction, fat distribution, and serum fatty acid composition during VLCD;

v) To evaluate the influence of vitamin A on body weight and energy metabolism;

vi) To investigate whether the increase in vitamin A intake could affect energy metabolism, substrate oxidation and expression of selected genes of energy and lipid metabolism in adipose tissue of obese patients;

vii) To evaluate the association between calcium consumption and body weight changes;

viii) To verify whether psychobehavioral and nutritional markers could be used as predictors of body weight change during a comprehensive weight reduction program including pharmacotherapy with sibutramine.

2. METHODS

Note: Only those methods which were closely related to my personal work are described in this chapter. For detailed description of all methods used in the projects see enclosed publications.

Human subjects

All studies were approved by the Ethics Committee of the General Faculty Hospital and the First Faculty of Medicine, Charles University, Prague. Protocols of the studies were fully explained to the subjects and their informed voluntary consent was obtained.

Subjects were recruited from outpatients who were referred to the Obesity Management Centre or from inpatients hospitalized in 3rd and 4th Departments of Internal Medicine of the General Faculty Hospital, Prague. Before the beginning of the studies, a comprehensive examination was performed, which included medical history, physical examination, routine blood and urine tests and an electrocardiogram. In study 1, zygosity of identical twins was determined by history, physical appearance, and identity of blood groups, red cell antigens, HLA-antigen system of A, B and C loci, and apolipoprotein B 3 hypervariable region.

Weight reduction regimen

Inpatients (studies 1, 2, 4)

Subjects received VLCD *Redita*® (Promil, Nový Bydžov, Czech Republic) providing 1.6 MJ/day with 37 g of protein, 50 g of carbohydrate and 3.8 g of fat (studies 1, 2) or 2.2 MJ/day with 40 g of protein, 70 g of carbohydrate and 9 g of fat (study 4). The weight management included also daily physical activity of light to moderate intensity.

Outpatients (studies 5-8)

Patients were prescribed a 5-6 MJ/day diet (study 8) or a diet with 500 kcal energy deficit related to calculated total energy expenditure (studies 5-7), with approximately 50-60 % carbohydrate, 15-20 % protein, and 25-30 % fat. They were advised to divide their daily intake over 4-5 meals. Before the beginning of the studies 5 and 6, patients followed the dietary recommendations for at least 6 months in order to achieve long-term weight maintenance. Patients were instructed to increase their daily physical activity by at least 30 minutes walking, 5 days a week.

Patients regularly recorded their dietary intake and had monthly check-ups by a dietician. The average daily intakes of energy, macronutrients and selected micronutrients were calculated using the software "*Nutrition*", which covers about 2500 food items common in the Czech Republic. One-week dietary records were analyzed. The food quotient (FQ), which is the theoretical RQ produced by the diet,

was calculated from macronutrient intake expressed in percent, according to the formula described by Toubro [1]:

FQ = [0.207 * carbohydrate (%) + 0.159 * fat (%) + 0.193 * protein (%) + 0.137 * alcohol (%)] / [0.207 * carbohydrate (%) + 0.226 * fat (%) + 0.243 * protein (%) + 0.206 * alcohol (%)].

Body composition and regional tissue distribution

Body weight, body fat mass and fat-free mass were assessed using bipedal bioimpedance method (Tanita Body Fat Analyzer TBF-105, Tanita, Tokyo, Japan). Total body energy was estimated from energy content of body fat (39.3 kJ per g) and fat-free mass (4.3 kJ per g). Anthropometric measurements were conducted using flexible tape measures, Best's caliper and pelvimeter according to a standardized procedure recommended at the Airlie Conference [2]. They included ten skinfolds, waist and hip circumference and sagittal abdominal diameter at the L4/5 level.

Markers of energy metabolism

In order to determine the resting metabolic rate and respiratory quotient, open-circuit indirect calorimetry (Deltatrac®, Datex, Helsinki, Finland) under basal conditions was used. The measurement was performed for 30 min. in recumbent position after a 16-hour overnight fast. Patients were asked to avoid smoking, caffeine intake and vigorous physical activity 24 hours prior to indirect calorimetry. The mean intraindividual coefficient of variation from repeated measurements was 3.0 % for RMR.

Human tissues

Samples of subcutaneous adipose tissue from the abdominal region were obtained by needle biopsy under local anesthesia (1% Mesocain) after an overnight fast (study 6). Tissues samples were preserved using *RNAlater*™ (Ambion, Austin, TX, USA) and stored at -70°C.

Animals

The study protocols were approved by the Animal Care and Use Committee at the Institute of Physiology.

3-4 month-old male C57BL/6J mice were housed in 20°C with 12 h light-dark cycle, 5 mice per cage, with free access to water and standard chow diet. Two experimental protocols were used: A, all mice were caged singly for one week at 20°C and than sacrificed between 9:00-10:00 a.m., while some of these mice were denied access to food for 6, 12, and 24 h before the sacrifice, respectively, and controls were allowed free access to food. B, mice were caged singly for two weeks either at 20°C or at 30°C and at 5:30 p.m. of the day of the experiment, mice were denied food or not and both groups were sacrificed 6 h later under dimmed red light. In both protocols, mice were sacrificed by cervical dislocation and

pididymal and subcutaneous dorsolumbar WAT depots were dissected. The tissue amples were stored in liquid nitrogen. Blood was collected by tail bleeds just ıefore sacrifice.

RNA analysis

Total RNA was isolated using TRIzol Reagent (Invitrogen, Carlsbad, CA). RNA was treated with RNase-free DNase. Gene expression was analyzed by everse transcription followed by real-time quantitative polymerase chain reaction PCR) (LightCycler Instrument, F. Hoffman-La Roche Ltd., Basel, Switzerland) vith primers specific for sterol regulatory element-binding protein-1 (SREBP-1), JCP2, fatty acid synthase (FAS), and phosphoenolpyruvate carboxykinase PEPCK). Levels of β-actin mRNA were used to correct for inter-sample 'ariations.

Statistics

Statistical significance of differences between groups was evaluated using -tests, Wilcoxon Signed Rank Test, or analysis of variance (ANOVA). Multiple egression analysis was applied in studies 2, 4, and 8. Correlation coefficients were :alculated in studies 1, 4, and 7. The level of significance of all tests was set at $^{>} = 0.05$.

3. RESULTS

This thesis is based on the following articles, referred to in text by their Roman numerals as indicated here:

I. Hainer, V., Kunešová, M., Stunkard, A.J., Pařízková, J., Štich, V., Mikulová, R., and Stárka, L. (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

II. Hainer, V., Kunešová, M., Pařízková, J., Štich, V., Mikulová, R., and Slabá, Š. (2000) Respiratory quotient in obesity: Its association with an ability to retain weight loss and with parental obesity. *Sb. Lek.* 101, 99-104

III. Šponarová, J., Mustard, K.J., Horáková, O., Flachs, P., Rossmeisl, M., Brauner, P., Bardová, K., Thomason-Hughes, M., Braunerová, R., Janovská, P., Hardie, D.G., and Kopecký, J. (2005) Involvement of AMP-activated protein kinase in fat depot-specific metabolic changes during starvation. *FEBS Lett.* 579, 6105-6110

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V. Mikulová-Braunerová, R., Hainer, V., Kunešová, M., Pařízková, J., Slabá, Š, and Wagenknecht, M. (2003) Influence of vitamin A consumption on resting metabolic rate and fasting respiratory quotient in severely obese subjects. *Med. Princ. Pract.* 12, 189-192

VI. Hainer, V., Kunešová, M., Bellisle, F., Hill, M., Braunerová, R., Wagenknecht, M., and STO Study Group (2005) Psychobehavioral and nutritional predictors of weight loss in obese women treated with sibutramine. *Int. J. Obes. (Lond)* 29, 208-216

Results of two studies presented as posters at 12th European Congress on Obesity, 2003, Helsinki and 13th European Congress on Obesity, 2004, Prague are also included (chapters 3.6 and 3.7).

3.1. The within-pair resemblance in serum levels of androgens, sex hormone-binding globulin and cortisol in female obese identical twins - effects of negative energy balance induced by very low calorie diet

Cortisol and androgens are known to play a role in the regulation of body fat distribution in women [3]. Therefore, changes in steroid hormones secretion could partly mediate the positive effects of negative energy balance on body fat distribution. The first aim of this study was to assess the changes in serum levels of steroids and sex hormone-binding globulin (SHBG) caused by weight reduction, which was induced by treatment with a very low-calorie diet (VLCD). The second aim of the study was to reveal any within-pair correlations in the serum levels of steroid hormones and SHBG before and after the treatment of obesity, as well as in the changes in their serum levels in response to VLCD (**aim i**).

Twelve pairs of obese female monozygotic twins (age 39.4 ± 7.4 years, body mass index (BMI) 34.9 ± 8.2 kg/m^2, weight 96.2 ± 21.9 kg) stayed for 40 days on an inpatient metabolic unit. The study consisted of three parts: 7 days for adaptation to the hospital and for baseline measurements, 28 days of weight reduction regimen and 5 days for final examination after weight reduction. Weight reduction regimen was based mainly on a very low-calorie diet Redita® (see Methods). During the first and third parts of the study, the following measurements were performed: Anthropometry and hydrodensitometry for assessment of body composition, and ultrasonography for evaluation of intraabdominal fat. Blood samples were taken to determine serum levels of cortisol, testosterone, dehydroepiandrosterone (DHEA), dehydroepiandrosteron sulphate (DHEA-S), and SHBG.

Significant reduction of all anthropometric and body composition characteristics was observed after VLCD treatment of obesity (Paper I, Table 1). In response to the negative energy balance, serum levels of testosterone and SHBG were increased, whereas serum levels of cortisol, DHEA and DHEA-S did not change significantly. Significant within-pair resemblances before and after the treatment were observed in serum levels of DHEA-S and testosterone; in cortisol at 7 a.m. (before the treatment) and in cortisol at 1 p.m. (after the treatment; Paper I, Table 2). As for the changes of the measured hormone levels, only changes in serum cortisol at 7 a.m., 1 p.m. and 9 p.m., and SHBG exhibited significant within-pair resemblance (Paper I, Table 3, Figures 1 and 2). With the exception of testosterone, no significant associations were found between the changes in serum levels of steroids and SHBG, and changes in body composition and fat distribution. In the case of testosterone, a significant correlation was observed between the increase in the serum level of the hormone and the decrease in fat free mass.

In conclusion, significant within-pair resemblance in serum androgens and cortisol might suggest an important influence of hereditary factors in the control of their serum levels. In contrast, genotype plays probably a minor role in the regulation of SHBG level, because no within-pair correlation was observed in SHBG baseline concentrations.

3.2. Respiratory quotient in obesity: its association with an ability to retain weight loss and with parental obesity

A high respiratory quotient (RQ) is associated with a susceptibility to weight gain in obese and postobese subjects [4]. The first aim of this study was to reveal the role of the degree of fat oxidation (expressed as respiratory quotient) in weight regain. The second aim of our study was to evaluate the effect of hereditary factors on the ability to oxidize fat (**aim ii**).

Obese subjects were treated by VLCD (see Methods) for 4 weeks. During the 2nd, 3rd and 4th week of this initial phase, resting metabolic rate (RMR) and fasting RQ were assessed by indirect calorimetry. Average values of these measurements were calculated. Subjects were classified according to their weight changes during the next two-year follow-up into four groups: Weight regainers, weight losers, weight cyclers (exhibited two or more cycles of weight loss >10kg with subsequent weight regain), and weight non-cyclers (without these cycles). During the treatment with VLCD, a significantly higher RQ was observed in weight regainers vs. weight losers and in weight cyclers vs. weight non-cyclers. Therefore, a high fasting RQ could represent a metabolic abnormality which predicts weight regain and weight cycling. On the contrary, no significant differences in RMR (expressed per kg fat free mass) among the four groups were found (Paper II, Table 3).

In the second part of the study, which was focused on genetic background of obesity, obese patients were divided into four groups according to the parental history of obesity: Mother obese, father obese, both parents obese, and none of them obese. There were no significant differences in the average age, body weight and BMI between the groups. RMR and fasting RQ were measured by indirect calorimetry. Daily intakes of energy and macronutrients were calculated from one-week dietary intake record. Body composition was assessed by bipedal bioimpedance method (see Methods). No significant differences in RMR (expressed per kg fat free mass) between the groups were revealed. On the other hand, a significantly higher fasting RQ was observed in subjects with obese mother or both parents, when compared with subjects without parental history of obesity (Paper II, Table 4). Patients from the group with obesity in both parents were 3.3-times more frequently classified as low fat oxidizers than as high fat oxidizers. These results suggest a role of hereditary factors in the ability to oxidize fat in severely obese subjects.

3.3. Involvement of AMP-activated protein kinase in fat depot-specific metabolic changes during starvation

Metabolic properties of white adipose tissue (WAT) depend on its anatomical

localization in the body, and health consequences of obesity are also affected by the site of fat accumulation [5]. However, mechanisms controlling the fat depot-specific differences in WAT metabolism are poorly understood. In this study, we focused on comparison of the responses of dorsolumbar and epididymal fat depots to starvation. The aim of the study was to investigate whether AMP-activated protein kinase (AMPK) could contribute to these differences (**aim iii**).

Three to four month-old male C57BL/6J mice were used (see Methods). Just before sacrifice, blood was collected by tail bleeds for estimation of plasma concentrations of non-esterified fatty acids (NEFA). Mice were killed by cervical dislocation, and dorsolumbar and epididymal fat depots were dissected. Fragments of the tissues were used for measurements of fatty acid (FA) oxidation and synthesis. Levels of uncoupling protein 2 (UCP2), sterol regulatory element-binding protein (SREBP-1), fatty acid synthase (FAS), and phosphoenolpyruvate carboxykinase (PEPCK) transcripts were quantified using real-time RT-PCR. Evaluation of kinase activity of α1 isoform of AMPK (α1-AMPK) and quantification of tissue lipids were performed in specific tissue extracts.

Starvation resulted in a progressive loss of body weight and reduction of the dorsolumbar (subcutaneous) fat weight. In agreement with the supposed increase in lipolysis within the adipose tissue, a rise in plasma NEFA was observed. Particularly in the epididymal fat depot, a decrease in SREBP-1 and FAS expression and increase in UCP2 and PEPCK expression, as well as drop in FA synthesis and rise in FA oxidation were detected. Likewise in the epididymal fat, a significant stimulation of α1-AMPK activity was revealed.

In this study, we confirmed the hypothesis that starvation results in differential responses of dorsolumbar and epididymal fat depots. Metabolic changes were more pronounced in the epididymal fat, whereas weight loss in the dorsolumbar fat. Changes in the epididymal fat were accompanied by corresponding activation of AMPK. Control of lipid and glucose metabolism in WAT by AMPK may thus contribute to regional differences in the metabolic properties of white adipose tissue depots and consequently to the development of adverse health consequences of obesity. Observed increase in UCP2 expression might be both cause and result of AMPK activation.

3.4. 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

It was demonstrated that long chain n-3 polyunsaturated fatty acids (PUFA) enhanced fatty acid oxidation in healthy humans [6], and led to reduction of adipose tissue accumulation in rats fed high-fat diet [7]. The aim of this study was to assess the influence of n-3 PUFA added to VLCD in comparison with VLCD

only on weight loss and composition of fatty acids during three-week weight reduction regimen (**aim iv**).

20 severely obese women participated in the study. They were randomly divided into two groups treated with either VLCD and n-3 PUFA supplement (n-3VLCD) or VLCD and placebo (VLCD). The groups did not differ significantly in basal BMI and age (Paper IV, Table 1). The study began with one-week eucaloric outpatient stabilization, which was followed by three-week weight reduction period. During this period, patients received VLCD Redita® (see Methods) and n-3 highly unsaturated fatty acids of fish origin in a dose of 2.8 g/day (contained eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids in the ratio of 2:1, and 0.9 mg vitamin E per 500 mg capsule to prevent fatty acid oxidation), or VLCD and placebo (saline solution). Daily moderate physical activity was included in the program. Blood samples were obtained at baseline and on the day 3, 7 and 21. Serum fatty acid analysis was carried out at baseline and on the day 21. Body composition and regional tissue distribution were assessed (see Methods). Fatty acid composition of serum lipid fraction was determined by gas chromatography.

BMI and hip circumference decreases were significantly higher in the n-3VLCD group than in the VLCD group (Paper IV, Table 1). Also β-hydroxybutyrate increase was significantly higher in the n-3VLCD group, standing for a higher ketogenesis (Paper IV, Figure 1). A strong positive correlation between the increase of arachidonic acid in serum phospholipids (PL), triglycerides (TG) and cholesterol esters (CE), and the change of β-hydroxybutyrate was found (Paper IV, Figure 2D). The PL DHA elevation significantly correlated with the BMI decrease (Paper IV, Figure 3B).

The main finding of this study is that the addition of n-3 PUFA of fish origin to VLCD caused a significantly higher decrease of BMI and hip circumference. The secondary finding is a significantly higher increase of serum β-hydroxybutyrate after n-3 PUFA treatment. The stimulation of ketogenesis is probably a result of a higher fatty acid oxidation. The significant correlation between BMI loss and change in PL DHA suggests that DHA is the active substance of the n-3 PUFA supplement.

3.5. Influence of vitamin A consumption on resting metabolic rate and fasting respiratory quotient in severely obese subjects

Retinoids are implicated in the regulation of fat stores by influencing adipocyte differentiation, thermogenesis and fat oxidation. Because of their potential role in energy metabolism, retinoids could be used in the treatment of obesity. For this reason, we aimed to investigate whether the amount of vitamin A could influence RMR and fat oxidation at rest (evaluated by RQ) in severely obese patients (**aim v**).

239 obese patients participated in the study (age 42.9 ± 12.0 years, weight 110.2 ± 22.7 kg and BMI 39.1 ± 7.5 kg/m^2). Body composition was assessed using bipedal bioimpedance method and anthropometry. RMR and RQ were determined

by indirect calorimetry. Average daily intakes of energy, vitamin A and macronutrients were calculated from one-week dietary records. Food quotient was determined from macronutrient intake expressed in percent (see Methods).

The patients were divided into quintiles according to their vitamin A consumption. Average daily intake of vitamin A in the whole cohort was 670 ± 430 IU; in the upper quintile ("vitamin A high consumers") >842 IU and in the lower quintile ("vitamin A low consumers") <382 IU. RMR and fasting RQ of high and low vitamin A consumers did not differ significantly. Body weight, BMI, body fat, waist circumference and FQ were also similar in both groups. A significantly higher energy intake was observed in high consumers when compared with low consumers of vitamin A, due to a higher intake of carbohydrates and proteins.

In conclusion, we did not confirm the hypothesis that vitamin A influenced resting metabolic rate or fasting respiratory quotient in obese patients. But although the high consumers of vitamin A exhibited a significantly higher energy intake, they had no higher BMI or body weight.

3.6. The effect of vitamin A on energy metabolism and the expression of UCP2 and FAS genes

This study complemented testing of the hypothesis that retinoids could affect energy metabolism. However, this study involved obese patients with low vitamin A intake, which were then supplemented with vitamin A. The aim was to determine the influence of increased vitamin A consumption on RMR, RQ, and expression of selected genes of energy and lipid metabolism (UCP2 and FAS) (**aim vi**). FAS was chosen because of its key role in fatty acids synthesis, UCP2 for its association with body fat mass and resting energy expenditure.

Subjects consisted of 9 obese women (age 39.8 ± 4.6, weight 99 ± 4.9 and BMI 36 ± 1.7) with decreased intake of vitamin A (<500 I.U./day) in their dietary records. Patients were administered retinol in a dose of 30 000 IU/day for 4 weeks. All examinations were performed both at the beginning and at the end of the study. They included bipedal bioimpedance, anthropometry, and indirect calorimetry (see Methods). Vitamin A and vitamin E levels were measured using HPLC. An average daily intake of energy, fat, carbohydrate, protein and vitamin A were calculated from one-week dietary records. Subcutaneous adipose tissue samples were obtained by needle biopsy, and UCP2 and FAS expression determined by real time RT-PCR.

After four-week administration of vitamin A, RMR expressed per kilogram FFM was significantly increased. UCP2 expression tended to increase and FAS expression tended to decrease, but the results were not statistically significant. No effect of increased vitamin A consumption on RQ, and retinol and γ-tocoferol serum levels was found.

To conclude, supplementation of vitamin A increased RMR expressed per kilogram FFM, but did not affect RQ or UCP2 and FAS gene expression in obese women.

3.7. Association of changes in calcium and macronutrient intakes with body weight change in obese subjects

The influence of calcium intake on the regulation of body weight was demonstrated in many animal studies [8]. On the other hand, results of human studies on the relationship between dietary calcium and body weight are not so clear. The aim of this study was to evaluate the association between body weight change and changes in consumption of macronutrients, calcium and phosphorus in response to the weight reduction regimen (**aim vii**).

A total of 208 obese subjects (BMI 40.0 ± 7.6 kg/m^2, body weight 112.8 ± 25.6 kg, age 46.7 ± 12.3) participated in the comprehensive weight reduction program. They were surveyed for 3-6 months. The program consisted of a diet with 500-kcal daily deficit (according to calculated total energy expenditure), moderate physical activity (walking) in duration of around 30 minutes/day, and monthly check-ups by dietician. The patients regularly recorded their food intake. At the beginning and at the end of the program, one-week dietary records were analyzed and nutrient intake was calculated (see Methods).

The weight reduction regimen caused a significant weight loss as well as decrease in energy, all three types of macronutrient, and phosphorus intake. Intake of calcium at the end of the regime also tended to be lower than at the beginning, but the result was not statistically significant. Body weight change negatively correlated with the change in calcium and protein intake. A positive correlation was found between the change in body weight and the change in consumption of fat and phosphorus. No significant correlation was found between the change in carbohydrate intake and body weight change.

In conclusion, changes in calcium and protein intake in the cohort of 208 obese subjects were negatively related to the body weight change. On the contrary, changes in fat and phosphorus consumption positively correlated with the change in body weight.

3.8. Psychobehavioral and nutritional predictors of weight loss in obese women treated with sibutramine

The amount of weight loss depends not only on genetic and metabolic characteristics, but also on psychobehavioral and nutritional ones. Psychobehavioral factors play an important role in the adherence to weight control strategies and thus influence weight loss maintenance. The aim of this study was to evaluate whether baseline BMI, and psychobehavioral (depression score, dietary restraint, disinhibition, and perceived hunger) and nutritional markers (energy and

macronutrient intakes) were significant predictors of weight loss observed after 4 and/or 12 months of a weight reduction program (**aim viii**). This program consisted of low-energy diet, increased physical activity, cognitive behavioral therapy, and sibutramine.

In total, 80 obese women (age 43.9 ± 10.6 years, BMI 36.7 ± 4.8 kg/m^2) participated in the study. First phase was double-blinded, placebo-controlled. After randomization, 38 patients received sibutramine (10mg/day) and 42 received placebo for 4 months. After this phase, an open period continued until month 12 during which sibutramine was administered to all subjects. Low energy diet (5-6 MJ/day, see Methods) was recommended to all patients. They were also advised to increase their daily physical activity by at least 30 min walking, 5 days a week.

At baseline and at months 4 and 12 patients filled out the Eating Inventory (EI) and Beck Depression Inventory (BDI). At the same time, one-week dietary records were analyzed (see Methods). Anthropometry, blood pressure and pulse rate was evaluated every month, as well as occurrence of side effects. Blood samples were obtained and ECG was recorded at screening, baseline, and at months 4, 8 and 12.

Body weight was significantly decreased at month 4 in both groups (Paper VI, Figure 1). Nevertheless, weight loss in the sibutramine group was significantly higher than in the placebo group (9.0 versus 4.9 kg). At month 12, a significant weight loss in comparison with baseline was observed in both groups. Significant changes in BDI score and in all EI parameters were demonstrated at month 4 in sibutramine group (Paper VI, Figure 2). Restraint was increased, whereas disinhibition and hunger were decreased. Between the beginning and the end of the study, the mean energy intake decreased by 23 %, that is by 1729 kJ (from 7530 to 5801 kJ/day). Multiple regression analysis demonstrated that BMI loss at month 4 was significantly influenced by mode of treatment and baseline BMI (Paper VI, Table 1). Predictors of weight loss at month 12 were initial BMI, depression score, dietary restraint and energy intake, which accounted for 43.8 % of the variance in the change in BMI (Paper VI, Table 2). On the other hand, the only factor changed in relation with the changes in BMI was the score of disinhibition.

Based on this study, psychobehavioral and nutritional characteristics could be used as predictors of weight loss in response to a comprehensive weight management program including treatment with sibutramine.

4. CONCLUSIONS

i) A significant within-twin-pair resemblance in serum androgen and cortisol levels was demonstrated, which suggests an important influence of hereditary factors in the control of their serum levels. As for the changes in hormonal levels in response to VLCD, a significant correlation was observed only between the increase in serum testosterone and decrease in fat free mass.

ii) Patients with mother obese or both parents obese had significantly higher fasting RQ than patients without family history of obesity, which supports the influence of genetic factors on the ability to oxidize fat. High fasting RQ during the treatment of obesity by VLCD represented an increased risk of weight regain and weight cycling.

iii) Starvation resulted in differential responses of dorsolumbar and epididymal fat depots. Metabolic changes were more pronounced in epididymal fat, while weight loss in dorsolumbar fat. These changes were accompanied by corresponding activation of AMPK. AMPK thus could contribute to regional differences in the metabolic properties of adipose tissue depots. Four times higher stimulation of UCP2 expression in epididymal fat could be related to the AMPK activation.

iv) Addition of n-3 PUFA to VLCD caused a significantly higher decrease of weight, BMI and hip circumference as well as a significantly higher stimulation of ketogenesis.

v) We did not confirm the expected association between the amount of consumed vitamin A, and RMR and fasting RQ in obese patients.

vi) The administration of vitamin A to obese patients caused significant increase in RMR when expressed per kilogram FFM. No change was observed in fasting RQ. On the metabolic level, UCP2 expression tended to increase and FAS expression tended to decrease in subcutaneous white adipose tissue, but the results were not statistically significant.

vii) Changes in calcium intake during the weight reduction program were negatively related to the body weight change.

viii) Based on this study, psychobehavioral and nutritional characteristics could be used as predictors of weight loss.

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6. ABBREVIATIONS

AMPK	AMP-activated protein kinase
BDI	Beck Depression Inventory
BMI	body mass index
DHA	docosahexaenoic acid
DHEA	dehydroepiandrosterone
DHEA-S	dehydroepiandrosterone sulphate
EI	Eating Inventory
EPA	eicosapentaenoic acid
FAS	fatty acid synthase
FQ	food quotient
NEFA	non-esterified fatty acids
PEPCK	phosphoenolpyruvate carboxykinase
PUFA	polyunsaturated fatty acids
RMR	resting metabolic rate
RQ	respiratory quotient
RT-PCR	reverse transcription polymerase chain reaction
SHBG	sex hormone-binding globulin
SREBP-1	sterol regulatory element-binding protein-1
UCP	uncoupling protein
VLCD	very low calorie diet
WAT	white adipose tissue

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