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EFFECTS OF HEALTH-PROMOTING NUTRITIONAL EDUCATION AND CHANGE IN DIETARY HABITS ON VISCERAL FATTY TISSUE CONTENTS AND ON CONCENTRATIONS OF INSULIN AND CORTISOL IN MENOPAUSAL WOMEN

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Key words: obese women, visceral fat, cortisol, insulin, hepatic steatosis

The experiment was aimed at determining effects of health-promoting nutritional education and changes in dietary habits of menopausal women on their visceral fatty tissue contents and concentrations of insulin and cortisol.

A four-month-long period of nutritional education resulted in a reduction of dietary energy content, total protein consumption (including uptake of animal proteins), total fat (including saturated fats), cholesterol, and sucrose. On the other hand, an increase was recorded in the consumption of plant protein, starch, dietary fibre, vitamin B_6, PP and beta-carotene as well as mineral components (Ca, Mg, and Zn). Those changes were accompanied by a slow, but steady, reduction of body weight (by 7.85±1.81 kg throughout the period of study, *i.e.* 0.49±0.11 kg per week) caused by the loss of both subcutaneous (0.38±0.15 cm, abdominal region, as measured at the L4 vertebra level) and visceral (1.0±0.56 cm, as measured at the L4 vertebra level) fatty tissue. The changes were accompanied by a reduction in BMI (from 33.2±4.4 to 29.9±4.3) and WHR (from 0.87±0.09 to 0.84±0.07).

The 4-month-long period of changed nutrition was found to significantly reduce blood concentrations of glucose (from 104.8±27.3 to 82.7±8.6 md/dL), insulin (from 180.1±51.1 to 123.4±58.2 pmol/L), and cortisol (from 548.2±186.3 to 368.1±87.3 nmol/L); the women affected by hepatic steatosis exhibited arrested fatty infiltrations, as evidenced by increased liver electron density (from 33.7±6.7 to 47.3±4.7 H.u.).

INTRODUCTION

It is well-known at present that the fatty tissue, particularly that deposited in the abdominal cavity, with is metabolically very active, its triacylglycerols constantly releasing fatty acids that affect various functions of the body [Bjorntorp, 1997a; Vallette *et al.*, 1991].

Numerous authors have shown the body fat content, including the intra-abdominal fat, to increase with age [Beaufrere & Morio, 2000; Ito *et al.*, 2001]. The resultant metabolic implications of the increase may be important for insulin resistance, diabetes type 2, and/or for cardiac-vascular disorders [Beaufrere & Morio, 2000; Bjorntorp, 1996, 1997b; Tessari, 2000].

The long-term health-promoting nutritional education, carried out by the present author among menopausal women has resulted in a change in the subjects' dietary habits that involved, *i.a.* body weight loss (averaging 6.0–12.5 kg over 4 months) and a reduction in both the Body Mass Index (BMI) and Waist/Hip Ratio (WHR). In this context, it was deemed desirable to find out which type of fatty tissue (subcutaneous or visceral) was reduced and to what extent.

MATERIALS AND METHODS

With the approval of the Local Bioethics Commission, the study involved 24 women aged 47–53 years, the BMI values of which ranged from 29.9 to 44.6.

The women volunteered to participate in a 4-month-long health-promoting nutritional-awareness course that spanned from October until the end of January. Classes (two 45-min lessons) were held once a week. The curriculum covered information on the fundamentals of alimentary tract function and the resultant dietary recommendations; the role in physiology, sources of, and demand for basic nutrients, vitamins, micro- and macroelements; the importance of water uptake for the alimentary system and for the total water budget of the body; acid-alkali equilibrium in nutrition; nutrition-based prophylaxy of heart disease, insulin-independent diabetes, and cancer (including diet-dependent cancer, with a particular attention to colon and breast cancer). The classes focused also on numerous other aspects of human physiology, nutrition physiology, dietetics, and correct meal arrangement and preparation.

In the second week of the course, the subjects' diets were analysed in detail and modified for each women individually, the modifications involving timing, magnitude, and type of meals and adjustment of various nutrients to individual demands. It was stipulated that daily protein consumption amounted to 0.8 g/kg body weight current, ±30% being supplied with animal protein; due to the second and third degree obesity among the women, it was recommended that fats contribute not more 25%, but not less than 20% of the daily food energy content and that carbohydrates account for 60–65% of the daily food energy content. Diet composition was changed by substituting poultry and fish for fatty

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meat and meat products, low-fat cottage cheese and yoghurts for fatty dairy products (hard cheeses, spread cheeses, fatty yoghurts, *etc.*). Sweetened dairy products (*e.g.* flavoured yoghurts and spreads) were substituted with natural ones; candies and sucrose-containing products were substituted with increased amounts of coarse grits, pasta, brown rice, beans, pea, whole-flour bread, *etc.* It was made sure that the diets contain natural sources of choline, involved in mobilisation and transport of neutral fats, particularly from the liver. It was also stipulated that the diets contain higher amounts of vegetables and seasonal native fruits, particularly apples. The patients were recommended to drink more fluids in the form of clean, boiled water in place of 3–4 cups of coffee or sweetened and sparkling soft drinks. All the recommendations that involved both the principles of nutrition physiology and individual needs of each subjects, were in agreement with the programme of food consumption and nutrients for the inhabitants of Poland for 2000 [Sekuła *et al.*, 1991] and with nutrition standards for the inhabitants of Poland [Ziemlański *et al.*, 1995]. No attempt was made to touch a subject on reducing the amount of calories in the subjects' diet due to two reasons. Firstly, the trained women had repeatedly and unsuccessfully employed low-caloric diets and this produced bad mental condition in them. Secondly, it was found that caloricity of diet gets reduced almost automatically with its correction, and with changes in sourcing essential nutrients as well as with changes in food preparation.

Methods. Information on diets and dietary habits of the subjects was collected twice. Having received appropriate instruction, the women recorded the timing, type, and amount of food they consumed on 3 randomly selected days (24-h periods) of the week. The written records were supplemented with information obtained from interviews with each subjects. The diets were recorded in early October; once the diet was modified and a few basic principles adopted, another set of records was obtained in early December. The food ration amount was assessed according to the "Album of photographs of food products and dishes" [Szponar *et al.*, 2000]. The data were processed with a computer software Dietetyk® ver. 2.5, manufactured by JuMaR.

The blood for assays was collected from fasted subjects at the Endocrine Laboratory of the Independent Public Hospital in Szczecin before and after the 4 months of education, before 7.30 and 8.30 a.m. The following assays were performed: glucose (enzymatic colorimetric method of Tietz, [1995], in an Integra closed-system biochemical analyser, using Roche reagents); cortisol and insulin (radioimmunological technique, using a reagent kit manufactured by the POLATOM Isotope Research and Development Centre, Świerk, Poland).

The thickness of a fold of skin and subcutaneous fat layer was measured with a special device over the brachial biceps muscle, brachial triceps muscle, underneath the scapula's inferior angle, and over the iliac ala in the medio-axillal plane.

The subjects were weighed, once a week, on a medical scales, each time in an identical way. The BMI values were calculated from the formula: body weight (kg)/[height (m)]^2; the WHR values were calculated as a ratio of the waist to hip circumferences. During the course, the participants did not change their physical activity level.

Fat tissue thickness was measured tomographically, having secured subjects' written consent, in 18 women affected by various stages of hepatic steatosis who would have been subjected to it, on their own cost, during the routine medical check-up.

Liver electron density as well as thickness of the intra-abdominal and subcutaneous fat layer (in the anterior abdominal cavity wall) were measured before and after the nutrition course at the Image Analysis Diagnostic Laboratory of the Independent Public Hospital in Szczecin, with a Somatom Plus 4 Expert computer tomographs; four 1 mm-thick profiles at the L4 vertebra level were taken.

The state of the liver was assessed from its electron density and expressed in Hounsfield units (H.u.).

Thickness (cm) of the intra-peritoneal fat was calculated from the difference between the thicknesses of layers as measured from the external surface of the subcutaneous fat in the anterior wall of the abdominal cavity to the L4 vertebra and from the internal surface of the fat layer to the L4 vertebra.

Data treatment. The data were subjected to statistical treatment (paired variables test) with the computer Statistica® software.

RESULTS

As analysed before modification, the mean energy content of the daily food ration was clearly higher than that recommended by nutritional standards [Ziemlański *et al.*, 1995]. It was associated with an excessive intake of animal proteins, sucrose, total fats (including saturated fats) and cholesterol (Table 1) and was reflected in distribution of energy contributed by individual nutrients (Table 2). The modified diets were found to be lower in energy content and fat contribution to it, the contribution of complex carbohydrates being increased. The uptake of vitamins, mineral components, and fluids changed for the better as well. The change in the dietary energy content and in proportions of basic nutrients resulted from altered amount, type, and source of nutrients consumed. A statistically significant increase in the consumption of grain and dairy products, potatoes, vitamin C and beta-carotene-containing fruits and vegetables, and pulses was recorded. On the other hand, the intake of eggs, meat, meat products, sugar and candies was significantly reduced (Table 3).

The reduction in body weight of the course participants, steady but varying in intensity between the women, was reflected in the changes of their BMI and WHR (Table 4).

Due to the change in body "texture", resulting from the loss of the subcutaneous fatty tissue, changes in the skin-fat fold thickness were not analysed. The fold thickness after the experiment was higher than that before it. On the other hand, the comparison of intra-peritoneal and subcutaneous fat layers measured at the L4 vertebra level evidenced not only a statistically significant dietary effect, but also a significantly higher loss of intra-abdominal fat (by 1.0±0.56 cm), compared to the loss of the subcutaneous fat (by 0.38±0.15 cm). The changes were accompanied by a significant ($p<0.01$) reduction in concentrations of glucose, cortisol, and insulin (Table 5).

An analysis of the liver electron density showed the parameter, as measured prior to the experiment, to be within the range of standard values (50–60 H.u.) in as few as

TABLE 1. Energy value and basic nutrients levels in daily food rations of women before and after the nutritional education ($\bar{x}$±SD, n = 72).

Components	Before education	After education
Energy (kJ)	10412±1952	9245±867*
(kcal)	2603±488	2311.3±216.8
Total protein (g)	92.8±22.3	74.2±18.3*
Animal protein	64.0±28.6	31.6±17.1**
Plant protein	28.8±7.5	42.6±9.2**
Total carbohydrates (g)	314.8±59.2	336.2±51.4
Starch	192.2±64.6	278.1±52.1**
Sucrose	110.2±18.8	31.3±6.2**
Lactose	12.4±6.4	26.8±5.1**
Dietary fibre (g)	20.8±8.3	41.6±16.3**
Total lipid (g)	96.9±23.6	63.9±18.6**
Saturated fatty acids	71.5±22.6	33.7±17.9**
Unsaturated fatty acids	25.4±19.3	30.2±18.3*
Cholesterol (mg)	383. 7±118.4	198.4±78.5
Vitamins:		
Beta-carotene (mg)	3.72±3.12	5.87±1.42*
E (mg)	10.1±5.0	10.9±2.1
C (mg)	85.6±51.1	82.4±21.9
B6 (mg)	1.41±0.41	2.08±0.49**
PP (mg)	11.25±3.87	15.16±4.12**
Mineral components:		
Ca^{++} (mg)	560.0±350.0	810.0±301.0**
Mg^{++} (mg)	216.45±77.54	320.6±65.4**
Zn^{++} (mg)	8.98±3.24	12.3±4.7*

*,** difference significant at p=0.05 and p=0.01

TABLE 2. Per cent contribution of protein, carbohydrates, and lipids to the total energy content of daily food rations of women before and after the nutritional education (n = 72).

Component	Before education	After education
Protein	15.0	13.5
Carbohydrates	50.8	61.1
Lipids	34.2	25.4
Saturated	25.3	13.4
Unsaturated	8.9	12.0

3 out of the 18 women tested; 11 subjects showed a higher density, the value being significantly lower than the standard in 4 women (diagnosed with hepatic steatosis). After 4 months of following a modified diet, favourable changes in the liver electron density were recorded in 14 women. All the women diagnosed with hepatic steatosis showed a significant increase in the electron density (still, however, below the standard), while the density significantly decreased to the standard range in 10 subjects (Table 6). Over the period of the course, the women did not adhibit any medicines or vitamins and minerals components supplementation.

DISCUSSION

The results concerning effects of the 4-month-long health-promoting nutritional education in obese women showed a number of nutritionally-beneficial changes, described in detail in earlier papers [Friedrich, 1997, 1998].

TABLE 3. Consumption of the selected groups of products by women before and after the nutritional education ($\bar{x}$±SD, n=72).

Component	Before education	After education
Cereals (g) [1)]	152.5±108.5	301.5±64.5**
Dairy products (g) [2)]	180.0±122.0	600.0±85.5**
Eggs (g)	21.0±18.0	20.0±5.5
Meat and sausages (g) [3)]	280.0±151.0	75.0±20.5**
Butter and cream (g) [4)]	45.0±24.5	10.5±5.5**
Other fats (g)	36.0±45.0	35.0±8.0
Patatoes (g)	51.0±52.0	150.0±35.0**
Vitamin C-containing fruit and vegetables (g)	150.0±126.0	310.0±110.0**
Beta-carotene-rich fruit and vegetables (g)	60.0±82.5	108.0±42.5*
Other fruit and vegetables (g)	102.0±61.5	750.0±50.0**
Pulses (g)	0.7±1.2	28.3±5.0**
Sugar and sweets (g) [5)]	108.0±79.0	15.0±3.5**

[1)] converted to flour; [2)] converted to milk; [3)] converted to meat with bones; [4)] converted to butter; [5)] converted to sugar; *,** – difference significant at p=0.05 and p=0.01

TABLE 4. Changes in body weight, BMI, and WHR of women exposed to 4-month-long health-enhancing nutritional education ($\bar{x}$±SD, n=24).

Index	Before education	After education
Body weight loss (kg)		7.85±1.81
Body weight loss rate (per week)		0.49±0.11
BMI	33.2±4.4	29.9±4.3
WHR	0.87±0.09	0.84±0.07

TABLE 5. Effects of health-promoting nutritional education and changed dietary habits on concentrations of glucose, insulin, and cortisol and on thickness of subcutaneous and intra-abdominal fat layer ($\bar{x}$±SD).

Index	n	Before education	After education
Glucose (mg/dL)	24	104.8±27.3	82.7±8.6**
Insulin (pmol/L)	24	180.1±51.1	123.4±58.2**
Cortisol (nmol/L)	24	548.2±186.3	368.1±87.3**
Subcutaneous fat (cm)	18	3.05±0.57	2.67±0.59**
Intra-abdominal fat (cm)	18	11.0±1.69	10.0±1.53**

** – difference significant at p=0.01

TABLE 6. Effects of health-promoting nutritional education and changed dietary habits on liver electron density ($\bar{x}$±SD).

Index	n	Before education	After education
Liver electron density (H. u.)	14	62.5±2.5	57.2±2.4**
Liver electron density (H. u.)	4	33.7±6.7	47.3±4.7**

** – difference significant at p=0.01

Generally, the changes involved a reduction in dietary energy uptake, an appropriate distribution of energy among various basic nutrients, an increased consumption of vegetables, fruits, and non-processed complex carbohydrates, which was reflected in an increased uptake of vitamins, minerals, and dietary fibre. The uptake of fluids increased as well.

The reduction in dietary energy content and composition, obtained as a result of the education, produced a slow albeit steady reduction in the subjects' body weight, associated with the low content of subcutaneous and visceral fat tissue. This was directly correlated with the reduction in BMI and WHR. Hence, not only did the total amount of the subjects' body fat decrease, but their hormone-metabolic status improved. As shown by a study of Krotkiewski *et al.* [1983] on obese women, insulin release is proportional to BMI, while insulin removal by the liver is inversely correlated with WHR.

The process of body weight loss during the 4 months of education proceeded at various pace and produced varying results; it was primarily related to: 1) the magnitude and type of changes in incorrect dietary habits (*e.g.*, restriction of sugar uptake from 12 to 2 spoonfuls; reduction of butter consumption, butter still being regarded by the women as the best vitamin A source; increased vegetable consumption); 2) reduction in the dietary energy uptake; 3) a change in mental attitudes and motivation behind starting the education and its practical implementation.

The body weight loss rate, particularly during the first two months, was related to adoption of various dietary recommendations and their individual effects on the women [Friedrich, 1997a]. During the two subsequent months, the rate of the body weight loss depended on observance of the recommendations, but also with numerous metabolic pathways reverting to their normal state as a result of a higher consumption of water, vitamins, minerals, and other regulating agents (*e.g.* dietary fibre) and due to changes in sources of and proportions between the basic nutrients.

Considering the body weight loss, the location of the fat tissue lost seems to be more important than the actual amount lost. This is related to the faster rate of metabolic transformations in visceral fat adipocytes than in the subcutaneous adipocytes, and the effects of the difference [Bjorntorp, 2000].

Lipogenesis in the body involves primarily transformations of glucose and transient compounds such as pyrogronate, lactate, and acetyl-CoA into fat. The entire lipogenesis is controlled by the nutritive status, and also by the actual composition of the diet. In the women subjected to this study, of a key importance was the excess of fat which, particularly at an excessive dietary energy, is always – as demonstrated by Bjorntorp [1996] – deposited in the visceral fatty tissue. Also of importance is the uptake of excess sucrose and easily assimilated starch originating from refine and processed products. Because they are rapidly digested and due to release of high glucose amounts, the dietary components mentioned enforce the release of insulin that activates acetyl-CoA carboxylase responsible for carbohydrate transformation into acetyl-CoA. The process is enhanced by cortisol, released in response to an increase in insulin concentration and inhibiting glucose utilisation by muscles. The reduction in glucose, insulin, and cortisol concentrations, observed in the experiment as an effect of diet modification, could have affected the slowdown in the rate of those transformations.

Fatty tissue deposition control is a complex problem. As demonstrated by physiological and clinical studies, it proceeds at the cellular and molecular level. In terms of physiology, the site of fatty tissue deposition is decided upon by sex hormones. Because of that, during the menopause, when the oestrogen concentrations decrease, also the women show increased deposition of fat around the waist, which is otherwise typical of men [Bjorntorp, 1997b]. However, excessive accumulation of fatty tissue, particularly its visceral type, is primarily associated with an increased release of cortisol which stimulates lipid accumulation pathways and is responsible for redistribution of fat to the perivisceral tissue [Bjorntorp, 1997a], and with increased release of lipopoietic insulin [Bjorntorp, 1997a; Tessari, 2000]. This would be supported by the author's earlier studies [Friedrich, 1997] that showed the normalisation of insulin and cortisol concentrations in the blood of obese women to be more conducive to the normalisation of WHR than the absolute body weight loss. In the present experiment, this contention is supported by the higher (by the factor of about 2.5) visceral fatty tissue loss, compared with the loss of the subcutaneous fatty tissue.

The type of fatty tissue lost could have been also significantly affected by an increased consumption of vitamins and minerals, both those having antioxidant properties and those directly involved in carbohydrate-lipid metabolism. Masini *et al.* [1994] are of the opinion that increased deposition of perivisceral fatty tissue is frequently correlated with weakened aerobic metabolism; once that is improved, the rate of oxidative phosphorylation in mitochondria increases and the amount of perivisceral fatty tissue is clearly reduced. Zemel [2002] demonstrated dietary calcium deficiency to stimulate calcitriol release, thus increasing the influx of calcium ions to cells, lipogenesis rate, and lipid accumulation, not only in the perivisceral fatty tissue, but also in the muscle tissue. This finding is also supported by the most recent results reported by Parkish & Yanowski [2003]. A similar effect was observed by Okada *et al.* [1997] in vitamin B (particularly B_6,) deficiencies; vitamins of the B group participate in glucose metabolism as co-enzymes. On the other hand, Sano *et al.* [1998] showed dietary zinc deficiency to result in hepatic glucose synthesis and glucose transformation into fatty acids in the subcutaneous fatty tissue.

The processes studied could have been also affected by the increased consumption of dietary fibre. Long-term observations demonstrated pectins and guar gums present in the modified diets in, *i.a.* pulses and fruits, to flatten the glycaemic and insulin responses as a result of prolonged starch digestion and glucose absorption from the intestine [Frantz, 1993]. It seems also important that the complex carbohydrates in the modified diets originated in coarse grits, non-husked rice, peas, beans, whole-flour bread, and vegetables, *i.e.* the so-called low glycaemic index products.

An analysis of changes in the liver electron density showed the density to increase, after the 4 months of modified nutrition, in all the women tested which were affected by hepatic steatosis. That would be clearly indicative of arrested fatty infiltration (a medical diagnosis) and normalisation of liver function.

Hepatic steatosis results from disturbed equilibrium between triacylglycerol formation rate and export. Lipids can be accumulated in the liver for a number of reasons [Lieber, 1991]. As serum lipoprotein formation was metabolically blocked in none of the women and none of them was chronically alcoholic, hepatic steatosis emerged as a result of excessive fat consumption and could have been enhanced by the history of drastic slimming dieting.

Considering the problems involved in measurement of skin-fat fold measurements in the subjects, it has to be acknowledged that such measurements are generally largely biased. In their study on young women with BMI ranging from 16.5–32.5 kg/m^2, Kitano *et al.* [2001] demonstrated that, comparing with bioimpedance and dual X-ray energy absorptiometry, the measurement of skin-fat fold thickness in the assessment of body composition suggested the lowest fat content and was the most biased technique.

CONCLUSIONS

Analysis of the results obtained allows concluding that health-promoting nutritional education for obese menopausal women significantly changed their dietary habits. In consequence, in addition to improved body functions and enhanced feeling of well-being, the subjects showed:

1. a slow but steady body weight loss, resulting from the loss of not only the subcutaneous, but also visceral fatty tissue;
2. a significant reduction in blood insulin and cortisol concentrations;
3. fatty infiltration arrest in the hepatic steatosis-affected subjects.

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WPŁYW PROZDROWOTNEJ EDUKACJI ŻYWIENIOWEJ I ZMIAN NAWYKÓW ŻYWIENIOWYCH NA ZAWARTOŚĆ WISCERALNEJ TKANKI TŁUSZCZOWEJ ORAZ STĘŻENIA INSULINY I KORTYZOLU U KOBIET W OKRESIE MENOPAUZY

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W przeprowadzonym doświadczeniu badano wpływ prozdrowotnej edukacji żywieniowej i zmian nawyków żywieniowych na zawartość wisceralnej tkanki tłuszczowej oraz stężenia insuliny i kortyzolu u kobiet w okresie menopauzy.

Stwierdzono, że czteromiesięczna edukacja żywieniowa spowodowała obniżenie wartości energetycznej diety, obniżenie spożycia białka ogółem, w tym białka zwierzęcego, tłuszczów ogółem w tym tłuszczów nasyconych, cholesterolu i sacharozy. Wzrosło natomiast spożycie białka roślinnego, skrobi, błonnika pokarmowego, witamin B_6, PP i beta-karotenu oraz składników mineralnych Ca, Mg i Zn (tab. 1). Zmianom tym towarzyszyła powolna ale systematyczną redukcja masy ciała oraz obniżanie się wartości wskaźników BMI i WHR (tab. 4), spowodowane utratą nie tylko podskórnej ale też wisceralnej tkanki tłuszczowej (tab. 5). Stwierdzono, że czteromiesięczna poprawa sposobu żywienia istotnie wpływała na obniżenie stężenia glukozy, insuliny i kortyzolu we krwi (tab. 5), a u kobiet z objawami stłuszczenia wątroby, cofanie się nacieczenia tłuszczowego czego objawem był wzrost elektronowej gęstości wątroby (tab. 6).